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Neuroendocrine stress response is moderated by sex and sex hormone receptor polymorphisms

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ABSTRACT

Sex hormones are significant regulators of stress reactivity, however, little is known about how genetic variation in hormone receptors contributes to this process. Here we report interactions between biological sex and repeat polymorphisms in genes encoding sex hormone receptors, and their effects on salivary cortisol reactivity in a sample of 100 participants (47 men & 53 women; 24.7 ± 3.23 years). Three genes were investigated: estrogen receptors alpha (*ESR1*) and beta (*ESR2*), and the androgen receptor (*AR*). Participants were classified as carrying 'Short' or 'Long' alleles based on median splits of the repeat distribution for each gene. Measures of physiological reactivity were collected before and after exposure to a canonical laboratory stressor and converted to traditional summary measures for analyses. Overall, men exhibited greater cortisol ($p = 0.001$) and mean arterial pressure reactivity ($p = 0.002$), while women displayed elevated heart rate throughout the session ($p = 0.02$). The effect of polymorphisms on salivary cortisol was sex sensitive. *ESR1* was associated with differential reactivity in men ($p = 0.04$), but not women ($p = 0.24$). *ESR2* genotype interacted with sex such that each additional 'Long' allele was associated with a 6.4% decrease in salivary cortisol in men, but a 9.5% increase in the levels of women ($p = 0.02$ for interaction). For the X-linked *AR*, the 'Long' allele was associated with decreased cortisol levels in men ($p = 0.047$), but in women had no effect ($p = 0.75$). Together, these results provide evidence for the saliency of genetic variation in sex hormone receptors on stress reactivity in humans and highlight their important role as mediators of hormonal activity.

1. Introduction

Since the original characterization by Walter Cannon and Hans Selye, the dynamics of stress and its relation to health and disease have received growing attention in the literature. The physiological stress response is well conserved across vertebrate taxa, and involves a signaling cascade along the sympathetic-adrenal-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes of the neuroendocrine system. The HPA signaling cascade results in the release of glucocorticoids (cortisol in humans) from the adrenal cortex into the bloodstream to produce systemic effects throughout the body. Dysregulation of the stress response along this axis has been implicated in an array of diseases including diabetes, depression, cancer, and cardiovascular disease (Cohen et al., 2007). Importantly, individual differences in the timing and magnitude of the stress response contribute to differential incidence and prevalence of stress-associated disorders (McEwen, 2008).

Substantial differences in individual stress physiology can be attributed to genetics. Twin studies have associated up to 62% of the variability in circulating cortisol to genetic background (Bartels et al., 2003). Stress reactivity, the increased cortisol secretion, heart rate, and blood pressure in response to threatening stimuli, is also partially heritable (Federenko et al., 2004), and nearly a dozen different genes have been shown to moderate cortisol responses to acute psychosocial stressors (Foley and Kirschbaum, 2010). Another example of the genomic influence on stress reactivity is sex differences, where the genetic background differs by a full chromosome, i.e. XX vs. XY. Men typically exhibit elevated salivary cortisol secretion during stress as compared to women, whereas in women the magnitude of the salivary cortisol response to stress varies across the menstrual cycle (Kirschbaum et al., 1999). Women in the luteal phase display patterns of salivary cortisol reactivity most similar to those of men, while patterns of women in the follicular phase are significantly lower than men as well as women in the luteal phase (Kirschbaum et al., 1999).

Thus, sex hormones, which deviate in a rhythmic pattern across menstrual cycles, may be important mediators of stress reactivity, at

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least in women. Estrogens have been implicated as a stress buffer in women. For example, estradiol is higher in the follicular phase, when cortisol reactivity is also diminished (Kajantie and Phillips, 2006). Furthermore, the stress reactivity in pregnant women, whose estradiol levels have spiked, is lower than age matched, non-pregnant counterparts, while the stress reactivity in post-menopausal women is higher than that of young-adult women (Kajantie and Phillips, 2006). The role of estrogens in the male stress response is less established and may actually increase reactivity when estrogen is applied transdermally (Kirschbaum et al., 1996).

Instead, testosterone appears to be the primary mediator of the stress response in males. Studies in male rodents have implicated testosterone as an upstream dampener of the HPA axis, inhibiting CRH release by neurons in the hypothalamus (Handa et al., 1994), effectively curbing the stress response before cortisol is secreted and thereby decreasing cortisol release into the periphery. Cortisol and testosterone may have counteracting effects. For example, the link between testosterone and dominance behavior is conditional on low cortisol (Mehta and Josephs, 2010; Popma et al., 2007). Furthermore, circulating testosterone levels decrease following cortisone administration (Cumming et al., 1983). Like estrogen, testosterone may have contrasting effects between sexes. Specifically, testosterone replacement therapy was associated with increased cortisol reactivity in elderly women, but not men (Kudielka et al., 1998).

