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Refining the multisystem view of the stress response: Coordination among cortisol, alpha-amylase, and subjective stress in response to relationship conflict

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Abstract

This study investigated associations among young adults' hypothalamic-pituitary-adrenal axis activity, autonomic nervous system activity, and subjective stress in response to interpersonal conflict to better characterize coordination across stress systems. Seven saliva samples were collected from 199 young adult opposite-sex couples before, during, and after they discussed an unresolved relationship conflict. Samples were later assayed for cortisol and alpha-amylase (sAA). Couples rated anticipatory stress prior to the conflict and perceived stress immediately following the task. Growth curve modeling was used to examine two possible levels of within-person coordination across physiological systems: alignment between cortisol and sAA responses throughout the sampling period ("matched phase coordination"), and association between overall levels of cortisol and sAA in response to conflict ("average level coordination"). Whereas both partners showed the former type of coordination, only women showed the latter type. Positive anticipation of the stressor predicted stronger cortisol-sAA matched phase coordination for women. Pre-task ratings related to women's sAA, and post-task ratings related to both partners' cortisol responses. Implications for a multisystem interpretation of normal and pathological responses to daily stress are discussed.

Keywords

cortisol; alpha-amylase; HPA axis; ANS; stress; couples

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1. Introduction

Multiple psychophysiological systems are involved in perceiving, reacting to, and recovering from threat and challenge (Weiner, 1992). Specialized responses from different systems enable more finely calibrated management of stress than reliance on a single system but depend on well-tuned coordination between systems, as well as between psychological and physiological states. Stress research has typically focused on the activation of individual subsystems—in particular, either the hypothalamic-pituitary adrenal (HPA) axis or the sympathetic branch of the autonomic nervous system (ANS)—during a standardized physical or psychological challenge task. This leaves an incomplete picture of how multiple stress response systems work together within individuals, particularly in the context of naturalistic interpersonal stressors encountered in daily life (Granger et al., 2012; Powers, 2011). During adolescence and early adulthood, sensitivity to interpersonal stressors, particularly among females, increases (Stroud, Salovey, & Epel, 2002; Stroud et al., 2009). Given that miscalibrated responses to interpersonal threat are thought to underlie mental health risk emerging during this period (e.g., Fernandez-Guasti et al., 2012; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Powers et al., 2006), it is important to understand how young adults typically respond to such stressors, and how these responses do or do not map onto subjective stress.

The present study was designed to further a multisystem view of the stress response and its implications for well-being by answering the following questions in a community sample of dating couples: (1) Are young adults' cortisol (HPA marker) and salivary alpha-amylase (sAA; ANS marker) trajectories in response to interpersonal stress coordinated across systems? In what way/s does this coordination occur? and (2) How does subjective stress going into and following the stressor relate to cortisol and sAA response trajectories, as well as the degree of within-person cortisol-sAA coordination? Answering these questions promises to both advance basic research on the nature of the stress response and inform stress interventions aimed at young individuals or couples.

1.1 Stress System Specialization and Connections

The two main components of the physiological stress response are the HPA axis and the sympathetic branch of the ANS (Chrousos & Gold, 1992). The ANS is designed to produce a rapid response, preparing the body to actively cope with a stressor through effects on the cardiovascular and respiratory systems and the release of stored catecholamines. The HPA system activates a slower cascade of secretory signals that culminate in the release of cortisol from the adrenal gland, adapting the body to stress conditions by inhibiting non-emergency vegetative processes such as sleep, sexual activity, and growth (Weiner, 1992). While both respond to psychosocial stress, the meaning of response within each system may differ; ANS activity is thought to be less valenced and reflective of both approach- and withdrawal-related arousal, whereas HPA activity is more specifically associated with negative affect and withdrawal (e.g., Buss, Davidson, Kalin, & Goldsmith, 2004; Nigg, 2006). In line with this view, ANS measures have been associated with an “effort” or “challenge” component of stress, and HPA measures with a “distress” component (Lundberg & Frankenhauser, 1980).

Salivary cortisol offers a noninvasive measure of HPA activity (Hellhammer, Wüst, & Kudielka, 2009), and sAA, an enzyme produced in response to activation of ANS innervation of the salivary glands, is used as a surrogate marker of autonomic activity (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Nater & Rohleder, 2009). The HPA and ANS are interrelated at the neural level, allowing each system to potentially influence the response of the other (Boyce & Ellis, 2005; Bremner & Vermetten, 2001; Sapolsky, Romero, & Munck, 2000; van Stegeren et al., 2007). Functional relations between the two systems remain under debate, with arguments existing for permissive, suppressive, stimulating, as well as preparative effects (Munck, Guyre, & Holbrook, 1984; Sapolsky, et al., 2000).

There have been numerous calls for studies to empirically examine coordination of these systems within the same individuals, particularly when responding to psychological stressors encountered in daily life (Bauer, Quas, & Boyce, 2002; Donzella, Gunnar, Krueger, & Alwin, 2000; Granger & Kivlighan, 2003; Granger et al., 2006; Spinrad et al., 2009; van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Salivary measures of both HPA and ANS activity promise a minimally invasive method of stress system assessment that is unlikely to generate additional stress. However, information on the nature of response across systems remains sparse and is often limited by restricted measurement and analysis of response trajectories.

