

Sex Matters: Anxiety and Aggression Predict Cortisol Responsivity in Men but Not Women

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Abstract

BACKGROUND: Sexual dimorphism in the hypothalamic pituitary adrenal (HPA) axis can influence sex-specific patterns of response to stressors. While a host of findings exist on sex differences in stress-induced activity of the HPA axis and associated mechanisms in rodents, less is known about the intricacies of sex differences in stress responsivity in humans. Accordingly, the overall aim of the present study was to investigate psychological variables that may account for differences in the cortisol stress response between men and women.

METHODS: Eighty-six participants filled out self-report measures of anxiety (STAY), aggression (BPAQ), and happiness (SHS). We then exposed all participants to a one-minute Cold Pressor Test (CPT) that was maintained between 3–5° C. Cortisol and pain ratings were assessed. We focused on the 20-minute time point for cortisol since that is when cortisol is near its peak post-stress.

RESULTS: Women reported higher pain ratings compared to men. Women also showed a positive relationship between pain ratings and cortisol. Aggression was significantly related to cortisol levels in men, but not in women. Similarly, trait anxiety was positively related to cortisol levels in men, but not in women. Happiness was unrelated to cortisol levels in women and men. Follow-up regressions were conducted separately for men and women. A significant model was found for cortisol in men only with trait anxiety, aggression, and the interaction between trait anxiety and aggression.

CONCLUSIONS: The current study builds on previous reports by showing that aggression and anxiety differentially influence the cortisol response to an acute stress in men and women.

INTRODUCTION

Sexual dimorphism in the hypothalamic pituitary adrenal (HPA) axis can influence sex-specific patterns of response to stressors (Asher *et al.* 2017; Heck & Handa 2019; Oyola & Handa 2017). While a host of findings exist on sex differences in stress-induced activity of the HPA axis and associated mechanisms in rodents, less is known about

the intricacies of sex differences in stress responsivity in humans. In contrast to rodent research, research in humans suggests that males have a higher cortisol response to an acute stress relative to females (Collins & Frankenhaeuser 1978; Frankenhaeuser *et al.* 1976; Frankenhaeuser *et al.* 1978; Kirschbaum *et al.* 1995a; Kirschbaum *et al.*

1995b; Stroud *et al.* 2002). Since complex and human-specific psychological factors such as rumination can influence stress responses (Faravelli *et al.* 2012; Herman & Cullinan 1997), it is possible that sex differences on several psychological variables relate to sex differences in HPA axis responsivity. Accordingly, the overall aim of the present study was to investigate key psychological variables that may account for differences in stress responses in men and women. We also aimed to discover how these variables relate to cortisol responses in men and women.

Aggression

Relative to women, men are more aggressive, scoring higher on self-report measures of aggression, and are more likely to be the target of aggressive acts (Borhart & Terrell 2014; Wilkowski *et al.* 2012). This difference has been proposed to be related to both sexual selection and social learning (Nivette *et al.* 2019; Nivette *et al.* 2014; Wilkowski & Robinson 2012). There is also evidence that high testosterone, low cortisol, and low serotonin are associated with increased trait aggression (Montoya *et al.* 2012). Given the higher levels of testosterone in males, this can also relate to sex differences in aggression. Sex-differences in aggressive behavior are reported to be related to concomitant increases testosterone and cortisol (Montoya *et al.* 2012). It is notable, however, that although unprovoked men are more aggressive than women, provocation attenuates the impact of sex on aggression (Bettencourt & Miller 1996). Previous research has shown that aggression in general can influence a behavioral response to stress. For example, in response to a stressor, individuals with high trait aggression demonstrate increased human-human approach behavior, while those with low trait aggression demonstrate increased avoidance behavior (Vogel & Schwabe 2019). In addition, low trait aggression is also associated with increased heart rate and electrodermal response to acute psychological stress (Zimmermann-Viehoff *et al.* 2008).

Trait Anxiety

Trait anxiety refers to the long-term experience of adverse feelings such as discomfort, worry, and tension (Spielberger 2010). Trait anxiety is another candidate variable that is known to be different between men and women and can also influence stress responsivity (Asher & Aderka 2018; Asher *et al.* 2017). Women are more likely than men to suffer from multiple forms of anxiety such as panic disorder (Sheikh *et al.* 2002; Kessler *et al.* 2012). It is possible that trait anxiety can relate to sex differences in stress responses. For example, in men, but not women, there is a cortisol-induced return of autonomic and amygdala-mediated fear responses (Kinner *et al.* 2018). In men, trait anxiety and heart rate are positively correlated with higher cortisone levels in response to the trier social stress test (Bae *et al.* 2019).