Importantly, the physiological effects of estrogens and androgens are mediated through cytosolic receptors. Lipid soluble hormones permeate the cell membrane and bind these receptors, which then translocate to the nucleus to modulate gene expression (Beato and Klug, 2000). As a result, differences in sex hormone concentrations between individuals do not necessarily imply differential hormonal activity. Differences in the abundance of receptors and efficiency of hormone-receptor binding also impact function. Notably, DNA sequence variation in polymorphic genes encoding receptors can significantly influence their abundancy and binding affinity (Comings, 1998). Therefore, the lack of consensus regarding the effects of sex steroids could be attributed, in part, to a failure to account for polymorphic genetic variations that modulate receptor activity, availability, and function.

Estradiol binds to two receptors, estrogen receptor alpha (*ESR1*) and estrogen receptor beta (*ESR2*), which are differentially distributed throughout the brain and periphery (Taylor and Al-Azzawi, 2000). Both genes contain repeat polymorphisms thought to play a role in gene expression, although this has yet to be validated; a dinucleotide $(TA)_n$ repeat near the promoter region of *ESR1* (del Senno et al., 1992), and a tandem $(CA)_n$ repeat within the fifth intron of *ESR2* (Tsukamoto et al., 1998). Testosterone acts through the X-linked androgen receptor (*AR*), which, like the estrogen receptors, is non-uniformly distributed throughout the brain and periphery (Sar et al., 1990), with notable differences between men and women (Fernandez-Guasti et al., 2000). One polymorphism of especial interest is a three-nucleotide $(CAG)_n$ repeat within exon one of the gene coding region. This polymorphism changes the receptor structure, and may significantly influence testosterone transactivation (Kazemi-Esfarjani et al., 1995). Specifically, removal of the CAG repeat in both the rat and human *AR* gene resulted in greater transcriptional activity of androgen dependent genes (Chamberlain et al., 1994).

Despite existing literature investigating associations between biological sex, sex hormone concentrations, and stress reactivity, studies investigating how genotypic variability contributes to sex differences in stress reactivity are limited (Pausova et al., 2010). In the current study, we investigated the association between repeat polymorphisms in *ESR1*, *ESR2*, and *AR* and stress reactivity in both men and women. As mediators of the predominate sex hormone in females, we hypothesized that polymorphisms in *ESR1* and *ESR2* would be more strongly associated with differential reactivity in women than men; and accordingly, for *AR* we hypothesized the opposite trend would be observed.

2. Methods

2.1. Sample recruitment

Young men and women were recruited by word of mouth and on-campus advertising from the college student population at Hebrew University of Jerusalem, Israel. Participants were excluded if they were under the age of 18 or over the age of 35, had a medical history of endocrine or psychiatric illness, smoked, were pregnant in the last year, or were taking any medication (excepting oral contraceptives). In total, 107 individuals participated in the study. Three participants for whom cortisol measurements were not available were excluded from analyses. Another four participants for whom genotypes on neither *ESR1*, *ESR2*, nor *AR* were unavailable were also excluded, bringing the final sample size to 100 (47 men, 53 women). The study was approved by the IRB of Herzog Hospital, Jerusalem and all participants provided written informed consent.

2.2. Stressor procedure & psychological indices

Testing was done in a discrete time window, 3:00–6:00 pm, to minimize noise from diurnal changes in cortisol. To further control for variation, participants were asked to refrain from physical activity for at least 2 h before the session, and refrain from brushing their teeth, eating, or drinking anything except water for 1.5 h before the session. Participants feeling ill were rescheduled. The procedure to induce stress was conducted in the Department of Psychology following the Trier Social Stress Test (TSST) paradigm, as previously described (Shalev et al., 2009). Briefly, participants were told they would be interviewing for their dream job while on video, given 5 min to prepare notes, and taken to the testing room. Notes were taken away from participants immediately before the interview began and interviewers were instructed to appear stern and disapproving. Following the five-minute interview, participants completed a serial subtraction cognitive task.

2.3. Biological sampling and cortisol assay

Saliva samples for cortisol assays were collected using salivette plugs (Sarstedt, Germany) at eight time points across the 90-minute session: 10 min prior to testing, 1 min prior to testing, immediately after testing, and 10, 20, 30, 45, and 60 min following testing. Saliva samples were stored at room temperature during the session, centrifuged at 4000 rpm at 24 °C for 10 min, and then assayed in an Elecsys 2010 Analyzer using an electrochemiluminescence immunoassay salivary cortisol kit (Roche Diagnostics, USA). The lower detection limit of the assay was 0.5 nM/L. Subject heart rate and blood pressure were collected 10 min prior to testing, 1 min prior to testing, immediately after testing, and 10 min after testing using an automatic blood pressure wrist monitor (Omron R7).