1.2 Intra-individual Coordination of HPA and ANS Responses

Very few studies have examined coordination between HPA and ANS responses, and the means of operationalizing such coordination has remained fairly simplistic—typically, correlations between post-stress cortisol and sAA levels or pre-post stress difference scores. We propose two possible forms of coordination (see Figure 1 for illustrations). “Average level coordination” (Figure 1A and 1C) occurs when the overall strength of HPA activation, reflected by an individual's mean cortisol across stress phases (including pre-stress anticipation, stress-related reactivity, and recovery), predicts the level of his/her sAA response trajectory. This could attest to a balance of threat and challenge reactions to stress, based on parallel calibration of the systems as a whole and/or uniform sensitivity to a particular type of stressor. Interestingly, previous research has generally shown nonsignificant correlations between cortisol and sAA in adults (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Nater et al., 2006; Stroud et al., 2009; van Stegeren, et al., 2006) and in children (Granger, et al., 2006), although a few studies have found significant correlations when sub-groups based on sex (Kivlighan & Granger, 2006) or maltreatment status (Gordis, Granger, Susman, & Trickett, 2008) were analyzed. Single time-point correlations, as opposed to measures of association involving the entire response trajectory may also have contributed to null findings.

“Matched phase coordination” occurs when changes in HPA activation across different phases of the stress response are aligned with changes in ANS activation (Figures 1B and 1C). In other words, a person's relative increases in cortisol from one time point to the next predict increases in sAA, and response trajectories unfold in a parallel fashion (see Laurent, Ablow, & Measelle, 2011, for further explanation of cross-system coordination). This may

offer a more situational measure of how threat reactions (indexed by HPA activity) trigger effort/challenge reactions (indexed by ANS activity) to confront the stressor within a given stress episode. It should be noted that whereas peak ANS response is reflected in sAA within a few minutes, there is an approximately fifteen to twenty minute lag between peak cortisol release in the blood and salivary measures (Schlotz et al., 2008). This means that cortisol-sAA associations in saliva samples likely represent a lagged effect of earlier HPA on later ANS response. A study testing both forward- and backward-lagged effects in an all-male sample yielded mixed evidence for positive vs. negative associations between cortisol and sAA (Engert et al., 2011). The only previous study of matched phase coordination as operationalized here found positive cortisol-sAA associations only among non-depressed women and their infants (Laurent et al., 2011). Given this and other evidence that discordance between HPA and ANS responses may signal mental health problems (Ali & Pruessner, 2012), a key task at this point is to determine how these systems are functionally related in real-life stress contexts and what predicts coordination vs. discordance.

One potentially important predictor is subjective stress preceding and following the stressor. Stress appraisals have been shown to mediate effects of self-perceived stress reactivity on actual physiological response, and their modification may help to explain impacts of cognitive behavioral stress management on cortisol reactivity (Gaab et al., 2003; Schlotz, Hammerfald, Ehlert, & Gaab, 2011). Recent conceptualizations of stress adaptation point to a constellation of response across physiology and behavior that may offer greater insight into adjustment than measuring either domain on its own (Towe-Goodman, Stifter, Mills-Koonce, & Granger, 2012). To fully appreciate the conditions for response within and across systems, and ultimately to make recommendations for stress regulation, the links between anticipatory/perceived stress and physiological responses must be clarified.

1.3 Subjective Stress and Physiological Responses

Past research offers evidence for variable coupling between subjective stress and stress physiology. For example, a number of studies have related anticipatory appraisals of high threat and/or low coping ability to higher cortisol responses to psychosocial stress (Gaab, Rohleder, Nater, & Ehlert, 2005; Juster, Perna, Marin, Sindi, & Lupien, 2012; Wirtz et al., 2007), though studies in adolescents have either shown no effect of pre-stress ratings (Oldehinkel et al., 2011) or related low coping appraisals to *lower* cortisol responses (Slattery, Grieve, Ames, Armstrong, & Essex, 2012). Research has also linked anticipatory stress appraisals to markers of ANS response (i.e., blood pressure; Juster et al., 2012), yet other researchers showed no effect on ANS markers (i.e., norepinephrine and epinephrine; Wirtz et al., 2007). Similarly, appraisals during and following stress have shown inconsistent associations with physiology. Several studies have linked perceived stress to HPA and/or ANS responses (Al'Absi et al., 1997; Oldehinkel et al., 2011; Robles et al., 2011), but others found no effect of post-stress appraisals or pre-post change in stress ratings (Engert et al., 2012; Gaab et al., 2005). Although most of these studies investigated effects on stress reactivity, some showed effects on post-stress recovery (Juster et al., 2012; Schlotz et al., 2011).

