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Anxiety Mediates the Effect of Acute stress on Working Memory Performance when Cortisol Levels are High: A Moderated Mediation Analysis

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Anxiety disorders are among the most prevalent psychiatric disorders, and 26.9 million Americans will suffer from anxiety at some point in their lives (Olatunji, Cisler, & Tolin, 2007). With such a high prevalence, it is necessary to determine how anxiety affects different aspects of everyday functioning. Although anxiety disorders can negatively impact health and relationships (Senaratne, Van Ameringen, Mancini, & Patterson, 2010; Teesson et al., 2011), Yerkes and Dodson's seminal work first demonstrated that there is an inverted U relationship between arousal/anxiety and performance. The theory suggests that performance is best under conditions of optimal arousal. However, performance suffers when this level of arousal is not achieved or when arousal exceeds the ideal (Calabrese, 2008).

Heightened arousal may inhibit performance by disrupting the ability to concentrate. Two theories explain why anxiety predicts inhibited performance and processing inefficiency in working memory (Derakshan & Eysenck, 2009; Eysenck & Calvo, 1992; Johnson & Gronlund, 2009). Baddeley and Hitch's (1974) original conceptualization of working memory had three components: the central executive and two "slave" systems, the phonological loop and the visuo-spatial sketchpad. The episodic buffer is a recently added fourth component that is thought to integrate information between working memory components (Baddeley, 2012). The processing efficiency theory (PET) states that anxiety impedes the working memory system by disrupting the central executive component, which is involved in complex functions, such as strategy selection, planning and attentional control

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(Derakshan & Eysenck, 2009). The attentional control theory (ACT) builds on the PET and states that anxiety disrupts the inhibition and switching functions of the central executive system (Eysenck & Calvo, 1992).

Much research supports the PET and ACT predictions of anxiety-related task performance (for a full overview see Derakshan & Eysenck, 2009). Eysenck et al. (2005) found that participants with high trait anxiety performed significantly worse during cognitively stressful working memory tasks than those with low trait anxiety. Likewise, through a series of four studies, Hayes et al. (2009) concluded that anxiety was linked to inefficient task performance when the task required a greater reliance on working memory. Recent research has also contributed physiological evidence in support of the PET and ACT theories. Neuroimaging data from Fales et al. (2008) showed that compared to non-anxious individuals, participants with high anxiety displayed decreased processing efficiency in the dorsolateral prefrontal cortex (DLPFC) and ventrolateral prefrontal cortex (VLPFC) during an anxiety-induced working memory-task.

Other research has demonstrated that independent of its susceptibility to anxiety, working memory performance can be worsened by psychosocial (Elzinga & Roelofs, 2005; Wolf, Schommer, Hellhammer, McEwen, & Kirchbaum, 2001), chronic (Kewman, Vaishampayan, Zald, & Han, 1991; Libon et al., 2010), and acute experimental (Duncko, Johnson, Merikangas, & Grillon, 2009; Schoofs, Wolf, & Smeets, 2009) stress and the subsequent release of cortisol. However, in a few studies, cortisol release has enhanced working memory when combined with a distractor task (Oei, Tollenaar, Spinhoven, & Elzinga, 2009), in aging veterans (Yehuda, Harvey, Buchsbaum, Tischler, & Schmeidler, 2007), and in elderly, but not young men (Wolf et al., 2001). It has been suggested that time of testing (morning vs. afternoon), time of cortisol administration (before vs. after testing), and age of participants (old vs. young) may explain some of the contradictory findings (Het, Ramlow, & Wolf, 2005).

The release of cortisol during its response to stress is a well-defined neuroendocrine mechanism activated as part of the negative feedback loop in the hypothalamic-pituitary-adrenal (HPA) axis (Sauro, Jorgensen, & Pedlow, 2003). Importantly, the prefrontal cortex (PFC) is a brain region involved in higher-order executive cognitive processes (Arnsten, 2009), affective processing (Posner et al., 2009), and has high densities of glucocorticoid receptors, which make it sensitive to changes in the concentration of cortisol induced by stress. As the PFC mediates working memory processes, this may explain why working memory is susceptible to increased cortisol levels (Barseganyan, Mackenzie, Kurose, McGaugh, & Roozendaal, 2010).

In support of this contention, Lupien et al. (1999) found significantly impaired working memory performance in young men when they were administered high doses of hydrocortisone (40 mg); however, at the same high doses, declarative fact-based memories were not significantly impaired. Another line of evidence suggests that cortisol levels affect processing, such that inhibition of emotional information (e.g., angry faces) occurs at lower doses of hydrocortisone (10 mg), but not for higher hydrocortisone doses compared to placebo. Of particular note, this effect differed for anxious participants, as they exhibited

inhibition of angry faces at higher, but not lower doses (Taylor, Ellenbogen, Washburn, & Jooper, 2011). Although these studies differed in their dose dependent findings, overall the research indicates increased levels of cortisol effect executive cognitive functioning.

There is a small body of research demonstrating that anxiety levels play an important role in working memory capacity when individuals are exposed to a stressor. For instance, Sorg (1992) found that a video game competition diminished working memory in individuals with high trait anxiety. Robinson et al. (2008) showed that working memory was preserved during anticipation of a naturalistic stressor (escape from a water submerged helicopter), but significantly impaired after stress exposure. Further, the men had significantly higher state anxiety levels and cortisol elevations following stress exposure. Similarly, a recent investigation conducted on the effects of parachuting stress for both novice and experienced parachutists found working memory impairments for novice and experienced parachutists 10 minutes before jumping compared to controls. In addition, novice parachutists had working memory impairments after landing (Leach & Griffith, 2008).

These studies provide important evidence for the disruptive influence of anxiety and a stressor on working memory. However, they were limited by the lack of control over the stressor and by small single-sex samples comprised of experts, which may not generalize to other populations. Further, due to the nature of the stressors, these studies could only test working memory before and not during exposure to stress. In the present work, we aimed to address these issues and tested working memory during exposure to acute stress in order to see if concurrent exposure impaired working memory performance. In line with Leach et al. (2008), we also tested working memory after stress, as in that study the combination of anxiety and cortisol increases produced working memory impairments. Finally, we measured cortisol at baseline and after acute stress or the control procedure in order to assess any changes.

