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Genetic and environmental influences on cortisol reactivity to a psychosocial stressor in adolescents and young adults

Chelsea Sawyers [a,*], Christina Sheerin [a], Meridith Eastman [a], Jason Burchett [a], Paul Howell [b], Gretchen Neigh [b], Ananda B. Amstadter [a], John Hettema [a], Roxann Roberson-Nay [a]

[a] Virginia Institute for Psychiatric & Behavioral Genetics, Virginia Commonwealth University, USA
[b] Department of Anatomy and Neurobiology, School of Medicine, Virginia Commonwealth University, USA

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ABSTRACT

Individuals vary in their response to psychological and physiological stressors, and this reactivity can be captured using measures of cortisol. Previous research suggests cortisol reactivity is under some degree of genetic control; however, the measures used have varied widely. This study (N = 524) examined potential differences in heritability across varying cortisol metrics of stress reactivity following the Trier Social Stress Test (TSST) and whether these measures are genetically or environmentally interrelated. Participants included twins aged 15–20 years (56% female). Cortisol reactivity to the TSST was assessed via serial salivary cortisol samples collected pre- and post-TSST. Modest to moderate heritability estimates (12% [95CI: 1–36%] - 45% [95CI: 16–69%]) were observed across measures purported to capture stress reactivity (peak, area under the curve [AUC], baseline-to-peak change). Findings also demonstrate both shared and unique genetic and environmental influences between baseline cortisol and cortisol reactivity. Minimal to no additional genetic innovations above and beyond the contributions of peak cortisol were found for other measures of cortisol reactivity such as AUC. This study is one of the largest twin-based samples to examine the heritability of cortisol reactivity, and results suggest that simpler measures of cortisol reactivity demonstrate higher heritability compared to more complex measurements.

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is a primary system responsible for responding to stress. The HPA axis acts through a cascade of brain and hormonal processes that leads to a release of glucocorticoids (e.g., cortisol; Bale and Vale, 2004) in response to either psychological or physiological stressors. Cortisol impacts a number of systems in the body and is influenced by subjective experiences of various affective states (Dickerson and Kemeny, 2004). HPA axis function is studied from two perspectives, basal (i.e., baseline or resting) activity and stimulated activity (i.e., reactivity). Although associations between basal activity and stress reactivity in the HPA axis have been noted (Chen et al., 2017), these associations are often weak, and it has been suggested that HPA dysregulation might only be detectable when the system is challenged (Kudielka and Wüst, 2010).

HPA axis activity is assessed through single samples collected at predefined times with the resulting values interpreted as an index of unstimulated HPA activity (Wüst et al., 2000). Reactivity is typically assessed using repeated cortisol sampling during exposure to a standardized stressor such as the Trier Social Stress Test (TSST; Kirschbaum et al., 1993). The TSST is one of the more common challenge paradigms designed to elicit an HPA-axis stress response and often is considered a gold standard psychosocial stressor given that it reliably induces cortisol change (Khoury et al., 2015) as it combines elements of uncontrollability and social-evaluative threat which are associated with the largest HPA axis stress response (Dickerson and Kemeny, 2004). A noted complication in the extant cortisol literature is that numerous indices of HPA reactivity have been used (Khoury et al., 2015) (e.g. area under the curve versus baseline to peak change), making synthesis of this literature difficult (Atkinson et al., 2013; Dickerson and Kemeny, 2004). The most commonly used cortisol indices after some form of challenge are post-stressor time points (usually 20 and 40 min) and derivatives thereof such as peak cortisol (peak of the post-stressor indices), percent change baseline-to-peak (BtP), and area under the curve with respect to increase

* Correspondence to: Chelsea Sawyers, Department of Psychiatry, PO Box 980126, Richmond, VA 23298-0126, USA.
E-mail address: sawyersc@vcu.edu (C. Sawyers).

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(AUCi). BtP is the percent change between the pre-task and peak cortisol collections. AUCi calculates the area under repeated measures of cortisol based on the time of each measure since the first measure and captures changes in measurements over time (Pruessner et al., 2003).

While stress-inducing stimuli will reliably result in a physiological and neuroendocrine response in most individuals, there is notable interindividual variability in HPA axis functioning in response to challenges (Almeida et al., 2009; Hruschka et al., 2005). Given the marked interindividual variability in both basal (i.e., resting) and stimulated (i.e., in response to stressors) activity of the HPA axis, and the association of HPA axis dysregulation with the onset and maintenance of a number of stress related conditions, examination of etiological determinants of this variability is important. Twin studies provide a classic approach to understanding the genetic and environmental influences on individual variability of HPA axis responding. The establishment of heritability of a phenotype is needed to justify the conduct of genetic association analyses on the phenotype itself and the estimation of its genetic covariation with psychopathological outcomes of interest.