Positive Affect

Positive affect is a subcomponent of the broader category of subjective well-being and refers to enjoyable engagement with the environment and positive feelings (e.g., happiness, excitement, joy, and contentment) (Tomkins 1963; Clark *et al.* 1989). While men and women do not report differences in subjective happiness (Crowley & Knowles 2014), there are sex differences in the variables they link to happiness. Men report happiness in having personal time, mental control, and active leisure while women report social affiliation, passive leisure, and goal pursuit as major contributing factors to their happiness (Tkach & Lyubomirsky 2006). Happiness, or increased positive affect, is also an important candidate psychological factor for the present investigation, given that happiness is overwhelmingly linked to increased stress resilience and lowered stress responsivity (Panagi *et al.* 2019; Pressman & Cohen 2005). Indeed, the “broaden and build” theory of positive emotions (Fredrickson 2004) specifically posits that positive affect can broaden one’s perspective which can result in increased psychological resources that lead to increased stress resiliency. Positive affect is generally thought to promote resilience in response to a challenge or stressful event (Salovey *et al.* 2000).

Pain Perception

The current study also examined the extent to which stress activates pain sensitivity. There are established human sex differences in the subjective experience of pain. In studies which experimentally administer pain across a variety of methods (shock, pressure, heat, cold), women report lower pain thresholds and require larger doses of analgesia in response to pain relative to men (Nasser & Afify 2019).

In line with these findings, we predicted that women would show higher levels of self-reported pain ratings in response to cold stress (the cold pressor test) relative to men. We also hypothesized that, consistent with previous work, men would have significantly higher levels of self-reported aggression than women while women would have significantly higher levels of self-reported anxiety than men. Based on previous cold-pressor test cortisol data from our lab and others (Banks *et al.* 2015; Banks *et al.* 2014; Alomari *et al.* 2015; Schwabe *et al.* 2008) we further aimed to show that physiological stress responsivity would be differentially related to aggression, trait anxiety, and positive affect/happiness in men and women when cortisol levels were at their peak post-stress (~ 20 min post-stress).

MATERIALS AND METHODS

Participants

Participants were recruited by flyers distributed on the Nova Southeastern University campus. Eighty-six undergraduate students participated in exchange for a \$10.00 gift card (45 women, 31 men, M age =30.92

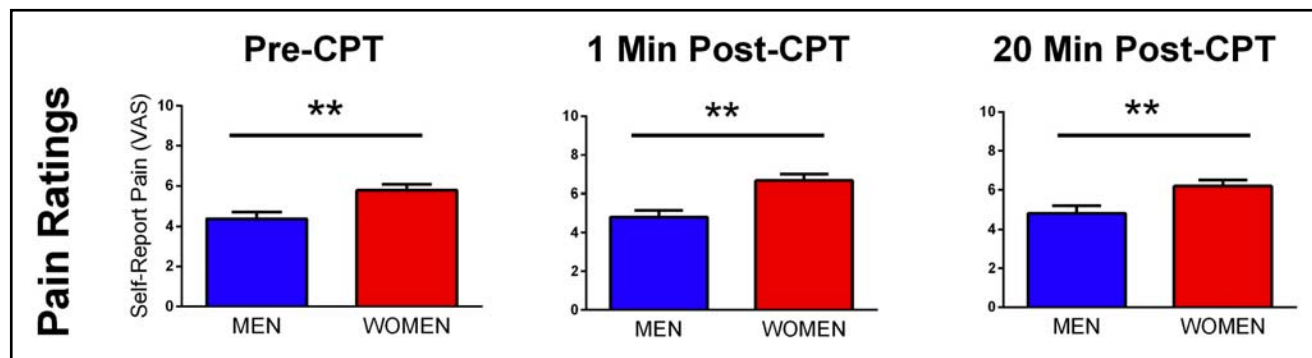


Fig. 1. Self-Reported Pain ratings by Sex and time in response to the Cold Pressor Task (CPT).