To allow for direct comparisons between working memory during and after the acute stress or the control procedure we used the Letter-Number Sequencing Test (LNS; Wechsler, 1997). The Digit Span (Wechsler, 1997) was used as a baseline measure of short memory to reduce potential LNS practice effects, because a lot of the variance in the LNS can be attributed to the Digit Span (Crowe, 2000), and because previous research has found a strong correlation ($r = .71$) between the LNS and Digit Span (Luo, Chen, Zen, & Murray, 2010). However, the LNS was chosen as it has been argued that the LNS requires more executive processing than the Digit Span (Engle, Tuholski, Laughlin, & Conway, 1999), and it has low practice effects (Beglinger et al., 2005). Additional strengths of the LNS are that it was constructed to fit Baddeley's working memory framework (Hudetz, Hudetz, & Klayman, 2000), it has been shown to be the best predictor of fluid intelligence (Shelton, Elliott, Hill, Calamia, & Gouvier, 2009), and it was a strong predictor of a working memory criterion that was comprised of subtests that included the n-back and listening span tasks (Hill et al., 2010).

Drawing on insights from the PET and ACT, and the established relationships between working memory and stress, the current study investigated the relationship between anxiety, acute stress, and working memory. The aforementioned studies suggest a complex

relationship between stress, anxiety, and working memory. Therefore, the aim of the present study was to better understand these relationships and determine how anxiety, which can be conceived as a preexisting vulnerability to stress, influences working memory after the addition of a stressor. Specifically, we suggest a mediation model, where anxiety explains the underlying relationship between exposure to acute stress and working memory. Further, acute stress results in higher cortisol levels (Duncko et al., 2009; Schoofs et al., 2009) and the effect of cortisol on working memory may be dependent on whether increases in cortisol are low or high when exposed to stress (Lupien et al., 1999; Taylor et al., 2011). As such, we test a model in which cortisol is a moderator of the mediated relationship (i.e., anxiety mediating the relationship between exposure to stress and working memory). To our knowledge, we are the first study to test these associations.

Hypotheses

We tested a model of moderated mediation, which, by definition, integrates two assumptions into one model. We conducted two preliminary analyses; first, whether exposure to forehead cold pressor (i.e., being in the stressed vs. control condition) influenced working memory performance through existing anxiety levels (simple mediation) for working memory assessed after acute stress or a control procedure. Next, we tested whether the strength of acute stress and anxiety depends on cortisol levels (moderation). In particular, we hypothesized that mediation of working memory performance by anxiety happens only in individuals who had high cortisol levels after exposure to acute stress (moderation mediation). This conceptual model was chosen, as we believe that the influence of cortisol release was dependent on the experiencing stress and anxiety to impair working memory (see Figure 1.) Previous research has demonstrated gender differences in cortisol response (Kirschbaum & Hellhammer, 1994) and in working memory performance during a stressor (Hood, Pulvers, & Spady, 2013). Further, as more women are diagnosed with anxiety disorders than men (McLean, Asnaani, Litz, & Hofmann, 2011) we will analyze the moderated mediation model with and without gender as a covariate.

2. Methods

2.1 Participants

One hundred and twenty-six university students volunteered for this study; however, one hundred and three participants met all inclusion/exclusion criteria and constituted the healthy non-smoking study sample ($M_{\text{age}} = 21.04$, $SD = 4.39$, range 18–49 years; 49% male) that gained course credit. Data from a subset of this sample has been published previously in relation to gender differences in working memory and tested hypotheses unrelated to anxiety (Hood, Pulvers, & Spady, 2013). A standardized interview checked for inclusion criteria. Participants were excluded ($N = 18$) if they had circulatory problems (e.g., Reynaud's disease), peripheral neuropathy, thyroid problems, diabetes, lupus, other connective tissue disorders, cardiovascular disorders, high blood pressure, and/or hypertension, or currently were taking any pain or psychotropic medications. In addition, participants could not have a history of fainting or seizures, significant trauma or history of pain disorders, significant weight loss or major surgery within the last 6 months, substance abuse, a neurological condition, be pregnant, and could not eaten or drank anything but water for one hour before

the study. Five participants data were excluded from analyses because one participant could not tolerate the CPT for the entire test and four participants were excluded due to administration errors on the Letter-Number Sequencing Test.

2.2 Measure and Apparatus

2.2.1 Beck Anxiety Inventory—The Beck Anxiety Inventory (BAI; Beck, Epstein, Brown, & Steer, 1988) is a twenty-one item self-report inventory for measuring the severity of anxiety. Items are measured on a four-point Likert scale, ranging from 0 “Not at all” to 3 “Severely—It bothered me a lot.” The total BAI score ranges from 0 – 63 and a higher score represents a higher degree of anxiety. Previous research demonstrated the BAI has good test-retest reliability over one week (.75) and high internal consistency ($\alpha = .92$) (Beck et al., 1988). The Cronbach’s alpha in this study was .89. One participant did not complete the entire BAI and so their data is not included in analyses using this measure.

2.2.2 The McGill Pain Questionnaire- Short-Form—The McGill Pain Questionnaire-Short Form (MPQ-SF) is a pain rating scale that consists of 15-descriptor items, 11 items relate to sensory pain dimensions (e.g. shooting), and four items relate to affective pain dimensions (e.g. fearful) (Melzack, 1987). Participants rated items on a 4-point scale, ranging from zero (no pain) to three (severe). Total scores can range from 0 – 45, with higher scores representing higher pain levels. The Cronbach’s alpha in this study was .89.

2.2.3 Pain Rating—Immediately after the cold pressor task participants verbally indicated the pain of the cold pressor task using a 0 – 100 pain index, with zero being no pain at all, and 100 the worst pain imaginable.

