J Abnorm Child Psychol
DOI 10.1007/s10802-014-9958-6

Autonomic and Adrenocortical Interactions Predict Mental Health in Late Adolescence: The TRAILS Study

Esther Nederhof · Kristine Marceau · Elizabeth A. Shirtcliff · Paul D. Hastings · Albertine J. Oldehinkel

Abstract The present study is informed by the theory of allostatic load to examine how multiple stress responsive biomarkers are related to mental health outcomes. Data are from the TRAILS study, a large prospective population study of 715 Dutch adolescents (50.9 % girls), assessed at 16.3 and 19.1 years. Reactivity measures of the hypothalamic pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) biomarkers (heart rate, HR; respiratory sinus arrhythmia, RSA; and pre-ejection period, PEP) to a social stress task were used to predict concurrent and longitudinal changes in internalizing and externalizing symptoms. Hierarchical linear modeling revealed relatively few single effects for each biomarker with the exception that high HR reactivity predicted concurrent internalizing problems in boys. More interestingly, interactions were found between HPA-axis reactivity and sympathetic and parasympathetic reactivity. Boys with high HPA reactivity and low RSA reactivity had the largest increases in internalizing problems from 16 to 19 years. Youth with low HPA reactivity along with increased ANS activation characterized by both decreases in RSA and decreases in PEP had the most concurrent externalizing problems, consistent with broad theories of hypo-arousal. Youth with high HPA reactivity along with increases in RSA but decreases in PEP also had elevated concurrent externalizing problems, which increased over time, especially within boys. This profile illustrates the utility of examining the parasympathetic and sympathetic components of the ANS which can act in opposition to one another to achieve, overall, stress responsivity. The framework of allostasis and allostatic load is supported in that examination of multiple biomarkers working together in concert was of value in understanding mental health problems concurrently and longitudinally. Findings argue against an additive panel of risk and instead illustrate the dynamic interplay of stress physiology systems.

Keywords Public speaking task · Mental disorders · depression · Anxiety · Antisocial behavior · Psychological problems · Prospective study

E. Nederhof (✉) · A. J. Oldehinkel
University of Groningen, University Medical Center Groningen, Interdisciplinary Center Psychopathology and Emotion Regulation, Groningen, The Netherlands
e-mail: e.nederhof@umcg.nl

K. Marceau
Center for Alcohol and Addiction Studies, Department of Behavioral & Social Sciences, Brown University School of Public Health, Providence, RI, USA

K. Marceau
Division of Behavior Genetics, Department of Psychiatry, Rhode Island Hospital, Providence, RI, USA

E. A. Shirtcliff
Department of Human Development and Family Studies, Iowa State University, Ames, IA, USA

P. D. Hastings
Department of Psychology, University of California Davis, Davis, CA, USA

Published online: 25 November 2014

In the past decade many researchers studied the concept of allostatic load in the context of behavioral problems, as reflected by several special issues on this topic (e.g., Cicchetti 2011; Peters and McEwen 2012; current special issue). Allostatic load refers to the cumulative wear and tear on the body as a result of chronic response patterns in which physiological systems are over-activated or dysregulated, potentially leaving the individual vulnerable for (psycho)pathology (Juster, McEwen, and Lupien 2010; McEwen 1998). Adaptation to chronic challenges is different from adaptation to acute challenges in the sense that it can result in altered set-points of the system (Berntson and

Cacioppo 2007). The body continuously adapts to acute challenges, whether predictable or unpredictable, by the dynamic coordination of various physiological regulatory systems, a process called allostasis. In some chronic challenges, however, it might be advantageous to alter internal set-points in order to adapt to the enduring demands of the situation. While altered set-points can be adaptive in the current situation, such adaptations may have trade-offs, including detrimental effects on the long run (McEwen 1998, 2005). A major advantage of the theory of allostasis and allostatic load is that it integrates across stress responsive physiological indicators, to provide a summative view of the individual rather than espouse examination of a single biomarker to capture stress. Disadvantages are that it is not always clear whether each biomarker unidirectionally indicates health risk and whether this risk adds cumulatively across systems. The present study examines how multiple stress responsive biomarkers interact dynamically in relation to mental health outcomes in adolescents.

Physiological Indicators of Allostatic Load

Physiological regulatory systems commonly examined in terms of allostatic load include the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. The ANS is designed to help the body deal with stressors on a relatively fast time-scale. It is comprised of the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). The role of the PNS is to maintain homeostasis, or rest and digest, and thus a decrease of PNS activity is necessary to mount a stress response. PNS activity is often measured by respiratory sinus arrhythmia (RSA), which captures fluctuations in heart rate driven by the respiratory (or breathing) cycle (Alkon et al. 2003; Bush et al. 2011a). Much of this literature examines how tonic RSA levels inhibit sympathetic arousal to induce a calm, restive, social psychological state (Hastings et al. 2008a). High RSA levels may be protective against the development of psychopathology, especially in adverse contexts, likely as a function of this vagal brake (Hastings et al. 2008b). The present study focused on RSA reactivity, because RSA levels are assumed to decline during a threat or challenge to help mobilize defensive reactions, working quickly with the sympathetic branch of the ANS to initiate a stress response. In line with the idea that low RSA levels might be a risk factor for psychopathology, failure to withdraw PNS activity during a challenge is thought to indicate allostatic load. PNS withdrawal has been associated with better emotion regulation and fewer mental health problems (El-Sheikh et al. 2001; El-Sheikh and Whitson 2006; Gentzler et al. 2009; Keller and El-Sheikh 2009), although findings are inconsistent (Gordis et al. 2006; Hastings et al. 2008a, 2008b).

If a withdrawal of the PNS is not sufficient to manage a stressor, increasing SNS activity is expected to mount the fight or flight response, including increases in blood flow and respiration in order to prepare the body for action. SNS activity can be measured by the pre-ejection period (PEP), the duration of isovolumic contraction of the heart (Bush et al. 2011a). A shorter PEP indicates higher SNS activation. SNS and PNS are assumed to be in a state of dynamic equilibrium, working coordinately to influence cardiac output, among other things, expressed in the heart rate (HR). Increasingly, there have been calls for action to examine their coordinated interaction (Bauer et al. 2002; Del Giudice et al. 2011). Indeed, several studies have shown that children are at risk for mental health problems when the PNS and SNS are not in dynamic equilibrium (El-Sheikh et al. 2009; Gordis et al. 2010; Keller and El-Sheikh 2009), emphasizing the need to study these systems together.

The third stress response system, the HPA axis, is primarily activated during situations that contain unpredictable or uncontrollable elements (Dickerson and Kemeny 2004; Koolhaas et al. 2011), but in a different manner from the ANS (Andrews, D'Aguiar, and Pruessner 2012). The HPA axis is thought to become activated when a stressor is of sufficient magnitude or duration to overwhelm the capacity to recruit resources of the ANS (Sapolsky et al. 2000). A stressor activates a cascade of events (e.g., hypothalamus stimulation, release of corticotrophin releasing hormone, pituitary gland stimulation and release of adrenocorticotropin hormone, adrenal gland stimulation and subsequent release of cortisol). Cortisol, the major output hormone of this axis, aids the body in dealing with a stressor and also suppresses the signal to release corticotrophin releasing hormone, thus regulating the HPA axis via negative feedback. Both high and low cortisol reactivity to specific stressors, are thought to contribute to allostatic load (Del Giudice et al. 2011; Shirtcliff et al. 2014). Previous work on cortisol-behavior associations has been equivocal: generally there is evidence that high cortisol reactivity may be associated with more internalizing problems whereas low cortisol reactivity may be associated with more externalizing problems (Dickerson and Kemeny 2004), although sometimes the opposite pattern of association, or no association, is found (see also Alink et al. 2008).

Allostatic Load Considering Multiple Physiological Systems

Especially within young populations or prior to when the wear and tear is obvious on these systems, it may be necessary to take a nuanced approach toward considering the interplay of multiple physiological systems (Bush et al. 2011b). The coordinated action of two systems is often conceptualized in terms of symmetric or asymmetric responses to stressors. Whereas the coordinated activation of the ANS and HPA systems in response to stressors has generally been supported, more

research is needed to draw conclusions about the patterns of symmetry/asymmetry in response to stressors and the behavioral correlates of asymmetric vs. symmetric activation (Schumacher et al. 2013). Studies investigating interactions between the two branches of the ANS in childhood generally suggest that co-operation between the two branches of the ANS (i.e., vagal withdrawal in combination with SNS activation or vice versa) seems protective against externalizing problems (El-Sheikh et al. 2009; Keller and El-Sheikh 2009; Gordis et al. 2010).