From a genetic perspective, *basal* cortisol has been repeatedly studied and overall, moderate estimates of heritability (45–62%) have been established in children and adults (Bartels et al., 2003; Gustafsson et al., 2011; Kirschbaum et al., 1992; Kupper et al., 2005; Linkowski et al., 1993; Van Hulle et al., 2012). However, many early twin studies of basal cortisol heritability suffered from relatively small sample sizes (N = 29–300). Within the molecular genetics literature one genome-wide association study was able to find a single genetic locus within SERPINA6/SERPINA1 that was significantly associated with morning plasma cortisol levels in adults (Bolton et al., 2014). Another study did not find significant single nucleotide polymorphism (SNP) heritability estimates (i.e., phenotypic variance explained by additive effects of common autosomal SNPs) across basal saliva and plasma cortisol samples (Neumann et al., 2017) Comparatively fewer studies of the heritability of salivary cortisol *reactivity* have been conducted, primarily in adults, and results are mixed. As previously reviewed by our group (Savage et al., 2017), heritability estimates of cortisol response to various stressors have ranged widely (8–44%), with negligible estimates in early, smaller studies (Inglis et al., 1999; Steptoe et al., 2009). However, the low heritability estimates may be attributable to contextual effects, with studies demonstrating increases in heritability estimates after repeated exposure to stressors (Federenko et al., 2004; Wust et al., 2005). Different heritability estimates of cortisol levels also have been observed across specific time points (e.g., studies that have examined reactivity as well as levels at specific, individual time-points post-stressor), the latter of which were shown to have greater heritability (Wust et al., 2005). Variability in heritability estimates may be due, in part, to the above-mentioned varied metrics of cortisol reactivity, suggesting the need to determine and compare heritability across various measurements and examine potential etiological overlap between them.

The limited literature appears to support moderate heritability of the cortisol response in adults, albeit measured in different ways. To our knowledge, there has been no examination of the heritability of cortisol reactivity in adolescents and young adults. Greater understanding of HPA reactivity in this population is of interest given hypotheses that suggest pubertal changes in stress reactivity may increase vulnerability to psychiatric disorders (Spear, 2009) and possibly explain the rising rates for psychopathology during this developmental period among vulnerable adolescents (Gunnar et al., 2009a, 2009b). The present study seeks to fill this gap and further examine potential differences in heritability across varying cortisol metrics of stress reactivity in a general population sample of adolescents and young adults. Thus, the primary aims of the present study were to 1) determine the broad-based heritability of baseline (i.e., pre-task) cortisol activity and three common measures of cortisol reactivity (peak cortisol, AUCi, and BtP) following the TSST and 2) examine whether these indices share genetic and environmental influences. Given the current genetic and phenotypic literatures on cortisol reactivity, we expect to find two primary, semi-independent measures (pre-task baseline, and a post-task peak), with the etiology of the other two measures being derived from those. Additionally, within the cortisol literature more broadly, it is also recognized that there will be a small, but notable proportion of healthy participants (e.g. 17% in an adolescent sample (Herbison et al., 2016) who are considered "non-responders" to stress induction tasks as determined by a lack of HPA axis reactivity (Miller et al., 2013). While some researchers have sought to explicitly examine responder status and identify correlates of non-responding, this has not been investigated in a twin study. Thus, an additional exploratory aim of this study was to determine whether inclusion of individuals considered non-responders impacts model fit. To provide context and comparison to the previous cortisol work we also report our findings for each post-TSST time points.

2. Methods

2.1. Participants

Twins included in analyses participated in the Adolescent/Young Adult Twin Study (AYATS) recruited by the Mid-Atlantic Twin Registry (Lilley and Silberg, 2013). AYATS enrolled an unselected sample of twins aged 15–20 years. Exclusion criteria included: serious medical conditions, intellectual disabilities, psychosis, psychotropic medication use, or non-psychotropic mediations with similar effects (e.g. beta-adrenergic blockers). Participants were invited for a laboratory visit where they completed a variety of assessments (described in detail elsewhere (Cecilione et al., 2018). Zygosity was determined via parent-report about the physical similarity of the twins and showed a high degree of concordance with DNA testing of blood samples in a subset of the sample (N = 82 pairs, $\kappa = 0.95$, 95% CI [.88, 1.0]).

