Individual differences in early adolescents' latent trait cortisol: Interaction of early adversity and 5-HTTLPR

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Abstract:

The present study aimed to examine the interaction of 5-HTTLPR and early adversity on trait-like levels of cortisol. A community sample of 117 early adolescent girls (M age = 12.39 years) provided DNA samples for 5-HTTLPR genotyping, and saliva samples for assessing cortisol 3 times a day (waking, 30 min post-waking, and bedtime) over a three-day period. Latent trait cortisol (LTC) was modeled using the first 2 samples of each day. Early adversity was assessed with objective contextual stress interviews with adolescents and their mothers. A significant 5-HTTLPR × early adversity interaction indicated that greater early adversity was associated with lower LTC levels, but only among individuals with either L/L or S/L genotype. Findings suggest that serotonergic genetic variation may influence the impact of early adversity on individual differences in HPA-axis regulation. Future research should explore whether this interaction contributes to the development of psychopathology through HPA axis functioning.

Keywords: early adversity | salivary cortisol | latent trait cortisol | 5-HTTLPR | adolescence

Article:

1. Introduction

At the core of the allostatic load framework is an attempt to explain biological mechanisms in the effects of cumulative stress on health and human development (McEwen, 1998). Within this framework, environmentally induced alterations in the activity of the hypothalamic-pituitary-adrenal (HPA) axis are considered key mediators in the pathway linking adversity to differential outcomes (Danese & McEwen, 2012; Gunnar & Quevedo, 2007; McEwen, 2000). More recently, researchers have begun to explore the role of gene-by-environment (G x E) interactions in the allostatic load model. One of the candidate genes under investigation is a functional polymorphism

located in the promoter region of the serotonin transporter gene (SLC6A4; also known as 5-HTT). Research suggests that serotonin transporter-linked polymorphic region (5-HTTLPR) conveys sensitivity to stress (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010). This is supported by the largest and most recent meta-analysis (Sharpley, Palanisamy, Glyde, Dillingham, & Agnew, 2014), although earlier, smaller meta-analyses drew negative conclusions (e.g., Risch et al., 2009).

Several studies have revealed the moderating effect of this serotonin transporter genotype in the relationship between early adversity and indicators of allostatic load (e.g., Alexander et al., 2009, Mueller et al., 2011; Willner, Morris, McCoy, & Adam, 2014). Findings such as these raise intriguing questions about genetic susceptibility to allostatic load in altering HPA-axis functioning. Recent research has primarily focused on the association between cumulative adversity or stressful life events, 5-HTTLPR, and stress-related HPA reactivity (Alexander et al., 2009, Mueller et al., 2011). However, allostatic load manifests not only in dynamic responses to acute stress but also in changes in the overall typical diurnal patterns of the HPA-axis. Latent trait cortisol (LTC) provides an index of variation in HPA-axis functioning that is independent of state-specific change (Doane, Chen, Sladek, Van Lenten, & Granger, 2015). LTC has been associated with early adversity (Stroud, Chen, Doane, & Granger, 2016a), recent stress (Stroud, Chen, Doane, & Granger, 2016b), problem behavior (Shirtcliff, Granger, Booth, & Johnson, 2005), and cardiovascular risk factors (Yeung et al., 2016). In the current study, we begin to address an important knowledge gap by exploring whether early adversity interacts with allelic variation in 5-HTTLPR to influence LTC level.

1.1. Early adversity and the HPA-axis

Child abuse and neglect (i.e., childhood maltreatment) have received considerable attention in research examining the impact of early adversity on HPA-axis activity (Alink, Cicchetti, Kim, & Rogosch, 2012; Cicchetti & Rogosch, 2001; Rogosch, Dackis, & Cicchetti, 2011; Tarullo & Gunnar, 2006). Not surprisingly, childhood maltreatment is associated with a broad range of adverse outcomes later in life, including posttraumatic stress disorder (e.g., Widom, 1999), major depression (e.g., Vrshek-Schallhorn et al., 2014; Widom, DuMont, & Czaja, 2007), and substance use (e.g., Scott, Smith, & Ellis, 2010). Furthermore, childhood maltreatment is associated with alterations in HPA-axis functioning, as indexed by diurnal cortisol profiles (i.e., the daily pattern of cortisol secretion), and cortisol reactivity (i.e., changes in cortisol level in response to a stressor; Alink et al., 2012; Cicchetti, Rogosch, Gunnar, & Toth, 2010; Neigh, Gillespie, & Nemeroff, 2009). Under the allostatic load framework, childhood maltreatment leads to "wear and tear" in the HPA-axis and alters its function, which in turn contributes to a variety of adverse health outcomes (McEwen, 2000).

