Cortisol stress reactivity can be shaped by control, support and threat in surprising ways – Illustrating HPA axis complexity

by

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A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of Bachelor of Science

With Honors in Biopsychology, Cognition, and Neuroscience from the

University of Michigan 2015

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Acknowledgements

First and foremost, I would like to thank Professor Nestor Lopez-Duran for allowing me to be part of his lab and giving me the opportunity to embark on such an experiential learning journey. His guidance, support, and knowledge of the field has been critical in fostering my interest in this thesis. My deepest thanks and gratitude also goes out to my mentor Stefanie Mayer, graduate student in Prof. Lopez-Duran's lab, who worked alongside me and was instrumental in my completing of this thesis. Her thoughtful comments, questions, mentoring skills, interest and knowledge in the subject matter all helped to make this thesis possible. I couldn't have had a better mentor than Ms. Mayer on this project! Additionally, I would like to thank Prof. Dr. James L Abelson for allowing me to use and apply my inquisitive questions to his TSST Data. He has been supportive not only of my thesis but of my future plans as well. For that, I am very grateful.

Lastly, I would like to thank all the people outside of work, whose love and encouragement inspired me to take on the challenge of completing an honors thesis. I am most thankful and grateful to my mother, Grace Adu, and sister, Sarah Adu, for their emotional and financial support as well as inspiring me through perseverance of their own struggles. Without them, I wouldn't be here. Finally, I would like to thank my best friend Natalie for her encouragement and all the sleeping hours of the night she spent awake with me as I was working on this thesis. To all a big thanks!

Ann Arbor, April 2015

Abstract

Research on the effects of psychosocial factors on HPA functioning has been well documented. Little is known, however, about how these factors differentially impact specific components of the HPA response to stress (i.e., activation and recovery phases). In this study, we use a new analytic approach to examine the impact of perceived social support, control, fear of negative evaluation and novelty on HPA activation, peak, and recovery phases. **Methods:** We conducted secondary analyses from a study by Abelson and colleagues (2014), using Growth Curve Modeling (GCM) with Landmark Registration (LR) (Lopez-Duran et al., 2014) to examine how psychological variables, assessed via visual analogue scale (VAS) ratings, impact cortisol activation, peak, and recovery. **Results:** All VAS variables except novelty impacted cortisol secretion, but in surprising ways. Greater sense of support and lower fear of negative evaluation predicted steeper activation slopes. Greater sense of control predicted steeper activation slopes and higher peak levels. Traditional GCM analyses produced similar results – although LR yielded more robust findings. **Discussion:** Results likely illustrate complexity within the HPA axis, with our most striking finding being lower cortisol release with lower sense of control, which contrasts with prior work (Dickerson & Kemeny, 2004). The way in which the VAS instruments were used must also be examined. The new analytic approach may prove useful in further disentangling the complexity of the HPA axis.

Keywords: stress, cortisol, HPA axis, TSST, VAS, support, novelty, control, negative evaluation

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Stress is associated with negative health consequences (Chrousos & Gold, 1998; McEwen, 1998). Although the link between excessive stress exposure and illness is complex, the Hypothalamic-Pituitary-Adrenal (HPA) axis, with its end product cortisol, is believed to be one biological mechanism by which stress impacts health. For example, HPA dysfunction has been associated with the development of stress-related disorders such as anxiety (Faravelli et al., 2012; Kallen et al., 2008), depression (Johnson, Kamilaris, Chrousos, & Gold, 1992; Lopez-Duran, Hajal, Olson, Felt, & Vazquez, 2009; Marin et al., 2011), and post-traumatic stress disorder (Marin et al., 2011). Because of the negative health consequences of stress, full understanding of the psychobiology of the HPA-axis is critical in efforts to reduce stress-related illness, and to promote resilience.

