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Coordination of Cortisol Response to Social Evaluative Threat with Autonomic and Inflammatory Responses is Moderated by Stress Appraisals and Affect

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Abstract

Recent approaches to stress regulation have emphasized coordination among multiple biological systems. This study builds on evidence that hypothalamic-pituitary-adrenal (HPA) axis activity should be considered in coordination with other stress-sensitive biological systems to characterize healthy responses. Healthy African-Americans (n=115) completed the Trier Social Stress Test, and biological responses were assessed through salivary cortisol, dehydroepiandrosterone-sulfate (DHEA-S), alpha amylase (sAA), and C-reactive protein (sCRP). Multilevel modeling demonstrated that cortisol responses typically aligned with changes in DHEA-S, sAA, and sCRP across the session. At the same time, the degree of cortisol coordination with sAA and sCRP varied by participants' subjective stress following the task; participants with higher secondary stress appraisals showed greater cortisol-sAA alignment, whereas those experiencing more negative affect showed greater cortisol-sCRP alignment. Results highlight the importance of a multisystem approach to stress and suggest that positive HPA axis coordination with the autonomic response, but not with the immune/inflammatory response, may be adaptive.

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Keywords

stress; coordination; cortisol; alpha-amylase; C-reactive protein; DHEA-S; Trier Social Stress Test

Advancing knowledge of individual differences in the reactivity and regulation of environmentally sensitive biological systems is critical to understanding how the social environment impacts health and human development. Multiple biological systems involved in the stress response—i.e., the hypothalamic-pituitary-adrenal (HPA) axis, the autonomic nervous system (ANS), and immune/inflammatory systems—were identified by pioneers in stress research (see Weiner, 1992 for a review), but it is only recently that technological advances have enabled the noninvasive measurement of these systems in the context of everyday life. Researchers assessing multiple systems in response to social challenge have begun to appreciate ways in which different components of the stress response may mitigate or enhance one another (e.g., Gordis, Granger, Susman, & Trickett, 2006; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007), and most recently attention to the *coordination* among environmentally sensitive biological systems has further expanded the concept of adaptive reactivity and regulation (Laurent, Powers, & Granger, 2013; Lucas et al., under review).

Contemporary theorists now speculate that change in any one system, studied individually, may offer an incomplete picture of risk/resilience processes, and it is through the study of coordinated change across systems that insights into paths from adversity exposure to negative mental and physical health outcomes will be revealed (e.g., Bauer, Quas, & Boyce, 2002; Hastings et al., 2011). As used here, “coordination” refers to the linkage of activation across biological systems, which may be present in the sense of similar absolute levels of activation (*average level coordination*) and/or alignment of increases and decreases in activation over time (*matched phase coordination*; see Laurent, Ablow, & Measelle, 2011 for further explanation and illustration). Typically, we expect coordinated systems to be positively associated, though they may also show negative associations (inverse coordination). Subjective stress—represented both by cognitive appraisals of stress and affective states—can further provide an indication of whether a given coordination pattern characterizes successful coping, and previous research has demonstrated distinct subjective stress correlates of different forms of coordination (Laurent et al., 2013). The present study was designed to advance a multisystem conceptualization of the biology of the stress response by addressing the following questions in a sample of healthy adults exposed to the Trier Social Stress Test (TSST), a standard laboratory based social evaluative threat task (Kirschbaum, Pirke, & Hellhammer, 1993): (a) Are cortisol responses coordinated in time with other adrenal, autonomic, and immune/inflammatory responses? and (b) Does the degree of such coordination vary according to a person’s subjective experience of stress (i.e., self-reported stress appraisals and affect following stress)?

Biological Systems Involved in the Stress Response

One of the major systems driving the mobilization of biological resources to manage threat is the neuroendocrine cascade of the HPA axis, which itself comprises different components

thought to balance one another. The release of cortisol inhibits non-emergency processes such as sleep, sexual activity, and growth; while useful in the short term, sustained cortisol elevations pose a risk to the integrity of central and peripheral tissues (Weiner, 1992). Dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are cosecreted with cortisol by the adrenal gland during stress and exerts neuroprotective effects via antiglucocorticoid action (Kimonides, Spillantini, Sofroniew, Fawcett, & Herbert, 1999; Taylor, 2012). There is evidence that the DHEA/-S response to acute psychosocial stress is correlated with the cortisol response, suggesting normative coordination of these adrenal outputs (Izawa et al., 2008; Lennartsson et al., 2012).

