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Acute cortisol response to a psychosocial stressor is associated with heartbeat perception

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Abstract

The aim of the present study was to examine the effect of an acute increase in cortisol in response to a psychosocial stressor on heartbeat perception, in a laboratory environment. Thirty-six participants (20 women, 16 men, mean age = 21.7 years, standard deviation = 1.7 years) completed a heartbeat counting task (Schandry paradigm) before and after exposure to an acute psychosocial stressor (Trier Social Stress Test; TSST). Heartbeat counting performance was compared between participants who exhibited strong cortisol responses (>15.5% increase in cortisol from baseline; responders) and those who did not (non-responders). Responders showed increased heartbeat counting accuracy following the TSST, which was not observed in non-responders. The two groups did not differ in their responsivity to subjective anxiety ratings or heart rate. These results indicated that acutely elevated cortisol in response to a psychosocial stressor is associated with increased interoceptive accuracy. The results provide a possible explanation for inconsistent findings on the effect of stress exposure on interoception.

Keywords : Interoception, Stress, Heartbeat perception, Cortisol, Anxiety

1. Introduction

Interoception refers collectively to the processing of internal bodily stimuli by the nervous system (Khalsa et al., 2018). It has been postulated to play an important role in the subjective experience of emotion (Critchley and Garfinkel, 2017). More specifically, physical arousal plays a pivotal role in the formation of anxious feelings; therefore, interoception, particularly regarding heartbeat, has been suggested to be significant in the pathogenesis of anxiety disorders (for a review, see Domschke et al., 2010). Enhanced interoceptive accuracy in anticipation of public speaking has been observed among individuals with high levels of social anxiety (Stevens et al., 2011; Durik et al., 2014) and may cause the spurious belief that their arousal is visible to others. In addition, enhanced interoceptive accuracy has been observed among individuals with panic disorder (Ehlers et al., 1988; Ehlers et al., 1995) and may play a role in inducing panic attacks. However, it has also been suggested that the increased interoceptive accuracy in anxiety disorders is rather equivocal (e.g., Van der Does et al, 2000). When trying to understand these equivocal findings, we should consider that most studies have mainly focused on differences in interoception among individuals with and without anxiety symptoms. Considering the

substantial heterogeneity even within the same disorder (e.g., Roberson-Nay and Kendler, 2011), the mechanism underlying changes in interoception in individuals with anxiety symptoms remains unclear, and further investigation is needed. Specifically, investigation of the biological basis of interoception changes in response to anxiety-provoking situations (stressors) would complement previous findings and provide an insight into this mechanism.

There is substantial, but not conclusive, evidence that exposure to psychosocial stressors affects interoceptive accuracy (for a review, see Schulz and Vögele, 2015). A specific way that stress might enhance interoceptive accuracy is *via* sympathetic activation. Interoceptive accuracy is enhanced following physical exercise (Richards et al., 1996), and positive correlations are observed between interoceptive accuracy assessed by Schandry's paradigm (1981), which requires participants to count their own heartbeat and cardiovascular sympathetic responses (heart rate, systolic blood pressure, cardiac output, and pre-ejection period) following physical exercise (Pollatos et al., 2007a). Furthermore, pharmacological manipulation (isoproterenol infusion) of sympathetic activation is associated with increased cardiorespiratory sensations (Hassanpour et al., 2016), and sympathetic activation engages interoceptive circuitry in the brain, including the insula (Hassanpour et al, 2018). One study has examined the effect of anticipation of public speaking using the heartbeat counting method and showed that interoceptive accuracy is enhanced in anticipation of public speaking when compared with the resting state (Schandry and Specht, 1981). However, Fairclough and Goodwin (2007) directly examined the effect of psychological stressors on interoceptive accuracy, by using a paradigm from Whitehead et al. (1977), which requires participants to discriminate between synchronous (true) and asynchronous (false) feedback of the heartbeat, and by manipulating a psychological state (mental arithmetic *vs*. yogic breathing). The authors found that interoceptive accuracy was not enhanced by stress; in fact, perception decreased following stress in women. Another study examined the effect of the socially evaluative cold-pressor test on interoceptive accuracy, using both Schandry and Whitehead tasks. Interoceptive accuracy was enhanced when assessed with the Schandry task, and diminished following stress testing using the Whitehead task (Schulz et al., 2013a). The heterogeneity of methods to induce stress in the laboratory and assess interoceptive accuracy may be responsible for mixed findings and illustrate the necessity to reveal the underlying psychobiological mechanisms.