Note: ** $p < .01$

years, $SD = 4.77$, range 18–40). Exclusion criteria included: a history of cardiovascular disease, Reynaud's phenomenon, seizures, frostbite, fracture, or open cut/sores on the non-dominant hand. To control for circadian fluctuations in cortisol secretion, participants were tested between 16:00 and 18:00 h, when cortisol secretion, while not at the circadian nadir, is at a low, declining value. We also determined through self-report that women were either on oral contraceptives or were in the luteal phase of their menstrual cycles at the time of testing. The Nova Southeastern University Institutional Review Board approved all aspects of this study. Written consent was acquired from all subjects before participating in the study procedure.

Procedures

Participants were provided with a written and verbal explanation of the study procedures. Once written consent was obtained, participants filled out self-report measures and provided background information, including completing a demographic questionnaire and a questionnaire asking about history of violence or aggression. We exposed all participants to a one-minute Cold Pressor Test (CPT) that was maintained between 3–5 °C, within the first 15 min of the study procedure. The participants were told that they would place their non-dominant hand in the water up to the

wrist for one minute while the experimenter recorded the time with a stopwatch. At one minute the participants were told that they could remove their hand from the water. To quantify subjective pain intensity, participants were asked to rate their anticipated pain intensity (1 min before the CPT), their experienced pain intensity (during the CPT), and their recalled pain intensity (20 min following the CPT). This subjective pain scale ranged from 1 to 10 (1 = little pain to 10 = strongest pain imaginable). Saliva was collected by unstimulated passive drool procedure described previously (Murphy *et al.* 2010). Baseline saliva was collected after the participants filled out the consent form and at least 15 minutes after arriving in the lab (to control for events prior to arrival) but before they were instructed about the CPT (in order to avoid anticipatory stress effects). Saliva samples were also collected 1 minute and 20 minutes following the CPT immediately following the pain ratings. Immediately after collection, the sample tubes were stored in a –20 °C freezer. State and trait anxiety were measured before the CPT was administered using the STAI-Y.

STAI-Y

The STAI-Y consists of two self-assessment questionnaires where participants select one response reflecting their feelings based on a 4-point Likert scale. Self-report

Tab. 1. Psychological factors by Sex

	Men		Women		t (df)
	Mean	(SD)	Mean	(SD)	
Aggression Total	70.29	16.62	59.56	12.71	3.19 (74) **
Physical Aggression	22.26	6.07	17.71	4.96	3.58(74)***
Verbal Aggression	14.71	4.63	12.71	3.81	2.06 (74)*
Anger	14.87	4.61	14.13	4.86	0.66 (74)
Hostility	18.00	6.88	15.00	4.64	2.27 (74)*
Trait Anxiety (STAI)	19.48	10.37	19.40	6.96	0.04(74)
Happiness (SHS)	18.06	3.45	18.29	3.65	-0.27 (74)

* $p < .05$, ** $p < .01$, *** $p < .001$, **** $p < .0001$

Tab. 2. Correlations between cortisol response and predictors of stress response (Females above the diagonal and Males below the diagonal)

	Cortisol- 20 minutes	Aggression	Trait Anxiety	Happiness
Cortisol- 20 minutes	--	-0.07	-0.07	0.10
Aggression	0.49**	--	-0.03	0.05
Trait Anxiety	0.39*	0.47**	--	-0.03
Happiness	-0.34+	-0.40*	-0.58***	--

Note: Values for Males (n= 30 for correlations with cortisol and n= 31 for correlations without cortisol) below the diagonal and values for Females (n=45) above the diagonal. * $p < .05$, ** $p < .001$, + $p = .065$

responses are (almost never, somewhat, often, almost always) (Spielberger & Gorsuch 1983). The rating scale used was the TAI-Y subscale comprised of 20 questions. The scale is made up of questions such as (I feel pleasant, I am happy, I lack confidence, and I worry too much over something that really doesn't matter).

The Buss-Perry Aggression Questionnaire (BPAQ)

The Buss-Perry Aggression Questionnaire (BPAQ) is a self-report measure looking at the subject's ranking on different factors of aggression. (Buss & Perry 1992). The Aggression scale consists of 4 factors: Physical Aggression (PA), Verbal Aggression (VA), Anger (A) and Hostility (H). The total score for Aggression is the sum of the factor scores. The scale is a 29-question survey, using a 5-point Likert scale (1 = extremely uncharacteristic of me, 3 = neither uncharacteristic nor characteristic of me, 5 = extremely characteristic of me). Subjects are asked to rate how characteristic each statement is of them. Example statements include: "I have become so mad I have broken things, at times I feel that I have gotten a raw deal out of life, there are people who pushed me so far that we came to blows."