2.2.4 Forehead Cold Pressor—The forehead cold pressor (Anderson et al., 2012; Logan, Gedney, Sheffield, Xiang, & Starrenburg, 2003) regulated temperature and maintained a continuous $0 \pm 1^\circ$ Celsius. The cold pressor apparatus used (The Polar Care 500 unit: Breg®) includes a low voltage submersible pump with in-line thermometer and flow valve for temperature control. The unit contained 2.3 kg of ice and 10.5 liters of water. A pad had cold water distributed throughout the entire surface, and researchers strapped the pad on the participants’ forehead while they completed the Letter-Number Sequencing test. Participants in the control condition wore the apparatus, but the pad did not contain any water. The Letter-Number Sequencing test took less than five minutes, which supported safety guidelines for exposure in cold pressor tasks.

2.3. Working Memory Tests

2.3.1. Letter-Number-Sequencing Test—For the LNS test (Wechsler, 1997), the researcher verbally presented different sets of increasingly longer sequences of intermixed letters and numbers at a rate of one per second. After each sequence, participants repeated the numbers in numerical order and letters in alphabetical order. The LNS test consists of 21 trials with sequences that range from two stimuli (e.g., B-4) up to a maximum length of eight stimuli. Researchers presented three trials at each length and discontinued the test when the participants failed on three consecutive trials of the same length. To minimize the possibility for practice effects, researchers administered alternate forms of the test (first and

second working memory tests). Researchers used formulated scaled scores for each individual based on his or her age. The subtest has a norm-referenced mean of 10 and a standard deviation of 3 (Wechsler, 1997).

2.3.2. Digit Span Test—The Digit Span backwards (Wechsler 1997) test assesses capacity and manipulation in short-term memory. Individuals repeated a series of orally presented digits in reverse order. Individuals immediately repeated the digits back after presentation. If successful, they are given a longer series. The longest series is the individual's digit span. The maximum score possible was 14. Researchers used formulated scaled scores for each individual based on his or her age. The subtest has a norm-referenced mean of 10 and a standard deviation of 3 (Wechsler, 1997).

2.4. Cortisol Assessment

To collect saliva samples for cortisol measurement, research assistants had participants passively salivate for one minute through a short straw into a cryovial tube. Participants chewed on a waxed sheet (Parafilm®) to stimulate saliva and provided salivary samples at baseline and following the second working memory test. After collection, researchers capped, labeled and froze samples at -20°C in a non-self-defrosting freezer. Before the assay, samples thawed at room temperature. Saliva was diluted 1:2 or 1:4 in B3 dilution buffer and free cortisol concentrations were measured using a high sensitivity cortisol immunoassay kit (Enzo® Life Sciences, Plymouth Meeting, PA) and an ELx800 plate reader (Bio-Tek, Winooski, VT) following the manufacturer's protocol. The intra-assay coefficient of variation was $< 15\%$ and the corresponding inter-assay coefficient was $< 15\%$. Due to intra-assay coefficients of greater than 30% ($N = 8$), cortisol values were only analyzed for 95 participants.

2.5 Procedures

Before arrival, researchers randomly assigned participants to either a control or an experimental condition. After participants arrived at the laboratory, a research assistant told participants that they may or may not experience discomfort although participants did not have prior knowledge about condition assignment. A research assistant then explained the procedure and obtained written informed consent. At the beginning of the session, a research assistant obtained the first salivary sample. Immediately afterwards, participants completed the baseline short-term memory test (Digit Span) and completed a counterbalanced questionnaire packet that contained measures of demographics, and the BAI. Next, participants completed the first LNS working memory test while wearing the forehead apparatus for no longer than 5 minutes. Participants were either in the stress condition (freezing water in the forehead apparatus) or in the control condition (no water in the forehead apparatus). Immediately following the first LNS and after removal of the apparatus, participants gave the verbal pain rating; they then completed the McGill Pain Questionnaire. Following a 15-minute break, in which participants read neutral magazines, participants completed the second LNS working memory test without the forehead apparatus. After the second LNS, a research assistant obtained a second salivary sample. The entire study took about one hour (see Table 1 for detailed timeline). All testing took place between 10am and 3pm to control for the diurnal variation of the stress hormone

cortisol (Jukic et al., 2008; Wegienka & Baird, 2005). The California State University San Marcos Institutional Review Board approved the protocol.

Statistical Analyses—Independent samples t-tests assessed the differences between the stress and control conditions. Cohen's d was used as the measure of effect size. Pearson correlations evaluated the associations between anxiety, salivary cortisol values, and short-term memory (Digit Span) at baseline, and between anxiety, salivary cortisol values, and the first and second LNS working memory tests separately for the stress and control conditions after the stress or control procedure. Due to positively skewed distributions, cortisol values were $\log_{10}$ -transformed for Pearson correlation analyses. In addition, we added a constant of one as some baseline cortisol values were close to zero: $\log(X_i + 1)$ (Howell, 2012).

In order to test the relationships between condition (stressed vs. control), anxiety, salivary cortisol values, and working memory performance we used the PROCESS procedure for SPSS (Hayes, 2012). PROCESS produced direct and indirect effects for mediation, conditional effects in the moderation models, and conditional indirect effects in moderated mediation model (Hayes, 2012). We tested models with and without statistically controlling for gender. In order to test for indirect effects, PROCESS utilizes bootstrapping, which is a non-parametric resampling procedure. As the sampling distribution of the statistic is formulated through resamples from the data set, there are no assumptions based on normality theory, it avoids power problems associated with non-normally distributed variables, and can be applied to small samples with more confidence (Preacher & Hayes, 2004, 2008; Preacher, Rucker, & Hayes, 2007).