2.4. Genotyping

Samples for genotyping were collected prior to stress exposure using two 10 mL sterile tubes containing 10 mL of Aquafresh mouthwash. Both samples were pooled for DNA extraction using the Master Pure kit (Epicentre, Madison, WI). The $(TA)_n$ repeat of *ESR1*, $(CA)_n$ repeat of *ESR2*, and $(CAG)_n$ repeat of the *AR* gene were amplified by polymerase chain reaction (PCR) on a Rotor-Gene 3000 (Corbett life science, Australia). The PCR reaction was conducted in a total volume of 10 mL containing 50 ng DNA (~ 1 mL), 5 mL Thermo-Start Master Mix (Thermo scientific), 2 mL primers (2.5 mM), 1 mL SYTO9 dye, and water to 10 mL. Primers were designed as previously described (Tsezou et al., 2008), and are listed for each gene in Supplementary Table A.1. The forward primer was labeled with 6-FAM and used together with a reverse primer for each gene. The temperature profile was 95 °C for 15 min followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C

for 30 s, with a final incubation at 72 °C for 7 min. Labeled DNA fragments were distinguished by size with automated capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer with GeneMapper Software (Applied Biosystems). Alleles for each gene were classified as "Short" or "Long" following standard procedures using median split of number of repeats for that gene (Tsezou et al., 2008). Three individuals not successfully genotyped for *ESR1*, one not successfully genotyped for *ESR2*, and another not successfully genotyped for *AR* were removed from analyses considering those specific genes. Repeats for *ESR1* ranged from 8 to 24 repeats with a median value of 14 ($N = 97$), for *ESR2* ranged from 5 to 15 with a median value of 12 ($N = 99$), and for *AR* ranged from 7 to 20 with a median value of 13 ($N = 99$). The range of repeats for *ESR1* was consistent with previously established reports (Tsezou et al., 2008). However, the range of repeats for *ESR2* and *AR* were lower than those previously reported for Caucasian populations (Tsezou et al., 2008).

2.5. Statistical analysis

All statistical tests were carried out using SPSS version 24 (Windows). Regression imputation using cortisol samples at all time points was used to generate values for two missing cortisol samples (Participant 17, sample 3 & Participant 65, sample 8). No statistical differences were observed between analyses with and without these participants, and the results using imputed values are reported here. Sampled cortisol (log-transformed) was converted to two supplementary measures of cortisol production using eight sampling time points; area under the curve with respect to ground (AUCg), and area under the curve with respect to increase (AUCi). While AUCg represents total output across time, AUCi is thought to capture sensitivity and rate of change with respect to baseline measurements. Both measures were calculated as previously described using cortisol measures and the time duration between measures (Pruessner et al., 2003). Briefly, AUCg was calculated using the formula $AUC_g = \sum_{i=1}^{7} \frac{(m_{i+1} + m_i)t_i}{2}$, where m_1–m_8 are the cortisol levels at each sampling time point and t_1–t_7 are the durations between sampling time points. Since AUCi is identical to AUCg except for the removal of the area between the ground and the baseline measure, it can be calculated from AUCg using the formula $AUC_i = AUC_g - (m_1 * T)$, where T is the total time between the first cortisol sample and the last cortisol sample.

Mean arterial pressure (MAP) was derived from sampled diastolic and systolic blood pressure (MAP = [2 * diastolic + systolic]/3). Both MAP and sampled heart rate were log-transformed and converted to AUCi and AUCg using the first four sampling time points to evaluate sympathetic reactivity. Summary AUCi and AUCg was subjected to univariate ANOVA analyses to investigate the effects of sex (men and women), genotype (Short/Short, Short/Long, and Long/Long), and two-way interactions between variables (e.g. Sex**ESR1*). Post-hoc comparisons were evaluated using Tukey's honestly significant differences. Effect sizes are reported as partial eta squared (η^2).

3. Results

3.1. Sample demographics and self-report measures

Independent *t*-tests indicated no significant sex differences in mean age (men = 24.8 ± 2.46; women = 24.62 ± 3.92) or body max index (BMI) (men = 23.01 ± 3.14; women = 22.54 ± 3.07). Genotype distributions were in Hardy-Weinberg equilibrium and there were no significant sex differences in the polymorphism distribution for each gene (Table 1). F-tests were conducted on age and BMI as a function of *ESR1*, *ESR2*, and *AR* genotype for women and men. No significant differences in age or BMI as a function of genotype were observed for either sex.

Table 1
Polymorphism distribution among sample.