The final sample for the current study analyses consisted of 206 monozygotic (MZ) twins and 318 dizygotic (DZ) twins (56% female; 10% African American, 2% Hispanic, and 88% white, non-Hispanic; mean age = 17.2 years [SD = 1.3]) who had sufficient data to calculate AUCi and whose cortisol samples passed all quality control criteria (described below). A twin model power analysis (Verhulst, 2017) confirmed this study as > 99% power to detect the effect sizes presented in this study. Informed consent was obtained by adult participants and the legal guardians of minors, and assent was obtained by all minors.

2.1.1. Laboratory task

Each twin participated in the Trier Social Stress Test (TSST), which consists of four parts: an anticipatory stress phase (5 min preparation period prior to a speech), a 5 min speech phase, a 5 min serial subtraction phase, and a 45 min recovery phase. The TSST is a valid and reliable task used to induce moderate psychosocial stress within a controlled laboratory setting (Kirschbaum et al., 1993). Participants were instructed to refrain from eating or drinking 30 min prior to sample collections. Salivary cortisol was measured prior to the beginning of the task, and collected in 15 min increments after the task for a total of 4 post-task time points (0 (immediately after the conclusion of the serial subtraction), 15, 30, and 45 min after task completion).

2.2. Measures

2.2.1. Cortisol

During saliva collection participants were asked to hold a cotton swab under their tongue for 2 min. Saliva samples were stored in − 20 °C freezer within 2 h of collection. After thawing samples were centrifuged at 1500 x g for 15 min to remove particulate matter and all samples were run in duplicate. Cortisol concentrations were determined using the Salimetrics salivary cortisol enzyme immunoassay kit with sensitivity of 0.007 μg/dL. Inter-assay and intra-assay CVs were < 17% and < 7%, respectively. Mean and standard deviation for each cortisol measure are shown in Table 1.

Table 1
Demographics and summary statistics.

Measure	Mean [SD]	
	MZ	DZ
N	206	318
Age	17.2 [1.2]	17.2 [1.3]
Sex	61% Female	52% Female
Ethnicity		
White (N)	185	272
Hispanic (N)	11	2
Black (N)	10	43
BMI	22.8 [4.20]	22.5 [4.20]
Pre-TSST	4.40 [4.95]	4.10 [3.48]
Post-TSST 1	5.74 [4.36]	5.04 [3.39]
Post-TSST 2	7.70 [5.99]	6.81 [5.02]
Post-TSST 3	5.80[4.55]	5.74 [4.90]
Post-TSST 4	4.39 [3.33]	4.53 [4.04]

Note: SD: standard deviation, MZ: monozygotic twins, DZ: dizygotic twins, BMI: Body Mass Index, TSST: Trier Social Stress Test. There was no statistically significant difference between the means of the MZ and DZ twin pairs for the cortisol measures.

2.2.2. Covariates

Body mass index (BMI) was calculated based on reported height and weight, and data from one participant with > 40 BMI was excluded from subsequent analyses. Age, sex, race, ethnicity, number of TSST confederates present (two vs. three, 72% had three), and time of sample collection were examined as potential covariates. Further, self-reported measures of average daily caffeine consumption (mean = 255 mg per day), recent alcohol use (yes/no, in past 12 h, 1.7% endorsed), smoker status (yes/no, 2.2% smoker), and use of any non-exclusionary medications (12.1% endorsed) were assessed. Of these, only time of sample collection (pre-task mean start time = 14:00:00, SD = 2:03 h, range = 10:34:00–18:38:00) was significantly associated with AUCi, peak cortisol, or BtP in preliminary linear regressions. Therefore, time of collection was regressed out of each cortisol measure prior to all twin analyses, and in the case of AUCi and BtP time was regressed out from individual cortisol measures (i.e., post-task times 1–4) prior to calculation of the indices. As part of the larger AYATS study (Cecilione et al., 2018), twin pairs completed study tasks on the same day, but in a different order. To adjust for any study-related effects, we included task order in all of our analyses as a moderator on the means to control for this covariate.

2.2.3. Measures of cortisol reactivity

Prior to calculation of AUCi, several data cleaning protocols were implemented. Twins who did not have enough data to calculate AUCi (e.g. missing a post-task collection time point; n = 22), or who had time intervals between sample collections that deviated significantly from the study protocol were excluded (n = 48) bringing the total usable sample to 524 participants. Based on preliminary analyses to identify covariates, time of day was regressed out of each time point prior to creation of the AUCi measure to minimize the effects of diurnal variation on cortisol. AUCi was calculated from the residualized measures using the method by Pruessner and colleagues [6] with actual time between measures used to calculate AUCi. Peak cortisol response was identified as the highest adjusted post-task salivary cortisol measure for an individual. Percent change baseline-to-peak (BtP) was defined as the percent change between the adjusted pre-task and peak post-task cortisol collections.