From our original dataset of 102 cases, random sampling with replacement generated a bootstrap sample of 102 cases for the mediation models. The moderated mediation model had 95 cases to generate the bootstrap sample. Repeating the process 5000 times provided the basis for the bootstrap estimates and calculated means and standard errors. For both the moderation and moderated mediation analyses PROCESS mean centered the specific values of the moderator. PROCESS produced asymmetric bias corrected and accelerated (BCa) 95% confidence intervals (CI) to test for significance, as they adjust for any bias and skewness in the bootstrapped distribution. If zero was not within the 95% confidence interval, we concluded that the indirect effect was significantly different from zero at $p < .05$, two tailed (Preacher & Hayes, 2004). Kappa-squared (κ^2) was used as the measure of effect size in mediation analyses.

3. Results

3.1. Preliminary Analyses of Baseline Measures

Initially, all participants completed the Digit Span as a measure of short-term memory at baseline. Scores ranged from 2–12 ($M = 6.21$, $SD = 2.02$). First, we conducted correlations on the entire sample to determine whether there were any relationships between anxiety and short-term memory at baseline, cortisol and short-term memory at baseline, and anxiety and cortisol at baseline, prior to the acute stress or control procedure. There were no significant relationships between anxiety and short-term memory at baseline, and cortisol at baseline (all $ps > .05$).

Although participants were randomly assigned to condition, we also ran baseline analyses separated on condition to aid in interpretation of subsequent analyses conducted after the stress or control procedure. As expected, there were no significant relationships between anxiety, short-term memory at baseline, and cortisol levels at baseline for the control condition or for stress condition analyzed separately (all $ps > .05$). These results indicate that there were no associations between pre-existing anxiety, cortisol levels, and short-term memory prior to the addition of acute stress or the control procedure.

3.2. Group Differences After the Acute Stress or Control Procedure

We conducted analyses to assess differences between the stress and control condition. For all subsequent analyses, the Letter-Number Sequencing test (LNS) was used to assess working memory. Table 2 shows the descriptive statistics for cortisol and anxiety levels, working memory scores, self-reported pain, and pain ratings separated by condition. Turning first to cortisol levels, individuals exposed to acute stress had significantly higher cortisol levels than controls after the second LNS working memory test $t(94) = 3.41, p = .001, d = .85$. Elevated cortisol levels after the second LNS working memory test indicate a cortisol elevation triggered by acute stress for the stressed participants, as cortisol levels at baseline were not significantly different between stress and control conditions. Similarly, anxiety levels and both working memory scores were not significantly different based on condition (all $ps > .05$). Self-reported pain, $t(94) = 7.95, p < .001, d = 1.95$, and pain ratings $t(94) = 8.87, p = .001, d = 1.64$, were both significantly different based on condition, with the stress condition reporting more pain than controls (see Table 2). These results indicate that the stress manipulation was effective; stressed participants experienced a significant rise in cortisol and reported more pain (higher pain ratings) than controls.

3.3. Correlations After the Acute Stress or Control Procedure

Correlations determined relationships between cortisol levels, anxiety, working memory, self-reported pain, and pain ratings for the stress and control condition separately. For the stress condition, anxiety levels and first LNS working memory test were significantly negatively associated ($r = -.31, p = .03$) and anxiety levels and second LNS working memory test were significantly negatively associated ($r = -.34, p = .01$). Anxiety and cortisol values after stressor were significantly positively associated ($r = .47, p = .001$). Cortisol values after stressor were significantly negatively associated with second LNS working memory test ($r = -.33, p = .02$). None of the other comparisons were significant for the stress condition (all $ps > .05$). For the control condition, there were no significant correlations between cortisol levels, anxiety levels, working memory scores, self-reported pain, and pain ratings (all $ps > .05$).

3.4. Mediation

Because stressed participants' anxiety was significantly related to the second LNS working memory and cortisol after stress, and because the second LNS working memory test was significantly related to cortisol, we tested to see if anxiety mediated the relationship between condition (stressed vs. control) and the second LNS working memory test. Mediation analyses indicate if the total effect (weight c) of the independent variable (IV; condition) on

a dependent variable (DV; working memory) is comprised of a *direct effect* (weight c') of the IV on the DV and an *indirect effect* (weight $a \times b$) of the IV on the DV through a predicted mediator (anxiety). Weight a denotes the IV on the mediator, whereas, weight b is the effect of the mediator on the DV.

For the second LNS working memory test, the total effect (weight c) of condition on the working memory was $-.28, p = .55$. Evidence of a total effect before estimating direct and indirect effects is no longer considered necessary for mediation analyses (Hayes & Matthes, 2009; Rucker, Preacher, Tormala, & Petty, 2011; Shrout & Bolger, 2002; Zhao, Lynch, & Chen, 2010). The direct effect (weight c') of condition on working memory was $-.50, p = .29$. Current recommendations suggest that inferences should not be based on the statistical significance of paths a and b ; instead inferences should be an explicit quantification of the indirect effect (Hayes, 2012). PROCESS revealed that the indirect effect was positive and statistically different from zero, as evidenced by a 95% bias-corrected bootstrap confidence interval that was entirely above zero (.02 to .59) with a κ^2 of .05 and a 95% BCa CI of .01 – .14.

3.5. Moderated Mediation

Moderated mediation emphasizes the estimation of the extent to which an indirect effect of an IV (X) on the DV (Y) through a mediator M depends on a moderator (W). In this study, as mediation was evident only for the second LNS working memory test (after acute stress or control procedure), we tested whether the indirect effect of condition (X) on the second LNS working memory test (Y) through anxiety (M) depended upon cortisol levels after acute stress (W). Analyses were conducted with and without gender a covariate. Gender was not a significant covariate and so all subsequent analyses do not include gender. Evidence of moderation of the indirect effect by anxiety is found in a statistically significant interaction between condition and cortisol levels in the model of anxiety, coefficient = $-1.06, p = .01$. As the first stage phase of the mediation model was moderated, the indirect effect was also moderated. In this scenario, Hayes (2012) recommends the estimation of conditional indirect effects using a biased and corrected bootstrapped CI to determine if the indirect effects differ from zero at specific values of the moderator.