One possible explanation for the inconsistent findings could be that sympathetic activation alone does not fully explain the effects

of psychosocial stress on interoceptive accuracy. Another stress response system, the hypothalamic-pituitary-adrenal (HPA) axis, might also play an important role. The HPA axis reacts at three levels when a stressor is encountered. First, corticotropin releasing factor (CRF) is released from the hypothalamus into the bloodstream, and induces the release of adrenocorticotropic hormone (ACTH) by the pituitary gland. Second, in humans, ACTH acts on the adrenal cortex to increase the release of cortisol. Third, cortisol may inhibit the release of both CRF and ACTH, thus constituting a negative feedback loop. In this process, cortisol may also affect human information processing. Recent findings that cortisol rapidly affects thalamic activity suggest its impact on sensory information processing is mediated by non-genomic mechanisms (Strelzyk et al., 2012). Specifically, previous findings suggest that interoceptive information is relayed *via* the thalamus (Cameron, 2001; Critchley et al., 2004; Pollatos et al., 2007b). Considering these findings, cortisol could modulate interoceptive accuracy.

An effect of cortisol on interoceptive accuracy has been inferred from the study by Schulz et al. (2013b) who examined the effects of cortisol administration on heartbeat evoked potentials (HEP), a psychophysiological indicator of cortical processing of cardioceptive signals derived from electroencephalogram and electrocardiogram (ECG) analyses. The authors found that when eyes were open, increased HEP amplitudes were observed between 1 and 17 min after cortisol administration. However, this study had several limitations, including the fact that the generalizability of the results to active processes of body perception was unclear, and that there was no concomitant stress exposure. Indeed, exaggerated concerns associated with interoception, often observed in relation to stressful situations, would reflect active processes of body perception. Thus, to clarify these previous findings, we examined the effect of stress-induced cortisol on interoceptive accuracy using a heartbeat tracking task. We hypothesized that an enhancement in interoceptive accuracy would be observed in individuals who exhibit clear cortisol responses to a psychosocial stressor but not in those with no clear cortisol responses.

2. Methods

2.1 Participants

To determine a sufficient sample size for examining our primary hypothesis (i.e., an effect of cortisol on heartbeat perception), we

conducted an a priori power analysis using G*power 3.1. We aimed to achieve 80% statistical power for a medium effect size (Cohen's $f = 0.25$, $\eta 2 \approx 0.06$) on a two-way mixed ANOVA. The power analysis found that 34 participants in total were required. To meet this sample size requirement, 36 Japanese university students were recruited (20 women, 16 men, mean age = 21.7 years, standard deviation = 1.7). This sample size was larger than or comparable to those of previous studies examining the effects of stress-induced cortisol responses (e.g., Roelofs et al., 2005; Roelofs et al., 2007; Tsumura and Shimada, 2012; Tsumura et al., 2015). Participants were recruited through advertisements on the university campus. Individuals were deemed ineligible if they met any of following criteria: (a) current illness or physical disease; (b) history of a diagnosed psychiatric disorder; (c) stressful experiences just prior to the experiment; (d) history of smoking; (e) use of medications that could affect cortisol responses (such as oral contraceptives or β-blockers); (f) suffering from severe sleep disturbance or fatigue; and (g) irregular menstruation (for women). Women also provided menstrual phase information on the day of the experiment. The breakdown for the menstrual phase for women when participation was following: 30.0 % for early follicular phase, 10.0 % for late follicular phase, and 60.0 % for late luteal phase, according to coding criteria in Duffy et al. (2017). Participants were asked to refrain from vigorous exercise, alcohol, caffeine, and food for 1 hour before study participation. All participants provided written informed consent and were told they could withdraw from the study at any time. Participants were compensated for their participation with a book coupon worth 1500 Japanese yen. The study was approved by a local ethics committee and conducted according to the Declaration of Helsinki.

2.2 Psychological assessments

We assessed subjective state anxiety during the experiment using the visual analog scale. Anchor values of zero and 100 were defined as "not at all" and "extremely" anxious, respectively. Because previous studies have reported that social anxiety symptoms, negative beliefs about social evaluation, and depressive symptoms (Dunn et al., 2007; Stevens et al., 2011; Durlik et al., 2014) affect interoceptive accuracy, we assessed these variables in our participants. Levels of social anxiety were assessed by two measures, the Social Phobia Scale (SPS; Mattick and Clarke, 1998) and the Social Interaction Anxiety Scale (SIAS; Mattick and Clarke, 1998). The SPS and SIAS assess fear associated with performing in public and with specific social interactions, respectively. Each consists of 20 items rated on a

five-point Likert-type scale (total range: 0–80). Levels of negative beliefs regarding social evaluation were assessed with the Self-Beliefs related to Social Anxiety scale (SBSA; Wong and Moulds, 2011). The SBSA consists of 15 items rated on an eleven-point Likert-type scale and includes three subscales: high standard beliefs (e.g., "I have to get everyone's approval"; range: 0–40), conditional beliefs (e.g., "If I make mistakes, others will reject me"; range: 0–70), and unconditional beliefs (e.g., "People think I am inferior"; range: 0–40). The levels of depressive symptoms were assessed using the Center for Epidemiologic Studies Depression scale (CES-D; Radloff, 1977), a self-reported measure designed to assess depressive symptomatology within the general population, consisting of 20 items rated on a four-point Likert-type scale (total range: 0–60). We used the Japanese translated and validated versions of the SPS (Kanai et al., 2004), SIAS (Kanai et al., 2004), SBSA (Maeda et al., 2017), and CES-D (Shima et al., 1985).