The HPA stress response system has been shown to be activated and attenuated by a number of psychosocial factors. Although the relationships between psychosocial stress, HPA activation, and health outcomes have been extensively studied (Adam & Kumari, 2009), most reports have failed to determine how psychological variables differentially impact specific aspects of the stress response (i.e., activation vs. recovery). Thus far, most studies have examined the HPA response broadly (e.g., determining the total amount of cortisol production after stress-inducing stimuli). However, such efforts do not advance our understanding of specific components of the HPA system. Biological functioning within specific time frames of the HPA response, particularly activation phase (rate of initial response to a stressor) and recovery phase (rate of decline after a peak response) may actually reflect different important processes with unique

psychosocial meaning (Lopez-Duran, Mayer, & Abelson, 2014). In this thesis, we hope to advance these efforts by examining the influence of psychosocial variables, specifically perceived social support, control, social evaluative threat and novelty, on HPA axis activation, peak and recovery levels.

Psychosocial modulators of HPA-axis functioning

Traditionally, a number of studies have looked at the effect of psychological factors on HPA functioning. We focus on these four factors due to a sizable literature on the impact of these factors on HPA response and their potential in helping elucidate the underlying mechanisms of the HPA response.

Social Support. The stress-buffering effects of social relationships have been a major finding in stress research. In fact, the influence of social relationships on health is so profound that it has been suggested to impact life expectancy by as much as cigarette smoking, hypertension, obesity, or level of physical activity (Robert M Sapolsky, 2004). Several animal studies have observed the effects of social support on the neuroendocrine axis, noting a blunting of the HPA stress response to stress among animals with social support (Hennessy, 1984, 1986). Consistently, studies in squirrel monkeys (Stanton, Patterson, & Levine, 1985) and wild baboons (R. Sapolsky, 1997) showed increased cortisol secretion during social isolation. Human studies have also reported similar findings. Steptoe and colleagues (2004) also reported an overall increase in HPA reactivity in lonely individuals. Other human laboratory studies, such as those by Kirschbaum and colleagues (1995) have also shown that other-supported subjects, compared to subjects who were alone, had blunted cortisol responses to stress. Breast cancer patients with

greater quality of social support predicted lower overall daily cortisol levels (Turner-Cobb, Sephton, Koopman, Blake-Mortimer, & Spiegel, 2000). Despite these robust findings, further understanding of the impact of social support on the relevant components of the HPA axis is warranted. It has been proposed that availability of social support is part of a resilient stress response that keeps the activity of the HPA-axis within an "optimal range" during stressful encounters (activation phase) and the ability to quickly terminate the stress response upon cessation of the stressor (recovery phase) (Ozbay et al., 2007). This notion although intuitive, remains largely theoretical. The previous studies have not advanced our understanding of how social support affects the activation and recovery phases of the HPA response. Further elucidation of the impact of social support on specific aspects of the HPA axis can enhance stress prevention and interventions techniques.

Sense of Control. Controllability is another important factor that affects HPA responding. Increased control has been proposed to dampen cortisol secretion to acute stress (Abelson, Khan, Liberzon, Erickson, & Young, 2008; Abelson, Khan, Young, & Liberzon, 2010) while uncontrollability increases cortisol output (Dickerson & Kemeny, 2004; R. Sapolsky, 1997). A study by Abelson and colleagues (2008) observed the effects of perceived control and cognitive coping in response to pharmacological activation of the stress system. They found that participants with experimentally induced "sense of control" exhibited reduced total cortisol output. Additionally, Abelson and colleagues (2010) also showed a reduction in cortisol levels when participants were exposed to brief cognitive interventions designed to enhance sense of control and coping (as well as reduce novelty) in response to biological activators that directly stimulated the pituitary.

These findings are important and call for an in-depth understanding of the effect of control on the dynamics of HPA functioning.

Social Evaluative Threat. Contexts deemed threatening to the social self have been observed to activate the HPA system. Specifically, social evaluative threat, defined as "an important aspect of the self-identity [which] is or could be negatively judged by others (Dickerson & Kemeny, 2004)," has been shown to be a potent activator of the HPA axis. In a seminal paper by Kirschbaum et al., (1993), laboratory tasks, most notably public speaking and mental arithmetic in front of peer judgers, led to dramatic increases in cortisol levels. This laboratory method (the Trier Social Stress Test, TSST) has been extremely effective in eliciting an HPA response in laboratory settings due to its social-evaluative nature. Although social evaluative threat has been well observed as a potent activator of the HPA system, further studies are needed to fully understand its effects on the specific components of the HPA response curve.