Some of these differences may have to do with variable response measurement—i.e., post-stress HPA/ANS activity levels vs. change from pre-post stress vs. change from post-stress to recovery. Few researchers have addressed the entire response trajectory, though a more comprehensive investigation of subjective stress and cortisol across multiple timepoints revealed significant correlations between the two either contemporaneously (in a pharmacological challenge paradigm) or after a five-minute lag (in a psychosocial stress task)(Schlotz et al., 2008). There are also indications that the type of stressor makes a difference, with more interpersonally threatening tasks—i.e., public speaking—eliciting stronger psychological-physiological response associations (Al'Absi et al., 1997).

Together, these studies show that the quality of stress anticipation and perceptions may be decisive for HPA and ANS responses, but a clearer understanding of which cognitive processes predict which aspects of response requires a multisystem investigation of physiological stress trajectories. It is important to determine which cognitions underlie HPA and ANS response components, given that the degree of reactivity and/or recovery in each relates uniquely to mental health. In particular, blunted sAA responses and high cortisol levels/incomplete recovery following stress have been associated with depression and related risk factors (Burke, Davis, Otte, & Mohr, 2005; McGirr et al., 2010; Robles et al., 2011). As suggested by Burke and colleagues' (2005) review, the ability to recover efficiently after a stressor has terminated may be even more important than cortisol levels themselves in determining well-being. Much of this work has been based on responses to standard pharmacological challenge or performance tasks (i.e., the Trier Social Stress Test), which reliably elicit stress responses but may not offer a complete picture of the processes actually contributing to mental health.

1.4 The Case for Studying Responses to Interpersonal Stress

In childhood and adolescence, the ANS and HPA systems respond differently to interpersonal vs. performance tasks (Stroud, et al., 2009), and certain aspects of psychosocial adjustment (i.e., attachment styles) have been associated with HPA responses to interpersonal conflict but not to performance stress (Ditzen et al., 2008). Moreover, developmental increases in the sensitivity of stress responses are stronger for interpersonal than for performance tasks (Stroud, et al., 2009). This increased sensitivity to interpersonal stress parallels increases in developmental risks for depression, anxiety, and other disorders associated with stress dysregulation.

To begin to understand the basis for such disorders, it is important to examine young adults' regulation of stress systems as they respond to the interpersonal conflicts that naturally arise within close relationships. We are aware of no published studies that assess both cortisol and sAA responses to interpersonal stress within a close adult relationship, and of only three studies of parent-child relationships (Laurent et al., 2011, 2012; Granger et al., 2006). Thus, research on normative adolescent/young adult couples' HPA and ANS responses to interpersonal relationship stress is urgently needed to recognize the basis for emergent mental health vulnerabilities and inform intervention targets.

1.5 The Current Study

In this study, we set out to advance a multisystem conceptualization of the stress response by investigating links among cortisol and sAA trajectories and subjective stress in the context of a common interpersonal stressor. To this end, a community sample of young adult dating couples was recruited for a laboratory interaction study involving the discussion and attempted resolution of a relationship conflict (see Kiecolt-Glaser et al., 1997, Kiecolt-Glaser & Newton, 2001 for further background on and validation of the stress task). Saliva samples collected before and after the conflict were used to measure HPA (via cortisol) and ANS (via sAA) responses from pre-task anticipation through post-task recovery phases. To address the primary study questions posed above, we used multilevel growth curve modeling to test average level and matched phase coordination across cortisol and sAA response trajectories, as well as effects of pre- and post-task subjective stress ratings on cortisol and sAA trajectories and coordination between the two. On the basis of prior research, we hypothesized that stress responses would be coordinated across systems within individuals, particularly for those who gave more positive/less negative ratings before and after the conflict. We further hypothesized that negative anticipation of the conflict and perceived stressfulness and negativity post-conflict would predict heightened cortisol levels following stress but less dynamic reactivity/recovery, and lower sAA levels.

2. Material and Methods

2.1 Participants

Participants included 199 young adult heterosexual couples (total of 398) who had been involved in a relationship for at least 2 months (median length: 10-12 months). The final sample for analyses was 196 couples (after the removal of extreme cortisol outliers). Ages ranged from 18 to 21 ($M = 19$ years, $SD = 9$ months), and the ethnic distribution was representative of the New England community from which the sample was drawn. See Table 1 for further description of the sample. Participants were recruited through flyers, posters, and presentations in undergraduate courses and either paid \$80 or given research credit points toward their final grade. All human subjects procedures were approved by the University of Massachusetts Institutional Review Board.

2.2 Procedure and Subjective Stress Measurement

All procedures took place in a two-room suite in our university laboratory at 4:00 pm, a time of day when cortisol and sAA levels are relatively stable in their diurnal rhythms (Lovallo, Farag, & Vincent, 2010; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007), increasing the probability that any shifts were due to the experimental stress task. Couples were unaware of the conflict task when they entered the lab and gave their first saliva sample. A second saliva sample was collected following a vivid description of the conflict task, timed so as to measure anticipatory stress (see Engert et al., 2012). Each partner identified a topic that had been a source of heated and unresolved conflict in the romantic relationship during the past month. Immediately before the conflict discussion, participants rated on a 5-point scale how much they were (a) looking forward to, and (b) nervous about the upcoming task (Powers, Pietromonaco, Gunlicks, & Sayer, 2006). The researcher randomly selected one of the topics by flipping a coin and the couple was taken to an adjoining room, seated on a

couch, and asked to spend 15 minutes discussing the issue and attempting to resolve the problem. Researchers were not present during the conflict task, though it was videorecorded. After the task was completed, participants rated on a 9-point scale how (a) stressful, and (b) negative it had been (Powers et al., 2006). Five additional saliva samples were collected at regular intervals during an hour-long recovery period.