2.3 Interoceptive accuracy

To assess interoceptive accuracy, participants performed the Schandry heartbeat counting task (Schandry, 1981). We included the task's instructions in a handout with illustrations and confirmed that participants understood them before starting the task. During the task, participants counted how many heartbeats they felt over a period of time. This result was compared to the actual number of heartbeats measured. Throughout the assessment, participants were not allowed to take their pulse or perform other strategies, such as holding their breath. Further, participants were instructed not to guess their heartbeat, even if they could not feel it, and to report zero heartbeats in such case. Participants completed six heartbeat counting trials (twice, each for 25 s, 35 s, and 45 s), in a random order, before and after the Trier Social Stress Test (TSST). The actual heartbeats in each trial were recorded using a pulse oximeter (IWS920, I.W. Technology Firm, Inc.), placed on the participant's left index finger. Pulse oximetry has previously been used to monitor the actual heartbeat in heartbeat detection paradigms (e.g., Critchley et al., 2004; Terasawa et al, 2015; Betka et al., 2018), as well as in ECG. We used a soft finger sensor to avoid any pulsatile sensation at the fingertip, as in previous studies (e.g., Betka et al., 2018). To ensure that heartbeat counting was not confounded by ability to estimate time, participants also completed a time estimation task at the end of the experiment, consisting of three trials (for 25 s, 35 s, and 45 s) in which the participants estimated the duration of each trial.

2.4 Cortisol levels

Participants were asked to salivate for 2 min and drool into a specimen tube through a 4-cm long straw (passive drooling). The saliva samples were frozen at temperatures below –20 °C until further analysis. Salivary cortisol levels were measured by enzyme-linked immunoassay using a commercial kit from Salimetrics (State College, PA, USA). The inter-assay coefficient of variation across all assays was 3.9%, and the intra-assay coefficient of variation was 7.6%.

2.5 Procedure

All testing was performed in the afternoon (between 1 and 6 pm) to control for circadian variation in cortisol activity. We used a standard acute psychosocial stress test, the TSST, in which participants were required to deliver a speech and perform mental arithmetic in front of two audiences (Kirschbaum et al., 1993).

Figure 1 shows the outline of the experimental procedure. At the beginning of the experiment, participants provided written informed consent. Next, participants completed psychological assessment questionnaires, which took approximately 10 min. Participants then remained seated in a quiet room for 10 min to control for any potential confounds before initial cortisol sampling. After the baseline assessment, participants completed the Schandry task. Next, they were given instructions for the TSST. After preparing for a public speech for 10 min, participants delivered the speech for 5 min and then performed a mental arithmetic task for 5 min. Following the TSST, participants were given an additional 10 min to rest. Finally, they completed the Schandry task again and finished the experiment. This design was based on previous studies reporting that neural effects are first observed approximately 10–20 min after glucocorticoid administration (Makara and Haller, 2001). Further, as the heart rate is expected to recover to nearly the resting level within 10 min following the TSST (e.g., Kimura et al., 2013), any possible confounding effect of heart rate elevation due to stress exposure would be minimized at this time point. Throughout the testing period, participants refrained from eating and drinking anything other than a small amount of water. Saliva collection and assessment of state anxiety were conducted at four time points: baseline (T0), after speech preparation (T1), just after the TSST (T2), and after a 10-min rest following the TSST (T3). Accordingly, the post-stress measurement was conducted approximately 15 min after TSST offset, following a 10-min rest and two times of saliva sampling.

2.6 Statistical analyses

Cortisol values were log-transformed before statistical analysis to minimize skewness. To examine the effect of cortisol response on heartbeat counting accuracy, we classified participants into two groups: responders and non-responders. According to criteria proposed by Miller et al. (2013), we defined responders as participants whose largest response in cortisol values at T1, T2, or T3 was at least 15.5% higher than the value at baseline (T0). Non-responders were defined as participants who did not show these changes. To ensure that the TSST successfully served as a socially evaluative stressor, we conducted a 2 (Group: responders, non-responders) × 4 (Time: T0, T1, T2, T3) mixed design ANOVA on subjective anxiety. If the assumption of sphericity was not met for repeated measures analyses, the Greenhouse-Geisser correction for non-sphericity was applied.