Novelty. Lastly, studies on the effects of novelty has shown it to be a robust activator of the HPA axis (Dinces, Romeo, McEwen, & Tang, 2014; Mason, 1968). In animal studies, Simpkiss and Devine (2003) found ACTH responses to acute stressors in rats blunted if the rats were familiar with the stressor. Additionally, Hennessy and colleagues (1995) found that even nonthreatening increments in environmental novelty induced sustained release of cortisol in animals. Human studies have also found similar results. Deinzer and colleagues (1997) examined the adrenocortical response of novice parachute jumpers to 3 consecutive parachute jumps and found significant cortisol responses to the first two jumps and a reduced response to the third jump. Similar results were found showing reduced cortisol levels following repeated experiences with

parachute jumping (Ursin, 2012). Al'Absi and Lovallo (1993) and Davis and colleagues (1981) also demonstrated that exposure to novel (experimental) situations can enhance cortisol responses. Taken together, these studies suggest that novelty enhances HPA axis activation while familiarity or repeated exposure reduces HPA activation.

Taken together, the effects of these psychosocial variables on the HPA axis have been well investigated. However, the aforementioned studies have largely focused on differences at baseline, post-stress peak cortisol levels, or overall HPA responses (using repeated measures ANOVA and Area Under Curve approaches). Although these analyses provide us with much needed information on the impact of psychosocial variables on the HPA system, they do not disentangle the influence of psychosocial variables on specific components (i.e., activation, peak, and recovery) of the HPA axis response, which may reflect different and potentially important physiological processes (Lopez-Duran et al., 2014).

Successful adaption to stress likely involves both – the capacity to quickly respond to stress and the ability to modulate and shut down the response in a timely manner (Chrousos & Gold, 1992). A quick, robust HPA response to a stressor is likely beneficial, providing energy and resources to cope with the current challenge (Linden, Earle, Gerin, & Christenfeld, 1997; R.M. Sapolsky, Romero, & Munck, 2000). However, the rate of recovery after exposure to a stressor has been shown to be important as well (Dienstbier, 1989; Linden et al., 1997). Cortisol levels delayed in post-stress recovery phase can induce chronic hyper-activation of the HPA system, leading to stress-related disorders (Chrousos & Gold, 1998). Identifying how well-researched psychosocial variables, such as social support, control, evaluative threat, and novelty affect HPA

activation, peak, and recovery will expand our knowledge of the dynamics of HPA functioning and how to best influence the system to mitigate its negative health effects.

One reason for the lack of information regarding the effects of psychosocial factors on different aspects of HPA functioning is that, until recently, the analytical tools to properly model these effects were not commonly employed. For this thesis, we performed a secondary analysis of data from a previously published Trier Social Stress Test (TSST) study by Abelson and colleagues (2014). We used multilevel growth curve modeling with landmark registration to better examine changes in cortisol levels at various stages of the stress response (Lopez-Duran et al., 2014). This technique allows us more in-depth information on the underlying biological processes of the HPA response such as intensity of response and speed of recovery. By understanding how psychological predictors (i.e., social support, control, social evaluative threat, and novelty) influence HPA activation and recovery, we can better understand the psychobiology of the stress response system. This can help us design interventions to specifically target factors that buffer HPA activation, reduce magnitude of responses, and facilitate recovery to maximize health benefits.

Method

This study consists of a secondary analysis of a study investigating the effects of cognitive interventions on HPA axis responses to the social evaluative threat of the TSST (Abelson et al., 2014). Our current study aims to elucidate the effects of psychosocial factors on specific aspects of HPA functioning, particularly HPA activation and recovery.