		Men	Women	Total	*P*-Value ($\times^2$)
Estrogen receptor alpha (*ESR1*)	Short/Short	11	10	21	0.342
	Short/Long	28	27	55	
	Long/Long	7	14	21	
	Total	46	51	97	
Estrogen receptor beta (*ESR2*)	Short/Short	15	11	26	0.478
	Short/Long	21	27	48	
	Long/Long	11	14	25	
	Total	47	52	99	
Androgen receptor (*AR*)	Short/Short	27	18	45	0.910[a]
	Short/Long	*	23	23	
	Long/Long	19	12	31	
	Total	46	53	99	

* The androgen receptor gene is X-linked. Men carry Short or Long alleles.
[a] Statistic reported for differences in distribution of Short/Short and Long/Long between men and women.

3.2. Sex differences in summary stress reactivity

There were significant differences in summary stress reactivity between men and women. Men exhibited significantly greater increases in MAP relative to women (AUCi; $F_{1,97} = 8.88$, $p = 0.004$, $\eta^2 = 0.08$), while women had significantly higher heart rate than men across the session (AUCg; $F_{1,95} = 4.98$, $p = 0.03$, $\eta^2 = 0.05$). Overall, men displayed greater cortisol reactivity to the TSST compared to women, as previously observed (Shalev et al., 2009). This was supported by univariate tests of summary AUCi ($F_{1,98} = 11.57$, $p = 0.001$, $\eta^2 = 0.11$) as well as AUCg ($F_{1,98} = 10.45$, $p = 0.002$, $\eta^2 = 0.10$). Oral contraceptive use has been reported to significantly influence salivary cortisol measures derived from women following stress testing using the TSST paradigm (Kirschbaum et al., 1999). As previously reported (Shalev et al., 2009), no significant differences were found in women as a function of oral contraceptive in the current sample; however, both groups of women were significantly different from men. Therefore, contraceptive use was not included as a variable in current analyses.

Polymorphisms in *ESR1*, *ESR2*, and *AR* were not associated with differences in summary MAP, nor were they associated with differences in summary heart rate when men and women were analyzed together (not shown). Furthermore, there were no associations between of *ESR1*, *ESR2*, or *AR* and summary heart rate or summary MAP when men and women were analyzed independently. As such, the remaining results focus exclusively on analyses investigating the effects of each gene on salivary cortisol.

3.3. Salivary cortisol by ESR1

Univariate analyses in the full sample showed significant variability in AUCi as a function of *ESR1* ($F_{2,91} = 5.08$, $p = 0.01$, $\eta^2 = 0.10$), with post-hoc tests revealing a significantly greater response in heterozygotes (Short/Long) as compared to those with the Long/Long genotype ($p = 0.01$). The two-way Sex**ESR1* interaction was not significant ($p = 0.39$). To investigate this further, the association between *ESR1* and cortisol release were analyzed separately for each sex. There was no significant association between *ESR1* and AUCi in women ($p = 0.24$, Fig. 1B), whereas the pattern remained significant for men ($F_{2,43} = 3.60$, $p = 0.04$, $\eta^2 = 0.14$), with heterozygotes (Short/Long) showing significantly elevated AUCi relative to Long/Long homozygotes ($p = 0.04$, Fig. 1A). Univariate analyses of AUCg in the combined sample showed no differences in total cortisol production as a function of *ESR1* ($p = 0.44$), nor was the Sex**ESR1* interaction significant ($p = 0.46$). Similarly, no associations were observed in sex-stratified analyses of *ESR1* and AUCg ($p = 0.25$ for men; $p = 0.11$ for women; Fig. 1).

