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Stress reactivity in healthy child offspring of parents with anxiety disorders

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ABSTRACT

Several studies suggest that anxiety disorders (AD) involve dysregulation of the autonomic nervous system (ANS) and hypothalamic-pituitary (HPA) axis. However, it is unknown if alterations in these biological systems are premorbid markers of AD risk or a state-dependent feature of anxiety. This study examined ANS and HPA-axis response to a laboratory stressor in healthy child offspring of parents with (n = 55) and without (n = 98) a history of AD. High frequency heart rate variability (HF-HRV) was assessed during sitting and standing baseline conditions and during a speech task where participants remained standing. Salivary cortisol was measured at baseline and at 15, 30, 45 and 60 min post-speech. Subjective anxiety was assessed with a visual analogue scale. Children of parents with AD displayed reduced HRV and a blunted cortisol response to the speech task compared to children of non-anxious parents. No risk group effect was found for anxiety ratings. These preliminary data suggest that healthy children of anxious parents exhibit altered stress reactivity to an acute laboratory stressor. Further research is needed to confirm findings and identify mechanisms that may account for altered self-regulation processes to a stressor in children at familial risk for AD.

1. Introduction

Heart rate variability (HRV) reflects the ongoing modulation of the heart by the sympathetic and parasympathetic branches of the autonomic nervous system (ANS) (Kleiger et al., 2005). It is widely recognized as a physiological marker for flexible and adaptive responding to environmental demands (Thayer and Lane, 2000). In particular, high resting HRV, which reflects more flexibility in the interval between heart beats and dominance of parasympathetic over sympathetic influences, has been linked with resilience and optimal physiological, emotional, cognitive and behavioral regulation in response to environmental demands (Koenig et al., 2016), whereas reduced resting HRV is associated with poorer self-regulatory processes (Koenig et al., 2016; Thayer and Lane, 2000). It has been proposed that parasympathetically mediated HRV provides an index of the functional integrity of neural networks implicated in self-regulatory processes such as emotion regulation (Porges, 2007; Thayer and Lane, 2000). This framework is supported, in part, by neuroimaging data showing that HRV is tied to functioning of prefrontal-subcortical brain circuits (Thayer et al., 2012).

Reduced HRV is considered a transdiagnostic psychophysiological marker of psychopathology (Beauchaine and Thyer, 2015) and has been observed in several psychiatric conditions including anxiety disorders (AD) (Gorman and Sloan, 2000; Licht et al., 2009; Pittig et al., 2013). Indeed, many of the physical symptoms that are characteristic of pathological anxiety suggest the involvement of the ANS (Klein et al., 1995); these include palpitations, tachycardia, trembling, sweating and tachypnea. While studies of HRV in AD have yielded mixed findings (Chalmers et al., 2016), a meta-analysis of 36 studies involving 2086 patients indicates that HRV is reduced in AD versus healthy controls, with small-to-moderate effect sizes (Chalmers et al., 2014). A few studies of HRV have also been conducted in pediatric AD, with results generally mirroring those of adults (Paniccia et al., 2017). For example, several studies have found that HRV at rest and during a laboratory stress task is reduced in children with diverse AD compared to control participants (Blom et al., 2010; Dieleman et al., 2015; Kossowsky et al., 2012; Monk et al., 2001; Nikolić et al., 2018; Rozenman et al., 2017; Schmitz et al., 2011; Sharma et al., 2011).

Another physiological system that has been implicated in the pathogenesis of AD is the hypothalamic-pituitary-adrenal (HPA) axis

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(Faravelli et al., 2012). The HPA axis is a major neuroendocrine system that orchestrates stress responsiveness (Sapolsky et al., 2000). Life stress alters HPA axis function and is also a major risk factor for the development of AD (Faravelli et al., 2012). One index of the HPA axis is adrenal secretion of cortisol (Hellhammer et al., 2009). Investigation of basal and stressor-induced cortisol patterns have generally found that cortisol levels are altered in adults with AD versus psychiatrically healthy controls, although no discernable differences have also been reported (Bandelow et al., 2017; Faravelli et al., 2012). Only a few studies have evaluated cortisol patterns in pediatric AD and results are also variable. For example, in the context of an acute stressor, increased (Coplan et al., 2002), blunted (Funke et al., 2017), and normal (Kramer et al., 2012; Martel et al., 1999) cortisol secretion have been reported. Studies of resting state cortisol levels in anxious children also report mixed findings (Dieleman et al., 2015; Feder et al., 2004; Funke et al., 2017). Inconsistent findings in children may be due to variation in salivary collection and measurement protocols, sample characteristics, stress and trauma exposure, adjustment for confounders, and other factors that may influence the HPA axis (Evans et al., 2013; Keil et al., 2012).