2.3 Salivary Analyte Determination

Seven salivary samples were collected over the course of the session to measure stress reactivity to and recovery from the conflict discussion task. Given the dynamic differences between cortisol and sAA, with the former taking longer to show peak response in saliva, the interpretation of each sample point differs depending on which biomarker is under discussion. The T1 sample (collected soon after arrival at the lab) measured participants' HPA activity shortly before arriving and ANS activity upon entry to the lab. The T2 sample, collected 15 minutes following a vivid description of the conflict task and directly before the discussion, measured HPA activation in anticipation of the task and more immediate pre-conflict anticipatory stress in the ANS. Ten minutes after participants completed the conflict discussion, the T3 sample was collected; this represented HPA activation during the conflict itself and ANS activity post-conflict. Finally, T4-T7 samples were collected at 10, 20, 35, and 50 minutes following the T3 sample to measure the course of both HPA and ANS recovery after the conflict.

Whole unstimulated saliva samples were collected by passive drool. Specimens were sealed in cryogenic vials and immediately placed in frozen storage (-20°C) until shipped on dry ice to Penn State for analysis. All samples were divided into two aliquots and separately assayed for salivary cortisol and sAA. On the day of testing, all samples were centrifuged at 3000 rpm for 15 minutes to remove mucins.

Following Granger and colleagues (2007), samples were assayed for cortisol by enzyme immunoassay (Salimetrics, State College, PA). The test uses 25 μl of saliva, has a lower limit of sensitivity .007 $\mu\text{g/dl}$, range .007 - 3.0 $\mu\text{g/dl}$, and average intra- and inter-assay coefficients of variation less than 5 and 10 percent. Samples were assayed for sAA by kinetic reaction assay (Salimetrics, State College PA). The assay employs a chromagenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. Intra-assay variation computed for the mean of 30 replicate tests was less than 7.5 percent. Inter-assay variation computed for the mean of average duplicates for 16 separate runs was less than 6 percent.

2.4 Control Variables

Several procedures safeguarded the accuracy of cortisol and sAA measurements. Researchers gave participants written and phone instructions to refrain from drinking alcohol, using illegal drugs, or visiting the dentist within the 24-hour period prior to the laboratory session, and they were required to not exercise, eat, drink (except water), smoke cigarettes, or brush their teeth up to 2 hours prior to participation. Upon arrival at the lab, if participants had an elevated temperature, felt ill, or reported they had been unable to comply with the restrictions above, they were scheduled to return at a later date. Participants rinsed their mouths thoroughly with water 10 minutes before giving the first saliva sample to

minimize potential contamination by food residue. In addition to these procedural controls, other variables that potentially affect HPA/ANS functioning—i.e., medications and other substance use, amount of sleep, recent meals and exercise, illness, and menstrual phase—were assessed by questionnaires and examined as statistical controls. Blood contamination, which can falsely elevate analyte levels, was measured in the first saliva sample for use as a control. Given mixed evidence for the influence of salivary flow rate on sAA measures (i.e., Beltzer et al., 2010; Rohleder, Wolf, Maldonado, & Kirschbaum, 2006), sample flow rate (volume/time) was considered as a covariate in sAA analyses. Table 1 offers further information about questionnaire-based control variables.

2.5 Analytic Strategy

The data under investigation are dependent (i.e., cortisol and sAA scores over time clustered within an individual; individuals' scores clustered within a couple). Therefore, a multilevel modeling strategy—dyadic growth curve modeling as outlined by Raudenbush and colleagues (1995)—was selected to account for the interdependence of partners' cortisol and sAA trajectories throughout the sampling period. Level 1 modeled within-couple variation in men's and women's stress markers over time with partner-specific growth parameters that were allowed to vary across couples. At Level 2, between-couple variability in these growth parameters could be explained by characteristics of the couple (including characteristics of each partner).

Hierarchical linear modeling (HLM; Raudenbush & Bryk, 2002) was first used to fit unconditional models of cortisol and sAA that included no explanatory predictors, providing a description of partners' average HPA and ANS response trajectories. Next, intra-individual HPA-ANS coordination was tested with models in which each partner's cortisol predicted his/her sAA trajectory. At Level 1, cortisol scores were time-varying covariates indicating the extent to which changes in the partner's cortisol levels paralleled changes in his/her sAA from one time point to the next (matched phase coordination). At Level 2, a time-invariant effect indicated the extent to which the partner's mean cortisol level predicted his/her mean sAA level across the session (average level coordination). Cortisol scores at Level 1 were centered on the participant's own mean, whereas at Level 2 they were centered on the grand mean for men or women. Finally, each partner's subjective stress measures—pre-task or post-task ratings—were added as Level 2 predictors explaining his/her HPA and ANS response trajectories and HPA-ANS coordination.