Using a formula presented by Durlik et al. (2014), heartbeat perception scores were computed as follows:

$$\text{Perception score} = 1/6\ \Sigma\ (1 - (|\text{actual heartbeats} - \text{reported heartbeats}|)/\text{actual heartbeats}),$$

where the sum was taken over the six trials comprising one test session. The resulting scores varied between 0 and 1, with higher scores indicating better interoceptive accuracy. To examine the effect of cortisol response on interoceptive accuracy, we performed a 2 (group: responder, non-responder) × 2 (time: pre, post) mixed design ANOVA on heartbeat perception scores. In addition, to ensure that the effect of cortisol response on interoceptive accuracy was not confounded by heart rate, we also performed an ANOVA with the same design on heart rate (bpm). Heart rate was estimated from recorded heartbeats during the Schandry tasks using the formula:

$$\text{Heart rate} = 1/6\ \ \Sigma\ \ (\text{actual heartbeats}/\ \text{trial duration in s}) * 60.$$

Further, to supplement the ANOVA results with cortisol responsiveness as a dichotomous variable, we calculated the correlation between cortisol responsiveness (area under the curve with respect to increase (AUCi); Pruessner et al., 2003), heart rate responsiveness (post-assessment – pre-assessment), and interoceptive accuracy changes (post-assessment – pre-assessment).

All analyses were conducted in R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Sample characteristics

Descriptive statistics for demographic information and self-report questionnaires in responders and non-responders are shown in Table 1. There were no significant differences in age, sex ratio, social anxiety measures, negative beliefs, depression, or time estimation accuracy between responders and non-responders. The mean estimated durations for the 25-, 35-, and 45-s trials were 19.4, 26.8, and 33.8 s (SD = 3.03, 4.61, and 5.77), respectively.

Subjective anxiety and cortisol responses to the TSST

Subjective anxiety and cortisol levels at each time point are summarized in Table 2 and Figure 2. For subjective anxiety, a two-way mixed ANOVA revealed a significant effect of time [$F(3, 102) = 45.38$, $\varepsilon = 0.65$, $p < .001$, $\eta_p^2 = 0.57$], but no significant effect of group [$F(1, 34) = 0.40$, $p = .53$, $\eta_p^2 = 0.01$] or interaction [$F(3, 102) = 0.64$, $\varepsilon = 0.65$, $p = .59$, $\eta_p^2 = 0.02$] was observed. Multiple comparisons with Bonferroni corrections revealed that participants exhibited elevated anxiety in anticipation of the TSST (at T1; $p < .001$), which lasted even after they completed the test (at T2; $p < .001$).

A two-way 2 (group: responders, non-responders) × 4 (time: T0, T1, T2, T3) mixed design ANOVA on cortisol levels revealed a significant interaction [$F(3, 102) = 25.17$, $\varepsilon = 0.57$, $p < .001$, $\eta_p^2 = 0.43$]. Post-hoc analyses using Holm's correction revealed that cortisol levels were higher at T1, T2, and T3 than T0 ($p < .01$ for all) among participants of the responder group. In contrast, in participants of the non-responder group, cortisol levels were lower at T1, T2, and T3 than at T0 ($p < .01$ for all). These results indicated that cortisol significantly increased only in the responder group.

3.3 Heart rate

A two-way 2 (group: responders, non-responders) × 2 (time: pre, post) mixed design ANOVA on heart rate revealed no significant main effects or interaction ($F < 1.91$, $p > .18$ for all; Figure 3), indicating that heart rates were almost equivalent at each time point, regardless of group.

3.4 Interoceptive accuracy

A two-way 2 (group: responders, non-responders) × 2 (time: pre, post) mixed design ANOVA on interoceptive accuracy revealed a significant interaction [$F(1,34) = 5.80$, $p = .02$, partial $\eta^2 = 0.15$], as shown in Figure 4. Post-hoc analysis revealed that, in the responder group, interoceptive accuracy was higher in the post- than the pre-test ($t = 2.52$, $p = .02$, $d = 0.58$). In contrast, no significant changes in interoceptive accuracy were observed in the non-responder group ($t = 0.99$, $p = .34$, $d = –0.24$). These results indicate that enhanced interoceptive accuracy was observed in the responder, but not in the non-responder, group.

3.5. Supplemental analyses

The Pearson correlation between cortisol responsiveness (AUCi) and changes in interoceptive accuracy was positive ($r = .42$, $p = .01$), indicating that higher cortisol responsiveness is associated with increased interoceptive accuracy. This correlation was still significant when controlling for sex, menstrual cycle phase (0 = men, 1 = early follicular phase, 2 = late follicular phase, and 3 = late luteal phase), and the time of experiment (0 = all testing was conducted before 5 pm, 1 = testing after 5 pm was included) ($r_p = .42$, .42, and .43, ps $< .05$, respectively). The correlation between cortisol responsiveness and heartbeat responsiveness was not significant ($r = –.08$, $p = .63$), while the correlation between interoceptive accuracy changes and heartbeat responsiveness was negative, although it did not reach significance ($r = –.29$, $p = .09$).