While many studies implicate dysregulation of the ANS and the HPA-axis in the pathogenesis of AD, it is unknown whether altered functioning in these biological systems is a predictor of AD risk or a state-dependent feature of anxiety. Given the familial nature of AD (Hettema et al., 2001) and possible intergenerational transmission of biological risk markers (Bilodeau et al., 2015; Zwanzger et al., 2012), a few studies have examined if HRV and cortisol secretion are altered in unaffected offspring with familial AD. In a preliminary report of children with a parent with panic disorder (Srinivasan et al., 2002), spectral analysis of HRV during supine and standing positions were similar in high- and low-risk children. However, high-risk children exhibited significantly decreased chaos of heart rate time series in supine posture, suggesting impaired cardiac autonomic flexibility due to decreased cardiac vagal activity. Evaluation of the HPA-axis system has revealed that unaffected offspring with parental AD (Vreeburg et al., 2010) and PTSD (Liu et al., 2016) show a higher cortisol awakening curve than control participants, and unaffected offspring with parental PTSD exhibit a blunted cortisol reactivity to acute stress (Danielson et al., 2015). Infants of anxious mothers demonstrate higher basal cortisol levels than infants of non-anxious mothers (Warren et al., 2003), suggesting HPA axis abnormality may be an early appearing transmitted biological marker for anxiety risk.

The aim of the present study was to assess HRV and cortisol response to a social stress test in healthy child offspring of parents with and without an AD. We used power spectrum analysis for studying HRV and salivary cortisol as an index of HPA axis function. Based on research suggesting that AD and risk for anxiety may be linked with reduced parasympathetic activity, we predicted that offspring of parents with AD would demonstrate reduced HRV during the social stress task compared to those with no parental psychopathology. We also predicted that offspring of parents with AD would demonstrate an altered pattern of cortisol secretion (blunted or elevated) to the stress test compared to the control children.

2. Methods

2.1. Participants

Participants were children between the ages of 7 and 18 years of age with a biological parent with a history of DSM-IV AD (in the current sample only panic disorder with or without agoraphobia, social anxiety disorder or generalized anxiety disorder) (“high risk” children) and control participants with no parental history of psychiatric illness (“low risk” children). Families were recruited via advertisements placed in local newspapers, the Internet, and flyers posted on community and university bulletin boards. Separate recruitment flyers were used to recruit children with and without parental AD. Children in both risk groups were eligible to participate if they had no current or past history of any threshold or subthreshold psychiatric disorder, clinically significant and/or unstable medical conditions, or regularly used medications with peripheral and central nervous system effects. The study was approved by the institutional review board and written informed consent was obtained from the child's legal guardian as well as the child's assent. Participants 16 years and over provided their own consent. Families were financially compensated for their participation in the study.

2.2. Assessment

The parent who contacted the research unit underwent an initial telephone pre-screen with a research assistant who explained the purpose of the study and who obtained information about the psychiatric status of both biological parents and history of psychiatric symptoms, medical illness, and medication use in their offspring. If the family was potentially eligible, a second telephone screen was scheduled to formally evaluate the diagnostic status of the child's parents. Current or lifetime diagnosis of a primary AD in the parent(s) of high risk children and the absence of psychopathology in both parents of low risk children were confirmed with a DSM-IV based structured clinical interview (SCID; First et al., 1995). SCID interviews with affected parents were conducted by a licensed psychologist and trained research assistants carried out the interviews with unaffected parents under the supervision of the psychologist.

Once parental diagnostic status was confirmed, their children were invited to the research unit at the University of Ottawa for a face-to-face interview with the child version of the Anxiety Disorders Interview Schedule (Silverman and Albano, 1996) to confirm the absence of psychopathology. Eligible children completed self-report questionnaires and their body mass index (BMI) was calculated from their height and weight measured during the assessment visit. Pubertal status (pre-pubertal, early-to-mid puberty, and late-to-post puberty) was determined using Tanner's scale (Tanner, 1962).

2.3. Social stress test

Children were scheduled for the social stress test within two weeks of the first visit and were asked to not take any prescribed or over-the-counter medications or food that might influence physiological measures 24 h prior to the procedure. Pubertal girls completed the stress test during the follicular phase of the menstrual cycle. The stress test was scheduled between 2 and 3 p.m. and parents were not present during the procedure. The stressor consisted of an impromptu speech modified from the Trier Social Stress Test (Kirschbaum et al., 1993), which is a well-established protocol to evaluate stress reactivity. Upon arrival at the laboratory, five electrodes were placed on the child's chest to simulate leads V1 to V5 and a Seers MC (GE Medical Systems) ambulatory ECG monitor was used to measure HRV. A respiration rate belt (PASPORT Respiration Rate Sensor PS-2133) was attached to the chest area to measure respiration rate, which is known to affect HRV. Children were seated in a comfortable chair and baseline anxiety ratings (T1) and salivary cortisol samples (T1) were collected. HRV and respiration were monitored for a 20-min period during the resting sitting position (T1). Children were then asked to stand and HRV and respiration were monitored for an additional 10-min period (T2). After the standing resting condition, children were told that they would be required to give a 10-min speech while standing on three of four topics (school, their favorite books or movies, friends, and sports or hobbies they enjoy) and that they had 2 min to prepare the speech. Two research assistants observed the speech task and took notes. Anxiety ratings were completed immediately prior to the speech (T2) to assess anticipatory anxiety. HRV and respiration rate were continuously monitored during the 10 min speech (T3). Following the 10 min speech

the ECG monitor was stopped. Anxiety ratings were completed immediately following the speech (T3) and salivary cortisol samples were collected at 15 (T2), 30 (T3), 45 (T4) and 60 (T5) minutes post-speech.