Beyond the HPA axis, the most well recognized stress response system is the autonomic nervous system, which complements the slower-reacting HPA axis with a rapid shift from energy-conserving to energy-expending processes needed to actively cope with stress. The two systems are connected at multiple physiological levels that enable a variety of permissive, stimulatory, and suppressive effects (Sapolsky, Romero, & Munck, 2000). Although previous studies testing correlations between salivary alpha-amylase (sAA), a marker of autonomic activation, and cortisol during psychosocial stress have typically failed to support an association (e.g., Nater et al., 2006; Stroud et al., 2009), research designs examining sAA and cortisol responses to stress over time (instead of at individual sampling points) have revealed positive coordination across systems (Engert et al., 2011; Laurent et al., 2013).

Another issue requiring further consideration is how HPA axis activity relates to immune/inflammatory aspects of the stress response. Inflammatory cytokines stimulate the release of C-reactive protein (CRP), a well-known inflammatory marker implicated in the development of atherosclorosis (Pearson et al., 2003). There is mixed evidence for whether blood and/or salivary CRP responds to acute stress (see Black, 2003; Campisi, Bravo, Cole, & Gobeil, 2012; Veldhuijzen van Zanten, Ring, Carroll, & Kitas, 2005), with inconsistent findings likely driven in part by a delayed time course of response (see review by Slavish, Graham-Engeland, Smyth, & Engeland, 2015). Whereas some research examining both cortisol and CRP during stress suggests parallel responses (Kennedy, Cryan, Quigley, Dinan, & Clarke, 2014), other research demonstrates a divergence (e.g., cortisol but no CRP response—Pilger et al., 2014; inverse association between cortisol and CRP responses—Nijm, Kristenson, Olsson, & Jonasson, 2009).

The majority of studies cited above either assessed relations between absolute levels of activation across systems or (less commonly) the relation between changes in each system over time, but not both simultaneously. Elsewhere, we have articulated the need to consider both average level and matched phase coordination to determine the nature of linkages across stress systems (Laurent et al., 2013). To our knowledge, no previous research has investigated such multilevel coordination across HPA axis, ANS, and immune/inflammatory systems in response to stress (though see Lucas et al., under review, for a related approach).

Relations with Subjective Stress

Beyond establishing which systems typically coordinate with one another during acute psychosocial stress, it is important to understand whether such coordination aids in or detracts from regulation. There are different ways of defining a "regulated" stress response, but one yardstick is a person's subjective sense of stress as indexed by stress appraisals and affect following stress exposure, as well as longer-term well-being. As laid out in the Transactional Model of Stress and Coping (Lazarus & Folkman, 1987), the stressfulness of an event is determined by both a primary appraisal of how difficult or threatening the stressor is and a secondary appraisal of how well equipped one feels to cope with or control it; according to this model, the person-environment transaction characterized by high primary and low secondary appraisals makes an event particularly stressful. While perceived stress often fails to show a direct association with physiological stress levels (see Campbell & Ehlert, 2012; McLeod, Hoehn-Saric, & Stefan, 1986), there is reason to believe that subjective and physiological stress can be related in certain contexts and/or when different time delays are considered (Schlotz et al., 2008). Furthermore, researchers have found an effect of anticipatory (but not retrospective) stress appraisals specifically on the cortisol response to the TSST (Gaab, Rohleder, Nater, & Ehlert, 2005). However, these studies have typically examined only one stress marker at a time, and more nuanced investigations of subjective stress in relation to multisystem associations over time are needed.

Previous research suggests that a divergence of cortisol from DHEA-S responses is problematic, with higher relative amounts of cortisol predicting greater acute negative mood during stress and longer lasting depression (e.g., Izawa et al., 2008; Young, Gallagher, & Porter, 2002). To date however, such relationships have only been suggested using cortisol/DHEA-S ratios, which do not assess the potential for coordinated responses to vary over the course of a stressful episode. Similarly, the coordination of cortisol with sAA responses may mark good psychological functioning; whereas positive anticipation of a stressor predicted stronger cortisol-sAA associations across a stress session, such associations were absent in depressed mothers and their infants exposed to a laboratory stressor (Laurent, Ablow, & Measelle, 2011; Laurent et al., 2013). Finally, inadequate control of inflammation by the HPA axis—evidenced by a more positive association between cortisol and CRP during stress—is thought to give rise to health problems (i.e., cardiovascular disease; Nijm & Jonasson, 2009). With the exception of the sAA research, these studies have not addressed temporal coordination across systems, and none of this work has simultaneously examined all three stress systems. Thus, it is currently unknown how coordination among these systems during acute stress relates to psychological well-being.