Less severe, but more common, types of early adversity have also been linked to variation in HPA-axis functioning (e.g., Miller, Chen, & Zhou, 2007; Repetti, Taylor, & Seeman, 2002). Such early adversity often captures adverse experiences within the family environment, including, for example, exposure to marital conflict, financial hardship, and death or illness of family members (e.g., Miller et al., 2007, Repetti et al., 2002). Importantly, the allostatic load model emphasizes the cumulative effects of early adversity on regulatory systems (Lupien et al., 2006). Thus, even though some of these early adverse experiences may be relatively less severe when considered in isolation, it is posited that their cumulative effect over time can generate allostatic load. In support of this, studies have found that the cumulative effect of multiple early adversities was associated with alterations in HPA axis activity (Repetti et al., 2002, Stroud et al., 2016a;

Zalewski, Lengua, Kiff, & Fisher, 2012). For instance, a recent study showed that the cumulative effect of multiple adverse family environment factors (e.g., parental divorce, residential instability)—but not most of the individual effects of each adverse family environment factor—was associated with lower morning cortisol levels (Zalewski et al., 2012).

In studies examining the effects of early adversity on HPA axis functioning, investigators have operationalized HPA-axis functioning using several indicators of the diurnal cortisol rhythm, including the cortisol awakening response (CAR), the diurnal slope, and the area under the curve (AUC) (Almeida, Piazza, & Stawski, 2009; Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Stalder et al., 2016). As expected of an environmentally sensitive system, close evaluation of the psychometrics properties of these indicators suggests that they exhibit considerable day-to-day variation. For example, Ross, Murphy, Adam, Chen, and Miller (2014) reported that over 70% of the variability in the CAR, and between 50% and 75% of the variability in the diurnal slope, could be attributed to day-to-day variation. Similarly, Doane et al. (2015) collected salivary cortisol data multiple times within a day, over a three-day period, at three measurement occasions, and reported that 82.30% and 81.25% of the variance in the CAR and diurnal slope (respectively) were attributable to day-to-day variation. In an effort to index stable intrinsic individual differences in HPA axis functioning, rather than day-to-day variation, researchers have employed a latent variable approach to isolate a latent trait factor that represents stable individual differences in cortisol (e.g., Doane et al., 2015, Stroud et al., 2016a). Consistent with the allostatic load framework, the handful of studies to date have demonstrated associations between LTC level and early adversity and recent acute stress (Doane et al., 2015, Stroud et al., 2016a, Stroud et al., 2016b).

1.2. 5-HTTLPR as a moderator of the relationship between early adversity and LTC level

Cumulative early adversity is thought to get "under the skin" by altering individuals' biological stress systems, including HPA-axis activity. Factors that affect sensitivity to stress at the individual level are also likely to affect the influence of cumulative early adversity on HPA axis activity. Although no prior research has examined the heritability of LTC levels, twin studies support substantial genetic contributions to other cortisol indices, including cortisol reactivity and the diurnal rhythm, across multiple developmental stages (Bartels, Berg, Sluyter, Boomsma, & Geus, 2003; Federenko, Nagamine, Hellhammer, Wadhwa, & Wüst, 2004; Steptoe, Jaarsveld, Semmler, Plomin, & Wardle, 2009). This suggests that genetic factors may also contribute to LTC level. Furthermore, research suggests that variation in one such factor—a functional polymorphism, 5-HTTLPR—modulated individuals' sensitivity to stress (Caspi et al., 2010). More specifically, individuals who expressed the short (S) as opposed to the long allele (L) exhibited lower transcriptional efficiency, and reduced serotonin transporter function (Heils et al., 1996, Lesch et al., 1996), which has been linked with hypervigilance to environmental stimuli (Homberg & Lesch, 2011).

The serotonin transporter genotype may moderate the association between cumulative early adversity and HPA-axis functioning for at least three reasons. First, the 5-HTTLPR genotype has been linked to individual differences in the functioning of brain regions involved in emotion processing and regulation. For example, 5-HTTLPR S-carriers show heightened amygdala neuronal activity in response to fearful stimuli (Hariri et al., 2002, Heinz et al., 2005), and greater coupling between the amygdala and the ventromedial prefrontal cortex, which integrates input from amygdala to guide behavioral responses in decision making (Heinz et al., 2005, Pezawas et al., 2005). Additionally, research suggests that the amygdala may enhance cortisol secretion, and

there is increasing evidence supporting limbic-HPA interaction (Herman, Ostrander, Mueller, & Figueiredo, 2005).

Second, accumulating evidence indicates that the serotonergic system is involved in the development of HPA-axis. For example, findings from animal studies suggest that the serotonergic system affects early programming of the HPA-axis (for review see Andrews & Matthews, 2004). Furthermore, a meta-analysis of 11 studies with human participants revealed a significant association between the 5-HTTLPR genotype and cortisol stress reactivity, with individuals with S/S genotype displaying heightened levels of cortisol reactivity to acute stressors, as compared to individuals with S/L or L/L genotypes (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2013). Fewer studies have examined associations between the 5-HTTLPR and diurnal cortisol indicators. Chen, Joormann, Hallmayer, and Gotlib (2009) found that adolescent girls with S/S genotype had higher waking cortisol levels, as compared to L-carriers, a finding consistent with a prior study which demonstrated that the S carriers had higher morning cortisol levels (Goodyer, Bacon, Ban, Croudace, & Herbert, 2009).