A few studies have investigated interactions between ANS and HPA function, and suggest that in childhood, higher basal cortisol is related to higher internalizing and externalizing problems among youth with higher SNS activity (El-Sheikh et al. 2008) whereas the combination of higher basal cortisol and PNS withdrawal is protective against internalizing problems (El-Sheikh and Hinnant 2011). Further, several studies have examined how coordinated *stress reactivity* of the HPA axis and the ANS is associated with mental health problems. Because comorbidity is usually high in children and adolescents (Angold et al. 1999), Hastings and colleagues modelled the effects of physiological reactivity on internalizing and externalizing problems simultaneously in a study of 215 14-year-olds. First, they found that asymmetrical ANS (assessed via heart rate) and HPA reactivity (i.e., high HPA-axis reactivity in combination with low ANS reactivity) was associated with concurrent externalizing problems. Second, they found that symmetrical HPA and SNS reactivity (assessed using mean arterial blood pressure), high ANS activation in combination with high HPA-axis reactivity, predicted concurrent internalizing problems. This pattern of symmetrical HPA and SNS reactivity (using salivary alpha amylase as a measure of SNS reactivity) was also found to predict total problems in a sample of 56 youth aged 7 to 16 years (Allwood et al. 2011). Finally, Gordis et al. (2006) investigated coordination of HPA and SNS reactivity (using salivary alpha amylase) to a speech in relation to externalizing problems. In contrast to Hastings et al. (2011), they found that aggressive behavior was highest in youth with symmetrical low reactivity in a study of 67 10–14-year-olds.

Thus, findings are largely consistent for internalizing problems: symmetrically high HPA and SNS reactivity is associated with more internalizing problems concurrently. However, the findings for externalizing problems appear inconsistent. It may be that behavioral correlates of (a)symmetry of ANS and HPA reactivity may differ when global ANS vs. SNS-specific activity is assessed, at least for externalizing problems. The above studies suggest that asymmetry of global ANS (e.g., heart rate) and HPA reactivity but symmetrically low SNS and HPA reactivity may mark risk for externalizing problems. Given the findings suggesting that cooperation of the SNS and PNS branches of the ANS may be protective against externalizing problems, examining interactions of SNS, PNS, and HPA reactivity may better predict externalizing problems than two-way interactions among these systems.

Given that allostasis is the process of maintaining stability through change and allostatic load emerges across development, it is not surprising that longitudinal changes are not always compatible with concurrent associations. Hastings et al. (2011) found that different sets of interactions predicted changes in internalizing problems compared to concurrent problems. Specifically, symmetrically low or high ANS (heart rate) and HPA reactivity predicted internalizing problems longitudinally in girls whereas asymmetrical reactivity, high ANS (heart rate) in combination with low HPA, predicted an increase in internalizing problems boys. In direct opposition to the concurrent findings, asymmetrical SNS and HPA reactivity (as opposed to symmetrically high reactivity) predicted increased internalizing problems longitudinally in girls. This remarkable, although not unprecedented (see Ruttle et al. 2011; Shirtcliff and Essex 2008), reversal of the association between physiological functioning from contemporaneous levels of problem behaviors to longitudinal changes in problem behaviors is in utmost need of replication. Gender appeared to play a larger role in longitudinal versus concurrent findings in Hastings et al. (2011) though these patterns are also in need of replication.

Present Study

Thus, the goal of the present study was to replicate and extend earlier findings (El-Sheikh et al. 2009; Gordis et al. 2006, 2010; Hastings et al. 2011; Keller and El-Sheikh 2009). Specifically, we examine interactions of ANS and HPA reactivity in relation to internalizing and externalizing problems during adolescence. We extend the previous work by examining separately measures of global ANS reactivity (heart rate) and both SNS and PNS reactivity in combination with cortisol reactivity. We examine these interactions both concurrently and longitudinally, and examine sex differences, as done in Hastings et al. (2011). Thus, we also extend the conceptualization of symmetry to examine the role of both cross-system (e.g., ANS and HPA) and within-system (e.g., PNS and SNS) symmetry for the development of mental health problems.

Such a replication and extension effort is possible with the TRacking Adolescents' Individual Lives Survey (TRAILS; see De Winter et al. 2005 and Nederhof et al. 2012 for an overview of the study). Reactivity of the HPA-axis and both branches of the ANS was assessed in 715 adolescents. HPA-axis reactivity was assessed by means of pre-stress and 20 min post-stress salivary cortisol. Reactivity of the ANS was assessed using heart rates (HR), heart rate variability in the high frequency domain, which is equivalent to RSA, was used as a measure of PNS reactivity, and the PEP was used as a measure of SNS reactivity.

Based on findings from Hastings et al. (2011) and Allwood et al. (2011), we hypothesized that symmetric high activation of SNS and HPA (specifically, PEP x cortisol reactivity interactions) would predict internalizing problems. For externalizing problems, we expected that when using the global measure of ANS reactivity (heart rate), asymmetrical reactivity would predict more externalizing problems (Hastings et al. 2011), whereas when using specific measures of SNS and PNS activity, symmetrically low SNS and HPA activation would predict more externalizing problems (Gordis et al. 2006). Further, we expected that interactions of SNS and PNS reactivity reflecting symmetrically high or low activation would predict more externalizing problems (El-Sheikh et al. 2009; Gordis et al. 2010; Keller and El-Sheikh 2009). There was insufficient literature to formulate hypotheses for the three-way interaction of SNS, PNS, and HPA reactivity. Nonetheless, we expected that the three-way interaction would likely be associated with externalizing problems because SNS x PNS and SNS x HPA interactions have been associated with externalizing problems.

Methods

Sample

Data were collected in a general population study called TRAILS (TRacking Adolescents' Individual Lives Survey), a large prospective population study of Dutch adolescents with bi- or triennial measurements from age 11 to at least early adulthood generally representative of the Northern part of the Netherlands (Huisman et al. 2008; Oldehinkel et al. 2004). Detailed information about sample selection and analysis of non-response bias has been reported elsewhere (de Winter et al. 2005). Data presented in this article are from the third (called T1 in this paper; September 2005 to August 2008) and fourth (called T2 in this paper; October 2008 to September 2010) assessment waves of TRAILS (Nederhof et al. 2012). The full sample at the third wave consisted of 1816 respondents (81.4 % of all first wave participants; mean age 16.3, SD =0.7, 52 % girls), and of 1881 respondents at the fourth wave (mean age 19.1, SD =0.6, 52.3 % girls). All protocols were approved by the Central Ethical Committee (CCMO). Parents gave written informed consent and adolescents gave their asset before participation.

During the third wave, a subsample of 744 adolescents was invited to perform a series of laboratory tasks, in addition to the usual assessments, hereafter referred to as the laboratory session. It was during this laboratory session that participants were assessed on biological reactivity to stress. We slightly oversampled participants with high scores on frustration and fearfulness, low scores on effortful control, higher parental psychopathology (depression, anxiety, addiction, psychoses, or antisocial behavior), and living in a single-parent family. In total, these higher-risk adolescents represented 66 % of participants invited for the laboratory session, whereas they represented 58 % of the total TRAILS population (see details in Sijtsema et al. 2013). Of all invited adolescents, 715 (96.1 %) agreed to participate (50.9 % girls). Participants were 90.8 % Dutch ancestry. The socio-economic status of 20.1 % of participants' parents was in the lowest quartile of the total TRAILS population, whereas 27.8 % of participants were from families within the highest quartile.

Laboratory Procedure

During the laboratory session, participants' physiological responses to a variety of challenging conditions were recorded. These conditions included orthostatic stress (from supine to standing), a spatial orienting task, a gambling task, a startle reflex task, and a social stress test. The experimental protocol was approved by the Central Committee on Research Involving Human subjects (CCMO). The test assistants, 16 in total, received extensive training in order to optimize standardization of the experimental session. The experimental sessions took place on weekdays, in sound-proof rooms with blinded windows at selected locations in the towns where participants resided. The sessions lasted about 3 hrs and 15 min, and started between 08:00 and 09:30 a.m. (morning sessions, 49 %) or between 01:00 and 02:30 p.m. (afternoon sessions, 51 %). Participants were asked to refrain from smoking and from using coffee, milk, chocolate, and other sugar containing foods in the 2 hrs before the session. At the start of the session, the test assistant, blind to the participants' risk status, explained the procedure and administered a short checklist on current medication use, quality of sleep, and physical activity in the last 24 h. Participants were attached to the equipment for cardiac autonomic measurements at this time.

Sympathetic, parasympathetic, and HPA (re)activity were assessed in response to the Groningen Social Stress Task (GSST; see also Bouma et al. 2009), a standardized protocol inspired by the Trier Social Stress Task (Kirschbaum et al. 1993) for the induction of moderate performance-related social stress. During the GSST, cardiac autonomic function was recorded continuously. Participants were instructed, on the spot, to prepare a 6-min speech about themselves and their lives and deliver this speech, while sitting in front of a video camera. They were told that their videotaped performance would be judged on content of speech as well as on use of voice and posture, and rank-ordered by a panel of peers after the experiment. The risk of being judged negatively by peers was included to induce threat of social rejection. Participants had to speak continuously for the whole period of 6 min. The test assistant watched the performance critically, without showing empathy or encouragement. After 6 min of speech,

the participants were told that there was a problem with the computer and they had to sit still and be quiet. Subsequently, they were asked to perform mental arithmetic. The participants were instructed to repeatedly subtract the number 17 from a larger sum, starting with 13,278. A sense of uncontrollability was induced by repeated negative feedback from the test assistant (e.g., "No, wrong again, begin at 13,278"). The mental arithmetic challenge lasted for 6 min, again followed by a 3-min period of silence, after which the participants were debriefed about the experiment.

Measures

HPA-Reactivity Salivary cortisol was sampled with the Salivette sampling device (Sarstedt, Numbrecht, Germany) containing a small swab in a plastic tube on which the participants had to chew for 60 s, until the swab was soaked with saliva (for details on collection, storage, and assays, see Bouma et al. 2009). Participants were asked to collect two morning saliva samples on the day of the laboratory session, one directly after waking up (mean time of awakening =07:39 h, S.D. =1:10 h) and one 30 min later.