2.4. Measures

2.4.1. Heart rate variability

We used power spectrum analysis for HRV. This method maps HRV graphically along a frequency spectrum according to the relative share of total variance contributed by different R-R intervals (Akselrod et al., 1981; Kleiger et al., 2005). Distinct frequency bands that contribute to total HRV include the high frequency (HF) band (0.15–0.40 Hz) and the low frequency (LF) band (0.04–0.15 Hz). HF power is widely accepted as a reliable measure of parasympathetic ANS activity (Thayer et al., 2012; Laborde et al., 2017). Although some researchers have used LF power as a marker of purely sympathetic activity and the LF/HF ratio as a measure of sympathovagal balance, these metrics are controversial as there is no clear evidence that LF power is a reliable index of cardiac sympathetic activity (Goldstein et al., 2011; Shaffer et al., 2014). Therefore, we only report findings of HF-HRV.

Data from the ambulatory ECG monitor were sent to the Arrhythmia Monitoring Centre of the University of Ottawa Heart Institute for analysis. The MARSSPC system (GE Marquette) was used for spectral analysis of all frequency components of HRV. The data were evaluated using a MARS 8000 workstation version 4.0A (GE Marquette) by a technician who was unaware of the subject's risk status. Maximum, minimum and mean heart rates were determined along with total arrhythmia counts. Following thorough editing, spectral analysis was performed on a 20-min segment of the resting sitting recording, a 10-min segment of the resting standing recording, and a 10-min segment of the speech task recording. Power was calculated for the HF, LF, and VLF bands for each subject. HF-HRV is expressed in normalized values (nu) (calculated as HF/LF + HF; Burr, 2007) and absolute values (milliseconds squared (ms^2)). Normalization minimizes the effect of changes of total power on LF and HF and produces values that are easily comparable across different studies (Burr, 2007).

2.4.2. Salivary cortisol

Salivary cortisol collection is non-invasive and easy to procure in pediatric research and is robustly associated with free cortisol in blood and serum (Hanrahan et al., 2006). Saliva samples for cortisol extraction were collected via oral swabs placed in the mouth for three minutes. The swabs were stored in vials and kept at −20 °C, then centrifuged to remove particulate matter, and the supernatant liquid stored in Eppendorf tubes at −80 °C until analyses. A technician who was unaware of the child's risk status performed the analyses in duplicate using the protocol and enzyme-linked immunosorbent assay cortisol kits from Salimetrics, Inc.

2.4.3. Visual analogue scale of anxiety

A horizontal 100-mm visual analogue scale (VAS) was used to measure subjective anxiety, with anchors of “no anxiety at all” to “extremely anxious”. The VAS is a quick and reliable way to measure state anxiety in children and adolescents and it demonstrates good divergent and convergent validity (Hornblow and Kidson, 1976; Williams et al., 2010).

2.5. Statistical analyses

Data analysis was conducted using SPSS version 24. Differences between the risk groups on baseline characteristics were evaluated with t-tests and chi-square tests. Repeated measures of HRV, cortisol secretion and VAS anxiety ratings were analyzed using linear mixed effects regression models, with Risk Group (high versus low risk group), Time (T1, T2, T3 for HF-HRV and VAS anxiety ratings and T1 to T5 for cortisol levels), and Risk Group x Time interactions as fixed factors. Because age-specific bandwidths for HRV have not been sufficiently validated in younger children (Weiner and McGrath, 2017) we included age group (7–10, 11–14, 15–18 years) as a factor in the model to test for possible effects of different age group on HRV response to the stressor. Separate analysis of HRV was also conducted for the subgroup of adolescents (≥12 years of age). The models were estimated using Restricted Maximum Likelihood (REML), with an unstructured covariance matrix to account for correlations among the repeated measures over time. Family was included as a random effect in the models because some families enrolled more than one child. A significant Time by Risk Group interaction would suggest that changes in anxiety and physiological measures over time were different between the risk groups; significant interactions were further analyzed with pairwise least square mean comparisons. For HF-HRV, the pre-specified contrasts of interest were between T1 and T2 to assess differences in response to postural change and between T2 and T3 to assess the effects of the speech task on HF-HRV. Contrasts beween T1 and T3 were not examined as any observed difference could be attributed to postural change rather than an effect of the stressor on HRV. For cortisol, the pre-specified contrasts of interest were the difference between baseline levels (T1) and levels at T2, T3, T4 and T5. Total cortisol output was also examined using the area under the curve with respect to ground (AUCg) formula described by Pruessner et al., (2003).

Because a number of factors are known to influence HRV (Gąsior et al., 2015; Jarrin et al., 2015; Vazquez et al., 2016) pre-specified covariates of gender, BMI and pubertal status were included in the mixed models (gender and BMI were covariates in analysis of the adolescent subsample). Respiration rate (averaged across the 3 time point) was also included as a pre-specified covariate in the analysis of HRV as the action of speaking influences HRV through acute changes in respiration patterns (Brugnera et al., 2018). For cortisol reactivity, we included age, gender, BMI and pubertal status as pre-specified covariates based on findings that these variables can influence cortisol secretion (Colich et al., 2015; Evans et al., 2013; Hagan et al., 2011; Kudielka et al., 2004). We also analyzed the physiological data without covariates and results of models with and without covariate adjustment are presented.