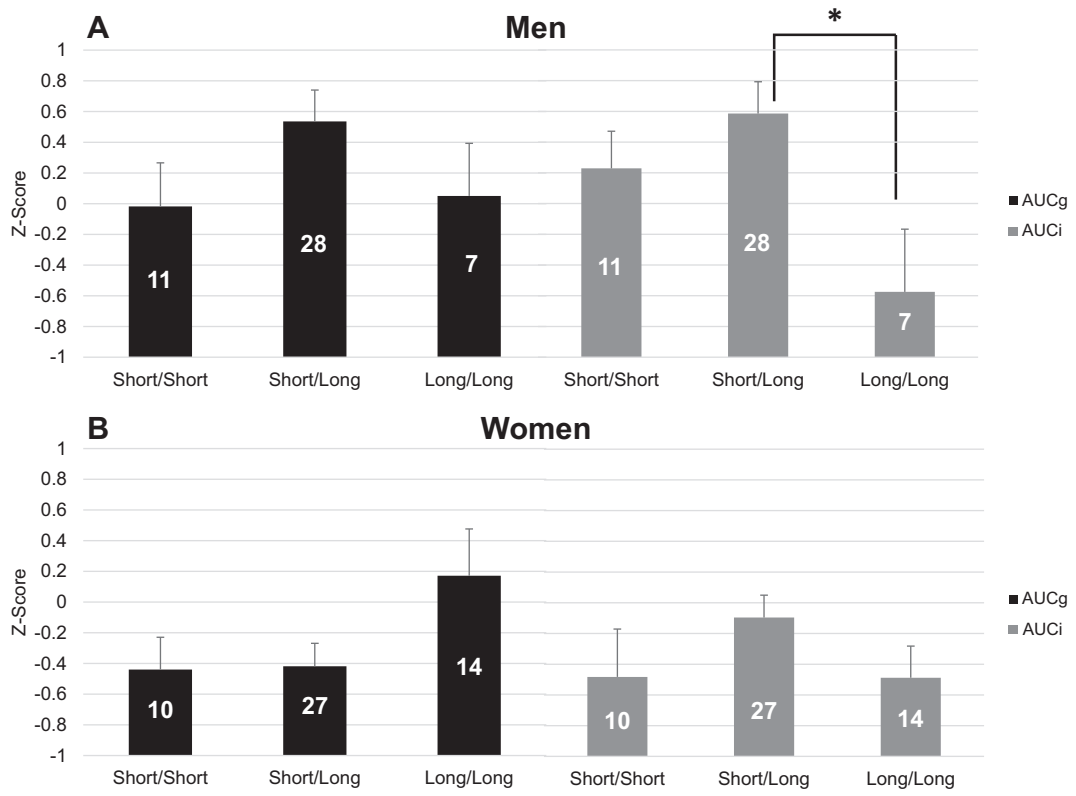

Fig. 1. Cortisol Reactivity by Estrogen Receptor Alpha. A) Men display no significant differences in AUCg as a function of *ESR1* genotype. There is significant variation in AUCi as a function of *ESR1* genotype, with Short/Long heterozygotes displaying significantly greater AUCi relative to Long/Long homozygotes ($p = 0.04$). B) Women display no significant differences in AUCg or AUCi as a function of *ESR1* genotype. Error bars indicate standard error of the mean. Significant differences ($p < 0.05$) between groups indicated with *. Numbers within bars represent the number of participants with each genotype. AUCg = area under the curve with respect to ground; AUCi = area under the curve with respect to increase.

3.4. Salivary cortisol by ESR2

Univariate analyses in the combined sample showed no significant variability in AUCi as a function of *ESR2* ($p = 0.17$). The two-way Sex**ESR2* interaction was also not significant ($p = 0.19$). Furthermore, there was no association between *ESR2* and AUCi in independent analyses of women ($p = 0.21$) or men ($p = 0.27$). Univariate analyses of AUCg showed no differences in total cortisol production as a function of *ESR2* when men and women were analyzed together ($p = 0.91$). However, the Sex**ESR2* interaction term was significant ($F_{1,95} = 5.76$, $p = 0.02$, $\eta^2 = 0.06$). Specifically, each additional Long allele was associated with decreased cortisol production in men (6.4% decrease per allele), and increased cortisol production in women (9.5% increase per allele) (Fig. 2). This pattern remained significant in independent analyses of women ($F_{1,50} = 4.16$, $p = 0.047$, $\eta^2 = 0.08$), but not men ($p = 0.17$).

3.5. Salivary cortisol by AR

The gene encoding the androgen receptor is located on the X-chromosome. As a result, all analyses involving *AR* polymorphisms were conducted in men and women independently following standard practices (Jonsson et al., 2001). Univariate analyses in women indicated no effect of *AR* on AUCi ($p = 0.78$) or AUCg ($p = 0.75$, Fig. 3B). In men, there was no effect of *AR* on AUCi ($p = 0.88$). There were, however, significant differences in total cortisol production (AUCg) as a function of *AR*, with carriers of the Long allele displaying significantly lower levels of cortisol relative to carriers of the Short allele ($F_{1,44} = 4.20$, $p = 0.047$, $\eta^2 = 0.09$; Fig. 3A).

4. Discussion

The current investigation explored associations between stress reactivity using the TSST paradigm, biological sex, and genetic variability in sex hormone receptors. The results herein strengthen existing literature on the differences in stress reactivity between men and women (Kirschbaum et al., 1999). Specifically, men displayed a more robust cortisol response following the stressor, coupled with increased mean arterial pressure, while women displayed greater heart rate throughout the testing procedure. This apparent distinction in the nature of reactivity between sexes is consistent with previous studies characterizing men as vascular responders and women as cardiac responders to stress (Allen et al., 1993; Kudielka et al., 2004), and is thought to be mediated by receptor density and sensitivity. For example, blood pressure is regulated by peripheral alpha and beta-adrenergic receptors, which are reported to have lower sensitivity to adrenergic agonists in women (Freedman et al., 1987). None of the three genes studied in the current investigation were associated with differential sympathetic reactivity. Instead, results showed evidence for estrogenic and androgenic modulation of the HPA axis during stress.