HPA-axis reactivity measures in girls using oral contraceptives (n =126 girls) were not valid in our sample. Bouma et al. (2009) showed that these girls do not show a cortisol response to the GSST, whereas girls who do not use oral contraceptives do show a response. Information on oral contraceptive use was missing for 6 girls. Cortisol values for these 132 girls were set at missing. Additionally, cortisol values for 5 participants were set at missing because they used corticosteroids or SSRIs. Cortisol values for another 18 participants were set at missing because they used pain medication at the day of the experiments. Finally, cortisol values of 4 participants who smoked before the experimental session were also set at missing.

Cortisol levels were also assessed just before the start of the GSST (C1) and directly after the end of the test (C2). Because there is a delay of approximately 20 min between the production of cortisol by the adrenal glands and the detectability of representative levels of cortisol in saliva (Kirschbaum et al. 1992), all samples reflect stress reactions about 20 min earlier. For most participants, C2 is peak cortisol, as it is taken immediately after cessation of the test, which is 20 min after the start. Cortisol values which deviated more than 2.5 standard deviations (SD) from the mean were windsorized. Because the cortisol variables were skewed, they were log transformed to normalize the distribution. The difference between C2, the post-GSST cortisol value, and C1, the pre-GSST cortisol value, was calculated for each individual as a measure of cortisol reactivity. Greater cortisol increases from pre- to post-GSST indicate higher cortisol reactivity. In total, cortisol reactivity was available for 537 participants.

ANS-Reactivity Cardiac autonomic function was assessed during speech (360 s) and 25 min posttest (300 s). A three-lead electrocardiogram and a four-lead impedance cardiogram was registered using 3 M/RedDot Ag/AgCl electrodes (type 2255, 3 M Health Care, Neuss, Germany), while the participant was sitting and breathing spontaneously. With a BIOPAC Amplifier-System (MP100, Goleta, CA), the signals were amplified and filtered before digitization at 250 samples/second. Dedicated software (PreCARSPAN, previously used in, e.g., Dietrich et al. 2007) was used to check signal stationarity, to correct for artifacts, to detect R-peaks, and to calculate the interbeat-interval (IBI) between two heartbeats. Blocks were considered invalid if they contained artifacts with a duration of more than 5 s, if the total artifact duration was more than 10 % of the registration, or if the block length was less than 100 s.

Heart Rate (HR) was calculated from the mean inter beat intervals (IBI) by HR=60,000/IBI. Heart rate reactivity was calculated by subtracting heart rate during rest from heart rate during speech. Greater HR increases from pre- to post-GSST indicate higher HR reactivity. Heart rate reflects the combined sympathetic and parasympathetic influence on cardiac function. Heart rate reactivity was available for 669 participants.

Respiratory Sinus Arrhythmia (RSA) was operationalized as the heart rate variability in the high-frequency band (0.15–0.40 Hz). Calculation of RSA was performed by power spectral analysis in the CARSPAN software program (Mulder, Van Dellen, Van der Meulen, and Opheikens 1988) using estimation techniques based on Fourier transformations of IBI series (Robbe et al. 1987). RSA mainly results from centrally mediated cardiac vagal activity (Camm et al. 1996). Higher scores (i.e., greater variability) indicate stronger influence of the parasympathetic nervous system on cardiac function.

RSA scores were highly skewed in part due to several extreme outliers. Outliers (six cases for RSA during speech and four cases for resting RSA) were winsorized at 12,000 and 20,000 ms^2 respectively (2.5 standard deviations), and then all scores were log transformed. The difference between the transformed measure of RSA collected during rest after the speech and RSA collected during the speech was calculated for each individual as a measure of RSA reactivity. Greater RSA decreases from pre- to post-GSST indicate higher RSA reactivity. RSA reactivity was available for 669 participants.

Pre ejection period (PEP) reflects the time interval between the onset of the electromechanical systole (Q-wave onset) in the ECG and the opening of the aortic valves co-occurring with the B-point in the ICG. Thoracic impedance was assessed with a BIOPAC NICO100C Noninvasive Cardiac Output Module. B-points were manually scored by an extensively trained rater using the VU-AMS interactive software. When there was doubt about the B-point, the scoring was discussed

with a second rater. Outliers were checked and quality of the PEP rates was subjectively scored on a 0–10 scale. PEP data were considered invalid if the quality of the PEP was low (i.e., score <6) or the signal contained too many artifacts (including participants with arrhythmias or extrasystoles). PEP reactivity was calculated by subtracting PEP during rest from PEP during speech. Greater PEP decreases from pre- to post-GSST indicate higher PEP reactivity. PEP reactivity was available for 584 participants.

Internalizing and Externalizing Behavior was assessed with the Youth Self-Report (YSR) and the parent-reported Child Behavior Checklist (CBCL; Achenbach and Rescorla 2001) at age 16 and with the Adult Self-Report (ASR; Achenbach et al. 2003) at age 19. At age 16 the internalizing and externalizing scores were created by saving the factor scores from a principal component analysis to combine parent and youth reports. Only the youth (young adult) report was available at age 19 years. These are commonly used questionnaires in current child and adolescent psychiatric research. The good reliability and validity of the YSR was confirmed for the Dutch translation (Verhulst, van der Ende, and Koot 1997). The YSR and CBCL contain a list of 112 behavioral and emotional problems, whereas the ASR contains a list of 102 behavioral and emotional problems, which participants or their parents can rate as 0=*not true*, 1=*somewhat or sometimes true*, or 2=*very or often true* in the past 6 months. The externalizing domain consists of the aggressive behavior and rule-breaking behavior syndrome scales (in the full TRAILS sample YSR Cronbach's α=0.87; CBCL Cronbach's α=0.90; ASR Cronbach's α=0.89). The internalizing domain consists of the withdrawn/depressed, anxious/depressed and somatic complaints scales (in the full TRAILS sample YSR Cronbach's α=0.89; CBCL Cronbach's α=0.87; ASR Cronbach's α=0.93). Data on internalizing behavior was available for 645 participants at age 16, and for 662 at age 19. Data on externalizing behavior was available for 654 participants at age 16, and for 661 at age 19.

Analytic Strategy

Hypothesis testing was conducted using structural equation models. Analyses were conducted first including only cortisol and HR because HR is an integration of SNS and PNS branches. Then a second set of analyses was conducted including cortisol, PEP, and RSA to attempt to separate the effects of SNS and PNS activation. Interaction variables of cortisol reactivity with heart rate reactivity, cortisol reactivity with RSA reactivity, cortisol reactivity with PEP reactivity, RSA reactivity with PEP reactivity, and a three-way interaction between cortisol, RSA, and PEP reactivity were created (by multiplying reactivity variables after centering) for use in hypothesis testing. First, contemporaneous models were conducted, wherein the measures of physiological change predicted internalizing and externalizing problems within the same wave of data collection. Second, longitudinal models were conducted, wherein the measures of physiological change predicted internalizing and externalizing problems at follow-up correcting for initial problem levels.

For each analysis we first fit a model wherein each path was allowed to differ for girls and boys was fitted to the data (unconstrained). Then, we used a nested approach and constrained the paths to be equal for boys and girls (constrained). The change in the chi-square test for model fit was calculated across the two models. A significant decrement in model fit indicated that constraining girls and boys to be equal resulted in a significant decline in the fit of the model, and therefore sex differences were significant. If the omnibus test revealed sex differences, each significant hypothesized path was constrained individually to determine whether there were sex differences for particular pathways of interest within the nested model framework. Missing data were handled using FIML estimation. In both models, the effect of age on internalizing and externalizing problems was modelled. Significant associations among the reactivity scores and two- and three-way interaction terms (Table 1) were also estimated in the SEMs in order to control for multicollinearity.

Results

Descriptive Statistics

On average, cortisol levels increased by 1.25 nmol/l (SD =2.73; untransformed data) during the stress test, as compared to pre-test levels. A total of 352 participants (65.9 %) showed the expected rise in cortisol in response to the stressor; 19 (3.5 %) showed no change, and the rest (166 participants, 30.6 %) showed a decrease in cortisol. The average heart rate increased by 13 beats per minute (SD =11). A total of 632 participants (94.5 %) showed the expected rise in heart rate in response to the stressor; the rest (37 participants, 5.5 %) showed a decrease. PEP decreased in response to the stressor (M=−16.01 ms, SD =17.31) on average; 514 participants (88 %) showed the expected decrease in PEP; 22 (4 %) experienced no change and 48 (8 %) experienced a rise in PEP in response to the stressor. The average RSA decreased slightly in response to the stressor (M=−258.84 ms^2, SD =2868.31; untransformed data), with half (49.9 %) of the participants experiencing the expected decline in RSA; the rest (50.1 %) an increase. Zero-order correlations among study variables are presented in Table 1. For each set of analyses, only significant findings are discussed in text. Model fitting results for each analysis are presented in Table 2.