Table 3 shows that in line with the assumptions, the indirect effect of condition on working memory through anxiety levels increased along with cortisol levels. This result indicated that anxiety mediated the effect of condition on working memory when cortisol levels were high (75th and 90th percentile), but not when cortisol levels were at or below average (the 50th percentile and below). Additionally, the strength of the conditional indirect effect (β) increased as cortisol levels increased. At the 75th and 90th percentiles, the 95% confidence intervals did not cross zero, which indicated that the conditional indirect effect was significantly different from zero at $p < .05$, two tailed (see Table 3).

Figure 2 elucidates that the 95% bootstrapped confidence interval for the conditional indirect effect is entirely above zero when cortisol values were at 6.20 nmol/l and above. Among individuals with cortisol values lower than this, the indirect effects were not different from zero as evidenced by a bootstrapped confidence interval that overlapped zero. For clarity, Figure 2 shows the indirect effect plotted at all values (uncentered) of the

moderator with a 95%-confidence band. Thus, anxiety levels mediated the effect of condition on working memory, only among those individuals who had high cortisol levels.

4. Discussion

The present research examined the relationship between condition (stressed vs. controls), anxiety, and working memory. Data revealed no significant differences in working memory between the stress and control condition. However, in the stress condition, there was a significant relationship between anxiety and working memory during and after acute stress, and between anxiety and cortisol levels after acute stress. These results indicate there is a link between high anxiety and working memory impairment both during and post acute stress. Subsequent analyses showed that anxiety mediated the relationship between condition and the second LNS working memory test. A moderated mediation analysis demonstrated that anxiety mediated the relationship only when cortisol levels increased. Thus, the moderated mediation model provides a description of the mechanisms by which stress influenced working memory.

Our data provide support for the attentional control theory (ACT). One prominent hypothesis of the theory postulates that anxiety impairs processing efficiency and performance effectiveness on tasks that involve updating and monitoring of working memory representations, but only under stressful conditions (Eysenck, Derakshan, Santos, & Calvo, 2007). Updating is a function controlled by the central executive, which codes incoming information and replaces no longer relevant information. Of importance here, updating is not just simple maintenance, it also requires a dynamic manipulation of the contents of working memory (Miyake, Friedman, Emerson, Witzki, & Howerter, 2000). In this study, participants with high anxiety appear to be vulnerable to distressing external stimuli unrelated to the task. Existing anxiety and the addition of a stressor may have impaired updating in working memory as the overall demands on the central executive increased. Our findings extend upon the small body of previous research that tested ACT using a stressor (Leach & Griffith, 2008; Robinson et al., 2008; Sorg & Whitney, 1992).

A critical aspect of this study is that increased cortisol levels moderated the relationship between condition (stressed vs. controls) and working memory via anxiety. This indicates that mere exposure to stress did not influence working memory in anxious individuals. Rather, our results imply that activation of the HPA axis and subsequent release of cortisol was necessary for working memory impairment. This finding is supported by the fact that moderated mediation only occurred for the second working memory test, which took place 25—30 minutes after the cold pressor task and coincided with the delayed cortisol response. Previous research has found that experimentally induced acute stress resulted in supraoptimal cortisol levels that may have reduced working memory-related DLPFC activity and reallocated neural resources away from executive functioning (Qin, Hermans, Van Marle, Luo, & Fernández, 2009). It is possible that this “resource depletion” occurred in the present study.

Cortisol released during acute stress may have reduced PFC functioning. However, it is worth noting that this process is potentially adaptive; it is a facilitation that trades the

accuracy of deliberate, higher-order cognition for speed in the “fight-or-flight” response induced by stress (Qin et al., 2009). Additionally, anxious individuals have lower pathway strength, which has been shown to be associated with lower levels of trait anxiety (Kim & Whalen, 2009) and decreased functional coupling between the ventromedial prefrontal cortex (VMPFC) and the amygdala (Pezawas et al., 2005). A weakened connectivity between amygdala and PFC coupled with a cortisol-induced reallocation of resources away from executive function networks, could explain the present study’s findings. More research is necessary, as this study did not include neuroimaging data. However, we do lay the groundwork for interesting future research.

Much of the research utilizing the ACT predicts that state anxiety interferes with cognitive functioning in the central executive of working memory. We proffer two plausible explanations for our findings, as the present work measured anxiety on arrival, but did not obtain a measure of state anxiety during the experimental paradigm. First, individuals higher in anxiety could have experienced more state anxiety during the task compared to those low in anxiety. Pre-existing anxiety could have led to more anxiety during the working memory test. Second, anxiety prior to testing may produce the same resource depletion of cognitive function as state anxiety. Pre-existing anxiety and state anxiety have both been shown to modulate neural activity to fearful distractors in a low perceptual load condition (Bishop, Jenkins, & Lawrence, 2007), so they could both result in similar working memory impairments. Indeed, previous research has found that individuals high in trait anxiety had reduced prefrontal activity and slower target identification even after controlling for state anxiety (Bishop, 2009). Moreover, there is some evidence that trait anxiety can produce working memory deficits (Leon & Revelle, 1985).

Although the present study provides new information of a moderated mediation model in the relationship between condition (stressed vs. controls), working memory, anxiety, and cortisol levels, we should also address some of the limitations. This study used the BAI, which has been described as a measure of “prolonged state anxiety.” The BAI was designed to measure clinical anxiety while minimizing the overlap between depression and anxiety (Beck et al., 1988). It might be helpful to use a purer measure of anxiety with this paradigm. Specifically, future researchers could use a measure of trait anxiety before testing and/or a measure of state anxiety after acute stress. Additionally, other neurochemical changes not tested in this study, such as the release of catecholamines, could have influenced working memory deficits. Catecholamines are hormones mainly produced by the adrenal glands and include dopamine, norepinephrine, and epinephrine. Previous animal research has found that catecholamine release after uncontrollable stress can modulate working memory functioning (Arnsten, 1998). These physiological responses could possibly be additional moderators in the tested mediation.