2.2.4. Non-responder status

In our sample "non-responders" were identified using a change threshold from baseline to peak response of 15.5% as recommended by Miller et al. (2013). Fifty-one individuals out of the 524 (9.7% of total sample; 42% of non-responders were MZ) who met all data cleaning criteria for this study were classified as non-responders. We performed sensitivity analyses in the univariate analyses by examining models that excluded "non-responders" from the whole sample to test for differences in heritability estimates.

2.3. Twin modeling

Biometrical modeling was used to estimate the influence of genetics and environment on various measures of cortisol reactivity. In this classic twin design, differences in correlations between twins in MZ and DZ twin types are leveraged to partition the phenotypic variance into underlying genetic and environmental influences by capitalizing on differences in genetic relatedness (Neale and Cardon, 1992). Additive genetics (A) reflects the latent cumulative effects of individual genetic loci influencing a trait. Common environment (C) captures non-genetic influences that make twins more similar to each other compared to the general population. Unique environment (E) describes influences that contribute to the differences seen between co-twins, including measurement error. Given the substantial effect of time of day on measured cortisol levels, collection time was regressed out prior to the twin analyses. We applied this model to each measure of post-task cortisol and cortisol reactivity (AUCi, peak, and BtP) individually as well as in a multivariate Cholesky decomposition that included the pre-task measurement. The multivariate Cholesky examines the degree to which the three measures of cortisol reactivity are genetically and environmentally interrelated to each other as well how they relate to overall cortisol level prior to exposure to a stressor (baseline). For each of these univariate and multivariate models, submodels were tested by dropping parameters and comparing the fit statistics to the full model to determine the best-fitting model. A full information maximum-likelihood approach for raw data implemented in the OpenMx software was used (Neale et al., 2016) and all analyses were conducted in the R environment (Team, 2015). Model fit was compared using Akaike Information Criterion (AIC) with lower or more negative values indicating a better fit (Akaike, 1987). AIC is an index balancing explanatory power with parsimony (Williams and Holahan, 1994).

3. Results

We fit univariate twin models to quantify the genetic and environmental influences on each measure of cortisol. For each of the three cortisol reactivity measures and pre-task baseline, individual parameters in the models were removed from the model to test their significance. Each section of Table 2 outlines the univariate models tested for each measure, including the measures of reactivity as well as each time point. Although not a primary aim, reporting of time point specific data allows for comparison of our data to previous research by Steptoe and colleagues (Steptoe et al., 2009). Models were compared to the full ACE model (1) in each section. Beginning with model 2 we tested the significance of each latent factor. The best fitting models for AUCi, peak, and BtP were AE models. For all three measures dropping C from the full model created the most parsimonious model that was not significantly different from the full model. Table 3 shows the proportion of variance estimates for each of the best fitting models. The best-fitting univariate model found AUCi to be 26% (95%CI: 5–45%) heritable with the remaining variance accounted for by unique environmental effects. Peak cortisol response had the highest heritability estimate of 45% (95%CI: 16–69%) with unique environmental effects accounting for the remaining variance. The baseline-to-peak had the lowest heritability estimate of 12% (95%CI: 1–36%) with unique environmental effects capturing the additional error variance introduced by mathematical manipulation and accounting for the remaining variance. Removing non-responders from the data did not significantly alter these parameter estimates or model fit. Therefore, to maximize power in the multivariate models all participants were included.

To examine the degree to which the three measures of cortisol reactivity are interrelated, we conducted a multivariate Cholesky

Table 2
Model fit statistics for univariate models of cortisol reactivity and each measured time point after TSST.