The Subjective Happiness Scale (SHS)

The Subjective Happiness Scale (SHS) is a 4-item scale of global subjective happiness. Two items ask respondents to characterize themselves using both absolute ratings and ratings relative to peers. On the contrary, the other two items offer brief descriptions of happy and unhappy individuals and ask respondents the extent to which each characterization describes them (Lyubomirsky & Lepper 1999). Participants rated the items using a 7-point Likert scale, ranging from 1–7. The questions on the 4-item scale are: "In general, I consider myself, not a very happy person – a very happy person", "compared with most of my peers, I consider myself less happy – more happy", "Some people are generally very happy. They enjoy life regardless of what is going on, getting the most out of everything. To what extent does this characterization describe you? Not at all – a great deal", "Some people are generally not very happy. Although they are not depressed, they never seem as happy as they might be. To what extent does this characterization describe you? Not at all – A great deal".

Cortisol

Saliva samples were run in duplicate and quantified via a human cortisol enzyme immunoassay (EIA) kit and a sAAKinetic Enzyme Assay Kit per the manufacturer's instructions (Salimetrics, LLC, Carlsbad, CA) Salimetrics LLC, USA) which has a 0.91 correlation with serum and a sensitivity < 0.007 ug/dL. The samples were immediately read in a BioTek ELx800 plate reader (BioTek Instruments, Inc., USA) at 450 nm with a correction at 630 nm. All samples were within the detection ranges indicated in the immunoassay kit, and the variations of sample readings were within the expected limits. Final concentrations for the cortisol were generated by interpolation from the standard curve in ug/dL.

RESULTS

Sex Differences in Response to Stressor

Based on our previously reported findings (Serrano et al. 2019), no effect of time was observed on cortisol levels but a significant increase in pain ratings was observed from 1 minute prior to the stressor to 1-minute following the stressor. Our prior analyses examining the effects of sex only examined the three-way interaction between sex, time, and genotype, given the focus of the prior work. However, we are interested in the effect sex differences may have without the effect of genotype. To determine if sex moderated any possible change in cortisol, such that the null effects observed previously may occur for one sex, we conducted a mixed model ANOVA. Consistent with previously reported findings, no main effect of time, sex, nor interaction between time and sex was observed, all p 's > .05. We previously reported a significant effect of time on pain ratings. To see if this effect was moderated by sex we conducted a mixed model ANOVA. In addition to previously reported main effect of time (Serrano et al. 2019), a significant effect of sex was found, $F(1, 73) = 13.22$, $p = .001$, partial $\eta^2 = 0.15$. No interaction between time and sex was observed. As seen in Figure 1, women reported higher levels of anticipated and rated pain prior to the stressor, $t(74) = 3.01$, $p = .004$, $d = 0.71$, immediately following the stressor, $t(74) = 3.76$, $p < .001$, $d = 0.89$, and 20 minutes following the stressor, $t(73) = 2.87$, $p = .005$, $d = 0.67$.