Table 1 Zero-order correlations among study variables

	T1 age	T1 IP	T1 EP	T2 IP	T2 EP	Cort Resp.	RSA Resp.	PEP Resp.	HR Resp.	Cort X RSA	Cort X PEP	Cort X HR	PEP X RSA	Cort X PEP X RSA
T1 age		−0.08	0	0	0.02	0	0.01	0.01	0.05	−0.01	0.01	−0.03	0.01	−0.02
T1 IP	0.01		0.46*	0.57*	0.36*	−0.03	0.06	−0.12 T	0.08	−0.07	−0.01	−0.06	0.05	−0.04
T1 EP	−0.01	0.53*		0.28*	0.49*	−0.02	0.09	−0.11	0.04	0.02	−0.03	−0.01	0.06	−0.19
T2 IP	−0.02	0.52*	0.23*		0.62*	0.06	0.10 t	−0.08	0.12*	−0.02	−0.01	−0.04	0.13*	−0.08
T2 EP	−0.01	0.33*	0.39*	0.63*		0.01	0.09 t	−0.06	0.05	−0.02	−0.11 t	−0.09	0.15*	−0.08
Cort Resp.	−0.04	−0.07	−0.16*	0.04	−0.08		0.18*	0.21*	0.30*	−0.16*	0.26*	−0.08	−0.03	−0.04
RSA Resp.	−0.03	0.01	−0.13*	−0.06	−0.08	0.18*		0.02	0.49*	0.19*	0.03	0.11*	0.08	−0.04
PEP Resp.	0.06	0.05	−0.08	0.05	−0.02	0.21*	−0.01		0.22*	−0.01	0.21*	0.09	−0.24*	0.10
HR Resp.	0.05	−0.03	−0.23*	−0.04	−0.12*	0.34*	0.59*	0.27*		0.11*	0.14*	0.18*	0.05	0.11 t
Cort X RSA	−0.13*	0.03	−0.06	0.03	0.01	0.32*	0.19*	−0.005	0.30*		−0.07	0.47*	−0.03	0.29*
Cort X PEP	0.10	0.07	−0.03	0.06	−0.07	0.40*	−0.01	0.10	0.07	−0.17*		0.39*	0.07	−0.29*
Cort X HR	−0.09	0.02	−0.10	0.04	−0.03	0.56*	0.28*	0.10	0.27*	0.77*	0.29*		0.06	0.14*
PEP X RSA	0.02	0.13*	0.02	0.06	−0.03	0.01	0.18*	0.21*	0.18*	0.34*	−0.031	0.22*		−0.14*
Cort X PEP X RSA	0	0.02	0.08	−0.02	−0.06	−0.11	0.28*	−0.03	0.16*	0.38*	−0.09	0.15*	0.12	

Correlations for boys presented in the bottom left, correlations for girls presented in the top right

X signifies interaction terms created by multiplying variables

IP internalizing problems, *EP* externalizing problems, *T1* the first assessment, *T2* the second assessment, *Cort* cortisol, *Resp*.responsivity, *RSA* respiratory sinus arrhythmia, *PEP* pre-ejection period, *HR* heart rate

*$p<0.05$, **$p<0.10$

Model Fitting

For each set of analyses, the unconstrained model fit the data well, but the constrained sub-model fit the data very poorly. Further, there was a significant decrease in model fit from the unconstrained to the constrained models, indicating the presence of significant sex differences generally, which were driven by measurement non-invariance (sex differences in means and variances) as well as by sex differences in path estimates (Table 2). Thus, in all cases findings from the unconstrained model are presented and interpreted.

ANS and HPA Reactivity Concurrently, high heart rate reactivity predicted internalizing problems in boys, but not girls

Table 2 Model fit statistics for all models

	χ^2	df	*p*-value	CFI	TLI	RMSEA	SRMR	χ^2 change	df	*p*-value
Concurrent HR										
Unconstrained	3.83	6	0.70	1.0	1.0	0.00	0.017			
Constrained	231.53	28	0.00	0.38	0.60	0.14	0.13	110.59	22	<0.001
Longitudinal HR										
Unconstrained	22.13	22	0.45	1.0	1.0	0.004	0.033			
Constrained	383.19	53	0.00	0.84	0.92	0.13	0.17	259.82	31	<0.001
Concurrent RSA & PEP										
Unconstrained	26.01	30	0.67	1.0	1.0	0.00	0.04			
Constrained	372.76	78	0.00	0.10	0.61	0.10	0.13	348.77	48	<0.001
Longitudinal RSA & PEP										
Unconstrained	61.09	62	0.51	1.0	1.0	0.00	0.046			
Constrained	1961.55	119	0.00	0.13	0.70	0.21	0.18	406.77	57	<0.001

Df degrees of freedom, *CFI* comparative fit index, *TLI* tucker-lewis index, *RMSEA* root mean square error of approximation, *SRMR* standardized root mean square residual. χ^2 is considered overly conservative for samples of this size. Rules of thumb for indexes of practical fit: CFI and TLI>0.95 indicates good fit. RMSEA and SRMR<0.05 indicates good fit. χ^2 change indicates the difference in model fit from the unconstrained to constrained models, and a significant p-value indicates a significant decrement in model fit

(Table 3). Constraining this single path to be equal for boys and girls revealed that this sex difference was statistically significant, χ^2 change (1) =8.98, p=0.02. Thus, the association of high heart rate reactivity and concurrent internalizing problems was significantly stronger for boys than girls.

Longitudinally, there was a significant association between low heart rate reactivity and externalizing problems in girls but not boys (Table 4). Constraining this single path to be equal between boys and girls resulted in a significant decrement in model fit, χ^2 change (1) =4.78, p=0.03. Therefore, the association of low heart rate reactivity and later externalizing problems was significantly stronger for girls than boys.

SNS, PNS, and HPA Reactivity Concurrently, there were two interactions for boys, but not girls (Table 5). First, there was an interaction of cortisol and PEP such that symmetric high reactivity of both was associated with the highest levels of externalizing problems. However, there was not a significant sex difference for this interaction, χ^2 change (1) =1.17, p= 0.28. Second there was an interaction of PEP and RSA predicting externalizing problems such that vagal withdrawal (decreases in RSA) in combination with SNS activation (PEP reactivity) predicted more externalizing problems. There was a significant sex difference in the interaction between PEP and RSA predicting externalizing problems, χ^2 change (1) =4.18, p=0.04. However, these interactions were qualified by a trend-level interaction of cortisol, PEP, and RSA for boys that was significant when examining boys and girls together (e.g., in the constrained model, β=−0.11, SE=0.05, t=−2.11, p= 0.04). Further, constraining the higher order interaction with cortisol, PEP and RSA to be equal for boys and girls did not result in a decrement in fit, indicating no sex difference in the three-way interaction, χ^2 change (1)=0.51, p=0.48. The data showed that youth exhibiting low cortisol reactivity, or even cortisol decreases, along with greater RSA decreases (PNS withdrawal), OR high cortisol reactivity along with large RSA increases (PNS augmentation), all in the context of greater PEP decreases (i.e., high SNS reactivity) had the most externalizing problems (Fig. 1). The interaction between cortisol and RSA reactivity was not present in the context of low SNS reactivity (i.e., small PEP decreases or even increases). Therefore, asymmetrical HPA and PNS reactivity predicted concurrent externalizing problems in the context of high SNS reactivity, but not in the context of low SNS reactivity.

Longitudinally, there was a significant three-way interaction between RSA, PEP and cortisol predicting future externalizing problems in boys, but not in girls, χ^2 change (1) =7.07, p=0.01 (Table 6). We found that boys with low cortisol reactivity along with RSA reactivity (PNS withdrawal) in the context of PEP reactivity (high SNS reactivity) had the largest increases in externalizing problems (See Fig. 2). That is, asymmetric HPA and PNS reactivity predicted increases in externalizing problems in the context of high SNS but not low SNS reactivity, paralleling the concurrent findings, but for boys only. There was also a cortisol X RSA interaction that predicted changes in internalizing problems in boys, such that boys with high HPA-axis reactivity and high RSA scores (i.e., PNS augmentation or minimal PNS withdrawal) had the largest increases internalizing problems between age 16 and 19 (see Fig. 3). Constraining the association between the cortisol - PNS reactivity interaction and internalizing problems to be equal between boys and led to a decrease in model fit, χ^2 change (1) =4.45, p=0.03, signifying that there was a significant sex difference such that asymmetric HPA and PNS activation predicted increased internalizing problems specifically in boys. There was also a main effect of RSA reactivity (decreases) predicting increased externalizing problems longitudinally in girls, although constraining path from RSA reactivity to externalizing problems to be equal for boys and girls did not result in a decrement in model fit either, χ^2 change (1) =1.65, p= 0.20, thus there was no real sex difference in this effect.