HF-HRV and cortisol values were skewed and were logarithmically transformed to improve normality. For HF-HRV, transformed and untransformed variables yielded similar results, therefore we report results of untransformed data for ease of interpretation (Tabachnick and Fidell, 2007). We did not impute missing values because our analytical strategy using REML allowed the estimation of reliable parameters without the need for imputation of the data under an assumption of missing at random (MAR) (Little and Rubin, 2002).

All statistical tests were considered significant at a two-tailed p-value of 0.05. A Bonferroni adjustment was applied for the pairwise comparisons.

3. Results

3.1. Participant characteristics

One hundred and sixty-eight children completed the face-to-face assessment visit. Of these, 14 were excluded (6 high risk; 8 low risk) because they met threshold or subthreshold criteria for a current or past psychiatric disorder and four (3 high risk, 1 low risk) withdrew from the study after the baseline visit. One hundred and fifty-three children completed the speech task including 55 children with a biological parent with a primary AD and 98 with no parental history of psychiatric illness. Table 1 presents baseline and demographic characteristics of the sample. With the exception of pubertal status, which differed across the risk groups (*chi-square* = 6.86, *df* = 2, p = .03), no differences in demographic or baseline characteristics were found.

Table 1
Characteristics of high and low risk children.

Variable	High risk children	Low risk children
Age (mean ± SD, years)	13.05 ± 2.9	12.14 ± 3.3
Gender (% Female)	49.1%	48.0%
Pubertal status (%)		
Pre-pubertal	15.1%	34.7%
Early-mid puberty	30.2%	20.0%
Late-post puberty	54.7%	45.3%
Body Mass Index (mean ± SD)	18.85 ± 3.3	19.17 ± 4.0
Respiration Rate (mean ± SD)	52.36 ± 5.9	52.98 ± 6.9

3.2. Heart rate variability

Five children (2 high-risk, 3 low-risk) had no HRV data, therefore analyses of this measure was based on 148 children who had at least one measurement of HRV.

Fig. 1a and b display the least squared means and standard errors for normalized and absolute HF-HRV, respectively. The groups did not differ on baseline (T1) HF-HRV. A significant Time by Risk Group interaction was found for normalized HF-HRV ($F = 4.65$, $df = 2, 141.15$, $p = .011$ with covariate adjustment and $F = 4.66$, $df = 2, 141.10$, $p = .011$ without covariate adjustment). There was no significant Time x Age Group interaction ($F = 1.71$, $df = 4, 141.17$, $p = .15$ with covariate adjustment and $F = 1.70$, $df = 4, 141.12$, $p = .15$ without covariate adjustment) or Time x Age Group x Risk Group interaction ($F = 2.12$, $df = 6, 134.05$, $p = .11$ with covariate adjustment and $F = 2.18$, $df = 1, 136.15$, $p = .13$ without covariate adjustment) (least square means (SEM) for normalized HF-HRV by age and risk group appear in the supplementary Table). For the significant Time x Risk Group interaction, pre-specified contrasts between T1 and T2 revealed that normalized HF-HRV decreased as a result of postural change, with the change being similar for high-risk (least square mean change = −0.12 [95% *CI*, −0.15 to −0.09], $p < .001$) and low-risk (least square mean change = −0.12 [95% *CI*, −0.14 to −0.10], $p < .001$) children. In contrast, high-risk children showed no significant change in normalized HF-HRV from T2 to T3 (least square mean change = 0.001 [95% *CI*, 0.04 to −0.04], $p = .95$), whereas low-risk children showed a significant increase in this HRV parameter during the speech (least square mean change = 0.06 [95% *CI*, 0.03 to 0.09], $p < .001$). Between group comparisons at T3 revealed a significant group difference, with normalized HF-HRV values being lower in the high-risk group (least square mean difference = −0.06 [95% *CI*, −0.10 to −0.02], $p < .003$).

Normalized HF-HRV results for the adolescent subsample mirrored those of the total sample (Time x Risk Group $F = 5.74$, $df = 2, 89.98$, $p = .005$ with covariate adjustment and $F = 5.74$, $df = 2, 90$, $p = .005$ without covariate adjustment); pre-specified contrasts between T1 and T2 revealed that normalized HF-HRV decreased as a result of postural change, with the change being similar for high-risk (least square mean change = −0.12 [95% *CI*, −0.15 to −0.08], $p < .001$) and low-risk (least square mean change = −0.13 [95% *CI*, −0.16 to −0.10], $p < .001$) adolescents. In contrast, high-risk adolescents showed no significant change in normalized HF-HRV from T2 to T3 (least square mean change = −0.01 [95% *CI*, −0.06 to 0.04], $p = 1.00$), whereas low-risk adolescents showed a significant increase in this HRV parameter during the speech (least square mean change = 0.07 [95% *CI*, 0.03 to 0.11], $p < .001$). Between group comparisons at T3 revealed a significant group difference, with normalized HF-HRV values being lower in the high-risk group (least square mean difference = −0.06 [95% *CI*, −0.11 to −0.01], $p < .015$).