The present study was designed to investigate the proposal that cortisol forms part of a coordinated multisystem stress response by exploring relations with additional stress markers during a standardized performance stressor. The Trier Social Stress Test has been shown to activate multiple stress-responsive systems—not only the HPA axis, but also the sympathetic nervous system and immune system (see review by Allen, Kennedy, Cryan, Dinan, & Clarke, 2014)—and thus offers an ideal backdrop against which to study multisystem stress response coordination. Other investigations using this dataset have focused on multisystem coordination during discrete phases of the stress response and

associations with perceived discrimination and racial identity (Lucas et al., under review), and how different aspects of the stress response relate to fit between justice beliefs and experienced injustice (Lucas et al., 2016). By contrast, the current study examines different levels of pairwise coordination among stress systems (i.e., within-person matched phase coordination and between-person differences in average level coordination) and probes the consequences of such coordination by relating individual differences in temporal coordination to post-stress appraisals and affect. Based on the research outlined above, we hypothesized that cortisol responses would typically show positive coordination with other adrenal and autonomic stress markers (DHEA-S, sAA) but not with inflammation (sCRP), and that the strength of cross-system alignment would vary across people. Participants reporting more positive states—i.e., lower primary stress appraisals/negative affect and higher secondary stress appraisals/positive affect—were expected to show greater cortisol alignment with DHEA-S and sAA, but less alignment (or inverse alignment) with sCRP, across the stress session.

Method

Participants

One hundred and eighteen African-Americans were recruited from metropolitan Detroit using posted and online advertisements. An online prescreen was used to determine eligibility, with the following exclusion criteria: (1) use of medications that would interfere with salivary stress response measurement, and (2) pre-existing medical or psychiatric condition that would make it inadvisable to undergo the stress induction. Of the eligible participants who enrolled in the study, three were excluded due to missing prescreen data, resulting in a final sample of 115 participants (35 male). Participants ranged in age from 18 to 63 (M = 31.51; SD = 13.74). Further information about the sample is provided in Table 1. All participants received modest financial compensation for participating in a single laboratory session lasting approximately three hours.

Task Procedure

The Trier Social Stress Test (TSST) was used to induce mild psychosocial stress and associated physiological responses (Kirschbaum et al., 1993). All stress induction sessions were conducted between 10:00 and 14:00 h (with the majority [66.1%] starting between 11:00 and 13:00 h) using two adjacent testing rooms in a laboratory located centrally on the campus of Wayne State University. Immediately following study consenting procedures, participants were given 10 minutes to acclimate. The remaining TSST protocol was then employed and included a task description phase, a 10-minute speech preparation period, and a 10-minute performance (5-minute speech and 5-minute arithmetic task) given in front of a 2-person panel (one male and one female). [1] Participants were allotted a 1 hour recovery

[1]This study employed two social behavior experimental manipulations that were administered just prior to the fourth saliva collection timepoint. One variation led participants to believe that their individual performance during the TSST was judged to be either satisfactory or unsatisfactory by a speech expert. A second variation called for a laboratory assistant to treat participants either politely or slightly impolitely just prior to the post-task recovery portion of the session. These manipulations were ancillary to the current interest in coordination of cortisol with other stress markers. To ensure that reported results were not affected, we also conducted analyses that covaried for experimental manipulations. The subsequently reported results and associated statistical significance did not meaningfully differ when these experimental manipulations were included.

period following task performance, during which time they completed a series of post task questionnaires, in addition to providing salivary samples.[2] Upon completion of the one hour recovery period, participants were debriefed and thanked for their participation.