Third, a few studies have explicitly examined the interplay between 5-HTTLPR, stress (early adversity or recent acute stress), and HPA axis functioning (e.g., Alexander et al., 2009, Willner et al., 2014). Two studies have focused on laboratory-based cortisol reactivity. First, Alexander et al. (2009) revealed that S/S young adults who self-reported higher degree of stressful life events based on the Life Events Checklist (e.g., motor vehicle accident, combat, the sudden unexpected death of a loved one) showed the greatest stress-related cortisol reactivity. Second, Mueller et al. (2011) also found a significant interaction between self-reported early adversity (i.e., the number of stressful life events during the first five years assessed with life history calendar) and 5-HTTLPR among young adults. Specifically, among young adults who were homozygous for L alleles, greater early adversity was negatively associated with cortisol reactivity, whereas among individuals with at least one S allele, early adversity was positively related to cortisol reactivity. However, Mueller and colleagues did not reveal a significant interaction between parent-reported early adversity and 5-HTTLPR among children.

In addition to these studies focusing on cortisol reactivity, a few studies have examined 5-HTTLPR in interaction with stress predicting diurnal cortisol indicators. For example, one study suggested that the 5-HTTLPR genotype moderated the effect of cumulative adversity (e.g., underweight at birth, mother's current depression, government assistance a year prior) on diurnal cortisol (Willner et al., 2014). The pattern of findings indicated that among L/L individuals, cumulative risk was negatively associated with the total output of cortisol (measured as AUCG) and waking cortisol level. However, a second study found no evidence that 5-HTTLPR moderated the effect of self-reported past year acute stress (e.g., serious marital problems, major financial problems) on average waking and evening cortisol levels in a sample of adult twins aged 30–50 years old (Vinberg, Mellerup, Andersen, Bennike, & Kessing, 2010). Collectively, this suggests that the impact of the 5-HTTLPR genotype on the association between environmental stress and HPA axis functioning likely depends upon a number of factors, including the measurement and classification of HPA axis functioning (e.g., cortisol reactivity vs. diurnal cortisol), the type of stress examined (e.g., recent stress versus early adversity), and potentially developmental stage. Moreover, none of these studies used contextual objective interview measures of stress. A meta-analysis showed that the association between 5-HTTLPR and stress sensitivity was stronger when stress was assessed with objective measure and in-person interview instead of self-reported questionnaires (Karg, Burmeister, Shedden, & Sen, 2011). Thus, more consistent findings may be revealed when using an objective contextual stress interview measure of early adversity, and an

indicator of HPA axis functioning that captures trait-like differences, as opposed to primarily stress reactive states or state-like functioning.

1.3. The present study

The present study was designed to build upon a prior investigation in which we demonstrated the accumulation of nine types of early adverse experiences within the family environment was negatively associated with individual LTC level in a sample of early adolescent girls (Stroud et al., 2016a). We drew data from the same project and extent our prior work by examining the moderating effect of 5-HTTLPR on the association between early adversity and LTC level. Given that the S allele of 5-HTTLPR is thought to convey sensitivity to environmental stress (Caspi et al., 2010), we predicted that 5-HTTLPR would moderate the impact of early adversity on LTC level, such that the magnitude of the negative association between cumulative early adversity and LTC would be most pronounced among individuals homozygous for the S allele.

2. Methods

2.1. Overview

Participants were early adolescent girls drawn from a larger study examining biopsychosocial predictors of emotional adjustment (see Stroud et al., 2016a for details). Briefly, during a laboratory visit, participants and their primary female caregivers (herein called mothers) provided informed assent and consent respectively and completed questionnaires and interviews. Adolescents used an Oragene saliva collection kit to provide DNA and were instructed how to self-collect saliva at home for cortisol. Within approximately one week of the laboratory visit, adolescents collected saliva samples three times a day (waking, 30 min post-waking, and bedtime) on three consecutive typical weekdays. On each saliva-sampling occasion, participants also completed a diary report including information about the timing of waking, affect, perceived stress, caffeine, and nicotine use.

2.2. Participants

Participants (M age = 12.39 years, SD = 0.77) and their mothers were recruited from two New England counties through advertisements or flyers, word-of-mouth, and local schools. Five of the original 122 participants were excluded from the current study due to noncompliance in salivary sample collection (no valid 30 min post-waking samples; see noncompliance definition in the section for HPA axis functioning), resulting in a final sample of 117 participants.1 Adolescents were mostly White (89%), and most families had annual household incomes of more than $61k (63%).

2.3. Early adversity

Adolescents' exposure to adverse family experiences up until the year before the laboratory visit was measured using the lifetime adversity section of the Youth Life Stress Interview (Rudolph & Flynn, 2007). Mothers and adolescents were interviewed by the same interviewer, and interviewers were blind to other data (see Stroud et al., 2016a for details). First, interviewers used a general

probe to assess exposure to particularly stressful events and circumstances. Second, interviewers probed about nine specific types of adversity, including death of a close family member or friend, long separation from parents (or primary caregivers), parental separation or divorce, exposure to serious marital conflict, chronic physical or mental illness of a close family member or friend, multiple family transitions (e.g., frequent moves between different caregivers), chaotic family living circumstances (e.g., neglect), legal problems of family members and financial difficulties. Finally, interviewers also probed about exposure to any other very difficult experience.