Estrogens and androgens exert primary effects on reproductive behavior and physiology, but are known to have systemic influences (Viau, 2002). Estrogens are thought to dampen HPA reactivity, primarily by enhancing the anxiolytic action of oxytocin (Klein and Corwin, 2002). Estrogenic activity is mediated by two receptors, *ESR1* and *ESR2*. Although both receptors are expressed throughout the brain, *ESR1* is overrepresented in the amygdala and hypothalamus (Osterlund et al., 2000b), while *ESR2* is predominately expressed in the thalamus and hippocampus (Osterlund et al., 2000a). In line with this, *Esr1*, the mouse homologue of *ESR1*, has been found to have a more critical role in the negative feedback of the HPA axis of female mice (Dorling et al.,

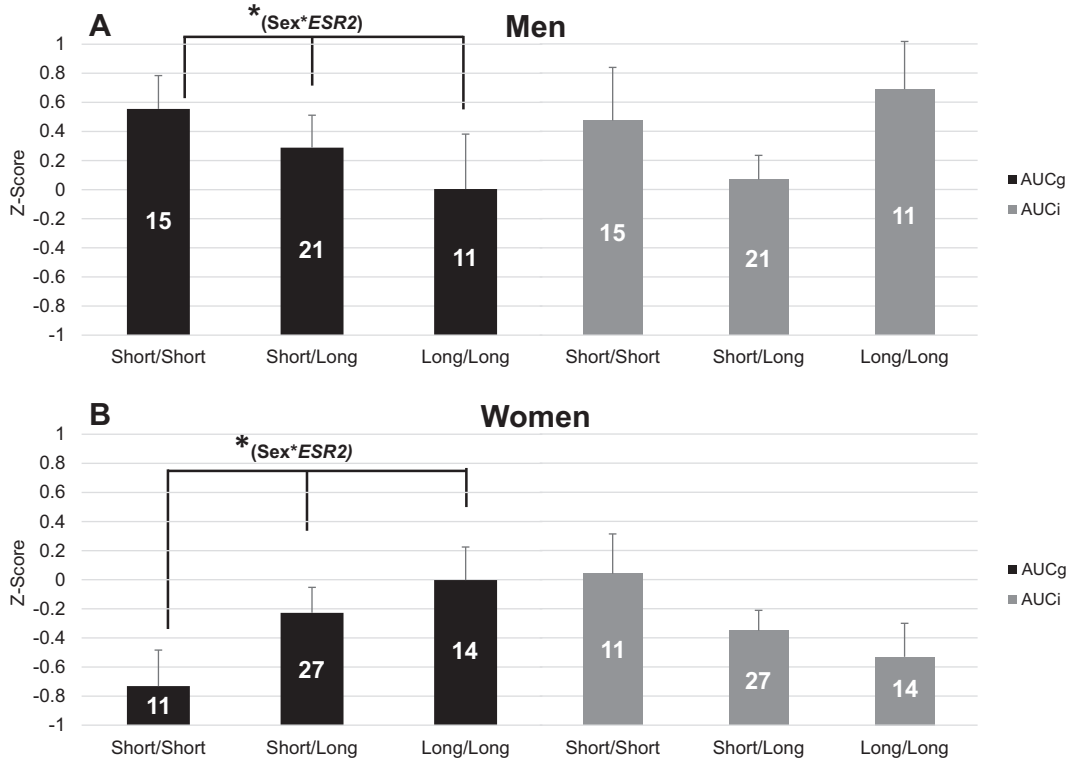

Fig. 2. Cortisol Reactivity by Estrogen Receptor Beta. There was a significant Sex**ESR2* interaction across sexes ($p = 0.02$), indicated by *(Sex**ESR2*), with men displaying lower summary AUCg per Long allele while summary AUCg increased per Long allele for women. This association remained significant in independent analyses of women (2B left), but not men (2A left). A) Men display no significant differences in AUCg or AUCi as a function of *ESR2* genotype. B) Women display significant differences in AUCg as a function of *ESR2* genotype ($p = 0.047$), but not AUCi. Error bars indicate standard error of the mean. Numbers within bars represent the number of participants with each genotype. AUCg = area under the curve with respect to ground; AUCi = area under the curve with respect to increase.