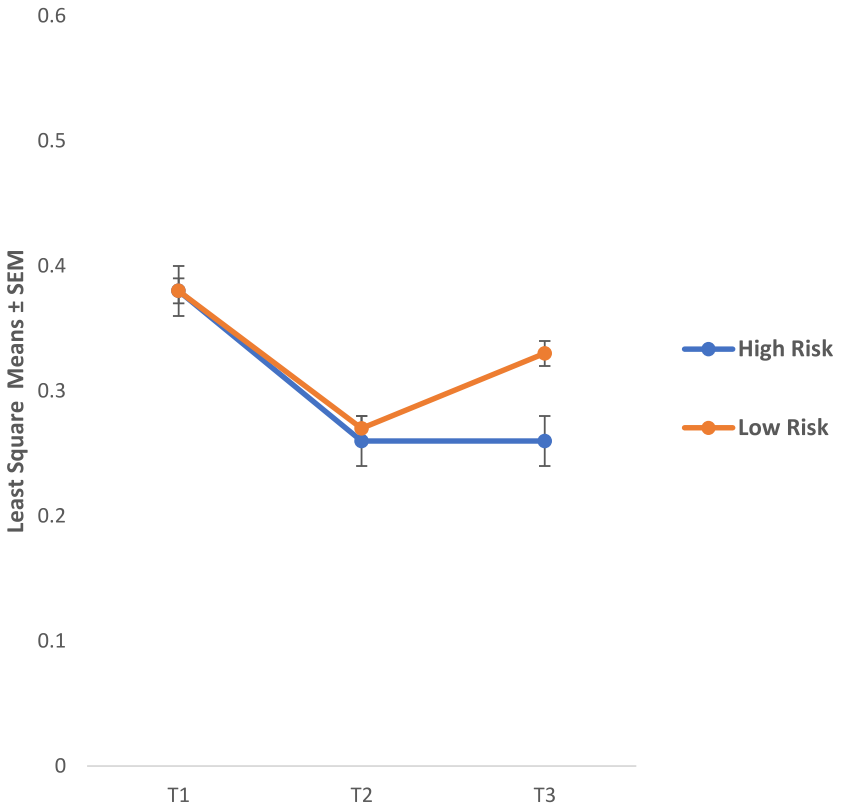

Fig. 1. (**a**). Least means squares for normalized High Frequency Power *Note:* T1 = sitting baseline, T2 = standing baseline, T3 = speech task. The Time by Risk Group interaction was statistically significant ($F = 4.65$, $df = 2, 141.15$, $p = .011$), after adjusting for the following covariates: gender, pubertal status, BMI and respiration rate.(**b**). Least means squares for normalized High Frequency Power (ms^2) *Note:* T1 = sitting baseline, T2 = standing baseline, T3 = speech task. The Time by Risk Group interaction was not statistically significant ($F = 1.82$, $df = 2, 141.38$, $p = .17$), after adjusting for the following covariates: gender, pubertal status, BMI and respiration rate.