For each adversity endorsed, participants provided information about the surrounding context and the consequence. A research assistant listened to audio-recordings of the interviews to prepare narratives for each adversity endorsed, which included information about the context and consequences, but not participants' subjective reactions. If mothers and adolescents endorsed the same adversity, the narratives reflected both of their reports. If only the mother or only the daughter endorsed the adversity, the narrative was based upon only one person's report (Rudolph & Flynn, 2007). An independent rating team who was blind to participants' subjective reactions and all other data coded the narratives on a 9-piont scale (from 1 = "no adversity" to 9 = "extremely severe negative impact") considering the likely impact of the adversity for a typical adolescent given the circumstances. The team rated each adversity endorsed and provided an overall severity rating. A second rating team who was blind to the original ratings re-rated a subset of participants (n = 60; inter-rater reliability: ICC = 0.99). The overall severity rating score was used as an index of cumulative early adversity in the current study.

2.4. DNA extraction and genotyping

The Oragene PrepIT L2P DNA Purification Kit was used to extract DNA from the oral fluid with minimal modification of the manufacturers recommended protocol. Following extraction, samples were quantified using PicoGreen (Life Technologies, Grand Island, NY), and normalized to 2 ng/μl. Because two independent groups (Martin, Cleak, Willis-Owen, Flint, & Shifman, 2007; Philibert et al., 2008) have been unable to replicate a finding that an addition adjacent polymorphism, rs25531, modifies the functioning of a subset of L alleles such that they behave like S alleles (i.e., "triallelic 5-HTTLPR genotype"; Wendland, Martin, Kruse, Lesch, & Murphy, 2006), we elected to examine traditional 5-HTTLPR genotype without recoding based on rs25531.

Per Wendland et al. (2006), genotyping of 5-HTTLPR used a modified fluorescent detection protocol. In a total reaction size of 20 ul, 12.5–25 ng of genomic DNA was amplified using 1X Multiplex Master Mix (Qiagen, Valencia, CA) and labeled oligonucleotide primers (5-HTTLPR-F: 5′ FAM-TCC GCT TTG GCG CCT CTT CC; 5-HTTLPR-R: 5′ HEX-TGG GGG TTG CAG GGG AGA TCC TG). Amplification consisted of an initial denaturation step at 95C for 15 min; 35 cycles of 94C for 30 s, 66C for 90 s, 72C for 60 s; and a final extension of 72C for 10 min. Fragment analysis was performed on an ABI 3730XL Genetic Analyzer (Life Technologies, Carlsbad, CA), and the resulting fragments were analyzed using GeneMarker v1.4 (SoftGenetics, State College, PA).

The 5-HTTLPR genotype was coded regarding the number of short alleles, where L/L was 0, S/L was 1, and S/S was 2. The distribution of the 5-HTTLPR genotype by race is reported in Table 1. Genotypes for the full sample did not deviate from Hardy-Weinberg Equilibrium, $\chi 2$ (1) = 0.59, p = 0.44. Five participants were missing on 5-HTTLPR genotype, but were retained in the analysis (missing data were handled with full information maximum likelihood; see Analytic Plan

below). Genotype frequencies did not significantly differ between White and Non-White girls ($\chi 2 = 4.15$, df = 2, p = 0.13), though the sample size of non-White girls was very small (n = 12).

Table 1. 5-HTTLPR genotypes frequencies.

Genotype	Full sample		White		non-White	
	N	**%**	**N**	**%**	**N**	**%**
LL	38	32.48	36	36.00	2	16.67
LS	51	43.59	46	46.00	5	41.67
SS	23	19.66	18	18.00	5	41.67
	112		100		12	

Note. Five participants had missing data on 5-HTTLPR, resulting in 112 instead of 117 cases. The five participants with missing data on 5-HTTLPR had non-missing data on other key variables and were therefore retained in analyses (see Analytic Plan for more details).

2.5. HPA axis functioning

Each participant supplied up to 9 unstimulated whole saliva samples (3 per day, on three consecutive days) by passive drool. On each day, samples were collected on waking, 30 min post-waking, and bedtime. Samples were returned via mail; stored at −20C until transported via courier on dry ice to the Biochemisches Labor at the University of Trier, Germany for assay. Saliva samples were assayed for cortisol in duplicate, using a solid phase time-resolved fluorescence immunoassay with fluorometric endpoint detection (DELFIA; Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). The intra-assay coefficients of variation ranged from 4.0% to 6.7%, and the inter-assay coefficients of variation ranged from 7.1% to 9.0%.

For the 30 min post-waking samples, those taken outside the time window of 23 and 37 min following waking samples were considered noncompliant. Five of the original 122 participants were excluded because they were noncompliant for all the 30 min post-waking samples and did not have any non-missing waking samples. Data points that were considered as noncompliant (n = 59) or as outliers (3 SD away from the corresponding mean for the time of day; n = 12) were treated as missing values and retained in the analyses. On average, each participant had 5.26 valid samples out of six total possible samples for the waking and 30 min post-waking samples over the 3-day sampling period.