Model	Parameters	EP	*df*	-2ll	AIC	*p*
AUCi						
1	ACE	5	477	4198.44	3244.44	–
2	**AE**	**4**	**478**	**4198.44**	**3242.44**	**1.00**
3	CE	4	478	4200.62	3244.62	.139
4	E	3	479	4204.13	3246.13	.057
BtP						
1	ACE	5	485	734.54	−235.46	–
2	**AE**	**4**	**486**	**734.54**	**−237.46**	**1.00**
3	CE	4	486	735.43	−236.57	.345
4	E	3	487	736.49	−240.50	.620
Peak						
1	ACE	5	485	768.77	−201.23	–
2	**AE**	**4**	**486**	**768.77**	**−203.23**	**1.00**
3	CE	4	486	776.72	−195.28	.004
4	E	3	487	778.07	−195.93	.009
Pre-TSST						
1	ACE	5	485	132.93	−837.07	–
2	**AE**	**4**	**486**	**132.93**	**−839.07**	**.999**
3	CE	4	486	136.68	−835.32	.053
4	E	3	487	147.41	−826.59	.000
Post- TSST 1						
1	ACE	5	480	117.97	−842.02	–
2	**AE**	**4**	**481**	**118.29**	**−843.71**	**.575**
3	CE	4	481	119.29	−842.70	.251
4	E	3	482	119.29	−842.70	.251
Post- TSST 2						
1	ACE	5	480	447.72	−510.27	–
2	**AE**	**4**	**481**	**447.72**	**−512.27**	**.999**
3	CE	4	481	458.89	−501.10	.000
4	E	3	482	488.95	−473.04	.000
Post- TSST 3						
1	ACE	5	480	315.41	−646.59	–
2	**AE**	**4**	**481**	**315.41**	**−648.59**	**.999**
3	CE	4	481	322.96	−641.03	.005
4	E	3	482	330.13	−635.86	.000
Post- TSST 4						
1	ACE	5	480	676.70	−283.30	–
2	**AE**	**4**	**481**	**676.70**	**−285.30**	**.999**
3	CE	4	481	680.95	−281.04	.039
4	E	3	482	681.75	−282.24	.079

Note. EP = number of estimated parameters in the model, df = degrees of freedom, − 2LL = negative two log-likelihood, AIC = Akaike Information Criterion, AUCi = area under the curve with respect to increase. BtP = percent change baseline-to-peak. Peak = highest cortisol reading from all post-task measures. Time = sample collected after Trier Social Stress Test, starting immediately after task conclusion and then in 15 min increments thereafter. ACE = model with additive genetic, shared environment, and unique environmental factors included. AE = model with additive genetic, and unique environmental factors included. CE = model with shared and unique environmental factors included. Best fitting models are designated in bold text for each section of analyses.

Table 3
Proportion of variance estimates for each best-fitting model.

Model	A [95% CI]	E [95% CI]
AUCi	0.26	0.74
	0.05–0.45	*0.55–0.95*
BtP	0.12	0.87
	0.01–0.36	*0.63–0.98*
Peak	0.45	0.55
	0.16–0.69	*0.31–0.84*
Pre-TSST	0.54	0.46
	0.31–0.68	*0.32–0.70*
Post-TSST 1	0.42	0.68
	0.25–0.56	*0.43–0.75*
Post-TSST 2	0.63	0.37
	0.40–0.65	*0.35–0.60*
Post-TSST 3	0.38	0.62
	0.19–0.54	*0.46–0.81*
Post-TSST 4	0.32	0.68
	0.04–0.58	*0.41–0.96*

Note. All parameter estimates are standardized and squared to reflect the percentages of variance accounted for by each source of influence. A= additive genetic factor, E = unique environmental factor. 95% confidence intervals are denoted in italics below each proportion of variance estimate. AUCi = area under the curve with respect to increase, BtP = percent change baseline-to-peak.

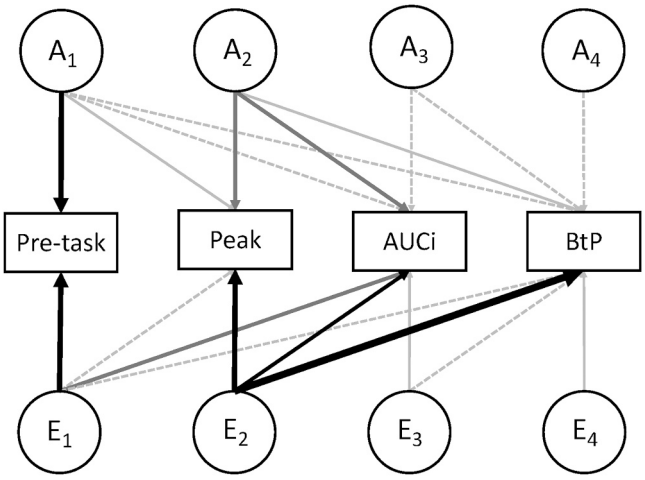

Fig. 1. Best fitting model for Cholesky Decomposition of Cortisol Reactivity. Only genetic and unique environmental factors were found to be significant and retained in the final Cholesky model of cortisol reactivity and a pre-task baseline measure. The darker and bolder lines indicate a stronger influence of that latent factor on the observed variable. Lighter dashed lines indicate a proportion of variance less than 5%. Table 5 provides the estimated proportion of variance accounted for by each of these pathways.

decomposition to assess the degree of shared genetic and environmental overlap as demonstrated by the cross-paths in Fig. 1 and off-diagonal values in Table 5. We used the same testing method as the univariate models discussed above and tested removing genetic and shared environmental influences to find the most parsimonious model. An AE (genetic and unique environment) model was the best fitting model as determined by lowest (most negative) AIC without a significant decrease in fit compared to the full model (Table 4). We also investigated whether the genetic and environmental overlap between these measures was significant by removing the cross-paths (shown in Fig. 1 and off-diagonal values in Table 5) from the model and found the model fit deteriorated (Models 2a-2c in Table 4).