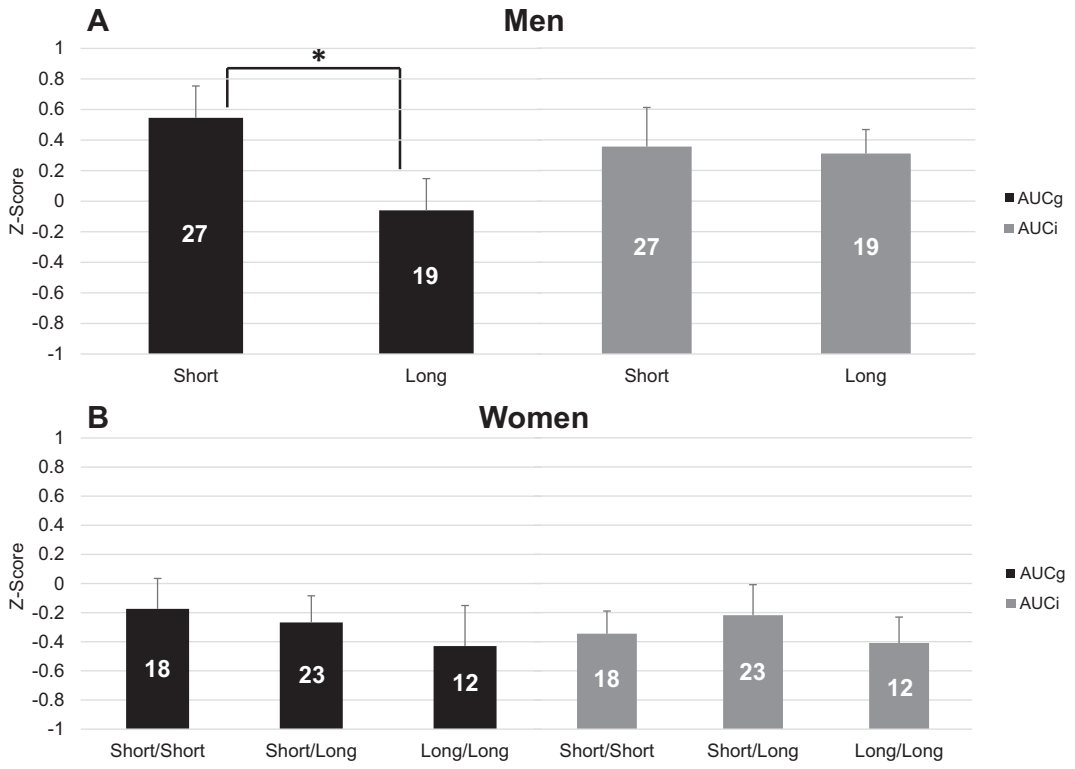

Fig. 3. Cortisol Reactivity by Androgen Receptor. A) Men carrying the Long allele in *AR* have significantly lower reactivity (AUCg, $p = 0.047$) than men carrying the Short allele. Men display no significant differences in AUCi as a function of *AR* genotype. B) Women display no significant differences in AUCg or AUCi as a function of *AR* genotype. Error bars indicate standard error of the mean. Significant differences ($p < 0.05$) between groups indicated with *. Numbers within bars represent the number of participants with each genotype. AUCg = area under the curve with respect to ground; AUCi = area under the curve with respect to increase.

2003; Weiser and Handa, 2009).

The $(TA)_n$ repeat polymorphism investigated here is located in the promoter region of the *ESR1* gene (del Senno et al., 1992). One notion is that the number of repeats influences transcription factor access, perhaps modulating receptor expression and subsequent estrogenic inhibition of HPA reactivity (Comings, 1998), and hence terminal cortisol levels. Although there were no significant differences in three-way comparisons of women by *ESR1* genotype (i.e. Short/Short vs. Short/Long vs. Long/Long), sensitivity analyses revealed significantly elevated total cortisol production (AUCg) in Long/Long homozygote women relative to women carrying the Short allele (i.e. Short/Short & Short/Long vs. Long/Long) ($F_{1,49} = 4.78$, $p = 0.03$, $\eta^2 = 0.09$; Fig. 1B). Thus, increased $(TA)_n$ may diminish estrogenic termination of the HPA response in women via decreased receptor availability, resulting in prolonged cortisol release into the periphery.

There was no association between *ESR1* and total cortisol production in men. Instead, *ESR1* appears to be related to their rate of reactivity. Both the main effect of *ESR1* on AUCi, as well as post-hoc differences between Short/Long heterozygotes and Long/Long homozygotes, remained significant upon independent analyses of men, but not women. Furthermore, sensitivity analyses revealed significantly diminished AUCi in Long/Long homozygote men relative to men carrying the Short allele (($F_{1,44} = 6.26$, $p = 0.02$, $\eta^2 = 0.12$; Fig. 1A). Effects of this polymorphism in men have been previously reported (Golubic et al., 2014; Kunnas et al., 2004; McIntyre et al., 2007; Rokach et al., 2005). Given AUCi reflects change with respect to baseline, a process mediated in the early stages of the stress response, this warrants further investigation into the effects of *ESR1* on HPA reactivity in males to complement its established role in the HPA negative feedback loop of females, and further highlight the importance of investigating individual differences in the stress response using both AUCg and AUCi.