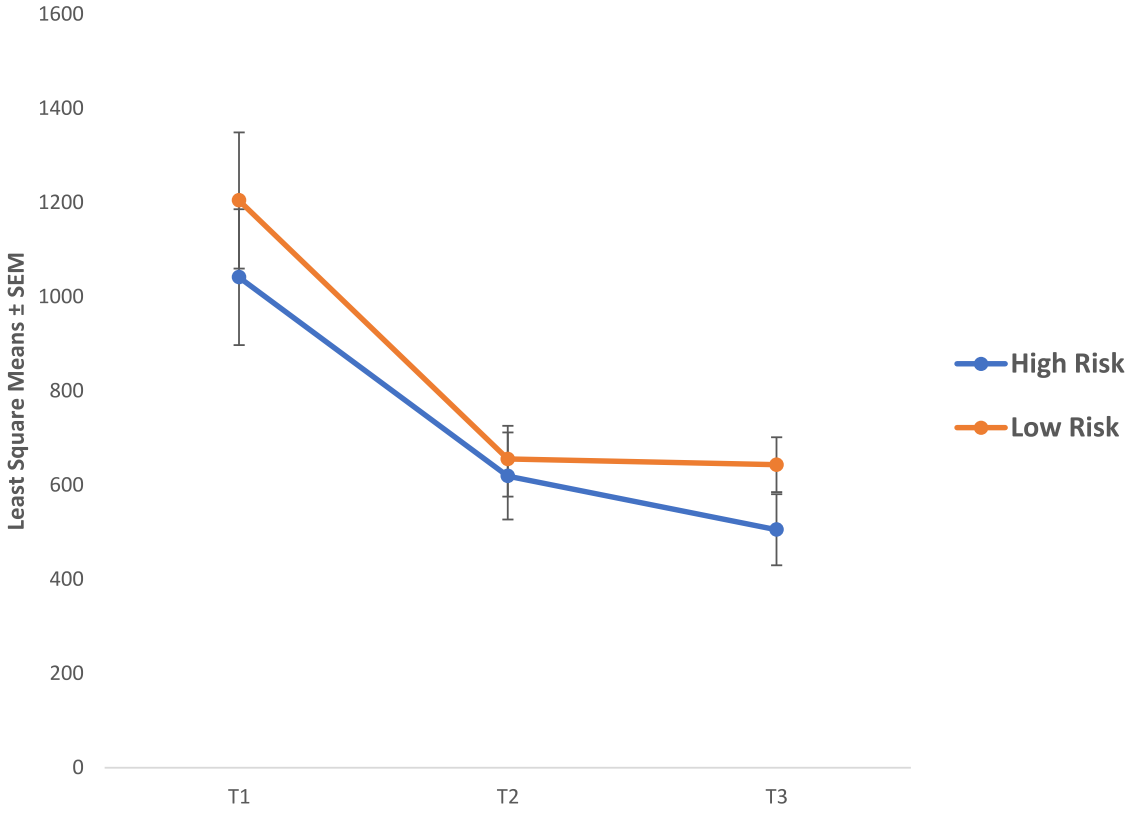

Fig. 1. (*continued*)

The Time x Risk Group interaction was not significant for absolute HF-HRV ($F = 1.82$, $df = 2, 141.38$, $p = .17$ with covariate adjustment and $F = 1.82$, $df = 2, 141.38$, $p = .17$ without covariate adjustment), indicating that the trajectory of change for this parameter did not differ between the risk groups. None of the interactions involving Age Group were statistically significant (Time x Age Group $F = 0.90$, $df = 4, 141.42$, $p = .47$ and Time x Age Group x Risk Group $F = 0.67$, $df = 6, 134.45$, $p = .68$ with covariate adjustments, and Time x Age Group $F = 0.90$, $df = 4, 141.43$, $p = .47$ and Time x Age Group x Risk Group $F = 0.70$ $df = 6, 136.15$, $p = .65$ without covariate adjustment) (least square means (SEM) for absolute HF-HRV by age and risk groups appear in the supplementary Table). Findings for the subgroup of adolecents paralleled those of the total sample.

3.3. Salivary cortisol

Ten children had no cortisol data (4 high risk, 6 low risk), therefore analysis was based on 143 children who had at least one measurement of cortisol. Missing cortisol data were primarily due to insufficient sampling volume. Fig. 2 displays the least square mean (± SEM) cortisol concentration values across time for high and low risk children. Baseline cortisol levels did not differ between the groups. Analysis of log transformed values revealed that the Time by Risk Group interaction was not statistically significant ($F = 1.99$, $df = 4, 130.95$, $p = .10$ with covariate adjustment and $F = 1.85$, $df = 4, 131.38$, $p = .12$ without covariate adjustment). Nevertheless, it is notable that cortisol levels did not significantly change over time from baseline in high-risk children (p values range from 0.33 to 0.73), whereas low-risk children displayed increased levels from baseline at T2 (least square mean change = 0.04 [95% *CI*, 0.02 to 0.06], $p < .001$) and T3 (least square mean change = 0.02 [95% *CI*, 0.04 to.10], $p = .001$) post-speech, with levels returning to baseline levels thereafter. Analysis of AUCg cortisol revealed no significant difference between the risk groups ($F = 0.48$, $df = 1, 82.24$, $p = .49$); the least square mean AUCg values were 0.32 [95% *CI*, 0.11 to 0.55] for high-risk children and 0.42 [95% *CI*, 0.25 to 0.50] for low-risk children.

3.4. VAS anxiety ratings

Table 2 displays the least square means for VAS anxiety ratings obtained at baseline, prior to beginning the speech task, and at the end of the 10-min speech task. The Time by Risk Group interaction was not significant ($F = 1.03$, $df = 2, 146$, $p = .36$), indicating that the trajectory of change in subjective anxiety was similar for high- and low-risk children. Correlation analysis for the total sample revealed that baseline anxiety ratings were not significantly associated with baseline cortisol ($r = -0.05$, $p = .56$) or baseline sitting HF-HRV ($r = 0.07$, $p = .40$ for absolute values and $r = 0.03$, $p = .75$ for normalized values) and standing HF-HRV($r = 0.10$, $p = .23$ for absolute values and $r = 0.12$, $p = .17$ for normalized values). Anxiety ratings obtained immediately prior to the speech (i.e., anticipatory anxiety) and post-speech did not correlate significantly with cortisol AUCg ($r = -0.06$, $p = .51$ and $r = 0.03$, $p = .71$), cortisol levels at each post-speech time point ($r = -0.02$, $p = .86$ to $r = -0.12$, $p = .17$), absolute HF-HRV during the speech ($r = -0.03$, $p = .69$ and $r = 0.10$, $p = .21$) and normalized HF-HRV during the speech ($r = -0.001$, $p = .99$ and $r = 0.07$, $p = .38$). When high and low risk groups were analyzed separately, non-significant correlations between anxiety ratings and physiological measures were also found.