Table 3 Parameter estimates for the unconstrained concurrent model of Heart Rate

	Age	Cort Resp.	HR Resp.	Cort X HR	T1 IP
Age	1				
Cortisol reactivity	NE	1			
HR reactivity	NE	0.33* (0.06) 0.28* (0.05)	1		
Cort X HR	NE	0.56* (0.05) −0.15* (0.06)	0.23* (0.07) 0.18* (0.06)	1	
T1 IP	−0.01 (0.05) −0.01 (0.06)	0.04 (0.09) 0.02 (0.06)	−0.06 (0.06) 0.13* (0.06)	0.02 (0.09) −0.05 (0.06)	1
T1 EP	−0.01 (0.05) 0.02 (0.06)	−0.06 (0.09) −0.02 (0.06)	−0.10 (0.06) 0.07 (0.06)	0.01 (0.08) −0.09 (0.06)	0.63* (0.03) 0.62* (0.04)

Un-standardized beta weights are presented, followed by standard errors in parentheses. Girls' estimates are presented above boys' estimates

X signifies interaction terms created by multiplying variables

NE not estimated, *IP* internalizing problems, *EP* externalizing problems, *T1* the first assessment, *Cort* cortisol, *HR* heart rate

*p<0.05, **p<0.10

Table 4 Parameter estimates for the unconstrained longitudinal model of Heart Rate

	Age	Cort Resp.	HR Resp.	Cort X HR	T1 IP	T1 EP	T2 IP
Age	1						
Cortisol reactivity	NE	1					
HR reactivity	NE	0.33* (0.06) 0.28* (0.05)	1				
C X HR	NE	0.56* (0.05) −0.15* (0.06)	0.23* (0.07) 0.18* (0.06)	1			
T1 IP	NE	NE	NE	NE	1		
T1 EP	NE	NE	NE	NE	0.63* (0.03) 0.62* (0.04)	1	
T2 IP	0.04 (0.05) −0.06 (0.05)	−0.10 (0.08) −0.09** (0.05)	0.01 (0.05) 0.06 (0.03)	0.06 (0.07) −0.06 (0.05)	0.53* (0.06) 0.57* (0.06)	−0.01 (0.06) 0.01 (0.06)	1
T2 EP	0.01 (0.05) −0.01 (0.05)	−0.09 (0.08) −0.03 (0.06)	−0.16* (0.05) 0.02 (0.05)	0.02 (0.08) 0.02 (0.05)	−0.01 (0.07) −0.05 (0.07)	0.37* (0.06) 0.53* (0.06)	0.53* (0.04) 0.43* (0.05)

Un−standardized beta weights are presented, followed by standard errors in parentheses. Girls' estimates are presented above boys' estimates

X signifies interaction terms created by multiplying variables

NE Not estimated, *IP* internalizing problems, *EP* externalizing problems, *T1* the first assessment, *T2* the second assessment, *Cort* cortisol, *HR* heart rate

*$p<0.05$, ** $p<0.10$

Discussion

The hypothesis in the present study was that symmetric high activation of SNS and HPA would predict internalizing problems. For externalizing problems, we hypothesized that symmetrically low activation of the SNS and HPA axes would predict more externalizing problems. Further, we expected that interactions of SNS and PNS reactivity reflecting symmetrically high or low activation would predict more externalizing problems. Our data revealed several main effects of physiological reactivity in association with internalizing and externalizing problems: 1) high ANS reactivity (HR increases)

Table 5 Parameter estimates for the unconstrained concurrent model of RSA and PEP

	Age	Cort Resp.	RSA Resp.	PEP Resp.	Cort X RSA	Cort X PEP	RSA X PEP	C X RSA X PEP	T1 IP
Age	1								
Cortisol reactivity	NE	1							
RSA reactivity	NE	0.17* (0.06) 0.18* (0.05)	1						
PEP reactivity	NE	0.18* (0.07) 0.19* (0.06)	NE	1					
Cortisol X RSA	NE	0.30* (0.06) −0.14* (0.05)	0.14* (0.07) 0.19* (0.05)	NE	1				
Cortisol X PEP	NE	0.36* (0.07) 0.22* (0.06)	NE	0.10 (0.08) 0.25* (0.05)	−0.13 (0.08) −0.06 (0.06)	1			
RSA X PEP	NE	NE	0.17* (0.06) 0.08 (0.06)	0.20* (0.05) −0.25* (0.06)	0.30* (0.07) 0.003 (0.06)	NE	1		
C X RSA X PEP	NE	NE	0.32* (0.07) 0.01 (0.06)	NE	0.40* (0.07) 0.31* (0.06)	−0.07 (0.07) −0.27* (0.06)	NE	1	
T1 IP	−0.02 (0.05) −0.01 (0.06)	0.03 (0.09) 0.07 (0.06)	−0.08 (0.07) 0.08 (0.06)	0.02 (0.07) −0.05 (0.07)	−0.02 (0.10) −0.001 (0.06)	0.04 (0.09) −0.04 (0.07)	0.08 (0.07) 0.10 (0.06)	−0.01 (0.10) −0.08 (0.07)	1
T1 EP	−0.01 (0.05) 0.02 (0.06)	−0.09 (0.09) 0.03 (0.06)	−0.03 (0.07) 0.08 (0.06)	0.01 (0.07) 0.02 (0.07)	0.08 (0.10) 0.004 (0.06)	−0.03 (0.08) −0.15* (0.07)	−0.05 (0.07) 0.14* (0.06)	−0.10 (0.09) −0.12** (0.07)	0.64* (0.03) 0.61* (0.04)

Un-standardized beta weights are presented, followed by standard errors in parentheses. Girls' estimates are presented above boys' estimates

X signifies interaction terms created by multiplying variables

NE not estimated, *IP* internalizing problems, *EP* externalizing problems, *T1* the first assessment, *Cort* cortisol, *RSA* respiratory sinus arrhythmia, *PEP* pre-ejection period

*$p<0.05$, **$p<0.10$

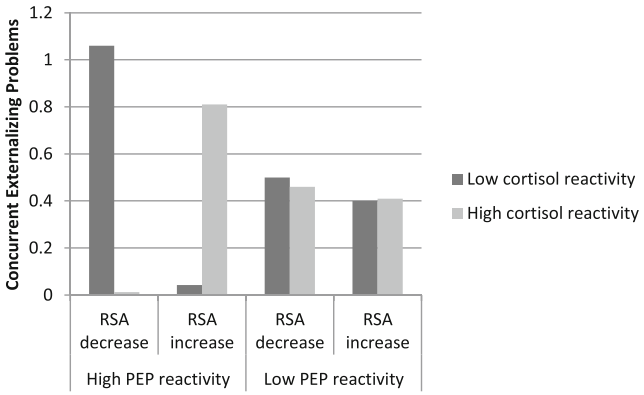

Fig. 1 Three-way interaction predicting boys' and girls' concurrent externalizing problems. Low and high refer to one standard deviation below and above the mean, respectively. Because the mean was near zero for RSA, one standard deviation below and above the mean are referred to as decrease and increase, respectively

was associated with more concurrent internalizing problems in boys, but not girls; 2) low ANS reactivity (HR increases) predicted increases in externalizing problems longitudinally in girls but not boys; 3) high PNS reactivity (RSA decreases) predicted increases in externalizing problems longitudinally. We did not find support for the hypothesis that symmetrically high activation of SNS and HPA would predict internalizing problems. Instead, this pattern of effects was associated with concurrent externalizing problems and was qualified by a three-way interaction: asymmetrical HPA and PNS reactivity predicted concurrent externalizing problems in the context of high SNS reactivity (PEP decreases), but not in the context of low SNS reactivity. We did find some evidence that interactions of SNS and PNS reactivity reflecting symmetrically high or low activation would predict more externalizing problems, but only in boys. Further, we found that asymmetric HPA and PNS activation predicted increased internalizing problems longitudinally in boys but not girls, a novel finding in the literature.

The TRAILS study is particularly well suited for testing these hypotheses, with multiple measures of physiological responses to stress and a range of levels of problem behavior. The theory of allostatic load highlights that there should be dynamic patterns of HPA/ANS activity across the population, regardless of whether individuals have heightened or normative levels of reactivity or problem behavior, but that particular patterns of symmetry/asymmetry may mark risk for (psycho)-pathology. As such, it was important to check whether the effects found here were driven by extreme groups or generalizable to the full sample. Notably, the interaction patterns were apparent whether examining only extreme groups or using more moderate groups to define high vs. low reactivity. Thus, we believe that the findings are not driven by extreme groups alone (although as expected, results were stronger for extreme groups; data available from authors upon request).

Interpretation of the Findings on Internalizing Problems

Regarding internalizing problems, we found that high HR reactivity predicted concurrent internalizing problems in boys, which is in line with other studies that found positive links between internalizing symptoms and HR reactivity (Dufton et al. 2011; Hastings et al. 2007) and with Hastings et al. (2011) who found symmetrically high HPA and HR reactivity was related to internalizing problems but that asymmetrical reactivity (high ANS in combination with low HPA) predicted an increase in internalizing problem. This is consistent with the idea that HR reactivity may serve as a global indicator of arousal and involuntary engagement with the adverse aspects of the stress task (Connor-Smith et al. 2000). It is interesting that HR was one of the few biomarkers to be linked with internalizing symptoms whereas associations for externalizing problems were observed using specific measures of sympathetic and parasympathetic reactivity.

Inclusion of the more specific measures of SNS and PNS activity via PEP and RSA, respectively, allowed us to probe this further. The low correlation between RSA and PEP was surprising. Given that heart rate increased in 95 % of our participants, either the SNS or the PNS must have worked to mount such a consistent heart rate reactivity pattern (Berntson and Cacioppo 2000). This increase in HR may have be driven by the SNS in a majority of participants, given that 88 % showed increases in SNS activation indexed by a decline in PEP during the public speech but only about half of the participants showed PNS withdrawal. In participants showing PNS augmentation (increased RSA), increased HR could be driven by SNS activation or sympathetically dominant coactivation of both autonomic branches (Berntson and Cacioppo 2000). Similarly wide individual differences were found in a prior study on RSA reactivity (El-Sheikh and Hinnant 2011). Or, this HR finding may be driven by the PNS given that HR reactivity was correlated higher with RSA reactivity than with PEP reactivity. Furthermore, we found an interaction between greater cortisol reactivity and PNS augmentation in prediction of rises in internalizing symptoms over time, largely within boys. This interaction is reminiscent of the longitudinal interaction uncovered by Hastings et al. (2011) where asymmetrical reactivity predicted a rise in internalizing problems although our finding is specific to high cortisol reactivity with PNS augmentation. Given that RSA and PEP did not interact in predicting internalizing problems, the HR effects observed are most likely due to RSA and PEP interacting uniquely within individuals, with the SNS driving HR effects within some individuals but the PNS driving HR within others.