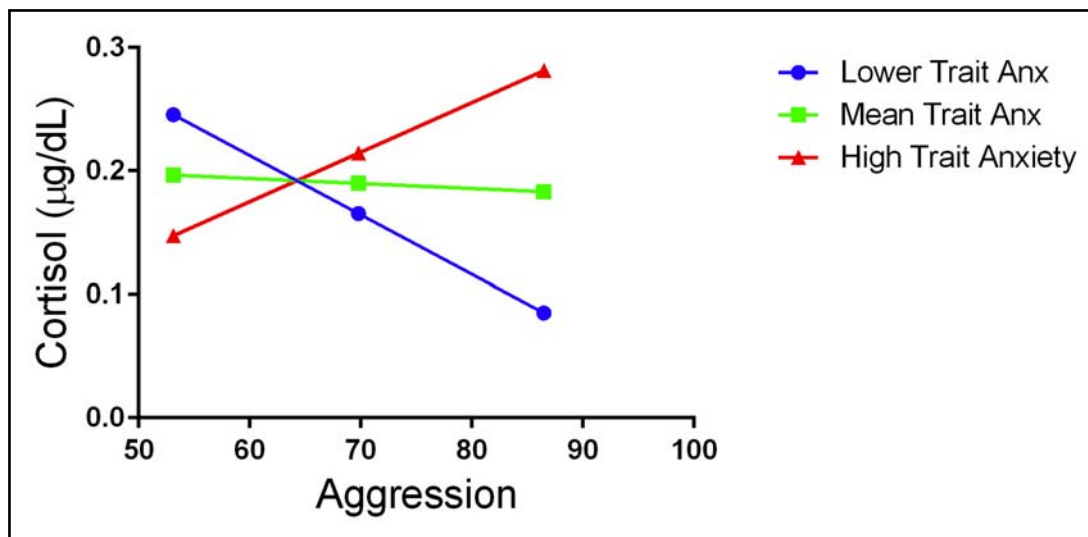


Fig. 2. Interaction between Aggression and Trait Anxiety predicting Cortisol 20-minutes post-Stressor (Cold Pressor Task) in men.

To determine if there are differences between men and women on the predictors of stress responsivity, a series of *t*-tests were conducted. As seen in Table 1, males reported significantly higher levels of overall aggression, $t(74) = 3.19, p = .002, d = 0.73$. Additionally, men reported higher levels on three of the subscales including physical aggression, $t(74) = 3.58, p = .001, d = 0.82$, verbal aggression, $t(74) = 2.06, p = .043, d = 0.47$, and hostility, $t(74) = 2.27, p = .026, d = 0.51$, but no difference was found on the anger subscale, $p > .05$. Further, no difference was found on levels of happiness or trait anxiety, p 's $> .05$.

To determine if ratings of pain immediately following the stressor were related to cortisol response either 1-minute following the stressor or 20-minutes following the stressor, we examined the correlations between the cortisol levels and 1-minute post stress pain rating in men and women. Pain ratings were unrelated to cortisol levels for males at 1-minute following the stressor, $r(30) = .05, p = .808$, and 20-minutes following the stressor, $r(29) = .08, p = .667$. Although the relationship between pain and cortisol levels was not statistically significant at 1-minute following the stressor in women, $r(44) = .26, p = .082$, the relationship was significant at 20-minutes, $r(44) = .32, p = .031$. These findings should be interpreted with caution because the significance would not hold if *p* values were adjusted for making multiple comparisons, but relationships between pain and cortisol in women were in a consistent direction for these time points. Anticipatory pain ratings were unrelated to cortisol levels at either time point in men or women, p 's $> .05$.

Predictors of Stress Responsivity by Sex

To test the hypothesis that predictors of stress responsivity may differ based on sex, we examined the relationship between peak cortisol response (20-minutes

post-stressor) and aggression, trait anxiety, happiness (Table 2). We chose to examine the 20-minute cortisol level due to timing of the cortisol response to a stressor. Aggression was significantly related to cortisol level in males, $r(29) = .49, p = .006$, but not in females, $r(44) = -.07, p = .626$, these correlations were statistically significantly different based on a fisher's *z* test, $z = 2.47, p = .014$. Similarly, trait anxiety was positively related to cortisol levels in men, $r(29) = .39, p = .035$, but not in women, $r(44) = -.07, p = .660$, the difference in these correlations approached significance, $z = 1.95, p = .051$. Happiness was unrelated to cortisol levels in women, $r(44) = .10, p = .532$, and did not reach statistical significance in men, $r(29) = -.34, p = .065$, but was in a direction consistent with the findings from anxiety and aggression.

Due to the relationships observed between cortisol and both trait anxiety and aggression, we conducted a regression to determine the unique effects of trait anxiety and aggression on cortisol. Additionally, we were interested in the interaction between trait anxiety and aggression. These regressions were conducted separately for men and women due to the differences observed in the prior correlation analyses. A significant model was found for men, $F(3, 26) = 8.68, p < .001, R^2 = .50$, with trait anxiety, $\beta = -.027, t = 3.14, p = .004$, aggression, $\beta = -.009, t = 2.43, p = .022$, and the interaction between trait anxiety and aggression, $\beta = .0004, t = 3.52, p = .002$. To understand this interaction, we plotted the relationship between aggression and cortisol (20-minutes post stressor) at three levels of trait anxiety (1-standard deviation below the mean, the mean, and 1-standard deviation above the mean). Additionally, we examined the condition effects of trait anxiety on the impact of aggression on cortisol. Consistent with what is seen in Figure 2, a significant positive effect was observed for aggression on cortisol at one standard

deviation above the mean for trait anxiety, $t = 3.04$, $p = .005$, a trend for a negative effect was observed at one standard deviation below the mean, $t = 1.84$, $p = .076$, but no effect was observed at the mean level of trait anxiety, $t = 0.24$ $p = .811$. A similar analysis was conducted for women, but no significant model was found.