Participants

The study included 54 healthy participants (33 males, 21 females) ranging from

18-45 years old (M_{age} = 23.41, SD_{age} = 6.06). Participants were recruited through multi-media advertisements. Research assistants performed initial telephone screenings to determine eligibility. Eligible participants were then invited for a face-to-face evaluation using several self-report measures and a Structured Clinical Interview for DSM-IV (SCID). The criteria for the study called for individuals age 18—45 years old, medically healthy, within 30% of ideal body weight, no exposure to psychoactive medication in the past two months, no history of substance dependence or abuse in the past six months, low levels of tobacco (less than 20 cigarettes/d) and alcohol use (mean 2.3 drinks/week), negative urine drug screens, and normal screening laboratory results. Participants had no psychiatric disorders or first-degree family history of affective disorders or anxiety (except specific phobia). Females were premenopausal, not pregnant or lactating, not using birth control pills, and studied during the luteal phase of the menstrual cycle (between days 18 and 27). Participants who were eligible for the study signed a written, Institutional Review Board-approved consent and were compensated $100 for participating in the study.

Procedures

The study took place at an academic medical research center. Participants reported to the laboratory at 1:00pm and intravenous access (IV) was established no later than 1:30 p.m. using an 18—20 gauge angiocatheter in an antecubital vein, kept open with normal saline drip. To accommodate to the research setting and IV, participants rested comfortably for one hour. Blood samples included in the current analysis were taken at 2:25 p.m. (immediately before the stress protocol initiation at 2:30 p.m.), at the end of the speech task (10 min after start of the TSST), at the end of the math task (15

min after start of the TSST) and at 25, 35, 45, 60 and 75 minutes after stress initiation (back in the accommodation room). After collection of the 2:25 p.m. sample, a research assistant escorted participants to a second room for the start of the TSST at 2:30 p.m.

TSST Challenge

Participants underwent the TSST, a standardized protocol in which participants give a 5-minute speech for a job interview and then orally perform a serial subtraction math test in front of a stern panel of "experts." Participants were also informed that they were videotaped and later evaluated by professionals trained in monitoring nonverbal behavior. The TSST has been described extensively previously (Kirschbaum, Pirke, & Hellhammer, 1993). Standard procedures were followed except for experimental variations in task instructions utilized for the parent study. Upon arrival in the laboratory, subjects were randomly assigned to receive one of four different instructions group: One group (SI; n = 15) was given standard TSST instructions as previously described (Kirschbaum, Pirke, & Hellhammer, 1993). Another group received standard instructions with a perceived ‘‘control" modification (SI Control; n=16). A third group received instructions aimed at increasing familiarity and helping participants to prepare coping strategies (CI Coping, n=12). The last intervention aimed to shift goal orientation from self-promotion to helping others (CI Compassionate Goals Orientation, n=11). Intervention effects are reported elsewhere (Abelson et al., 2014). We controlled for the effect of intervention instruction in our current analyses.

Subjective measures

Subjective states were recorded before, during and after the TSST. Specifically, Visual Analog Scales (VASs) were used to measure emotional states and cognitive

experiences prior to the TSST (5 minutes pre-TSST), at the end of the TSST task, and at 20 and 45 minutes post-TSST task. These ratings quantified emotions or cognitions on 100-mm visual analog lines (anchored from ‘‘not at all’’ to ‘‘most ever’’) and provided a measure of the supportiveness of the social environment (staff support) in which the experiment was conducted, the degree of control over what was happening to them, fear of negative evaluation by the TSST staff (to assess social evaluative threat) and the degree to which the laboratory experiences were perceived as novel. For this study, we calculated the average VAS rating across all four time points as a measure of overall support, control, fear of negative evaluation, and novelty.

Cortisol Assay and Processing

Cortisol was assayed using commercial kits. Cortisol was assayed using Coat-a-Count cortisol kits (Siemens, USA), a well-validated radioimmunoassay (RIA) with analy- tical sensitivity of 0.2 mcg/dl and inter-assay and intra-assay variabilities of less than 5%.