We also found that low cortisol reactivity predicted increases in internalizing problems in boys and girls longitudinally. Hastings et al. (2011) found that low cortisol reactivity predicted increased internalizing problems, although this did

Table 6 Parameter estimates for the unconstrained longitudinal model of RSA and PEP

	Age	Cort Resp.	RSA Resp.	PEP Resp.	Cort X RSA	Cort X PEP	RSA X PEP	C X RSA X PEP	T1 IP	T1 EP	T2 IP
Age	1										
Cortisol reactivity	NE	1									
RSA reactivity	NE	0.17* (0.06) 0.18* (0.05)	1								
PEP reactivity	NE	0.18* (0.07) 0.18* (0.06)	NE	1							
Cortisol X RSA	NE	0.30* (0.06) −0.13* (0.05)	0.13* (0.07) 0.20* (0.05)	NE	1						
Cortisol X PEP	NE	0.35* (0.07) 0.22* (0.06)	NE	0.10 (0.08) 0.25* (0.05)	−0.13 (0.08) −0.06 (0.06)	1					
RSA X PEP	NE	NE	0.17* (0.06) 0.09 (0.06)	0.20* (0.05) −0.25* (0.06)	0.30* (0.07) 0.000 (0.06)	NE	1				
C X RSA X PEP	NE	NE	0.32* (0.07) 0.01 (0.06)	NE	0.40* (0.07) 0.29* (0.06)	−0.07 (07) −0.26* (0.06)	NE	1			
T1 IP	NE	NE	NE	NE	NE	NE	NE	NE	1		
T1 EP	NE	NE	NE	NE	NE	NE	NE	NE	0.63* (0.03) 0.62* (0.04)	1	
T2 IP	0.03 (0.05) −0.05 (0.05)	−0.11 (0.08) −0.09** (0.05)	0.03 (0.06) 0.04 (0.05)	0.01 (0.06) −0.09** (0.06)	0.01 (0.08) −0.10* (0.05)	0.08 (0.07) 0.08 (0.06)	0.09 (0.06) −0.05 (0.05)	0.03 (0.08) 0.07 (0.06)	0.52* (0.06) 0.57* (0.05)	−0.02 (0.07) 0.02 (0.06)	1
T2 EP	−0.01 (0.05) −0.02 (0.05)	−0.07 (0.09) −0.02 (0.06)	−0.14* (0.06) 0.04 (0.05)	−0.08 (0.06) −0.08 (0.06)	−0.06 (0.09) 0.07 (0.05)	0.04 (0.08) 0.02 (0.06)	0.07 (0.07) −0.06 (0.05)	0.15** (0.09) −0.12* (0.06)	0.002 (0.06) −0.06 (0.07)	0.39* (0.06) 0.53* (0.06)	0.53* (0.04) 0.45* (0.05)

Un-standardized beta weights are presented, followed by standard errors in parentheses. Girls' estimates are presented above boys' estimates

X signifies interaction terms created by multiplying variables

NE not estimated, *IP* internalizing problems, *EP* externalizing problems, *T1* the first assessment, *T2* the second assessment, *Cort* cortisol, *RSA* respiratory sinus arrhythmia, *PEP* pre-ejection period

*$p<0.05$

**$p<0.10$

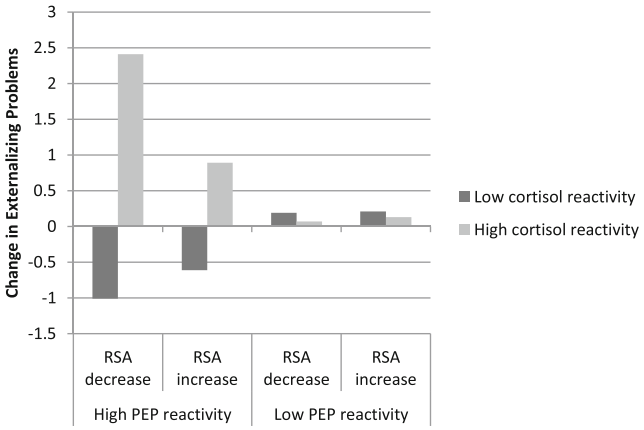

Fig. 2 Three-way interaction between cortisol, RSA, and PEP reactivity predicting changes in boys' externalizing problems. Low and high refer to one standard deviation below and above the mean, respectively. Because the mean was near zero for RSA, one standard deviation below and above the mean are referred to as decrease and increase, respectively

not reach significance without taking the interaction with the ANS into account. Low cortisol has predicted increased mental health problems, but not specifically in normative populations (Dorn et al. 1993; McCool and Susman 1994; Ouellet-Morin et al. 2011) or for internalizing symptoms (Burke et al. 2005; Essex et al. 2011; Shirtcliff and Essex 2008).

Interpretation of the Findings on Externalizing Problems

As with other studies (Hastings et al. 2011; Gordis et al. 2006), interactions between the separate branches of the ANS and the HPA-axis were more consistent regarding externalizing problems as opposed to internalizing symptoms although these symptom types are highly comorbid. Two patterns emerged of interest. We found that youth exhibiting low HPA reactivity (low cortisol changes or even decreases) along with increased autonomic activation characterized by both PNS withdrawal

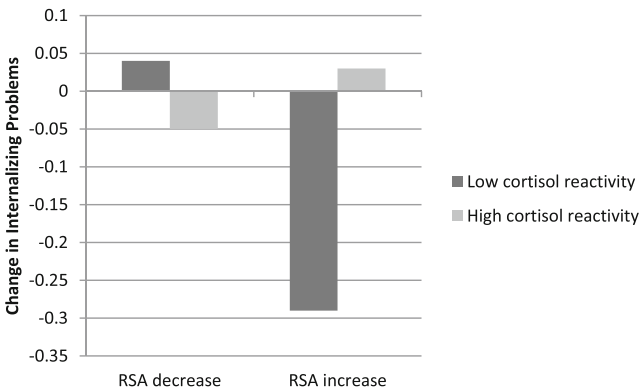

Fig. 3 Interaction between cortisol and RSA reactivity predicting changes in internalizing problems in boys. Low and high refer to one standard deviation below and above the mean, respectively. Because the mean was near zero for RSA, one standard deviation below and above the mean are referred to as decrease and increase, respectively

(RSA decreases) and SNS activation (i.e., PEP decreases) had the most concurrent externalizing problems. These findings do not fit with Gordis et al. (2006) finding of symmetrical reactivity predicting externalizing problems, but do fit at face value with Hastings et al. (2011) who found asymmetrical reactivity was related to concurrent externalizing problems. This first profile seems consistent with theories of hypo-arousal (Phillips 2011; Rottenberg et al. 2002), especially those that predict that mild hypo-arousal is not especially problematic (Del Giudice, et al. 2011), or is likely related to adolescent-limited externalizing behavior or sensation-seeking traits (Sijtsema et al. 2010). That is, the ANS is a low-threshold, fast component of the stress response whereas the HPA axis is much slower to respond. A profile of mild autonomic reactivity, especially efficient parasympathetic withdrawal, may be an effective strategy for maintaining physiological stability without mounting a full HPA response. Indeed, parasympathetic withdrawal has been described as adaptive when HPA nonresponse is observed (Gunnar et al. 2009; Taylor et al. 2008). Individuals who are capable of mainly using this physiological system might have to minimally activate other physiological systems, which might be advantageous in the long-run. In support of this interpretation, these boys with asymmetrical activation (low HPA-axis reactivity and autonomic activation characterized by both PNS withdrawal and short PEP) showed striking decreases in externalizing behavior over time, ending the study with some of the lowest externalizing symptoms by the time youth in this study were aged 19. Again, this is consistent with the notion that the concurrent symptoms we observed were adolescent-limited as symptoms did not persist into emerging adulthood and also with Hastings et al. (2011) who did not find this profile to be problematic over time.

The second profile of interest was the finding that youth with high HPA-reactivity along with PNS augmentation but SNS activation (i.e., low PEP) had the most concurrent externalizing problems as well as high externalizing problems over time from age 16 to age 19 years, at least within boys. Boys showing high HPA-axis reactivity, PNS augmentation and high SNS activation showed largest increases in externalizing problems. Translating these results to symmetric or asymmetric reactivity is not easy given that HPA, SNS and PNS were differentially implicated, with SNS activation observed (i.e., PEP decline) along with PNS augmentation rather than the PNS withdrawal typically expected during a stressor. It is unclear whether symmetrical or asymmetrical findings were observed given that, within the ANS, both autonomic branches diverged in their prediction of externalizing symptoms (Berntson and Cacioppo 2000). That is, findings are consistent with the asymmetrical reactivity observed by Hastings et al. (2011) if HR was driven by RSA, but is consistent with the symmetrical reactivity observed by Gordis et al. (2006) if HR was driven by the SNS (as might

be expected with the SNS biomarker of alpha-amylase used by Gordis et al. 2006).