This suggests that although some paths, and therefore the proportions of variance (displayed in Table 5), were estimated at zero, the model fit worsens when all cross-paths are removed. Consequently, there is a degree of overlap in genetic and environmental influences for these measures. These findings suggest that the heritability point-estimates found in the univariate models can best be accounted for by two main genetic factors, baseline (i.e., pre-task) cortisol levels (A_1) and peak cortisol levels (A_2). As expected, minimal to no genetic innovation was observed from the other two measures of cortisol reactivity, AUCi and percent change BtP (3% [95%CI: 2–11%] and 0% [95%CI: 0–3%], respectively). The largest source of genetic influence on AUCi was from the cross-path from Peak which accounted for 20% (95%CI: 1–30%) of the phenotypic variance of AUCi. This is illustrated in Fig. 1, which shows the stronger influences of two primary and semi-independent measures (A_1 and A_2) on their measures as well as the cross-loading onto AUCi and BtP, compared to the two derived measures (A_3 and A_4). Mathematical manipulation of measures to create other measures (e.g. BtP and AUCi) does increase the measurement error captured in E and helps explain, in part, the reduced heritability (A) estimates observed with these variables. Additionally, in the case of AUCi since time is not heritable any differences in the timing of sample collection will also contribute to the measurement error captured in the unique

Table 4
Model fit statistics for cholesky decomposition models.

Model	Parameters	EP	*df*	-2ll	AIC	*p*
1	ACE	38	1914	3604.36	-223.64	–
2	**AE**	**28**	**1924**	**3610.56**	**-237.44**	**0.798**
3	CE	28	1924	3612.18	-235.82	0.646
4	E	18	1934	3668.08	-199.92	< 0.001
2a	AE no A Correlations	22	1930	3644.95	-215.05	< 0.001
2b	AE no E Correlations	22	1930	4576.50	716.50	< 0.001
2c	AE no AE Correlations	16	1936	5834.68	2230.68	< 0.001

Note. EP = number of estimated parameters in the model, df = degrees of freedom, − 2LL = negative two log-likelihood, AIC = Akaike Information Criterion, AUCi = area under the curve with respect to increase. ACE = model with additive genetic, shared environment, and unique environmental factors included. AE = model with additive genetic, and unique environmental factors included. CE = model with shared and unique environmental factors included. Best fitting model is designated in bold.

variance (E), thereby further reducing the estimated heritability. Estimated proportion of variance due to E shows a similar pattern as the A factors with baseline and peak factors (E_1 and E_2, respectively) accounting for the majority of the unique environment and the AUCi and BtP E factors accounting for substantially smaller proportions of variance (14% [95%CI: 11–18%] and 5% [95%CI: 2–9%], respectively).

4. Discussion

This study represents one of the largest twin-based samples to date examining the heritability of cortisol reactivity, and the first to do so in an adolescent and young adult population. We aimed to 1) determine the broad-based heritability of three commonly used measures of cortisol reactivity and pre-task baseline and 2) examine whether the genetic and environmental influences on those measures were interrelated. An exploratory aim was to determine if etiologic influences differed among responders and non-responders. Results indicated modest to moderate heritability estimates depending on the metric with no etiological differences based on responder status, and these findings, and their implications, will be discussed in turn.

AE models provided the best fit in all models, suggesting that both genetic and unique environmental influences, but not shared environmental influences, contributes to the etiology of cortisol stress reactivity, which is consistent with the extant literature (e.g. basal cortisol (Bartels et al., 2003) and cortisol reactivity (Wust et al., 2005)). The first set of analyses demonstrate that heritability estimates, although somewhat overlapping, varied depending on which metric was used (i.e., lowest was 12% (95%CI: 1–36%) for percent change BtP, highest was 45% (95%CI: 16–69%) for peak). As discussed by Khoury et al. (2015), the lack of consistency across metrics that attempt to capture the same process is problematic, and present findings suggest this may be a factor in the wide range of heritability rates of cortisol response to stressors in the literature (Bartels et al., 2003).