4. Discussion

This study investigated the effect of cortisol response to a psychosocial stressor on interoceptive accuracy. Psychosocial stress testing, using the TSST, caused an acute rise in subjective anxiety, suggesting that it was successful as a socially evaluative stressor. The results indicated that interoceptive accuracy increases following psychosocial stress in participants who show strong cortisol responses but not in those who do not. This result is consistent with our initial hypothesis that cortisol responses increase interoceptive accuracy.

Our results are in accordance with the findings by Schulz et al. (2013b) that cortisol administration produces larger HEP. Furthermore, we did not observe any effect of cortisol response on heart rate itself. Therefore, we conclude that the observed effect on

interoceptive accuracy is not due to altered cardiac activation, which is also in line with the study by Schulz et al. (2013b). Compared with existing studies using concomitant stressors, our results are consistent with those that used the Schandry task (Schulz et al., 2013a), but inconsistent with those of studies using the Whitehead task. In fact, contradictory effects were observed in this study (Fairclough and Goodwin, 2007; Schulz et al., 2013a). The Schandry and Whitehead tasks are considered to pose different cognitive demands, while both assess interoceptive accuracy (Schulz et al., 2013a). The Schandry task requires attention only to visceral sensation; however, the Whitehead task requires concurrent attention to visceral sensation and exteroceptive stimuli (visual or auditory). Based on these assumptions, our results suggest that cortisol may enhance interoceptive accuracy *via* modulating attention to visceral sensation. Therefore, it may contribute to focusing attention on interoceptive signals at the cost of other (exteroceptive) signals.

Although the potential contribution of HPA axis activation on interoceptive accuracy has been suggested in a previous study (Schulz et al., 2013a), our experimental design provided plausible evidence for this. In existing studies, post-stress assessments of interoceptive accuracy have usually been conducted just after stress exposure (e.g., Fairclough and Goodwin, 2007; Schulz et al., 2013a) and considered to reflect sympathetic activation, and only a mild HPA axis activation due to the delay in HPA axis activation following stress exposure. The present study utilized a 10-min resting period to allow heart rate to recover to baseline levels and examine the influence of HPA axis activation. However, considering that neural effects are first observed approximately 10–20 min after glucocorticoid administration (Makara and Haller, 2001), and peak cortisol responses are usually observed at 10 min (or later) after the cessation of a stressor, our timing of post-stress assessments may still be suboptimal. A study including a longer resting period would further support our results.

We assume that the increased interoceptive accuracy observed in the present study is an acute, state-dependent elevation caused by an acute elevation in cortisol following stress exposure. While such elevation would disappear over time, amplification of heartbeat perception could cause prolonged elevation in anxiety and cortisol levels, which may eventually develop into a vicious circle (Barsky et al., 1988). That means that amplified somatic sensations may themselves serve as "stressors" and cause prolonged cortisol responses, which may in turn maintain enhanced heartbeat perception. From the predictive coding framework of interoception, the peripheral endocrine and immune changes brought about by HPA-axis hyperactivity may cause afferent interoceptive input decoupled from the

specific interoceptive predictions that are issued by the agranular visceromotor cortex, leading to increased prediction-error signals (Barrett and Simmons, 2015). In this case, prediction errors could be resolved by actively continuing to drive the body's systems to generate the predicted sensory input. Following this, the prediction error could be ignored but burdens to the body's systems could further accumulate. Such a failure in allostatic regulation would eventually lead to negative health outcomes, such as fatigue and depression (Stephan et al., 2016).

Although excessively high interoceptive accuracy in response to stressors would be maladaptive, attention to bodily sensations may serve an adaptive function to reduce physiological stress responses (Steptoe and Vögele, 1986). In this regard, we should also note that we observed a significant drop in cortisol levels relative to baseline in non-responders who did not show interoceptive accuracy increases. As this drop was more intense than expected based on diurnal changes during the afternoon (Edwards et al., 2001), we hypothesize that it likely reflects the recovery from an anticipatory stress response to the experimental situation (e.g., Lopez-Duran et al., 2015). As responders and non-responders did not differ in levels of depression and social anxiety, it is unclear which pattern of responses is more adaptive. However, the pattern in non-responders is not considered to be the typical response to stressors, and it is possible that both a hyper- and hypo-activity in the HPA axis in response to stressors could be associated with maladaptive conditions.

Although we observed enhanced interoceptive accuracy in responders, subjective anxiety did not differ between responders and non-responders. This result is not surprising, given the dissociation between subjective emotional stress and endocrinological stress responses, as observed even in a study using rigorous pharmacological manipulations (Ali et al., 2017). It may be partly due to insensitivity of the subjective measure to assess individual differences in psychological states. Nevertheless, it is possible that a difference between groups would have appeared in later time periods. As we did not examine the influence of the enhanced interoceptive accuracy on subsequent mood states, it would be interesting to investigate whether responders exhibit higher subjective anxiety than non-responders in later time periods.