To understand these effects, we focus on understanding PNS activity. We view it unlikely that the task failed to mobilize the PNS or withdrawal the vagal brake as the stressor successfully crossed the stress threshold for the other stress components. Furthermore, while we expected that RSA would decline as evidence of PNS withdrawal during a challenge, PNS augmentation was common (observed within about half of the participants) which may indicate that participants were actively disengaged from the task and were suppressing their thoughts and emotions about the task (Gillie et al. 2014). Thus, this profile might be interpreted as the most stressed boys (high HPA-axis reactivity in combination with PNS augmentation and SNS arousal) having externalizing problems, suggestive of vigilant or reactive aggression and externalizing symptoms (Frick and Shirtcliff 2014). High perceived arousal and unpleasantness have indeed shown to be associated with high physiological responsivity during the social stress test (Oldehinkel et al. 2011). Furthermore, these boys showed increases in externalizing behaviors from age 16 to age 19, hinting toward increasing long-term problems linked with high HPA-axis reactivity in combination with PNS withdrawal and high SNS reactivity. In sum, findings for externalizing symptoms are tentatively consistent with the symmetrical activation observed by Gordis et al. (2006) and extend this vigilant pattern across both concurrent and longitudinal externalizing problems in adolescents.

In conclusion, little support was found for the interaction between HPA-axis and ANS reactivity for concurrent levels or longitudinal changes in mental health when the ANS was conceptualized as an integrated, unidirectional biomarker. When the branches of the ANS were disentangled through RSA and PEP, however, dynamic interactions were apparent with the HPA axis. We found evidence for such interactions, pointing in the direction of both symmetrical and asymmetrical activations being linked with mental health problems depending on type and timing. Our results indicate that while reactivity of one system might suggest active coping, the others can simultaneously show signs of distress. Clear and simple replication of prior empirical studies was not apparent, but rather the study extends the contentions of prior research (Hastings et al. 2011; Gordis et al. 2006) and theory (Bauer et al. 2002; Beauchaine 2001) including allostatic load (McEwen 1998) across three distinct stress-responsive biomarkers: HPA, SNS and PNS. The inconsistencies may point to the underlying complexities of the concept of arousal or stress or health risk.

Acknowledgments This research is part of the TRacking Adolescents' Individual Lives Survey (TRAILS). Participating centers of TRAILS include various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen, and the Parnassia Bavo group, all in the Netherlands. TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 452-04-314 and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005; NWO Longitudinal Survey and Panel Funding 481-08-013), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), Biobanking and Biomolecular Resources Research Infrastructure BBMRI-NL (CP 32), the participating universities, and Accare Center for Child and Adolescent Psychiatry. We are grateful to all adolescents, their parents and teachers who participated in this research and to everyone who worked on this project and made it possible.

Conflicts of Interest None of the authors report any conflicts of interest.

References

Achenbach, T. M., & Rescorla, L. (2001). *ASEBA school-Age forms & profiles*. Burlington: Aseba.

Achenbach, T. M., Dumenci, L., & Rescorla, L. A. (2003). DSM-oriented and empirically based approaches to constructing scales from the same item pools. *Journal of Clinical Child and Adolescent Psychology, 32*, 328–340.

Alink, L. R. A., van Ijzendoorn, M. H., Bakermans-Kranenburg, M. J., Mesman, J., Juffer, F., & Koot, H. M. (2008). Cortisol and externalizing behavior in children and adolescents: mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Developmental Psychobiology, 50*, 427–450. doi:10.1002/dev.20300.

Alkon, A., Goldstein, L. H., Smider, N., Essex, M. J., Kupfer, D. J., & Boyce, W. T. (2003). Developmental and contextual influences on autonomic reactivity in young children. *Developmental Psychobiology, 42*, 64–78.

Allwood, M. A., Handwerger, K., Kivlighan, K. T., Granger, D. A., & Stroud, L. R. (2011). Direct and moderating links of salivary alpha-amylase and cortisol stress-reactivity to youth behavioral and emotional adjustment. *Biological Psychology, 88*, 57–64.

Andrews, J., D'Aguiar, C., & Pruessner, J. C. (2012). The combined dexamethasone/TSST paradigm–a new method for psychoneuroendocrinology. *PLoS ONE, 7*, e38994. doi:10.1371/journal.pone.0038994.

Angold, A., Costello, E. J., & Erkanli, A. (1999). Comorbidity. *Journal of Child Psychology and Psychiatry, 40*, 57–87.

Bauer, A. M., Quas, J. A., & Boyce, W. T. (2002). Associations between physiological reactivity and children's behavior: advantages of a multisystem approach. *Journal of Developmental and Behavioral Pediatrics, 23*, 102–113.

Beauchaine, T. (2001). Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, 13*, 183–214.

Berntson, G. G., & Cacioppo, J. T. (2000). Psychobiology and social psychology: past, present, and future. *Personality and Social Psychology Review, 4*, 3–15. doi:10.1207/s15327957pspr0401_2.

Berntson, G. G., & Cacioppo, J. T. (2007). *Integrative physiology: homeostasis, allostasis, and the orchestration of systemic*

physiology. Handbook of psychophysiology (p. 433). New York: Cambridge University Press.

Bouma, E., Riese, H., Ormel, J., Verhulst, F. C., & Oldehinkel, A. J. (2009). Adolescents' cortisol responses to awakening and social stress; effects of gender, menstrual phase and oral contraceptives. The TRAILS study. *Psychoneuroendocrinology, 34*, 884–893.

Burke, J. D., Loeber, R., Lahey, B. B., & Rathouz, P. J. (2005). Developmental transitions among affective and behavioral disorders in adolescent boys. *Journal of Child Psychology and Psychiatry, 46*, 1200–1210.

Bush, N. R., Alkon, A., Obradovic, J., Stamperdahl, J., & Boyce, W. T. (2011a). Differentiating challenge reactivity from psychomotor activity in studies of children's psychophysiology: considerations for theory and measurement. *Journal of Experimental Child Psychology, 110*, 62–79.

Bush, N. R., Obradovic, J., Adler, N., & Boyce, W. T. (2011b). Kindergarten stressors and cumulative adrenocortical activation: the "first straws" of allostatic load? *Development and Psychopathology, 23*, 1089–1106.

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., & Singer, D. H. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation, 93*, 1043–1065.

Cicchetti, D. (2011). Allostatic load. *Development and Psychopathology, 23*, 723–724.

Connor-Smith, J. K., Compas, B. E., Wadsworth, M. E., Thomsen, A. H., & Saltzman, H. (2000). Responses to stress in adolescence: measurement of coping and involuntary stress responses. *Journal of Consulting and Clinical Psychology, 68*, 976–992.

De Winter, A. F., Oldehinkel, A. J., Veenstra, R., Brunnekreef, J. A., Verhulst, F. C., & Ormel, J. (2005). Evaluation of non-response bias in mental health determinants and outcomes in a large sample of pre-adolescents. *European Journal of Epidemiology, 20*, 173–181.

Del Giudice, M., Ellis, B. J., & Shirtcliff, E. A. (2011). The adaptive calibration model of stress responsivity. *Neuroscience & Biobehavioral Reviews, 35*, 1562–1592. doi:10.1016/j.neubiorev.2010.11.007.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*, 355–391.

Dietrich, A., Riese, H., Sondeijker, F. E., Greaves-Lord, K., van Roon, A. R. I. E., Ormel, J., & Rosmalen, J. G. (2007). Externalizing and internalizing problems in relation to autonomic function: a population-based study in preadolescents. *Journal of the American Academy of Child and Adolescent Psychiatry, 46*, 378–386.

Dorn, L. D., Susman, E. J., & Petersen, A. C. (1993). Cortisol reactivity and anxiety and depression in pregnant adolescents: a longitudinal perspective. *Psychoneuroendocrinology, 18*, 219–239.

Dufton, L. M., Dunn, M. J., Slosky, L. S., & Compas, B. E. (2011). Self-reported and laboratory-based responses to stress in children with recurrent pain and anxiety. *Journal of Pediatric Psychology, 36*, 95–105. doi:10.1093/jpepsy/jsq070.

El-Sheikh, M., & Hinnant, J. B. (2011). Marital conflict, respiratory sinus arrhythmia, and allostatic load: interrelations and associations with the development of children's externalizing behavior. *Development and Psychopathology, 23*, 815–829. doi:10.1017/S0954579411000320.

El-Sheikh, M., & Whitson, S. A. (2006). Longitudinal relations between marital conflict and child adjustment: vagal regulation as a protective factor. *Journal of Family Psychology, 20*, 30–39. doi:10.1037/0893-3200.20.1.30.

El-Sheikh, M., Harger, J., & Whitson, S. M. (2001). Exposure to interparental conflict and children's adjustment and physical health: The moderating role of vagal tone. *Child Development, 72*, 1617–1636.

El-Sheikh, M., Erath, S. A., Buckhalt, J. A., Granger, D. A., & Mize, J. (2008). Cortisol and children's adjustment: the moderating role of sympathetic nervous system activity. *Journal of Abnormal Child Psychology, 36*, 601–611.

El-Sheikh, M., Kouros, C. D., Erath, S., Cummings, E. M., Keller, P., & Staton, L. (2009). Marital conflict and children's externalizing behavior: interactions between parasympathetic and sympathetic nervous system activity. *Monographs of the Society for Research in Child Development, 74*(1), 1–78.