To obtain objective saliva sample collection times, we asked all participants to store their saliva collection materials in a container with a MEMS 6TM (Aardex; Aardex Group, Richmond, VA) track cap that recorded the time when it was opened. However, only 87 of the 117 participants (74%) used the track cap. Self-reported sample times were considered consistent if they matched the track-cap recorded time within a 10-min time window. For the waking and 30-min post waking samples across three days, 80% of the self-reported time fell within the 10-min time window of the track cap recording time (84% and 80% for Day 1 waking and 30-min post waking; 81% and 83% for Day 2; 76% and 74% for Day 3).

2.6. Covariates

We examined various demographic and health variables as potential covariates. There were day-level covariates collected on each of the three study days and person-level covariates that remained the same across the study. Day-level covariates included: (a) hours since waking; (b) caffeine use in the past hour; (c) nicotine use in the past hour; (d) perceptions of stress in the past hour (1 =

"not at all"; 5 = "very much"); (e) previous night perceptions of stress (1 = "not at all"; 5 = "very much"); (f) average daily negative affect; (g) average daily positive affect. Average daily positive and negative affect were assessed using the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988). Adolescents were asked to report on the extent to which they felt a list of 10 positive (e.g., excited) and 10 negative (e.g., upset) emotions in the hour prior to the sampling on a scale from 0 (not at all) to 4 (extremely); mean daily negative and positive affect variables were computed.

Person-level covariates included race (White = 1), oral contraceptive use, age, and pubertal status. At the laboratory visit, pubertal status was assessed via adolescent self-report using the Pubertal Development Scale (PDS; Petersen et al., 1988). Adolescents reported on a four-point scale (1 = "no development", 4 = "completed development") about their growth spurt in height, skin and body hair changes, breast development, and age at menarche (note that age at menarche was rated at either 1 or 4). The mean of these five items was used in analyses. Oral contraceptive use (n = 2; 1.7%) and nicotine use (ranged from 0 to 0.9%) were infrequent and therefore not included in analyses.

2.7. Analytic plan

Analyses were conducted in Mplus 7 (Muthén & Muthén, 1998–2015). First, we modeled a latent trait level of cortisol. Consistent with prior work (Doane et al., 2015, Stroud et al., 2016a), the waking and the 30 min post-waking cortisol samples from the three days of the collection were used to form the LTC (Model 1; See Fig. 1). Correlations between all potential day-level covariates and 30 min post-waking cortisol levels were examined. Preliminary analysis showed that none of the day-level covariates were significantly correlated with the corresponding 30 min post-waking cortisol indicators, and thus, none were included in the measurement model. Second, we tested the main and interactive effects of early adversity and 5-HTTLPR genotypes on LTC levels. The resulting significant interaction was probed using standard simple slope estimates (Aiken & West, 1991; Cohen, Cohen, West, & Aiken, 2002). That is, we tested whether the association between cumulative adversity and LTC was significant among three different groups, including a group homozygous for L alleles, a group with one L allele and one S allele, and a group homozygous for S alleles. Levene's test revealed that variances of early adversity ($p = 0.85$) and LTC ($p = 0.37$) were not significantly different between the L/L, L/S, and S/S groups. Before conducting this analysis, we examined whether the potential person-level covariates were significantly correlated with both early adversity and LTC level. Because no covariates were significantly correlated with both variables, none of the potential person-level covariates were included. The bivariate correlation between the primary variables along with means and standard deviation are reported in Table 2.

Model fit was assessed with the $\chi 2$ test (a p-value >.05 suggests good fit), the comparative fit index (CFI; >.90 indicates good fit) and the root mean square error of approximation (RMSEA; <0.05 indicates good fit; Hu & Bentler, 1998). Models were estimated using the maximum likelihood method with robust standard errors, and cases with missing data on 5-HTTLPR (n = 5) were kept in the final analysis and were handled with the full information maximum likelihood (Allison, 2009).

Table 2. Bivariate Correlations (n = 117).

	1	2	3	4	5	6	7	8	9	10	11
1. cortisol Day1 waking (µg/dL)	1.00										
2. cortisol Day1 30 min post-waking (µg/dL)	0.39**	1.00									
3. cortisol Day2 waking (µg/dL)	0.41**	0.40**	1.00								
4. cortisol Day2 30 min post-waking (µg/dL)	0.43**	0.42**	0.36**	1.00							
5. cortisol Day3 waking (µg/dL)	0.26**	0.29**	0.50**	0.37**	1.00						
6. cortisol Day3 30 min post-waking (µg/dL)	0.20	0.56**	0.41**	0.54**	0.37**	1.00					
7. Latent trait cortisol a	0.61***	0.63***	0.82***	0.77***	0.70***	0.62***	1.00				
8. Early adversity	−0.14	−0.09	−0.27**	−0.15	−0.07	−0.14	−0.26**	1.00			
9. 5-HTTLPR (# of short alleles)	0.00	−0.07	−0.12	−0.08	0.02	−0.02	−0.08	0.07	1.00		
10. Pubertal status	0.00	0.06	−0.13	−0.01	0.18	0.20	−0.003	.20*	0.07	1.00	
11. Age	0.05	0.09	−0.09	0.01	0.15	−0.01	0.01	−0.03	−0.05	0.41**	1.00
Mean	0.25	0.38	0.24	0.35	0.27	0.33	0	4.12	0.87	2.69	12.39
SD	0.16	0.19	0.15	0.19	0.16	0.17	0.08	2.16	0.73	0.59	0.77

Note. a. Latent trait cortisol was a regression-based factor score from Mplus for purpose of descriptive statistics.
***p < 0.001.
**p < 0.01.
*p < 0.05.