Previous literature has associated fewer $(CA)_n$ repeats in *ESR2* with increased testosterone levels in women (Westberg et al., 2001), highlighting the potential for estrogenic-androgenic interactions. Higher numbers of $(CA)_n$ was associated with increased cortisol output for women in the current study, providing indirect support for previous studies in older women showing increased stress reactivity following testosterone administration (Kudielka et al., 1998). However, this notion is still unclear since variation in *AR*, which directly modulates androgenic action, was not associated with the stress reactivity of women in the current study. Therefore, androgenic action may have only marginal impacts in younger females, with estrogenic activity providing the dominant influence. Future studies with a wider age range may provide further clarification.

The $(CAG)_n$ polymorphism lies within exon 1 of the *AR* gene, and has been shown to negatively impact testosterone-dependent transcription (Kazemi-Esfarjani et al., 1995). Specifically, removal of the CAG repeat in both the rat and human *AR* gene resulted in greater transcriptional activity of androgen dependent genes (Chamberlain et al., 1994). Thus, the Long allele is associated with decreased androgenic activity. In line with this, greater (CAG)n has also been associated with higher serum testosterone levels (Stanworth et al., 2008), indicative of an adaptive response where testosterone production increases to accommodate limited androgenic activation. In the current study, men carrying the Long allele had significantly lower salivary cortisol production in response to the TSST compared to men carrying the Short allele. Given the antagonist properties of cortisol and testosterone (Viau, 2002), we can presume such a decrease would be accompanied and driven by increased testosterone levels necessitated by the Long *AR* genotype, with the polymorphism having little impact on the stress response itself. In support of this, the number of CAG repeats has been shown to modulate brain activity in the frontal gyrus and anterior insula (Mascaro et al., 2014), but had no effect on activity in the hypothalamus, amygdala, or other brain regions implicated in the human stress response.

We acknowledge limitations in this study. The homogeneity of our participants detracts from the generalizability of the current work, which was conducted in Caucasian individuals of a specific ethnicity at one university. Further, because our analyses investigate differences between genotypes defined according to a median split within a sample, we cannot make claims about the impact of the absolute number of repeats on stress physiology. Distinguishing this is important, given the median number of repeats we observed for *ESR2* and *AR* were below those previously reported (Tsezou et al., 2008). This may reflect sample bias, population differences (Ackerman et al., 2012), or differences in software used to quantify the number of repeats. Specifically, previous studies have reported consistently fewer repeats when samples are analyzed with GeneMapper software (used in the current study) versus other commercially available software (Mascaro et al., 2014).

Limited sample size, reflecting the complexity of the TSST design, may have also detracted from the ability to observe effects within each sex, or may have produced spurious findings. Future studies should employ larger sample sizes across heterogeneous ethnicities and ages to increase generalizability and statistical power. Even so, the moderate effect sizes ($0.06 \leq \eta^2 \leq 0.14$) observed in the current work are consistent with studies investigating the association between these polymorphisms and other health-related outcomes (Krithivas et al., 1999; Ogawa et al., 2000; Westberg et al., 2003), as well as those investigating the impact of other genetic variants on responses to the TSST (Alexander et al., 2009; Armbruster et al., 2011; Armbruster et al., 2016; Wust et al., 2009). Levels of circulating sex hormones were not assessed, so a full picture of estrogen and testosterone activity is not available. A better understanding would be accessible if genetic variability in receptors, including additional polymorphisms than those investigated here, as well as circulating concentrations of sex hormones, were assessed in tandem, in both men and women.

The importance of hormones as overall regulators of biology is extremely salient. Hormones are the primary mediators of cellular activity, standing at the gateway between genotype to phenotype by virtue of their multifaceted effects on gene expression (Cox et al., 2016). Despite their traditional association with one sex, estrogens and androgens are known to significantly influence the physiology of both sexes (Maney, 2017). Hormone activity is mediated through cellular receptors, which translocate to the nucleus to promote such expression changes. Importantly, it has been suggested that repeat polymorphisms such as the three genes investigated in the current study can significantly impact transcription, even when they occur in untranslated regions as is the case for $(TA)_n$ and $(CA)_n$ in *ESR1* and *ESR2* respectively (Comings, 1998). Numerous studies suggest that intronic variations modulate transcription (Oldridge et al., 2015; Pravica et al., 2000; Rosatto et al., 1999). The current study is the first to our knowledge that investigated the effects of these polymorphisms in relation to stress reactivity in men and women. Further studies are warranted to replicate the findings and reveal the underlying mechanisms mediating the role of these polymorphisms on receptor availability and function.

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Declaration of interest

None declared.

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