Growth curve models were centered at the T3 sample and included partner-specific intercepts (representing conflict-related cortisol or post-conflict sAA levels), slopes (the instantaneous rate of change of the curve at T3), quadratic terms (the acceleration/deceleration describing the rate of change in slope), and (for sAA only) cubic terms (describing the overall curvature of the trajectory's changing acceleration/deceleration rate). Whereas intercepts reflected levels of HPA/ANS activation following stress, the other terms offered insight into the dynamics of reactivity and recovery across the entire session. Examples of the multilevel models explaining (a) HPA response, and (b) HPA-ANS coordination are given below:

Post-Task Ratings Predict HPA Response

Level 1

Cortisol = male [$\beta_1 + \beta_2$ (linear slope) + β_3 (quadratic)] + female [$\beta_4 + \beta_5$ (linear slope) + β_6 (quadratic)] + error

Level 2

$\beta_1 = \gamma_{10} + \gamma_{11}$ (male stressful) + γ_{12} (male negative) + error

$\beta_2 = \gamma_{20} + \gamma_{21}$ (male stressful) + γ_{22} (male negative) + error

$\beta_3 = \gamma_{30} + \gamma_{31}$ (male stressful) + γ_{32} (male negative) + error

(similar equations explain β_4 -6, but with female partner ratings)

Post-Task Ratings Predict HPA-ANS Coordination

Level 1

sAA = male [$\beta_1 + \beta_2$ (cortisol)] + female [$\beta_3 + \beta_4$ (cortisol)] + error

Level 2

$\beta_1 = \gamma_{10} + \gamma_{11}$ (mean male cortisol) + γ_{12} (male stressful) + γ_{13} (male negative) + error

$\beta_2 = \gamma_{20} + \gamma_{21}$ (male stressful) + γ_{22} (male negative) + error

(similar equations explain β_3 -4, but with female partner cortisol and stress ratings)

To summarize, HLM models were set up to examine (a) average male and female partner cortisol and sAA trajectories across the session, (b) matched phase and average level coordination of partners' cortisol and sAA trajectories, and (c) individual differences in both (a) and (b) related to partners' anticipatory and post-task ratings of the conflict stressor.

3. Results

3.1 Transformations and Controls

Because cortisol and sAA distributions were skewed at each time point (i.e., positive skew due to the spread between fewer high values and a majority of low-moderate values), a natural log transformation was applied as recommended by Howell (2007), and the transformed scores were used in the following analyses. Variables significantly related to cortisol and sAA ($p < .05$) were included in unconditional models reported below, as well as in all explanatory models. These consisted of blood contamination (for men's and women's cortisol, as well as men's sAA), allergy medication (for men's cortisol and sAA), and antibiotic medication (for men's and women's sAA). Salivary flow rate also related significantly to sAA levels and was retained in analyses. None of the other control variables tested—i.e., demographics (participant age, ethnicity), relationship characteristics (status and length, satisfaction), other questionnaire variables outlined in section 2.4—were significant and thus were excluded from further analyses.

3.2 Descriptives

3.2.1 Subjective stress ratings—On average, partners indicated moderate positive (looking forward to) and negative (nervous about) anticipation of the task (M 's = 2.33 - 2.49

on a 5-point scale, SD 's = 1.00 - 1.08). Women reporting greater positive anticipation tended to report less negative anticipation ($r = -.28, p < .05$), but there was no such association for men ($r = .05, ns$). Post-task, partners reported that the conflict was moderately stressful (M 's = 4.17 - 4.20 on a 9-point scale, SD 's = 2.25 - 2.40), and somewhat less negative (M 's = 3.28 - 3.56 on a 9-point scale, SD 's = 1.80 - 1.62). Partners who perceived the conflict as more stressful also tended to perceive it as more negative (r 's = .39 - .48, $p < .05$); at the same time, the amount of variance in one rating dimension explained by the other (< 25%) suggested these remained separable constructs.

3.2.2 Average cortisol and sAA response trajectories—Paired samples t -tests were used to assess whether sample-wide cortisol and sAA levels changed significantly from one time point to the next, indicating reactivity (a rise associated with the conflict) and recovery (a decline following the conflict). Cortisol reactivity occurred mainly from the entry sample (T1) to anticipation (T2), $t(379) = -6.77, p < .001$, with recovery beginning in the interim from the anticipation to discussion task (T3) sample, $t(386) = 3.85, p < .001$, and continuing from the discussion to the first recovery sample (T4), $t(387) = 5.89, p < .001$ and beyond (T4-5 $t[387] = 6.91, p < .001$; T5-6 $t[389] = 6.38, p < .001$; T6-7 $t[389] = 4.47, p = .06$).