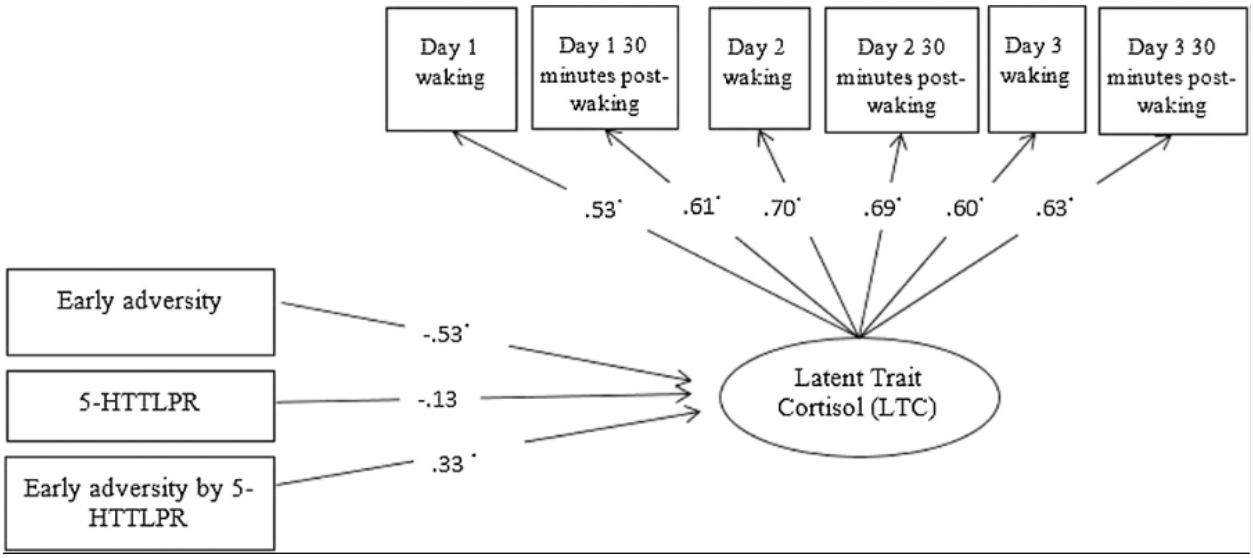

Fig. 1. Interaction of early adversity and 5-HTTLPR on latent trait cortisol (LTC). Standardized factor loadings and coefficients are presented.

3. Results

3.1. Latent trait cortisol (LTC) measurement model and descriptive statistics

The waking and 30 min post-waking samples were used to construct the LTC (see Stroud et al., 2016a). The measurement model of LTC showed a favorable solution, $\chi 2$ (9) = 18.14 (p = 0.04), CFI = 0.92, RMSEA = 0.09 (p = 0.12 for test of RMSEA < = 0.05).

3.2. Early adversity, 5-HTTLPR genotype, and latent trait cortisol (LTC) level

As shown in Table 3, all standardized factor loadings were significant (ps < 0.001) and above 0.5, suggesting that the waking and the 30 min post-waking cortisol were reliable measures for LTC level. The proportion of variance accounted for by the LTC for each sample ranged from 28% to 48% (see Fig. 1). The main effect of early adversity was significant (p = 0.003) but not 5-HTTLPR (p = 0.24). There was a significant interaction between early adversity and 5-HTTLPR genotype on LTC level (p = 0.03). Simple slope analyses showed that, counter to hypotheses, among either L/L or S/L genotypes, early adversity was significantly negatively associated with LTC level, but early adversity and LTC level were not significantly related among S/S homozygotes, who had a relatively lower LTC level (despite no significant main effect of 5-HTTLPR genotype on LTC level). At the low level of early adversity, the number of S alleles was negatively associated with LTC level at a marginally significant level (p = 0.053), but at the high level of early adversity, there was no significant association between 5-HTTLPR and LTC level (p = 0.54). Fig. 2 illustrates the simple effects of each genotype for the significant interaction. The model together explained 15% of the total variance of LTC.

Table 3. Model estimates for moderation model (n = 117).