5. Limitations

Our study has several limitations. First, we should be cautious regarding the validity of the interoceptive accuracy assessment. Although

the Schandry task is one of the most common methods to assess interoceptive accuracy, it has been subjected to criticism in respect to its construct validity. The major criticism is that it is susceptible to prior knowledge or beliefs about one's resting heartrate and IQ scores (e.g., Murphy et al., 2018; Ring et al., 2015). We tried to minimize the influence of these variables by instructing participants not to make any guess on their heartbeat, but a recent study suggested that the instruction against guessing does not necessarily assure a valid score (Desmedt et al., 2018). We also utilized the time estimation task to minimize group differences in interoceptive accuracy arising from the ability to estimate time; however, performance in the time estimation task could have also been differently affected by cortisol between groups (e.g., Yao et al., 2016). Recent studies have reported that heartbeat counting accuracy shows low test-retest reliability (Wittkamp et al., 2018) and does not correlate with other more rigorous measures of cardiac interoception (Ring and Brener, 2018), which further questions the construct validity of the task. Considering these issues, future replications using methodologies not subject to these concerns are desired.

In addition, we only assessed cortisol levels in terms of stress-induced biological changes. To our knowledge, no other study has examined the relationship between stress-induced cortisol response and active heartbeat perception. However, this relationship is correlational by nature, and it is possible that psychophysiological changes not measured in this study may have increased interoceptive accuracy. Specifically, regarding adrenergic activity, we assessed heart rate only during the Schandry task. Thus, the influence of adrenergic carryover after the TSST was not considered enough. Continuous monitoring of heartrate throughout the experiment would help address this issue. We monitored the heart rate using pulse-oximetry, although using ECG would be preferred to control for the possible influence of somatosensory feedback. In association to this point, we did not include a non-stress control group. Although our approach could reveal the effect of cortisol response on interoception, it does not necessarily reflect the effect of stressful experience. Utilizing the non-stress control group may provide further insights, including the effect of non-hormonal effect of stress on interoception, by comparing the non-responder group and the non-stress control. Further, we did not control for sex in the present study. A previous study suggests that females are less efficient in consciously detecting their heartbeat (Grabauskaitė et al., 2017). Moreover, males show clearer cortisol responses following stressors than females (Liu et al., 2017), and thus differential cortisol responses may explain differences between males and females. Although there was no difference in sex ratio between the groups in this study, future studies

should control for the effect of sex. Finally, the present results are derived from a non-clinical university student sample, and the generalizability of the results to clinical populations may be limited. Replication of the present study with an actual clinical sample (e.g., individuals with stress-related disorders, such as depression, who typically show alterations in the HPA axis) is desirable.

Notwithstanding these limitations, the present study suggests the stress-induced cortisol responses increase interoceptive accuracy. An important goal for future studies is to apply our findings for treating individuals suffering from exaggerated interoceptive sensations. To achieve this goal, the efficacy of psychological treatments for individuals with HPA-axis dysregulation should be addressed in future studies. While an improvement of heartbeat counting scores by cognitive behavior therapy in panic disorder has not been observed (Antony et al., 1994), recent findings suggest that interoceptive accuracy is subject to training (e.g., Fischer et al., 2017; Schaefer et al., 2014). Further, if our findings of extended and increased interoceptive sensation by cortisol are confirmed as transient in future studies, understanding the relationship between cortisol and interoceptive sensation would be an important element of psychoeducation. Understanding that increased interoceptive sensations in response to stressors are caused by cortisol and they disappear over time would contribute to alleviate exaggerated concerns about interoceptive sensations and break the vicious circle created by stressors, cortisol, and increased interoceptive accuracy.

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Table 1. Characteristics of responders and non-responders

	Responders	Non-responders	t/χ^2	p
Total (*n*)	19	17	-	-
Female: Male	9:10	11:6	1.09	.30
Age	21.7 (1.7)	21.8 (1.9)	0.13	.90
BMI	21.34 (2.55)	20.82 (2.07)	0.67	.51
SPS	24.42 (15.75)	22.00 (12.46)	0.51	.62
SIAS	35.00 (14.08)	33.53 (13.95)	0.31	.76
SBSA-CB	23.58 (12.92)	22.06 (14.08)	0.34	.74
SBSA-UCB	15.05 (8.76)	12.53 (7.18)	0.94	.36
SBSA-HS	17.26 (9.46)	16.53 (11.10)	0.21	.83
CES-D	13.58 (6.63)	12.47 (8.44)	0.44	.66
Time estimation	.74 (.12)	.79 (.11)	1.33	.19

Note: Data are represented by mean and standard deviation (*SD*).

SPS = Social Phobia Scale; SIAS = Social Interaction Anxiety Scale; SBSA = Self-Beliefs related to Social Anxiety Scale; CB = conditional beliefs; UCB = unconditional beliefs; HS = high standard beliefs; CES-D = Center for Epidemiologic Depression scale.