From the univariate models we moved forward to examine the genetic and environmental overlap between cortisol reactivity measures via a multivariate Cholesky decomposition model. We posit that the divergent heritability estimates are likely attributable to the introduction of greater error variance as metrics move further away from actual values and incorporate mathematical transformation. Another possibility for the increase in E seen in the univariate models for AUCi and BtP is that these measures are capturing a truer environmental signal. If that was the case we would expect the multivariate models to show an increase in E that is unique to those measures (E_3 and E_4 respectively). However, that is not the case as shown in Table 5 with the majority of the environmental variance of AUCi and BtP being accounted for by the environmental influences of Peak (off-diagonal paths in Table 5). As the peak metric had a more consistent, moderate heritability estimate compared to the other reactivity metrics, when attempting to capture accurate heritability estimates, this more basic metric is likely preferable. In our exploratory analysis we found estimates were not significantly altered whether non-responders were included or excluded from analyses. Not having an increase in salivary cortisol in response to a stressor is itself a response in the context of this study. Therefore, it stands to reason that exclusion of a specific reaction (i.e. non-response) to a stressor should not affect the etiological influences on indices that are capturing responses. However, the non-responder rate in this sample does limit the power to detect differences and this exploratory analysis is an avenue for future research.

Our findings suggest that there is overlap between the genetic and environmental influences on the three cortisol reactivity metrics denoted by the cross-paths in the multivariate model (off-diagonal values in Table 5). This was an expected result given that the cortisol reactivity measures are calculations of the same underlying data. The results suggesting the presence of unique genetic effects between the pre-task measure (A_1) and stress reactivity (as measured via peak, A_2) are in line with non-genetic examinations of the interrelations among baseline and reactivity indices (Khoury et al., 2015). This supports existing models that suggest HPA axis response patterns to acute stress and basal HPA axis activity are related and the relationship should be considered in the context of physical and mental health outcomes (Chen et al., 2017). The finding that the other, calculated, measures of reactivity did not further add unique information (i.e. A_3 and A_4 in Table 5) are not unexpected. Given that the calculated measures of reactivity compound the error variance of each base measure and that time is not heritable, it is reasonable to posit that the unique environmental influences for ACUi and BtP are inflated to a degree due to the nature of their calculation. This error inflation and lack of unique etiological information (A_3 and A_4) adds further support to the suggestion that peak may be the preferred measure of use in cortisol studies. This is a potentially useful finding for informing attempts to bridge between genetic studies of cortisol and decrease the redundant use of numerous cortisol indices (Khoury et al., 2015) and at minimum, suggests that attempts to

Table 5
Proportion of Variance Estimates for Best-Fitting Cholesky Model.

Measure	A_1	A_2	A_3	A_4	E_1	E_2	E_3	E_4
Pre-task	0.49	–	–	–	.51	–	–	–
	0.42–0.66				*0.34–0.61*			
Peak	0.14	0.21	–	–	0.10	0.55	–	–
	0.02–0.38	*0.01–0.38*			*0.03–0.27*	*0.44–0.77*		
AUCi	0.02	0.20	0.03	–	0.22	0.39	0.14	–
	0.01–0.13	*0.01–0.30*	*0.02–0.11*		*0.01–0.31*	*0.25–0.53*	*0.11–0.18*	
BtP	0.00	0.10	0.00	0.00	0.02	0.83	0.00	0.05
	0.00–0.00	*0.01–0.32*	*0.00–0.00*	*0.00–0.03*	*0.00–0.12*	*0.58–0.95*	*0.00–0.01*	*0.02–0.09*

Note: All parameter estimates are standardized and squared to reflect the percentages of variance accounted for by each source of influence. A= additive genetic factor, E = unique environmental factor. 95% confidence intervals are denoted in italics below each proportion of variance estimate. AUCi = area under the curve with respect to increase, BtP = percent change baseline-to-peak. Diagonal values represent variance of the trait, off-diagonal values represent proportion of variance accounted for by other variance components.

synthesize information on cortisol responsivity across datasets, must take into consideration the metric used.

Heritability estimates of cortisol reactivity in this and other studies are lower than what is found for basal salivary cortisol response (e.g., a large adult twin study of average daily salivary cortisol levels reported a heritability of 42–48% (Franz et al., 2010)). This pattern also has been found with regard to the modulation of startle response, in that while moderate heritability in overall basal startle response was demonstrated, genetic influences on fear potentiated startle were not found (Savage et al., 2019). The authors suggested that the strong dependency of fear potentiated startle on basal startle makes unique genetic influences difficult to disentangle and detect; it is possible that a similar phenomenon is occurring with cortisol response, and is worth further investigation. Indeed, it may be that most of the heritable influence on stress reactivity is determined by basal processes or that unique environmental effects, which may or may not leave their mark behind (i.e. epigenetic factors), become more important in reactivity to stress. Regardless, findings suggest that cortisol measures of baseline and peak responding are capturing distinct processes.