Saliva Collection and Preparation

A total of six salivary samples were collected from each participant. An initial sample was collected following the 10 minute acclimation period. The stress task was then introduced to participants, fidelity checks were administered, and they were given time to prepare their speech (approximately 35-minute interval). The second and third samples were collected immediately before and after the TSST performance. Samples 4 through 6 were collected during the recovery period 15, 30 and 60 minutes after task completion, respectively. Participants were provided with 2.5ml of water upon arrival to the laboratory, as well as after each salivary sample collection. To ensure the fidelity of samples, participants were asked to refrain from consuming food, caffeine, citric drinks and dairy, and to avoid vigorous exercise or brushing teeth in the 30 minutes prior to saliva collection and to report adherence to these guidelines (Granger et al., 2007). Participants donated 2 mls whole saliva by passive drool at each timepoint. Saliva samples were divided into approximately 1 mL aliquots to minimize the impact of multiple freeze thaw cycles on the salivary analyte data. Aliquoted samples were stored at −80 C until they were shipped frozen by overnight delivery to Salimetrics laboratories (State College, PA) where they were again stored at 80 C until the day of assay.

Measures

Cortisol—Saliva samples were assayed in duplicate to determine cortisol levels using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). The test used 25 μL of saliva per determination, has a lower limit of sensitivity of 0.007 μg/dL, standard curve range from 0.012 μg/dL to 3.0 μg/dL, an average intra-assay coefficient of variation of 5.42% and an average inter-assay coefficient of variation less than 10%.

Dehydroepiandrosterone-sulfate (DHEA-S)—Saliva samples were assayed in duplicate for DHEA-S using a highly-sensitive enzyme immunoassay (Salimetrics, State College, PA). The test used 100 μl of saliva per determination, has a lower limit of sensitivity of of 43 pg/mL, standard curve range from188.9 pg/mL to 15,300 pg/mL, an average intra-assay coefficient of variation of 5.35% and an inter-assay coefficient of variation less than 10%. DHEA-S scores were corrected for salivary flow rate—i.e., scores residualized with respect to sample volume/collection—time prior to analysis.

Alpha-Amylase (sAA)—Following Granger and colleagues (2007), all samples were assayed for sAA using commercially available kinetic reaction assays (Salimetrics, State College, PA) without modification to the manufacturers recommended protocols. Intra-assay variation (CV) computed for the mean of 30 replicate tests was less than 7.5%. Inter-assay

[2]Participants completed measures of stress appraisals and affect both before and after the stress task. Because the focus of the current study was on interpreting physiological response profiles based on participants' actual experience of the stressor, rather than predicting responses by anticipatory states, we used post-task ratings in the primary analyses reported below. Pre-task ratings were examined in supplementary analyses and found to be unrelated to stress response coordination, and including pre-task ratings in explanatory models also failed to change the primary model results.

variation computed for the mean of average duplicates for 16 separate runs was less than 6%. All sAA scores were corrected for salivary flow rate prior to analysis.

C-reactive Protein (sCRP)—Following Out and colleagues (2012), all samples were assayed for salivary CRP using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). The test used 50 μl of a 10x dilution of saliva per determination (15 μl of saliva), has a lower limit of sensitivity of 10 pg/mL, a standard curve range from 93.75 to 3000 pg/mL, an average intra-assay coefficient of variation of 3.69% and an average inter-assay coefficient of variation less than 10%.

Post-task Primary and Secondary Stress Appraisals—Cognitive stress appraisals following the task were assessed using a six item measure (Chang, 1998). Items were rated on a ten-point Likert-type scale ranging from 1 (*not at all*) to 10 (*very*). *Primary appraisal* was assessed by averaging four items that reflected importance ("how important was the task"), stress ("how much stress did the task evoke"), threat ("how threatening could negative consequences have been for you"), and challenge ("how challenging could have positive consequences been for you"). *Secondary appraisal* was assessed by averaging two items that reflect control ("how much control did you feel you had over the outcome') and effectiveness ("how effectively did you feel you were able to prepare"). Internal consistency was modest for the primary appraisal scale (α =.46) but was acceptable for secondary appraisal (α = .81).[3]

Post-task Positive and Negative Affect—All participants completed the positive and negative affect schedule immediately following the TSST to capture momentary arousal of positive and negative emotion resulting from the task (PANAS-X; Watson & Clark, 1994). Items were answered on a five-point Likert-type scale that ranged from 1 (*very slightly or not at all*) to 5 (*extremely*). Following recommended procedures, measures of positive and negative emotion were computed based on the 10 items tapping each dimension; scales were internally consistent for both post-stress positive affect (α =.88) and negative affect (α =.77).