Table 2. Mean subjective anxiety at each time point

	Responders				Non-responders			
	T0	T1	T2	T3	T0	T1	T2	T3
Subjective anxiety								
M	17.95	49.37	38.32	19.79	11.06	50.82	37.53	13.65
(*SD*)	(13.99)	(22.79)	(27.80)	(23.39)	(9.71)	(18.92)	(20.49)	(11.79)

Note: *M* = mean; *SD* = standard deviation.

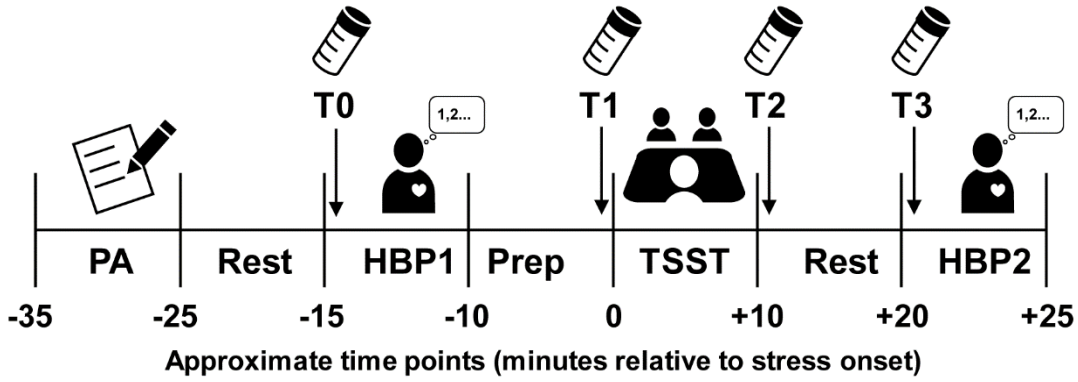

Figure 1. Overview of the testing timeline. At T0, T1, T2, and T3, subjective anxiety assessments, and saliva sampling were conducted. PA = psychological assessments, HBP = heartbeat perception task, Prep = preparation time for the speech task, TSST = Trier Social Stress Test.

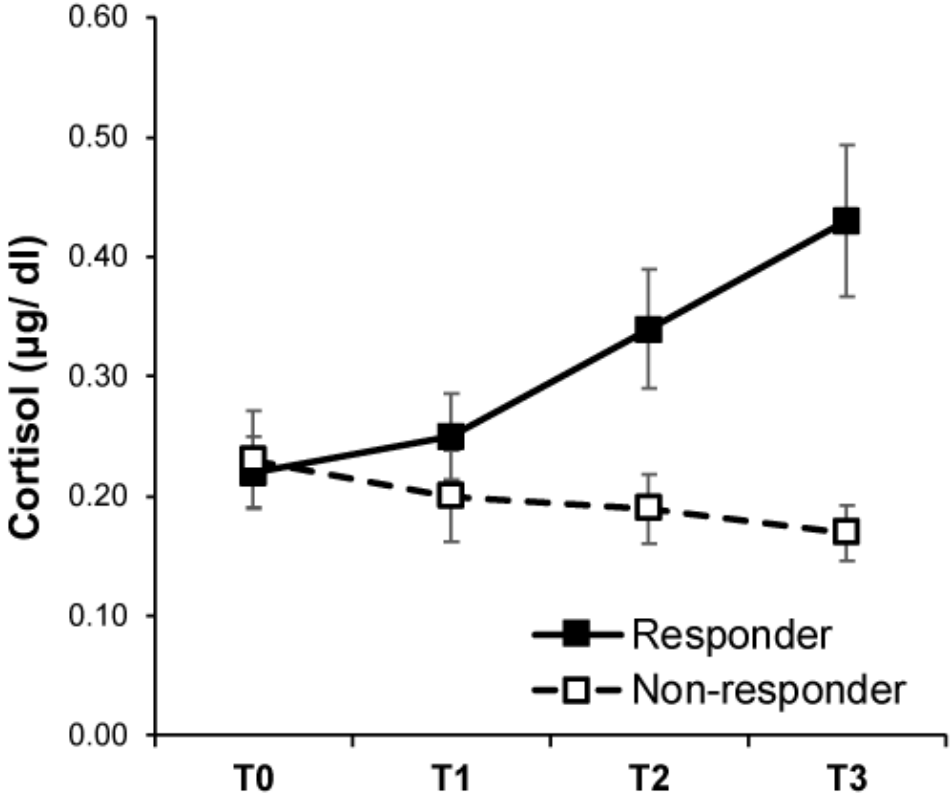

Figure 2. Cortisol levels at each time points in each group (Responders *vs*. Non-responders). Error bars represent standard error of the mean.