The examination of cortisol heritability in an adolescent and young adult population is of interest not only because this age range is lacking within the extant literature, but also given hypotheses that suggest pubertal changes in HPA activity, specifically increases in stress reactivity, may increase vulnerability to psychiatric disorders (Spear, 2009; Walker et al., 2001) and possibly explain the rates for psychopathology increasing with age among vulnerable adolescents. There is existing evidence that basal cortisol increases across puberty (Netherton et al., 2004; Spear, 2009), and some evidence for a marginal increase in cortisol reactivity during the TSST by age in a study of 9–15 year-olds (Gunnar et al., 2009a, 2009b). Developmental changes in cortisol reactions to stress associated with both age and pubertal maturation may coincide with potential shifts in the heritability of the stress response at this developmental stage. Comparing our findings of specific post-task time points to previous work showed our estimates are in line with those from a sample of children (Steptoe et al., 2009), but more work is needed in child and adolescent populations to determine whether changes in cortisol reactions across development are due to shifts in heritability, other biological shifts, environmental factors, or a combination. Longitudinal studies are needed to specifically test this question and additional research is needed to inform the potential association of increased reactivity with increased risk for a number of psychiatric disorders.

4.1. Limitations

The results of this study should be viewed in the context of several limitations. First, given the limited age range and the predominantly Caucasian sample, generalizability is impacted and further research is needed to determine whether patterns of reactivity and heritability differ in other developmental stages and racial/ethnic groups. However, this study does examine an age range that is overlooked in the current literature and begins to bridge the gap between child and adult studies. The present study also recruited a population-based sample of generally healthy twin participants. While this strengthens the generalizability of findings to a broader population, it is unknown how findings may translate to clinical populations or compare to findings from clinically ascertained samples. Second, although sex differences in HPA reactivity have been noted, particularly in relation to pubertal development (van Keulen et al., 2020) and associated with testosterone and progesterone, the present study did not assess pubertal development nor sex hormones (Stephens et al., 2016). The study was also not sufficiently powered to test for sex differences in heritability. Both areas represent important extensions of this work. Third, analyses were conducted on data collected during a single laboratory visit. Thus, findings cannot speak to the stability of the heritability estimates longitudinally. Due to the constraints of scheduling an extensive single laboratory visit, the start times of the TSST varied. However, we addressed this in our analyses by regressing time of day from each measure. Related to this point, we note that the pre-task, baseline measure of cortisol was measured at a single, variable time-point. Thus, it is best considered an unstimulated measure of cortisol reactivity and not a true basal value, which should be considered when comparing study findings to other heritability estimates of basal cortisol measured using the cortisol awakening response. Given the etiological nature of this study the variance in time would increase the noise in the data (represented by the E variance). Fourth, while all TSST procedures were carried out in the same room, there was some variation in the TSST audience for each twin pair. However, it is noted that analyses indicated no difference by number of confederates (two vs three). As noted in the methods section, twin pairs completed tasks on the same day, but in a different order (thus, the timing of cortisol measures was not the same). These effects were mitigated, however, by task order included as moderator on the means to control for the effects of this covariate, and time of day of the cortisol sampling incorporated into the models. Finally, from our original sample of 595 participants, 61 were excluded from analyses due to missing data and protocol deviations which could theoretically influence our analyses. However, there was no pattern to those with missing data, and protocol deviations were not more prevalent in either one twin type or twin order over the other leading us to believe the variance estimates obtained were not unduly influenced by these data cleaning efforts.

5. Conclusions

Although modest to moderate heritability estimates were found, results also highlight the notable range of heritability estimates across various measures that are purported to capture the same process. Findings also demonstrate both shared and unique estimates between baseline (pre-task) cortisol and cortisol reactivity, with larger estimates for the former. Although minimal to no additional genetic innovations above and beyond the contributions of peak cortisol measure were found for other measures of cortisol reactivity such as AUCi. Therefore, we recommend also reporting peak response in studies where AUCi is an appropriate approach for the main research aims to help bridge findings to the growing genetics literature. Continued work examining longitudinal outcomes to inform upon developmental changes in the heritability of cortisol reactivity, repeated assessments to determine the reliability of these estimates, and in clinical samples to determine the association with psychopathology, is also warranted.

Conflict of Interest Statement

The authors have no conflicts of interest.

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Author Disclosure

Sawyers, Sheerin, and Eastman substantially contributed to the conception, design, analysis, interpretation and drafting of this work. Burchett and Howell substantially contributed to the acquisition of data for this work. Amstadter, Hettema, Neigh, and Roberson-Nay substantially contributed to the conception, interpretation, and revising of this work.

Declarations of Interest

None.

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