Statistical Analysis

We examined the impact of psychosocial variables on specific aspects of the HPA-axis response using a two-piece multilevel growth curve modeling (GCM) with landmark registration as applied to neuroendocrine data (see Lopez-Duran et al., 2014). This approach has been shown to be more sensitive than traditional methods (rANOVA, examinations of AUC, etc.) in the identification of subtle differences in distinct aspects of the response (intensity of activation, recovery capacity, etc.), facilitates the partitioning of variance due to amplitude (e.g., peak magnitude) and timing effects (timing of peaks), and to more accurately reflect the underlying differences in ACTH and plasma cortisol

(Lopez-Duran et al., 2014). This approach involved three steps. First, individual post-stress peaks are identified from a visual analysis of the individual curves (Lopez-Duran et al., 2014). Second, the timing of each individual peak was identified and was used to create a new time axis reflecting minutes from peak. This entails the adjustment of the curves so that each peak falls on the same time point. For those without an identifiable peak, we used the +15 time point (the mode peak time) as their expected, but not observed, "peak time" in order to model their non-response. Finally, we created two spline time variables to represent minutes before (TimeBeforePeak) and after the peak (TimeAfterPeak). We then conducted a multilevel random effects model of the cortisol pre- and post-peak trajectory with peak levels as the intercept. All models included random intercepts and slopes. Group assignment (instruction groups used in the parent study) was added as a control variable to all models.

Results

Unconditional models: Modeling Cortisol Stress Response

We first examined unconditional growth curve models of cortisol responses. The model with linear and quadratic activation slope and linear recovery slope was the best fit to the cortisol data (AIC = 2187.63) compared to a linear activation and recovery model only (AIC = 2235.21). The unconditional time model indicated significantly accelerating cortisol levels towards peak (activation slope), TimeBeforePeak $b = 0.30$, $SE = 0.03$, $p < .001$, TimeBeforePeak^2 $b = 0.01$, $SE = .0007$, $p < .001$ and significantly decreasing cortisol levels after the peak (recovery slope), TimeAfterPeak $b = 0.01$, $SE = 5.81$, $p < .001$.

Conditional models: Modeling Cortisol Stress Response

In a conditional model that included the effect of group assignment, we found a significant effect of group on the linear activation slope $F(1, 206) = 3.96$, $p = .05$, and on the recovery slope $F(1, 53) = 4.51$, $p = .04$, therefore we control for group assignment in subsequent models. Detailed results of group are originally reported elsewhere (Abelson et al., 2014).

Perceived support: supportiveness of the laboratory staff. There was no effect of support on peak, $b = 0.01$, $SE = .037$, $p = .78$. Higher support predicted steeper activation (linear activation $b = 0.004$, $SE = .002$, $p = .045$) and greater acceleration (quadratic activation $b= 0.0001$, $SE = .00004$, $p = .01$), without affecting recovery slope ($b= 0.0002$, $SE = .0005$, $p = .73$).

Perceived control: the degree of control over what's happening. Greater control predicted greater peak levels, $b = .07$, $SE = .028$, $p = .011$, steeper activation (linear activation $b = 0.004$, $SE = .002$, $p = .03$), and marginal greater acceleration (quadratic activation $b = 0.0001$, $SE = .00004$, $p = .055$), without affecting recovery slope ($b = -.0006$, $SE = .0004$, $p = .13$).

Social evaluative threat: fear of negative evaluation by TSST staff. There was a marginal effect of negative evaluation on peak, $b = -.066$, $SE = .039$, $p = .095$, such that higher negative evaluation yielded lower peak levels. Negative evaluation also predicted flatter activation (linear activation $b = -.005$, $SE = .002$, $p = .01$) and less acceleration (quadratic activation $b = -.0001$, $SE = .00005$, $p = .04$), without affecting recovery slope ($b = 0.0004$, $SE = .0005$, $p = .44$).

Novelty: the degree to which the laboratory experiences were perceived as novel. Novelty did not impact peak, $b = -.040$, $SE = .028$, $p = .16$, activation (linear

activation b = -.001, SE = .002, p = .500; quadratic activation b = 0.00001, SE = .00003, p = .68), or recovery slope (b = 0.0004, SE = .00038, p = .35).

Discussion

The purpose of this study was to examine the effects of psychosocial variables, mainly perceived social support, control, social evaluative threat and novelty, on the activation, peak and recovery phases of the HPA response system. We found that psychological factors differentially impacted different components of HPA axis response to the TSST - in surprising ways.