In terms of our cortisol assessment, it would have been beneficial to have salivary samples at multiple time points to give a clearer picture of the moderating effect of cortisol. Further, it would have been advantageous to test participants over a shorter period to further control for the diurnal variation of cortisol. This would be especially useful as the timing of cortisol collection may be particularly important in terms of whether cortisol increases are detrimental or enhancing for working memory (Het et al., 2005). Finally, due to assay

collection problems, cortisol data was not available for all participants. Because of these limitations, our results should be interpreted cautiously until replicated.

Our results may have clinical implications. Leigh and Hirsch (2011) found that worry-prone individuals had less residual working memory capacity during worry compared to low worriers. Additionally, Hayes et al. (2008) found that high worriers had higher levels of anxiety. These studies are consistent with our findings and provide further support for ACT, as worrisome thoughts may capture attentional control. Although speculative, it is possible that residual working memory capacity is reduced for individuals with anxiety disorders. Our research may help elucidate how anxiety disorders influence working memory.

Conclusions

By showing that working memory deficits in healthy individuals under acute stress operate through anxiety only when cortisol levels are high, the present study provides new information into the underlying physiological mechanisms at work. Further, these relationships had not previously been tested when assessing the PET and ACT. Future studies could expand on the present work through the addition of brain imaging to assess neural correlates. Likewise, it would be worthwhile to test the same paradigm using a specific measure of trait and/or state anxiety, or test a clinical population diagnosed with an anxiety disorder. However, the present research is an important step in understanding the complex relationship between these variables.

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References

- Anderson RD, Mehta P, Wei J, Johnson BD, Petersen J, Handberg E, Azarbal B. Differential effects of cold pressor and acetylcholine during endothelial function assessment in women with no obstructive coronary artery disease: the NHLBI- sponsored women's ischemia syndrome evaluation (wise) study. *Journal of the American College of Cardiology*. 2012; 59:E1489–E1489.
- Arnsten AFT. Catecholamine modulation of prefrontal cortical cognitive function. *Trends in Cognitive Sciences*. 1998; 2:436–447. [PubMed: 21227275]
- Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews Neuroscience*. 2009; 10:410–422. [PubMed: 19455173]
- Baddeley AD, Hitch GJ. Working memory. *The psychology of learning and motivation*. 1974; 8:47–89.
- Barseganyan A, Mackenzie SM, Kurose BD, McGaugh JL, Roozendaal B. Glucocorticoids in the prefrontal cortex enhance memory consolidation and impair working memory by a common neural mechanism. *PNAS Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:16655–16660.
- Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: Psychometric properties. *Journal of Consulting and Clinical Psychology*. 1988; 56:893–897. [PubMed: 3204199]
- Beglinger LJ, Gaydos B, Tangphao-Daniels O, Duff K, Kareken DA, Crawford J, Fastenau PS, et al. Practice effects and the use of alternate forms in serial neuropsychological testing. *Archives of Clinical Neuropsychology*. 2005; 20:517–529. [PubMed: 15896564]

- Bishop SJ. Trait anxiety and impoverished prefrontal control of attention. *Nature Neuroscience*. 2009; 12:92–98. [PubMed: 19079249]
- Bishop SJ, Jenkins R, Lawrence AD. Neural processing of fearful faces: Effects of anxiety are gated by perceptual capacity limitations. *Cerebral Cortex*. 2007; 17:1595–1603. [PubMed: 16956980]
- Calabrese EJ. Stress Biology and Hormesis: The Yerkes-Dodson Law in Psychology—A Special Case of the Hormesis Dose Response. *Critical Reviews in Toxicology*. 2008; 38:453–462. [PubMed: 18568865]
- Cheng DT, Knight DC, Smith CN, Helmstetter FJ. Human amygdala activity during the expression of fear responses. *Behavioral Neuroscience*. 2006; 120:1187–1195. [PubMed: 17201461]
- Crowe SF. Does the letter number sequencing task measure anything more than digit span? Assessment. 2000; 7:113–117. [PubMed: 10868248]
- Derakshan N, Eysenck MW. Anxiety, processing efficiency, and cognitive performance: New developments from attentional control theory. *European Psychologist*. 2009; 14:168–176.
- Duncko R, Johnson L, Merikangas K, Grillon C. Working memory performance after acute exposure to the cold pressor stress in healthy volunteers. *Neurobiology of Learning and Memory*. 2009; 91:377–381. [PubMed: 19340949]
- Elzinga BM, Roelofs K. Cortisol-Induced Impairments of Working Memory Require Acute Sympathetic Activation. *Behavioral Neuroscience*. 2005; 119(1):98–103. [PubMed: 15727516]
- Engle RW, Tuholski SW, Laughlin JE, Conway ARA. Working memory, short-term memory, and general fluid intelligence: a latent-variable approach. *Journal of Experimental Psychology: General*. 1999; 128:309. [PubMed: 10513398]
- Eysenck MW, Calvo MG. Anxiety and performance: The processing efficiency theory. *Cognition and Emotion*. 1992; 6(6):409–434.
- Eysenck MW, Derakshan N, Santos R, Calvo MG. Anxiety and cognitive performance: Attentional control theory. *Emotion*. 2007; 7(2):336–353. [PubMed: 17516812]
- Eysenck MW, Payne S, Derakshan N. Trait anxiety, visuospatial processing, and working memory. *Cognition and Emotion*. 2005; 19(8):1214–1228.
- Fales CL, Barch DM, Burgess GC, Schaefer A, Mennin DS, Gray JR, Braver TS. Anxiety and cognitive efficiency: Differential modulation of transient and sustained neural activity during a working memory task. *Cognitive, Affective & Behavioral Neuroscience*. 2008; 8(3):239–253.
- Hayes S, Hirsch C, Mathews A. Restriction of working memory capacity during worry. *Journal of Abnormal Psychology*. 2008; 117(3):712. [PubMed: 18729625]
- Hayes, AF. PROCESS: A versatile computational tool for observed variable mediation, moderation, and conditional process modeling [White paper]. 2012. Retrieved from <http://www.afhayes.com/public/process2012.pdf>
- Hayes Andrew F, Matthes J. Computational procedures for probing interactions in OLS and logistic regression: SPSS and SAS implementations. *Behavior Research Methods*. 2009; 41:924–936. [PubMed: 19587209]
- Hayes S, MacLeod C, Hammond G. Anxiety-linked task performance: Dissociating the influence of restricted working memory capacity and increased investment of effort. *Cognition and Emotion*. 2009; 23(4):753–781.
- Het S, Ramlow G, Wolf OT. A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinology*. 2005; 30:771–784. [PubMed: 15919583]
- Hill BD, Elliott EM, Shelton JT, Pella RD, O’Jile JR, Gouvier WD. Can we improve the clinical assessment of working memory? An evaluation of the Wechsler Adult Intelligence Scale-Third Edition using a working memory criterion construct. *Journal of Clinical and Experimental Neuropsychology*. 2010; 32:315–323. [PubMed: 19657913]
- Hood A, Pulvers K, Spady TJ. Timing and Gender Determine If Acute Pain Impairs Working Memory Performance. *The Journal of Pain*. 2013; 14:1320–1329. [PubMed: 23972353]
- Hudetz JA, Hudetz AG, Klayman J. Relationship between relaxation by guided imagery and performance of working memory. *Psychological Reports*. 2000; 86:15–20. [PubMed: 10778243]
- Johnson DR, Gronlund SD. Individuals lower in working memory capacity are particularly vulnerable to anxiety’s disruptive effect on performance. *Anxiety, Stress & Coping: An International Journal*. 2009; 22:201–213.