DISCUSSION

Previous studies have shown sexual dimorphism in the HPA axis structure as well as HPA axis responsivity to stress (Sze & Brunton 2019). The current study builds on these reports by showing that the psychological variables, anxiety, and aggression, relate to an acute stress response in men, but not women.

Results from the current study confirm previous findings that women report lower pain tolerance than men. This was true immediately after the cold pressor test and 20 minutes later. We further showed women also reported greater anticipated pain. Cortisol levels were related to females' pain ratings 20 minutes after the CPT – a time when cortisol is at its peak post-stress levels. Pain is a subjective and emotional phenomenon that is associated with perceived or actual tissue damage and can be expressed in terms of such damage. Acute pain which is experienced suddenly and without warning has important implications for survival and recuperation. Chronic pain occurring with relative consistency, has no survival value and can become pathological, leading to dysfunction (Wiesenfeld-Hallin 2005). Here, only women showed a positive correlation between pain ratings and cortisol 20-minutes following to CPT. It will be important to further shed light on the extent to which differences in HPA axis responsivity relates to acute and chronic pain in men and women – especially since women greatly outnumber men as sufferers of chronic pain disorders (Maurer *et al.* 2016).

Sex differences in HPA axis activity emerge early on in development – even before puberty (Romeo 2010; Romeo & McEwen 2006). During puberty, increased testosterone secretion in males decreases the growth rate of the cortisol-secreting zona fasciculata of the adrenal gland (Viau 2002). Puberty is also the time when sex differences in stress responsivity emerge. Not only do sex hormones influence HPA axis structure and function, but sex hormones also influence limbic brain regions that are critical for stress perception and response (De Bellis *et al.* 2001). It is possible that sexual dimorphic changes in the HPA axis and limbic brain structures, such as the hippocampus and amygdala, at least partially exemplify why psychological factors examined in the current study differentially influence stress responses in men and women.

Our findings show that psychological variables, can also influence sexual dimorphic stress responses. A stress-induced cortisol response (20-minutes post-stressor) was positively associated with aggression

and trait anxiety in men, but not women. Follow up modeling, further showed a significant interaction between trait anxiety and aggression with stress induced cortisol in men. We did not find a significant difference in trait anxiety between men and women. It is perhaps surprising that we did not see any relationship between anxiety and stress responses in women given that previous work has shown a close relationship between stress responsiveness and mood and anxiety disorders in women (Altemus 2006; Parker & Brotchie 2010). Since we looked at the response to an acute physical stress, it is possible that a relationship would emerge in an investigation of longer-term stress. However, our study confirmed previous reports about general sex differences in aggression. Relative to women, men reported higher levels of aggression. The subscale measures showed they were specifically higher on measures of physical aggression, verbal aggression, and hostility anger subscale. Combined, these findings suggest a complex relationship between aggression, anxiety, and stress response – even in the absence of observable differences in trait anxiety.

Although happiness was not related to stress-induced cortisol response in men or women, we did find a non-significant negative trend in men ($p = .065$), that lower happiness was associated with higher post-stress cortisol levels. In agreement with previous reports, we also did not show any sex difference in self-reported happiness. Importantly, there are multiple measures of positive affect and it is possible we did not fully capture the extent to which happiness can be protective in this initial investigation. It is also possible happiness plays a role in long-term, but not short-term stress responses.

One possible limitation to the current study is that we did not address possible variability in cortisol responses related to women's menstrual cycle. However, this is mitigated by the general finding that while there are some menstrual cycle-related changes in cortisol, the differences between phases rarely reach statistical significance, unlike estradiol and progesterone (Montero-López *et al.* 2018; Gordon & Girdler 2014; Walder *et al.* 2012; Lustyk *et al.* 2010; Maki *et al.* 2015).

Given the preponderance of stress experienced in the daily lives of men and women, it is important to understand the variables at play in sex differences in stress responses. The current study shows aggression and anxiety differentially influence the cortisol response to an acute stress in men and women. It will be important for follow up work to further untangle the relationship between these factors in the response to ongoing stress and possible development of mood disorders.

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DISCLOSURE STATEMENT

The authors declare that they have no financial or non-financial conflicts to declare.

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