Couples showed sAA reactivity from the entry (T1) to post-discussion (T3) sample, $t(370) = -2.12, p = .03$, though the incremental increases from T1-2 and T2-3 failed to reach significance. Couples showed a significant decline in sAA from the post-discussion (T3) to first recovery sample (T4), $t(388) = 6.84, p < .001$ that continued from T4-5, $t(388) = 4.33, p < .001$. The change from T5-6 was nonsignificant, and from T6-7 there was a marginal increase in sAA, $t(390) = -1.92, p = .06$.

In keeping with the above, the best-fitting HLM model for cortisol trajectories was a quadratic function, with levels rising to a peak and then falling across the session in an expected reactivity/recovery pattern. The best-fitting model for sAA trajectories was a cubic function, with a prominent initial peak and recovery, followed by a tendency to rise at the end of the sampling period. Tentative explanations for this final rise include sAA resuming its diurnal pattern and/or couples' beginning preparation for leaving the laboratory. Whereas partners' cortisol levels related to one another over the course of the session, as indexed by the tau correlation ($\tau = .33$), partners' sAA levels were unrelated ($\tau = -.06$). This suggests cross-partner influences on cortisol (but not sAA) responses as measured in this context.

3.3 Cortisol-sAA Response Coordination

As described above, cortisol was entered as a predictor of sAA to assess both matched phase (Level 1) and average level (Level 2) coordination. The Level 1 cortisol-sAA association was significant for both men and women ($b = .20$ for men and $.31$ for women, $p < .01$), suggesting that earlier HPA activation reflected in cortisol consistently predicted subsequent ANS activity measured by sAA in the same saliva sample. At Level 2, women's (but not men's) mean cortisol predicted sAA levels throughout the sampling period ($b = .24, p < .05$ for women; $b = .06, ns$ for men). This means that women with higher cortisol also had higher sAA, whereas no such association existed for men. Figure 2 shows predicted men's and women's cortisol and sAA trajectories (expressed as Z-scores to allow a common scale).

3.4 Subjective Stress Effects

3.4.1 Pre-task ratings—Women's positive anticipation of the conflict task predicted higher sAA levels and stronger matched phase coordination with cortisol throughout the session (see Table 2, left panel). The model offered a significant improvement in fit compared to the baseline model, as demonstrated by change in the deviance statistic, $\chi^2(10) = 21.06, p < .05$. There were no effects of pre-task ratings on cortisol or on men's physiological responses.

3.4.2 Post-task ratings—For both partners, perceived stressfulness predicted higher conflict-related cortisol levels, and for men it also predicted steeper reactivity/recovery curves (see Table 3, right panel, and Figure 3). Perceived negativity predicted lower conflict-related cortisol levels (significant effect for women, trend for men) and flatter reactivity/recovery curves (significant effect for men, trend for women; see Figure 4). Finally, perceived negativity predicted a more positive sAA slope following the conflict for men; this meant that men who rated the conflict as more negative tended to recover less following the task (see Table 4, right panel). Whereas the explanatory model for cortisol yielded a significant improvement in fit over baseline, $\chi^2(12) = 22.83, p < .05$, the model for sAA did not, $\chi^2(16) = 15.19, ns$. There were no significant effects of post-task ratings on women's sAA.

4. Discussion

This investigation advances a multisystem characterization of the stress response by specifying links between HPA and ANS responses, and between physiology and subjective stress, in the context of a common interpersonal stressor. Findings from this study extend previous work in several ways. First, we found that both young men and women showed matched phase coordination—i.e., changes in cortisol predicted changes in sAA across the response trajectory—but only women showed average level coordination—i.e., similar magnitudes of cortisol and sAA response—across systems. Second, the quality of stress anticipation predicted cortisol-sAA coordination, whereas perceptions in reaction to the stressor itself predicted cortisol response trajectories. Finally, we found distinct effects of positive anticipation of conflict, perceived stressfulness and negativity that may help to understand the basis for mood-related physiological dysregulation. We elaborate on these points in the following sections.

Growth curve modeling revealed a synchronization of cortisol and sAA responses—matched phase coordination—for both men and women, but linked absolute levels of cortisol and sAA—average level coordination—for women only. These findings support the view that HPA and ANS stress response systems are connected in a positive or potentiating fashion, at least in response to the intimate interpersonal stressor used in this study. The nature of this connection appears to differ by sex, though, with more evidence of an overarching systems association in women. This fits with previous research showing stronger cortisol-sAA correlations in women (Kivlighan & Granger, 2006). It may be that the HPA and ANS systems are tuned in a more parallel fashion across development for women, making the overall sensitivity and output of these systems similar. An alternate explanation is that women's responses are closely calibrated specifically when confronting an interpersonal

stressor such as this one. This would be consistent with previous research highlighting the importance of interpersonal, as opposed to achievement, stress for young women (Stroud et al., 2002). More studies of men's and women's average level coordination in different types of stress contexts are needed to explore this idea and its implications.