- Jukic AMZ, Weinberg CR, Wilcox AJ, McConaughy DR, Hornsby P, Baird DD. Accuracy of Reporting of Menstrual Cycle Length. *American Journal of Epidemiology*. 2008; 167:25–33. [PubMed: 17928401]
- Kazama AM, Heuer E, Davis M, Bachevalier J. Effects of neonatal amygdala lesions on fear learning, conditioned inhibition, and extinction in adult macaques. *Behavioral Neuroscience*. 2012; 126:392–403. [PubMed: 22642884]
- Kewman DG, Vaishampayan N, Zald D, Han B. Cognitive impairment in musculoskeletal pain patients. *International Journal of Psychiatry in Medicine*. 1991; 21:253–262. [PubMed: 1955277]
- Kim MJ, Whalen PJ. The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *The Journal of Neuroscience*. 2009; 29:11614–11618. [PubMed: 19759308]
- Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*. 1994; 19:313–333. [PubMed: 8047637]
- Leach J, Griffith R. Restrictions in working memory capacity during parachuting: A possible cause of “no pull” fatalities. *Applied Cognitive Psychology*. 2008; 22:147–157.
- Leigh E, Hirsch CR. Worry in imagery and verbal form: Effect on residual working memory capacity. *Behaviour research and therapy*. 2011; 49:99–105. [PubMed: 21159327]
- Leon MR, Revelle W. Effects of anxiety on analogical reasoning: A test of three theoretical models. *Journal of Personality and Social Psychology*. 1985; 49:1302–1315. [PubMed: 4078678]
- Libon DJ, Schwartzman RJ, Eppig J, Wambach D, Brahlin E, Peterlin BL, Kalanuria A. Neuropsychological deficits associated with complex regional pain syndrome. *Journal of the International Neuropsychological Society*. 2010; 16:566–573. [PubMed: 20298641]
- Logan HL, Gedney JJ, Sheffield D, Xiang Y, Starrenburg E. Stress influences the level of negative affectivity after forehead cold pressor pain. *The Journal of Pain*. 2003; 4:520–529. [PubMed: 14636820]
- Lupien SJ, Gillin CJ, Hauger RL. Working memory is more sensitive than declarative memory to the acute effects of corticosteroids: A dose-response study in humans. *Behavioral Neuroscience*. 1999; 113:420–430. [PubMed: 10443770]
- McLean CP, Asnaani A, Litz BT, Hofmann SG. Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *Journal of Psychiatric Research*. 2011; 45(8): 1027–1035. [PubMed: 21439576]
- Melzack R. The short-form McGill Pain Questionnaire. *Pain*. 1987; 30:191–197. [PubMed: 3670870]
- Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A. The unity and diversity of executive functions and their contributions to complex “frontal lobe” tasks: A latent variable analysis. *Cognitive Psychology*. 2000; 41:49–100. [PubMed: 10945922]
- Oei NYL, Tollenaar MS, Spinhoven P, Elzinga BM. Hydrocortisone reduces emotional distracter interference in working memory. *Psychoneuroendocrinology*. 2009; 34:1284–1293. [PubMed: 19398277]
- Olatunji BO, Cisler JM, Tolin DF. Quality of life in the anxiety disorders: A meta-analytic review. *Clinical Psychology Review*. 2007; 27:572–581. [PubMed: 17343963]
- Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS, Egan MF, et al. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: A genetic susceptibility mechanism for depression. *Nature Neuroscience*. 2005; 8:828–834. [PubMed: 15880108]
- Posner J, Russell JA, Gerber A, Gorman D, Colibazzi T, Yu S, Wang Z, et al. The neurophysiological bases of emotion: An fMRI study of the affective circumplex using emotion-denoting words. *Human Brain Mapping*. 2009; 30:883–895. [PubMed: 18344175]
- Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behavior Research Methods, Instruments & Computers*. 2004; 36:717–731.
- Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behavior Research Methods*. 2008; 40:879–891. [PubMed: 18697684]
- Preacher KJ, Rucker DD, Hayes AF. Addressing moderated mediation hypotheses: Theory, methods, and prescriptions. *Multivariate Behavioral Research*. 2007; 42:185–227.