	β	SE	*p*
Measurement model for LTC			
cortisol Day1 waking	0.53	0.09	<0.001
cortisol Day1 30 minutes post-waking	0.61	0.09	<0.001
cortisol Day2 waking	0.70	0.10	<0.001
cortisol Day2 30 minutes post-waking	0.69	0.09	<0.001
cortisol Day3 waking	0.60	0.09	<0.001
cortisol Day3 30 minutes post-waking	0.63	0.0	<0.001
Path to latent trait cortisol			
Early adversity	−0.50	0.17	0.003
5-HTTLPR (# of S alleles)	−0.13	0.11	0.237
Early adversity x 5-HTTLPR	0.33	0.16	0.033

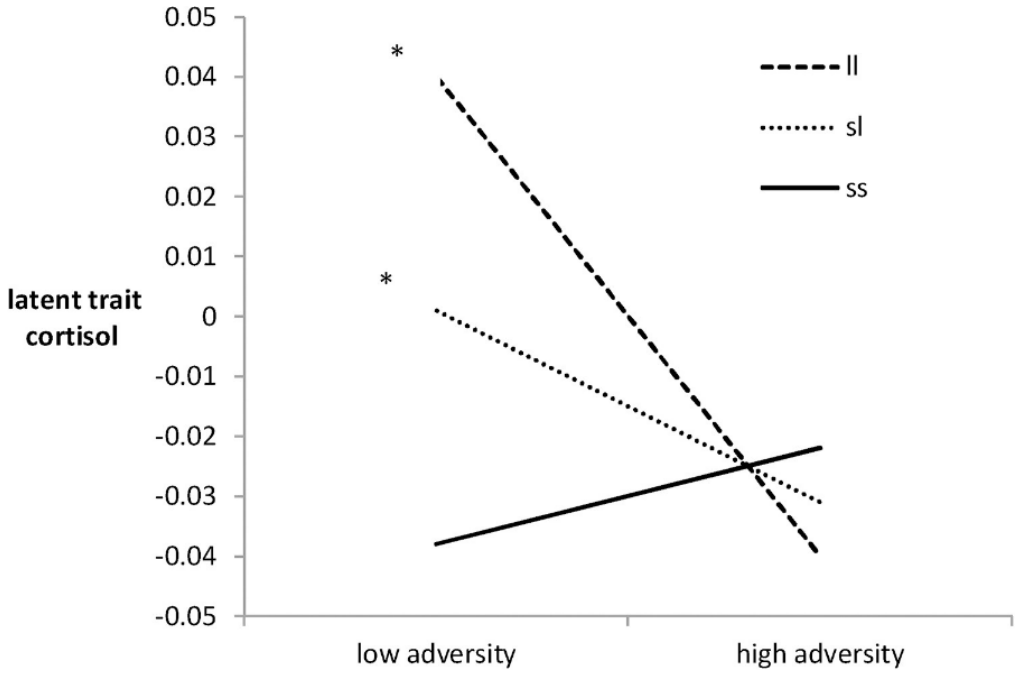

Fig. 2. Simple slope effect of early adversity on latent trait cortisol by 5-HTTLPR genotype.
Notes. High and low early adversity was defined as 1 SD above and below the mean. Latent trait cortisol was regression-based factor score output from Mplus. Asterisk indicates that the slope is significant at an alpha level of 0.05.

4. Discussion

The current study investigated whether the association between early adversity and individual differences in HPA-axis activity (indexed by LTC level) was moderated by 5-HTTLPR genotype. Consistent with predictions, 5-HTTLPR genotype moderated the association between early adversity and LTC level. However, the pattern of findings contradicted our a priori hypotheses: Among individuals with at least one L allele, greater early adversity was associated with lower

levels of LTC. By contrast, among individuals homozygous for S alleles, early adversity was not associated with LTC.

The present findings suggest that early adversity contributes to the individual difference in HPA-axis activity among L-carriers, but not among S-homozygotes. We hypothesized that the negative association between early adversity and LTC level would be most pronounced among S-carriers because S alleles are postulated to convey stress sensitivity in the development of depression, and one previous study (the only one to our knowledge) showed that S/S young adults with more lifetime stressful events showed the largest cortisol reactivity to a laboratory-based stressor (Alexander et al., 2009). Though unexpected, the present finding is consistent with some prior work. For instance, Mueller et al. (2011) showed that among young adults (aged 19–31), but not children (aged 8–11 years old) and older adults (aged 54–68), with L/L genotype, early adversity (i.e., stressful life events in the first five years of life) was negatively associated with cortisol reactivity to a laboratory-based stressor. Further, this finding occurred in the context of the main effect of genotype in all three groups, in which L/L individuals had greater cortisol reactivity. Mueller et al.'s findings suggest that relationship between early adversity, 5-HTTLPR genotype, and HPA axis activity may vary by age, but their study did not explicitly test whether the 5-HTTLPR Genotype × Early Adversity effect significantly differed across age groups. Furthermore, in a more recent study of adolescents (aged 9–17), results revealed that among youth with the L/L genotype, higher cumulative risk scores were related to lower levels of total secretion of diurnal cortisol indexed by area under the curve (Willner et al., 2014). Together with the present findings, this suggests that higher stress (early adversity or recent stress) was linked to hypoactivity of HPA-axis functioning among L-carriers.