Sex-independent temporal cortisol-sAA coordination (matched phase coordination) suggests an alignment of acute reactivity and recovery processes to a particular stressor is more broadly normative, and misalignment may represent a problematic response. Combined with earlier research showing matched phase coordination among non-depressed, but not depressed, women and their infants (Laurent et al., 2011), the current finding that women reporting more positive stress anticipation showed stronger cortisol-sAA coordination lends preliminary support to this idea. It may be that positive appraisals allowed active challenge reactions to more closely support threat reactions to the stressor. Further work in varied samples will be needed to determine if this benefit is specific to women, or extends to men, as well. Another question to be addressed in future research is whether the final rise we observed in sAA can be explained by cross-system effects; this could represent a delayed ANS response following earlier HPA activation, enhanced ANS sensitivity to incoming stimuli following HPA recovery, or some combination of stimulating and preparative effects (Sapolsky et al., 2000).

Differing effects of pre-stress anticipation and post-stress perceptions may offer further insight into how each phase of cognitive processing impacts physiological regulation. More positive (but not necessarily less negative) anticipation of the conflict discussion predicted sAA levels and coordination with the cortisol response, at least for women. On the other hand, perceptions of how stressful and negative the conflict actually was related most consistently to cortisol responses. The latter finding is consistent with previous reports of associations between perceived stress and magnitude of HPA response (e.g., Al'Absi et al., 1997; Oldehinkel et al., 2011). It may be that stress anticipation contributes more to ANS coordination with HPA activity, and post-stress perceptions better explain individual differences in HPA responses. An alternative explanation is that the specific rating dimensions used post-stress map better onto the threat appraisals commonly associated with HPA activity. Future studies should more systematically examine effects of different appraisal dimensions preceding and following stress on men's and women's response patterns within and across physiological systems.

Positive associations between perceived stressfulness of the conflict discussion and cortisol levels measured after the conflict offer further validation for this stress paradigm, and differing effects of stressfulness vs. negativity suggest these should be considered separately to understand stress dysregulation. Whereas stressfulness related to higher cortisol levels, negativity related to less dynamic reactivity/recovery curves. Each of these patterns has been implicated in depression, and this study offers a potential explanation for how each arises: a propensity to perceive conflict as stressful appears to promote HPA hyperactivity, and negatively biased perceptions appear to blunt HPA recovery. On the other side, more positive cognitions approaching conflict appear to promote sAA activation, which has also been associated with lower depressive symptoms. These patterns are consistent with the distinction between negative affect and threat-related HPA activation on the one hand, and

affectively neutral or even positive challenge-related ANS activation on the other. Though preliminary, these findings have implications for stress management interventions. Building a more positive approach to interpersonal conflict could help buffer young women from depression, and modifying perceptions of the conflict itself could benefit both men and women.

Several design limitations in the present study may have obscured further relations among psychophysiological stress measures. The saliva sampling times were originally dictated by the expected course of cortisol reactivity and recovery, and not sAA. Thus, there was no immediate post-task measure, and peak ANS response to the conflict discussion may have been missed. In addition, the subjective stress measures were fairly limited, comprising single-item ratings that could not cover all potentially important dimensions of stress anticipation and perception. Given these limitations, it is notable that we were still able to detect effects on sAA response and a variety of theoretically consistent pre- and post-task rating effects. Future studies should follow up on and expand these findings with more comprehensive physiological and subjective stress sampling techniques.

Conclusions about normative responses to interpersonal stress and adjustment-relevant differences should also be tempered by an acknowledgment of sample limitations. This study focused on young dating couples, and it is not possible to know whether the stress response patterns we identified might be different for older couples in longer relationships, or for individuals not in a romantic relationship. There are reasons to believe that discussion of interpersonal conflict could be more or less stressful for young dating couples as opposed to older marital couples (i.e., Cunningham, Braiker, & Kelley, 1982; Florsheim, Moore, & Edgington, 2003; Larson, Clore, & Wood, 1999), and further cross-sectional and/or longitudinal comparisons are needed to determine which is the case. Our sample comprised opposite-sex, mostly European-American dyads from a community sample. Further research is needed to judge the generalizability of these findings and applications to clinical mood disorders by examining physiological responses to interpersonal conflict in more diverse samples.

Despite these limitations, several aspects of the design of the study deserve comment. First, this study defined and assessed multiple types of stress response coordination, providing further insight into how these systems may be functionally related. Matched phase and average level coordination highlight the potential importance of both acute moment-to-moment links between HPA and ANS activity and overall linking of systemic response magnitudes, with subjective stress-related differences in the former pointing to its adaptive value. Second, this study examined cortisol and sAA trajectories in response to an understudied psychological stress event: real-life, unresolved conflicts that occur within the context of a close relationship. Given identified developmental differences in response to interpersonal vs. performance stress, it is important to delineate both normative responses to each type of stressor and cognition-related differences, and to begin to match these with health outcomes of interest. This work helps to broaden the lens for future research on normative and pathological responses to daily stress not as a single measure, but rather as a constellation of more or less integrated components.

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Highlights

- We investigated coordination of HPA and ANS responses to an interpersonal stressor.
- Responses were aligned across systems over time (matched phase coordination).
- Women's levels of HPA and ANS activation were related (average level coordination).
- Positive pre-task anticipation predicted stronger HPA-ANS coordination for women.
- Post-task stress appraisals related to HPA, but not ANS, response trajectories.