Perceived support by laboratory staff only had an impact on the activation phase of the HPA response system. Contrary to our hypothesis, we found that individuals who reported greater perceived staff support had a steeper cortisol activation slope with no differences on peak levels or recovery slope. This finding is inconsistent with previous studies showing that social support reduces HPA axis reactivity to stress (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Kirschbaum et al., 1995; Rosal, King, Ma, & Reed, 2004) and that lack of social support increases HPA reactivity in lonely individuals (Turner-Cobb et al., 2000). However, other research showing a link between social support and reduced cortisol responses have also found mixed and counterintuitive results (e.g., Smith, Loving, Crockett, & Campbell, 2009) or no associations between social support and HPA axis activity (e.g., Arnetz, Theorell, Levi, Kallner, & Eneroth, 1983). More consistent results linking social support and reduced cortisol responses are often obtained from studies that investigate the effect of familial social support (as suggested by: Rosal et al., 2004; Uchino, Cacioppo, & Kiecolt-Glaser, 1996). Our laboratory staff,

although friendly, was largely unfamiliar to the participants, which may explain our diverging findings.

Other potential explanations for our diverging results involve the timing and/or nature of our self-report VAS measure. A recent study by Hellhammer and Schubert (2012) examined associations between the psychological (via VAS measures) and physiological (cortisol secretions & heart rate) stress response. They found that physiological response to the TSST was more in line with subjective measures of stress during, but not before or after the stressor. Unfortunately, we were not able to measure perceived staff support during the TSST. Instead, we measured perceived staff support via VAS after the TSST. The TSST-induced stress might have altered the way in which individuals used the VAS measure. For example, greater staff support might have been reported as an attempt to cope with the previously stressful encounter, suggesting that VAS ratings might be very sensitive to the effects of stress exposure.

An alternative explanation for our finding is that rather than contradicting previous studies, this finding may be unmasking the underlying psychobiological mechanism by which social support mediates a healthy HPA axis response in healthy populations. Most studies have looked at the effect of social support on overall attenuation of the HPA system, while our study is unique in that we tested the effect of support on specific components of the HPA axis system. The greater HPA axis activation observed in our findings may reflect an initial and robust increase in cortisol secretion, mobilizing the necessary resources for coping with the presence of a stressor in our healthy population. Yet, we found no difference in peak levels or on the recovery phase. This suggests that those who experienced support have an acute activation of the axis that

rapidly deactivates reaching similar peaks to those who reported lower levels of support. This would be consistent with notions that successful adaption to stress likely involves the capacity to quickly respond to stress and rapid deactivation (Chrousos & Gold, 1992) and that a quick, robust HPA response to a stressor is likely beneficial, providing energy and resources to cope with the current challenge (Linden et al., 1997; R.M. Sapolsky et al., 2000). It is possible that individuals who showed such a quick and healthier HPA axis activation were also those who perceived the laboratory staff as more supportive, consistent with evidence that healthy individuals tend to feel more supported by others (Southwick, Vythilingam, & Charney, 2005), yielding our positive correlation of staff support and HPA axis activation.

Perceived control over what is happening also produced results contrary to our hypothesis. We found that individuals who reported greater feelings of control over what was happening showed steeper activation slope and higher peak levels without affecting the recovery slope of the HPA response axis. Previous studies on the sense of control-HPA response interaction have found opposite results. For example, participants with experimentally induced "sense of control" exhibited reduced cortisol output (Abelson et al., 2008, 2010). However in this study, we did not experimentally manipulate sense of control. Instead, we assessed individual differences in subjective sense of control via VAS measures before and after the stressor. As mentioned above, the use of our VAS measures might have been influenced by prior stress experience, reflecting psychological processes in an attempt to cope with the stressor especially for health populations.

Fear of negative evaluation by the TSST staff had an impact on the activation phase, a marginal effect on peak levels and no impact on the recovery phase of the HPA