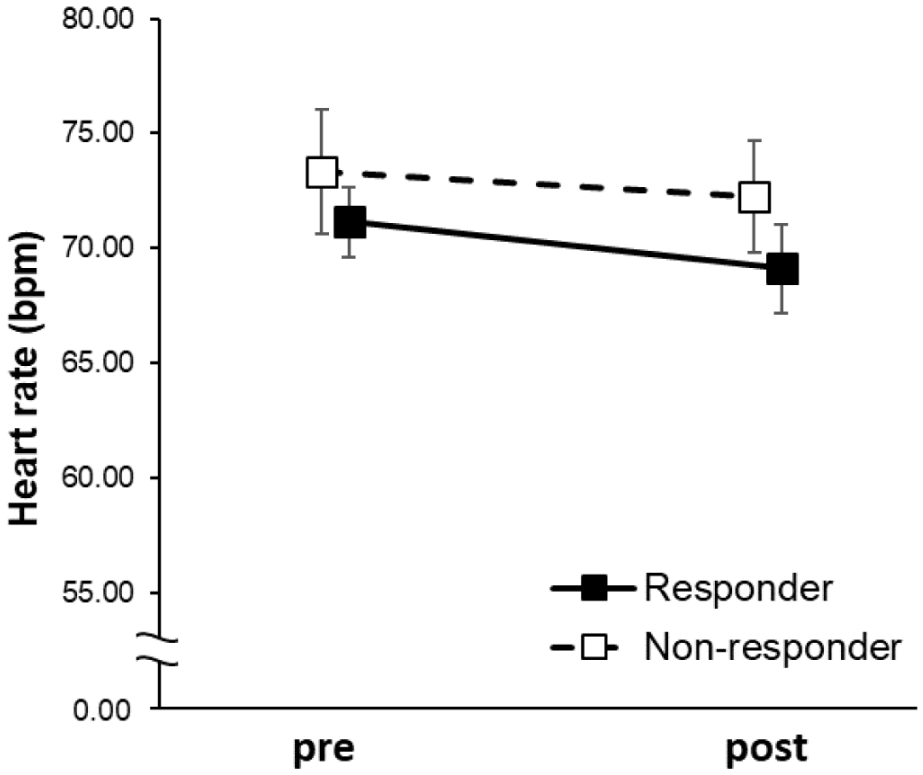

Figure 3. Heart rate (bpm) before (pre) and after (post) the Trier Social Stress Test (TSST) in each group (Responders *vs*. Non-responders). Error bars represent standard error of the mean.

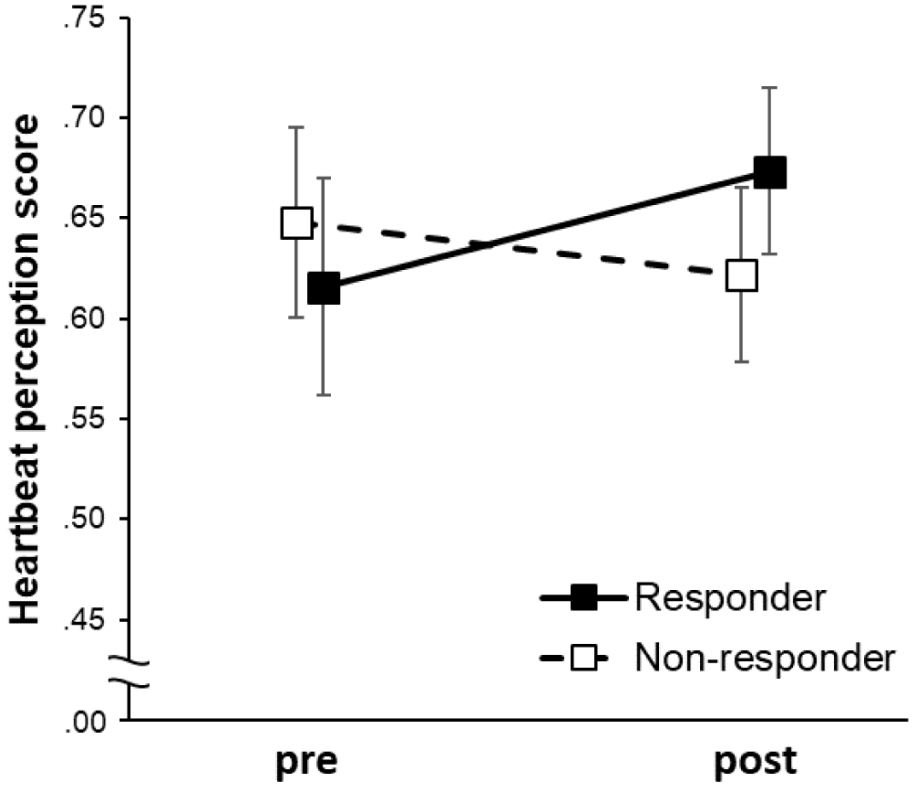

Figure 4. Heartbeat perception before (pre) and after (post) the Trier Social Stress Test (TSST) in each group (Responders *vs*. Non-responders). Higher scores indicate better performance in heartbeat perception. Error bars represent standard error of the mean.