Although controversial, meta-analytic evidence indicates that the S allele of 5-HTTLPR confers increased the risk for depression and related outcomes in the face of early adversity (Caspi et al., 2003, Karg et al., 2011, Sharpley et al., 2014). However, it is less clear whether the S allele of 5-HTTLPR confers increased the risk for altered HPA-axis functioning via a main effect or an interaction with early adversity. Research has suggested that the S allele is associated with heightened cortisol reactivity (Miller et al., 2013) and with increased amygdala reactivity to threatening and fearful stimuli (Canli et al., 2006, Rao et al., 2007, Viviani et al., 2010), but the S allele typically has not been found to be associated with baseline levels of HPA function (for a review, see Caspi et al., 2010).

It is possible that 5-HTTLPR interacts with early adversity to affect the pathways to HPA-axis functioning, and psychopathology in different ways. S-homozygotes, with their heightened amygdala reactivity to threat-related stimuli and enhanced cortisol reactivity to stressors (Miller et al., 2013, Viviani et al., 2010) may be vulnerable to depression in the face of recent stress as well as early adversity (e.g., Karg et al., 2011), and this interplay of genes and environment may not necessarily directly affect other intermediate endophenotypes, such as HPA-axis functioning. In line with this, others have posited that psychological vulnerability conveyed by the S allele may manifest in the heightened reactivity to stress that is not accompanied by subsequent downregulation of basal HPA-axis indicators (Willner et al., 2014). In partial support of this hypothesis, one study indicated that S-homozygotes had larger (i.e., greater peak) cortisol reactivity to laboratory-based stressors, which was stable over 18 months (Hankin, Badanes, Smolen, & Young, 2015). Thus, it is possible that S-homozygotes, with enhanced reactivity to fearful/threatening stimuli, were overall prone to depression, and the allostatic load framework may not be applicable in this case. Another possible explanation could be that for S/S individuals, because of their increased stress sensitivity (Caspi et al., 2010, Karg et al., 2011), only a small

degree of early adversity may be needed to push their LTC levels to be much lower, and as a result their LTC levels appear lower than L-carriers.

In contrast, the L allele of 5-HTTLPR, which typically is not regarded as a conferring risk for depression in the face of stress, may facilitate malleability in response to stress, by altering HPA-axis functioning. This is consistent with the findings of two previous studies which showed that L-carriers appear to show alterations in HPA-axis functioning, as indexed by total daily cortisol secretion (Willner et al., 2014) and cortisol reactivity in response to laboratory-based stressors (Mueller et al., 2011). The present study builds upon these findings by demonstrating that the accumulation of early adverse experiences in girls' everyday lives contributed to individual differences in HPA-axis activity among L-carriers. Although speculative, it may be that L-carriers were more likely to downregulate their basal HPA functioning to adapt to early adversity. This downregulation of HPA-axis may interfere with other physiological systems, such as the immune system, to increase the risk for physical and mental health problems (McEwen, 1998). Consistent with the allostatic load framework, the current study showed that L-carriers were most susceptible to the influences of early adversity in their HPA axis functioning. Future research should incorporate the mental health outcomes and examine whether this pathway of cumulative adversity to lower level of LTC among L-carriers were, in fact, lead to mental health problems (i.e., a conditional indirect effect on mental health problems). Given that previous research has only revealed the detrimental effect of the coupling between S allele of 5-HTTLPR and life stressful events on depression (Caspi et al., 2010, Karg et al., 2011), it is possible that the downregulation of the HPA-axis among L-carriers may serve as a protective mechanism from the development of depression and other adverse health outcomes. This hypothesis warrants exploration in future work.

The findings of the current study should be interpreted in light of several limitations. First, although we established the temporal precedence of early adversity to LTC level, the measure of early adversity was a retrospective assessment of early adverse experiences occurring over from birth through one year before the interview, and was collected on average less than a week before measurement of the LTC level. Thus, although both mothers and adolescents were interviewed about adolescents' level of early adversity, it is possible that their recollection of early adversity and LTC level were both affected by other potential confounding variables, such as current mood state or history of psychopathology. Future studies should collect measures of early adversity and LTC level at multiple different time points to establish temporal order, and rule out third variables. Second, the sample was small and comprised of mostly White adolescents drawn from the community. Thus, results may not generalize to adolescents from diverse racial and ethnic backgrounds, or to those experiencing a more severe level of early adversity. Third, only early adolescent girls were included. Given that there are gender differences in the diurnal cortisol rhythm when individuals enter puberty (Shirtcliff et al., 2012), future work is needed to evaluate whether the findings generalize to early adolescent boys as well as other developmental stages.

These limitations notwithstanding, the current study is the first to document an association between 5-HTTLPR, early adversity, and intrinsic individual differences in HPA axis functioning (modeled as LTC). Findings built upon the existing literature and highlighted the important role of the L allele of 5-HTTLPR in the allostatic load framework. Future longitudinal research is needed to elucidate the pathway from early adversity to mental health problems involving HPA axis functioning and/or the 5-HTTLPR genotype.

Disclosure statement

In the interest of full disclosure, DAG is Founder and Chief Scientific and Strategy Advisor at Salimetrics LLC and SalivaBio LLC and these relationships are managed by the policies of the committees on conflict of interest at Johns Hopkins University School of Medicine and the University of California, Irvine. No other author has conflicts to disclose.

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