4. Discussion

This study determined if stress reactivity differed in healthy children of parents with a history of AD and those with no parental psychopathology. When autonomic activity was assessed, no risk group difference was found for baseline sitting HF-HRV and both groups displayed a similar change in this HRV parameter during postural change. The lack of difference with postural change concurs with

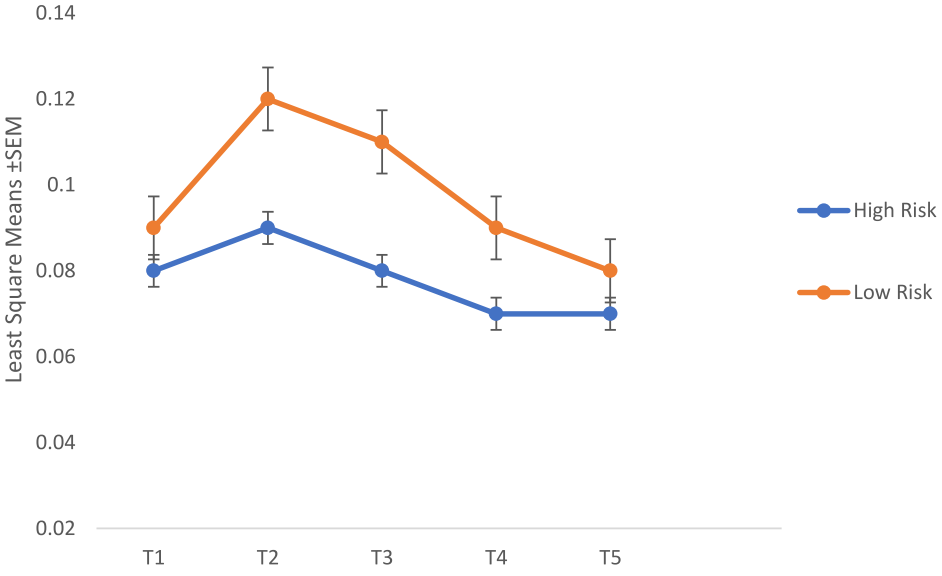

Fig. 2. Least square means for salivary cortisol. Values are log transformed. *Note:* T1 = baseline and T2 to T5 indicates +15, +30, +45 and +60 min post-speech, respectively. The Time by Group interaction approached significance ($F = 1.99$, $df = 4, 130.95$, $p = .10$), after adjusting for following covariates: age, gender, BMI and pubertal status.

Table 2
VAS anxiety ratings.

	High risk (LS mean, 95% CI)	Low risk (LS mean, 95% CI)	Between-group difference (LS mean, 95% CI)
Baseline (T1)	9.34 ± 1.8 (5.68, 12.90)	11.79 ± 1.4 (9.03, 14.55)	−2.47 (−7.06, 2.12)
Pre-speech (T2)	28.09 ± 3.4 (21.41, 34.78)	26.82 ± 2.5 (21.82, 31.83)	1.27 (−7.09, 9.63)
Post-speech (T3)	19.34 ± 3.0 (13.43, 24.26)	23.82 ± 2.2 (19.34, 28.26)	−4.48 (−11.88, 2.92)

VAS indicates Visual Analogue Scale; LS indicates least square.

findings of Srinivasan et al. (2002), who found that frequency domain indexes of HRV during supine and standing positions did not differentiate children with and without parental panic disorder, although they did report decreased parasympathetic tone in high risk children during supine position when non-linearity measures of HRV were used. Given the complexity of HRV, it is conceivable that our baseline results would differ if a non-linearity approach was used in addition to spectral analysis. Nevertheless, unlike the current study, Srinivasan et al.'s (2002) high-risk children included those with psychiatric conditions such as separation anxiety, which may have confounded findings. Overall, findings from the current study suggest that resting state HF-HR, a reliable index of adaptive functioning, is not altered in healthy high-risk children and that resting autonomic impairment observed in individuals with AD may be a consequence of pathological anxiety rather than a pre-existing risk factor.

In contrast to resting state HRV, we did find that normalized HF-HRV differed between the groups during the speech task. Low-risk children showed an increase in HF-HRV during the speech task, whereas high-risk children showed a blunted response. Although absolute HF power did not differ, values were numerically lower in the high-risk group during the speech task. The lower normalized HF-HRV values during the speech task in high-risk children suggests that they may have less flexibility in their cardiac autonomic response to an acute psychosocial stressor. Reduced HF-HRV during exposure to an acute stressor has also been described in children with AD (Kossowsky et al., 2012; Monk et al., 2001; Schmitz et al., 2011), and preliminary longitudinal data suggest that low vagal reactivity to stress may predict the development of anxiety symptoms in adolescents (Greaves-Lord et al., 2010). Reduced HRV in children with AD is also thought to represent an autonomic pattern associated with risk for future cardiac disease (Monk et al., 2001). Indeed, reduced HRV is a risk factor for cardiovascular morbidity and mortality and AD and cardiac disease are highly comorbid (Celano et al., 2016; Chalmers et al., 2014). Research suggests that treatment of AD can correct dysregulation of HRV (Cohen and Benjamin, 2006; Garakani et al., 2009) and it is conceivable that intervention strategies that improve autonomic cardiac reactivity to acute stress in unaffected high-risk children may reduce risk for future development of AD and possibly cardiac disease.