response system. Contrary to our hypothesis, we found that individuals who reported less fear of negative evaluation by the TSST staff had a steeper cortisol activation slope and slightly higher cortisol peak levels. This finding is inconsistent with previous studies showing that greater fear of social evaluative threat is an important determinant of increased cortisol secretion (Bosch et al., 2009; Dickerson & Kemeny, 2004; Gruenewald, Kemeny, Aziz, & Fahey, 2004; Rohleder, Beulen, Chen, Wolf, & Kirschbaum, 2007). However, situations that characterize social evaluative threat contain a broad array of cognitions and emotions, including shame and humiliation as well as anxiety. Our VAS measure "fear of negative evaluation" was focusing more on anxiety and worry related aspects rather than shame or humiliation. However, studies have found more consistent links between shame/humiliation and increased cortisol responses (Denson, Creswell, & Granville-Smith, 2012; Gruenewald et al., 2004). Linking subjective states such as fear/anxiety with increased cortisol responses has been rather difficult (Gruenewald et al., 2004; Schedlowski, Wiechert, Wagner, & Tewes, 1992) and might explain our inconsistent finding.

Perceived novelty, contrary to our hypothesis, had no impact on the activation, peak and recovery phases of the HPA response system. This finding is inconsistent with prior research showing novelty to be a robust activator of the HPA stress response system (Al'Absi & Lovallo, 1993; Davis et al., 1981; Deinzer et al., 1997; Ursin, 2012). However, we did not experimentally manipulate the experience of novelty in the current study. Instead, we assessed individual differences in perceived novelty via VAS measures throughout the stressor. Most participants had already visited the laboratory on a (separate) screening day. So when they came in for their TSST, they were already

familiar with most of the staff and the facilities. This may explain our finding of the lack of association between perceived novelty and HPA responding, which after the fact is not all that surprising. Another things is that we only assessed novelty 5 minutes prior to the stress task – we thereby potentially missed any effects of novelty right after entering the laboratory.

Overall, our results likely illustrate complexity within HPA axis functioning. The psychopathology literature has already shown parallel surprises. For instance, Petrowski and colleagues (2010; 2013) observed that panic patients showed flattened rather than heightened TSST responses. Additionally, Takahashi and colleagues (2005), Jezova and colleagues (2004) and Young and colleagues (2004) found that social anxiety patients do not show increased reactivity despite heightened sensitivity to social evaluative threat. Our findings may be a presentation of the complex and intricate functioning of the HPA axis. Additional work is needed to determine whether the observed pattern in healthy subjects have relevance to the parallel findings in patients.

Limitations

There are important limitations to this study. Participants received different TSST instruction groups in the parent study (Abelson et al., 2014), which may have impacted our findings. However, this seems unlikely as we controlled for the impact of group by adding it as a covariate to all our models. Additionally, the study did not assess for psychological states during the TSST. However, few selected studies (e.g., Hellhammer & Schubert, 2012) have looked at psychological states in the midst of the TSST. Future TSST studies utilizing psychological measures may want to look at this critical juncture. Assessing psychological stress measures during the stressor may provide more reliable

information than pre and post-TSST measures (Hellhammer & Schubert, 2012). Also, we only used subjective measures to assess psychological states, which might have been influenced by stress experience. However, VAS measures have been widely used in the neuroendocrine literature to assess similar psychological states. Lastly, we did not have the statistical power to control for the impact of sex in our models. Nonetheless, our findings provide guidance for future studies to apply similar strategies to disentangle the impacts of psychosocial variables on different components of the stress response.

Taken together, our findings, although inconsistent with some previous studies, were consistent across multiple domains (i.e., perceived staff support, sense of control over what is happening, and feelings of negative evaluation). Psychological factors that were hypothesized to buffer cortisol reactivity to the TSST were actually associated with increased HPA axis functioning. We found that perceived staff support, sense of control over what is happening, and feelings of negative evaluation by the TSST staff elicited steeper, rather than our predicted flatter cortisol activation slopes. Sense of control and fear of negative evaluation also predicted higher, rather than our projected lower cortisol peak levels. Our results were replicated when using more traditional mixed modeling approaches (data not presented here), although more robust results were seen using our new technique of growth curve modeling with landmark registration (Lopez-Duran et al., 2014).

Implications

Our findings have several important implications. First, the way in which the VAS instruments are used in current stress studies must be examined. Second, the link between psychological variables and HPA functioning is likely complex and surprising

results in healthy, well-adjusted populations might reflect different underlying mechanisms than those detected in symptomatic populations. Third, our new modeling approach may prove to be particularly useful in disentangling the complexity of the HPA axis in future stress studies.

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