Essex, M. J., Armstrong, J. M., Burk, L. R., Goldsmith, H. H., & Boyce, W. T. (2011). Biological sensitivity to context moderates the effects of the early teacher-child relationship on the development of mental health by adolescence. *Development and Psychopathology, 23*, 149–161.

Frick, P.J., Shirtcliff, E.A. (2014). Children at risk for serious conduct problems. In M. Cima (Ed.), *Forensic psychopathology and treatment*. London: Routledge, in press.

Gentzler, A. L., Santucci, A. K., Kovacs, M., & Fox, N. A. (2009). Respiratory sinus arrhythmia reactivity predicts emotion regulation and depressive symptoms in at-risk and control children. *Biological Psychology, 82*, 156–163. doi:10.1016/j.biopsycho.2009.07.002.

Gillie, B. L., Vasey, M. W., & Thayer, J. F. (2014). Heart rate variability predicts control over memory retrieval. *Psychological Science, 25*, 458–465. doi:10.1177/0956797613508789.

Gordis, E. B., Granger, D. A., Susman, E. J., & Trickett, P. K. (2006). Asymmetry between salivary cortisol and α-amylase reactivity to stress: relation to aggressive behavior in adolescents. *Psychoneuroendocrinology, 31*, 976–987.

Gordis, E. B., Feres, N., Olezeski, C. L., Rabkin, A. N., & Trickett, P. K. (2010). Skin conductance reactivity and respiratory sinus arrhythmia among maltreated and comparison youth: relations with aggressive behavior. *Journal of Pediatric Psychology, 35*, 547–558. doi:10.1093/jpepsy/jsp113.

Gunnar, M. R., Talge, N. M., & Herrera, A. (2009). Stressor paradigms in developmental studies: what does and does not work to produce mean increases in salivary cortisol. *Psychoneuroendocrinology, 34*, 953–967.

Hastings, P. D., Zahn-Waxler, C., & Usher, B. A. (2007). Cardiovascular and affective responses to social stress in adolescents with internalizing and externalizing problems. *International Journal of Behavioral Development, 31*, 77–87. doi:10.1177/0165025407073575.

Hastings, P. D., Sullivan, C., McShane, K. E., Coplan, R. J., Utendale, W. T., & Vyncke, J. D. (2008a). Parental socialization, vagal regulation, and preschoolers' anxious difficulties: direct mothers and moderated fathers. *Child Development, 79*, 45–64.

Hastings, P. D., Nuselovici, J. N., Utendale, W. T., Coutya, J., McShane, K. E., & Sullivan, C. (2008b). Applying the polyvagal theory to children's emotion regulation: social context, socialization, and adjustment. *Biological Psychology, 79*, 299–306. doi:10.1016/j.biopsycho.2008.07.005.

Hastings, P. D., Shirtcliff, E. A., Klimes-Dougan, B., Allison, A. L., Derose, L., Kendziora, K. T., & Zahn-Waxler, C. (2011). Allostasis and the development of internalizing and externalizing problems: changing relations with physiological systems across adolescence. *Development and Psychopathology, 23*, 1149–1165.

Huisman, M., Oldehinkel, A. J., de Winter, A., Minderaa, R. B., de Bildt, A., Huizink, A. C., & Ormel, J. (2008). Cohort profile: the Dutch 'tacking Adolescents' individual lives survey'; TRAILS. *International Journal of Epidemiology, 37*, 1227–1235.

Juster, R. P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience & Biobehavioral Reviews, 35*, 2–16.

Keller, P. S., & El-Sheikh, M. (2009). Salivary alpha-amylase as a longitudinal predictor of children's externalizing symptoms:

respiratory sinus arrhythmia as a moderator of effects. *Psychoneuroendocrinology, 34*, 633–643. doi:10.1016/j.psyneuen.2008.12.016.

Kirschbaum, C., Read, G.F., & Hellhammer, D. (1992). *Assessment of hormones and drugs in saliva in biobehavioral research* (p. 356). Seattle, Toronto, Bern, Göttingen: Hogrefe & Huber.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'–A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*, 76–81.

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Flugge, G., Korte, S. M., & Fuchs, E. (2011). Stress revisited: a critical evaluation of the stress concept. *Neuroscience and Biobehavioral Review, 35*, 1291–1301. doi:10.1016/j.neubiorev.2011.02.003.

McCool, W. F., & Susman, E. J. (1994). Cortisol reactivity and self-report anxiety in the antepartum: predictors of maternal intrapartal outcomes in gravid adolescents. *Journal of Psychosomatic Obstetrics and Gynecology, 15*, 9–18.

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine, 338*, 171–179.

McEwen, B. S. (2005). Stressed or stressed out: what is the difference? *Journal of Psychiatry & Neuroscience, 30*, 315–318.

Mulder, L. J. M., Van Dellen, H. J., Van der Meulen, P., & Opheikens, B. (1988). *CARSPAN: a spectral analysis program for cardiovascular time series. Computers in psychology: methods, instrumentation and psychodiagnostics* (pp. 39–47). Lisse: Swets and Zeitlinger.

Nederhof, E., Jörg, F., Raven, D., Veenstra, R., Verhulst, F. C., Ormel, J., & Oldehinkel, A. J. (2012). Benefits of extensive recruitment effort persist during follow-ups and are consistent across age group and survey method. The TRAILS study. *BMC Medical Research Methodology, 12*, 93.

Oldehinkel, A. J., Hartman, C. A., De Winter, A. F., Veenstra, R., & Ormel, J. (2004). Temperament profiles associated with internalizing and externalizing problems in preadolescence. *Development and Psychopathology, 16*, 421–440.

Oldehinkel, A. J., Ormel, J., Bosch, N. M., Bouma, E. M. C., Van Roon, A. M., Rosmalen, J. G. M., & Riese, H. (2011). Stressed out? Associations between perceived and physiological stress response in adolescents: The TRAILS study. *Psychophysiology, 48*, 441–452.

Ouellet-Morin, I., Odgers, C. L., Danese, A., Bowes, L., Shakoor, S., Papadopoulos, A. S., & Arseneault, L. (2011). Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children. *Biological Psychiatry, 70*, 1016–1023.

Peters, A., & McEwen, B. S. (2012). Introduction for the allostatic load special issue. *Physiology and Behavior, 106*, 1–4. doi:10.1016/j.physbeh.2011.12.019.

Phillips, A. C. (2011). Blunted cardiovascular reactivity relates to depression, obesity, and self-reported health. *Biological Psychology, 86*, 106–113. doi:10.1016/j.biopsycho.2010.03.016.

Robbe, H. W., Mulder, L. J., Rüddel, H., Langewitz, W. A., Veldman, J. B., & Mulder, G. (1987). Assessment of baroreceptor reflex sensitivity by means of spectral analysis. *Hypertension, 10*, 538–543.

Rottenberg, J., Kasch, K. L., Gross, J. J., & Gotlib, I. H. (2002). Sadness and amusement reactivity differentially predict concurrent and prospective functioning in major depressive disorder. *Emotion, 2*, 135–146.

Ruttle, P. L., Shirtcliff, E. A., Serbin, L. A., Ben-Dat Fisher, D., Stack, D. M., & Schwartzman, A. E. (2011). Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviors in youth: Longitudinal and concurrent associations with cortisol. *Hormones and Behavior, 59*, 123–132. doi:10.1016/j.yhbeh.2010.10.015.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*, 55–89.

Schumacher, S., Kirschbaum, C., Fydrich, T., & Ströhle, A. (2013). Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders? A review of preliminary findings and the interactions with cortisol. *Psychoneuroendocrinology, 38*, 729–743.

Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology, 50*, 690–703. doi:10.1002/dev.20336.

Shirtcliff, E. A., Peres, J. C., Dismukes, A. R., Lee, Y., & Phan, J. M. (2014). Hormones: commentary. Riding the physiological roller coaster: adaptive significance of cortisol stress reactivity to social contexts. *Journal of Personality Disorders, 28*, 40–51. doi:10.1521/pedi.2014.28.1.40.

Sijtsema, J. J., Veenstra, R., Lindenberg, S., van Roon, A. M., Verhulst, F. C., Ormel, J., & Riese, H. (2010). Mediation of sensation seeking and behavioral inhibition on the relationship between heart rate and antisocial behavior: The TRAILS study. *Journal of the American Academy of Child and Adolescent Psychiatry, 49*, 493–502.

Sijtsema, J. J., Nederhof, E., Veenstra, R., Ormel, J., Oldehinkel, A. J., & Ellis, B. J. (2013). Effects of family cohesion and heart rate reactivity on aggressive/rule-breaking behavior and prosocial behavior in adolescence: the tracking Adolescents' individual lives survey study. *Development and Psychopathology, 25*, 699–712.

Taylor, S. E., Burklund, L. J., Eisenberger, N. I., Lehman, B. J., Hilmert, C. J., & Lieberman, M. D. (2008). Neural bases of moderation of cortisol stress responses by psychosocial resources. *Journal of Personality and Social Psychology, 95*, 197–211.

Verhulst, F. C., Van der Ende, J., & Koot, H. M. (1997). *Manual for the youth self report (YSR)*. Rotterdam, the Netherlands, Erasmus University, Department of Child and Adolescent Psychiatry, Sophia Children's Hospital.