Examination of cortisol secretion revealed that high-risk children displayed a blunted cortisol response to the stressor, whereas low risk children showed an expected cortisol reactivity pattern. Also, mean AUCg values were lower in high risk children, although differences were not statistically significant. Alterations in HPA axis response to stress is a marker of risk for stress-related disorders including AD (Hankin et al., 2015). A blunted cortisol reactivity is thought to reflect down-regulation of the HPA axis due to excessive cortisol secretion associated with exposure to episodes of acute stress and chronic stress and a shift from hyper- to hyporesponsiveness of the HPA axis over time (Gunner and Vazquez, 2001; Heim et al., 2000). Although the factors that account for blunted cortisol activity in children remain unclear, some researchers suggest it may develop as an adaptation of the HPA axis to early-life adversity, especially childhood maltreatment (Bunea et al., 2017), adverse parenting (Kawai et al., 2017), exposure to interparental conflict (Davies et al., 2008) and parental response to children's negative emotions (Guo et al., 2017). Living with a parent with psychological distress can also be inherently stressful for children and may impact their stress physiology over time (Koch et al., 2010; Mahrer et al., 2014). Fetal exposure to maternal stress and anxiety is also hypothesized to influence the developing HPA axis and has been linked with impaired stress reactivity and elevated risk for anxiety in children and adolescents (Grant et al., 2009; O'Connor et al., 2005; Van den Bergh et al., 2008). This latter finding is important considering the greater prevalence of AD in women than in men (McLean et al., 2011). In light of the lack of research on cortisol reactivity to stressors in healthy children with familial AD, more research is needed to characterize HPA functioning in this high-risk group of children and explore mechanisms that may account for normal and dysregulated HPA axis activity.

Analysis of anxiety ratings revealed no significant interaction between time and risk group. Both groups demonstrated a small increase in subjective anxiety immediately prior to the speech, with levels decreasing somewhat but remaining higher than baseline values at the end of the speech. We also found no significant association between subjective ratings of anxiety and physiological measures for the total sample and each risk group individually, which is not unexpected as there is often poor concordance between subjective and physiological measures of stress and anxiety (Gramer and Saria, 2007). Unfortunately, we did not obtain repeated ratings of subjective anxiety during the speech because of concerns that this would interfere with the child's concentration and flow of speech. Although we asked children to rate their levels of anxiety immediately after the speech, we did not ask them to retrospectively rate their peak anxiety levels during the speech itself. It is therefore possible that group differences may have emerged if we had done so.

Study limitations should be noted. First, although we controlled for respiration rate during HRV measurement, our results might be the effects of the speech stressor on respiration. Because of its strong influence on the HF band (Brown et al., 1993) any changes in respiration due to talking may have affected the analysis of HRV. It is conceivable that the requirement for vocalization makes our stress task less appropriate for this sort of study compared to a non-verbal stressor. Some studies have reported that stress tasks involving vocalization produce less of a reduction in HRV than non-verbal equivalents or fail to change HRV at all (Bernardi et al., 2000; Sloan et al., 1991; Vuksanovic & Gal, 2007). Other groups however have presented data showing greater vagal withdrawal in anxious versus not anxious participants during a verbal task such as the one used in the current study (Garcia-Rubio et al., 2017; Grossman et al., 2001), even after controlling for respiration variation (Grossman et al., 2001). Second, there is a lack of pediatric HRV guidelines and the optimal bandwidth for frequency domain measures across different age groups is not well established (Weiner and McGrath, 2017). Although this study found no significant age effect for HRV response to the stressor and we used the same frequency bandwidth as other studies (e.g., Harrewijn et al., 2018), including a population-based study of children aged 6–8 years of age (Seppälä et al., 2014), future research would benefit from comparing high and low risk children and adolescents across a range of frequency bandwidths. Third, we did not measure HRV after the speech task ended and do not know if stress recovery of HRV would differ in high- and low-risk children. Fourth, while we controlled for a number of possible confounders of our physiological indices of stress, it is possible that other variables not accounted for influenced findings. Finally, although our overall sample size was relatively large, we had fewer participants in the high-risk group and may not have had sufficient power to detect small but clinically important effects on some measures. Despite these limitations, this study has a number of strengths, including the inclusion of children with no history of psychopathology based on structured clinical interviews and SCID confirmation of parental AD and no psychopathology.

In conclusion, this study is to our knowledge one of the first to examine stress reactivity in unaffected children with and without a parent with AD. Results suggest that while children of parents with AD exhibit normal HF-HRV during resting states, they have a less flexible autonomic response when exposed to a psychosocial stressor. While there was some indication that cortisol reactivity to the stressor was also altered in children with parental AD further research is needed to confirm this finding. Genetic studies suggest that HRV (Harrewijn et al., 2018) and cortisol (Steptoe et al., 2009) response to a laboratory stressor may be heritable and findings from the current study suggest that physiological stress reactivity could serve as an endophenotype for research on AD genes and gene x environment interactions. An interesting direction for future research would be to prospectively follow young at-risk offspring to assess the developmental trajectory of cardiac ANS and HPA axis function and factors that contribute to change such as pubertal process and environmental influences. Another fruitful area of research will be to assess the predictive value of physiological stress reactivity for future development of AD in children with genetic and other vulnerability factors for pathological anxiety.

Conflict of interest

The authors do not have any conflicts to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.12.171.

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