- Qin S, Hermans EJ, Van Marle HJF, Luo J, Fernández G. Acute psychological stress reduces working memory-related activity in the dorsolateral prefrontal cortex. *Biological Psychiatry*. 2009; 66:25–32. [PubMed: 19403118]
- Robinson SJ, Sünram-Lea SI, Leach J, Owen-Lynch PJ. The effects of exposure to an acute naturalistic stressor on working memory, state anxiety and salivary cortisol concentrations. *Stress: The International Journal on the Biology of Stress*. 2008; 11:115–124.
- Rucker DD, Preacher KJ, Tormala ZL, Petty RE. Mediation analysis in social psychology: Current practices and new recommendations. *Social and Personality Psychology Compass*. 2011; 5(6): 359–371.
- Sauro MD, Jorgensen RS, Pedlow CT. Stress, Glucocorticoids, and Memory: A Meta-analytic Review. *Stress: The International Journal on the Biology of Stress*. 2003; 6(4):235–245.
- Schoofs D, Wolf OT, Smeets T. Cold pressor stress impairs performance on working memory tasks requiring executive functions in healthy young men. *Behavioral Neuroscience*. 2009; 123:1066–1075. [PubMed: 19824773]
- Senaratne R, Van Ameringen M, Mancini C, Patterson B. The burden of anxiety disorders on the family. *Journal of Nervous and Mental Disease*. 2010; 198:876–880. [PubMed: 21135638]
- Shelton JT, Elliott EM, Hill BD, Calamia MR, Gouvier WD. A comparison of laboratory and clinical working memory tests and their prediction of fluid intelligence. *Intelligence*. 2009; 37:283–293. [PubMed: 20161647]
- Shrout PE, Bolger N. Mediation in experimental and nonexperimental studies: New procedures and recommendations. *Psychological Methods*. 2002; 7:422–445. [PubMed: 12530702]
- Sorg BA, Whitney P. The effect of trait anxiety and situational stress on working memory capacity. *Journal of Research in Personality*. 1992; 26:235–241.
- Taylor VA, Ellenbogen MA, Washburn D, Joobor R. The effects of glucocorticoids on the inhibition of emotional information: A dose-response study. *Biological Psychology*. 2011; 86(1):17–25. [PubMed: 20934481]
- Teesson M, Mitchell PB, Deady M, Memedovic S, Slade T, Baillie A. Affective and anxiety disorders and their relationship with chronic physical conditions in Australia: Findings of the 2007 National Survey of Mental Health and Wellbeing. *Australian and New Zealand Journal of Psychiatry*. 2011; 45:939–946. [PubMed: 21967412]
- Wechsler, D. WAIS-III: Wechsler adult intelligence scale. San Antonio: Psychological Corporation; 1997.
- Wegienka G, Baird DD. A Comparison of Recalled Date of Last Menstrual Period with Prospectively Recorded Dates. *Journal of Women's Health*. 2005; 14:248–252.
- Wolf OT, Convit A, McHugh PF, Kandil E, Thorn EL, De Santi S, De Leon MJ. Cortisol differentially affects memory in young and elderly men. *Behavioral Neuroscience*. 2001; 115:1002. [PubMed: 11584913]
- Wolf OT, Schommer NC, Hellhammer DH, McEwen BS, Kirchbaum C. The relationship between stress induced cortisol levels and memory differs between men and women. *Psychoneuroendocrinology*. 2001; 26:711–720. [PubMed: 11500252]
- Yehuda R, Harvey PD, Buchsbaum M, Tischler L, Schmeidler J. Enhanced effects of cortisol administration on episodic and working memory in aging veterans with PTSD. *Neuropsychopharmacology*. 2007; 32:2581–2591. [PubMed: 17392739]
- Zhao X, Lynch JGJ, Chen Q. Reconsidering Baron and Kenny: Myths and truths about mediation analysis. *Journal of Consumer Research*. 2010; 37:197–206.

Figure 1.
Conceptual moderated mediation model in which cortisol levels influence working memory through the combination of condition (stress or control) and anxiety.

Figure 2.

A plot of the moderated indirect effect of condition (IV) on working memory (DV) through anxiety (mediator) versus uncentered cortisol levels (moderator) with confidence bands. This plot indicates that at cortisol values above 6.20 nmol/l, anxiety levels mediated the effect of condition on working memory.

Table 1**Study Timeline**

From the beginning of the study	
0—5 minutes	Study procedure and informed consent
5—10 minutes	Beck Anxiety Inventory (BAI)
10 minutes	First salivary sample
10—15 minutes	Digit Span
15—20 minutes	First LNS (with forehead apparatus)
25—30 minutes	Verbal pain rating and McGill Pain Questionnaire
30—45 minutes	Break (read neutral magazines)
45—50 minutes	Second LNS (without forehead apparatus)
50 minutes	Second salivary sample
50—55 minutes	Debriefing

Note: LNS = Letter-Number Sequencing Test

Table 2
Descriptive statistics for cortisol and anxiety levels and working memory and pain scores separated by condition.

Condition	Cortisol at baseline (nmol/L)	Cortisol after LNS 2 (nmol/L)	BAI	LNS 1	LNS 2	McGill Pain	Pain Rating
Acute Stress	Mean	4.46	12.87	9.09	10.53	10.27*	31.85*
	SD	2.89	4.75	9.07	2.50	6.52	23.62
Control	Mean	4.90	9.63	9.80	10.26	2.17	1.83
	SD	3.29	7.27	2.52	2.24	2.55	3.90

Note: BAI = Beck Anxiety Inventory, LNS = Letter-Number Sequencing working memory test,

* indicates a significant difference ($p < 0.05$) from the control condition.

Table 3

Bootstrapped indirect effects of condition on working memory via anxiety levels at specific values of the moderator (cortisol levels).

Mediator: Anxiety					
Cortisol Percentiles	Cortisol Levels	Effect (β)	SE	Upper BCa CI	Lower BCa CI
10th	2.00	-.13	.18	-.55	.17
25th	3.02	-.04	.15	-.37	.25
50th	4.82	.10	.13	-.11	.42
75th	7.72*	.34	.18	.07	.78
90th	10.35*	.56	.27	.12	1.20

* Note: $p < 0.05$; 5000 Bootstrapping resamples;

SE = Standard Error; Upper BCa CI and Lower BCa CI = Lower level and Upper level of the bias corrected and accelerated confidence intervals.