Figure 1.

Proposed types of cross-system response coordination. *Note.* Panel A shows average level (but not matched phase) coordination; panel B shows matched phase (but not average level) coordination; panel C shows both types of coordination; panel D shows neither type of coordination.

Figure 2.
Predicted men's and women's cortisol and sAA trajectories.

Figure 3. Perceived conflict stressfulness predicts men's (top) and women's (bottom) cortisol trajectories, shown at “low” (25th percentile) and “high” (75th percentile) ratings.

Figure 4. Perceived conflict negativity predicts men's (top) and women's (bottom) cortisol trajectories, shown at “low” (25th percentile) and “high” (75th percentile) ratings.

Table 1
Sample Characteristics

Couple Variables	Percent of Sample or <i>M, SD</i>
Relationship Length	
2-6 months	26%
6-12 months	20%
1-2 years	36%
> 2 years	18%
Relationship Status	
Dating (non-exclusive)	4%
Dating (exclusive)	48%
Committed long-term relationship	47%
Engaged	1%
Individual Variables – Male, Female Partner	
Relationship Satisfaction ^a	17.66, 3.39; 18.28, 2.93
Ethnicity	
Caucasian	84%, 90%
Asian/Asian American	6%, 4%
Latino/a	5%, 4%
African American	2%, 1%
Other	3%, 1%
Medication Use	
Antibiotics	1%, 5%
OTC Pain Medicine	8%, 11%
Cold Medicine	1%, 4%
Allergy Medicine	4%, 2%
Asthma Medicine	7%, 3%
Birth Control Pills	0%, 65%
Cigarette Smoker	9%, 6%
Alcohol Use (past 24 hours)	6%, 1%
Drug Use (past 24 hours)	2%, 0%
Sleep (past 24 hours)	8.09, 1.55; 8.15, 1.56

^aPerceived Relationship Quality Components Satisfaction; Fletcher, Simpson, & Thomas, 2000.

Table 2
Predictive Models for Partners' Cortisol-sAA Coordination

Outcome	<i>Pre-Task Ratings</i>		<i>Post-Task Ratings</i>	
	Looking forward	Nervous	Stressful	Negative
	Estimate, SE	Estimate, SE	Estimate, SE	Estimate, SE
Men				
sAA Intercept	-.06, .06	-.05, .06	-.006, .03	.03, .04
Cortisol	.007, .03	-.009, .03	.001, .01	-.008, .02
(matched phase effect)				
Women				
sAA Intercept	.14, .06	-.03, .06	-.008, .03	.02, .04
Cortisol	.13, .04	.03, .04	-.02, .02	.05, .03
(matched phase effect)				

Note. Both cortisol and sAA scores have been natural log-transformed prior to analysis. Significant effects ($p < .05$) highlighted in bold.

Table 3
Predictive Models for Partners' Cortisol Trajectories

Outcome	<i>Pre-Task Ratings</i>		<i>Post-Task Ratings</i>	
	Looking forward	Nervous	Stressful	Negative
	Estimate, <i>SE</i>	Estimate, <i>SE</i>	Estimate, <i>SE</i>	Estimate, <i>SE</i>
Men				
Intercept	-.04, .04	.04, .04	.05, .02	-.04, .03
Slope	-.009, .02	-.02, .02	.005, .01	-.007, .01
Quadratic	.02, .03	-.02, .03	-.04, .02	.05, .02
Women				
Intercept	-.02, .04	.05, .05	.05, .02	-.09, .03
Slope	.008, .02	-.02, .02	.01, .009	-.02, .01
Quadratic	.04, .03	.006, .03	-.01, .01	.03, .02

Note. Cortisol scores have been natural log-transformed prior to analysis. Significant effects ($p < .05$) highlighted in bold. Trends ($p < .10$) italicized.

Table 4
Predictive Models for Partners' sAA Trajectories

Outcome	<i>Pre-Task Ratings</i>		<i>Post-Task Ratings</i>	
	Looking forward	Nervous	Stressful	Negative
	Estimate, <i>SE</i>	Estimate, <i>SE</i>	Estimate, <i>SE</i>	Estimate, <i>SE</i>
Men				
Intercept	-.08, .06	-.02, .06	.01, .03	.03, .04
Slope	.006, .06	-.05, .06	-.01, .02	.08, .03
Quadratic	.07, .04	-.04, .04	-.03, .02	.005, .03
Cubic	-.04, .09	.10, .09	.02, .04	-.11, .06
Women				
Intercept	.05, .05	-.02, .05	.03, .03	-.05, .04
Slope	-.02, .06	-.03, .05	-.008, .03	.02, .03
Quadratic	-.08, .05	.02, .05	-.04, .02	.02, .03
Cubic	.03, .10	.04, .09	.04, .05	-.05, .06

Note. sAA scores have been natural log-transformed prior to analysis. Significant effect ($p < .05$) highlighted in bold. Trends ($p < .10$) italicized.