Control Variables—Participants also reported on a number of variables that could impact salivary measures. These included age, sex, socioeconomic status (income, education, employment status), substance use (including nicotine and use of any other substances in the past 24 hours), acute (fever) and chronic health conditions (diabetes, thyroid disorder, other endocrine disorders), wake time that day, when they last ate (and whether dairy had been consumed in the past 30 minutes), oral health (recent tooth brushing, gums bleeding, dental work, and mouth bruising), body mass index, exercise frequency, and (for women) use of birth control medication, menstrual cycle phase, pregnancy, and menopause. Assignment to experimental condition was also tested for influence. Of these, only male sex (γ = -.31, p < .001) emerged as a significant predictor of cortisol levels and was retained in analytic models reported below. Oral health variables were also tested in relation to sCRP; no significant associations were found.

[3]Alternate versions of the post-task primary appraisal scale were computed with items removed to increase internal consistency. The results using the alternate scale did not differ from those using the full scale; therefore, in the interests of comparability with other research using the standard measure, reported results are based on the full scale.

Analytic Strategy

Hierarchical linear modeling (HLM; Raudenbush & Bryk, 2002) was used to model cortisol response trajectories and associations with other stress markers. This approach separates within-person variability in cortisol over time (Level 1) from between-person differences in cortisol response (Level 2). At Level 1 cortisol variability was modeled with an intercept and time-varying stress covariate (i.e., DHEA-S, sAA, or sCRP) centered around that participant's own mean; this captured within-person synchrony or alignment of responses across systems. At Level 2, the participant's mean level of the other stress marker (centered around the grand mean for the sample) was entered as a predictor of the cortisol intercept; this showed whether between-person differences in cortisol levels related to activation levels of other stress systems. Finally, post-task stress appraisals and affect were entered as predictors of the Level 1 stress covariate effect to determine whether within-person alignment of stress systems varied by subjective stress. In addition to these primary models addressing study hypotheses, piecewise growth models were fit with separate reactivity (samples 1–3) and recovery (samples 3–6) slopes to describe response trajectories in each of the stress systems.

Results

Variable Transformation and Initial Comparisons

All physiological stress marker variables showed significant positive skew, so they were log-transformed prior to analysis. For all reported results, $p < .05$ was considered significant. Before fitting multilevel models, a series of repeated-measures t-tests were run using SPSS 21 to determine if each marker changed from one sample to the next in the group of participants as a whole (note that the *df* vary based on missing samples, which did not impact HLM models below as full maximum likelihood estimation allowed missing data at Level 1). As described more fully above, samples 1 and 2 were collected prior to the stress task, approximately 35 minutes apart; sample 3 immediately afterward; and samples 4 through 6 during the recovery period.

These tests revealed that only sAA showed a consistent rise from study entry, with a significant increase from sample 1 to 2 ($t[115] = 5.85$, $p < .001$, $d = 1.09$); both cortisol and DHEA-S actually decreased from sample 1 to 2 ($t[117] = 3.87$, $p < .001$, $d = .72$ and $t[113] = 4.15$, $p < .001$, $d = .78$), indicating an initial anticipatory HPA axis stress response in participants as they acclimated to laboratory surroundings. sCRP did not change from sample 1 to 2. Cortisol, DHEA-S, and sAA all rose significantly from sample 2 to 3 ($t[117] = 3.09$, $p = .003$, $d = .57$; $t[113] = 3.20$, $p = .002$, $d = .60$; and $t[115] = 4.55$, $p < .001$, $d = .85$). The rise in sCRP from sample 2 to 3 did not reach significance but met the standard for a small-medium effect based on Cohen's d ($t[117] = 1.73$, $p = .087$, $d = .32$). These increases from pre- to post-stressor indicate immediate task-related reactivity for all markers except sCRP. Following the stressor, sAA decreased significantly from sample 3 to 4 ($t[115] = 5.49$, $p < .001$, $d = 1.02$), and all markers decreased from sample 4 to 5 ($t[117] = 3.19$, $p = .002$, $d = .59$ for cortisol; $t[113] = 2.60$, $p = .011$, $d = .49$ for DHEA-S; $t[114] = 2.02$, $p = .046$, $d = .38$ for sAA; and $t[115] = 2.25$, $p = .026$ for sCRP, $d = .42$). Only cortisol showed an ongoing decrease from sample 5 to 6 ($t[117] = 3.41$, $p = .001$, $d = .63$); DHEA-S and sCRP actually

increased from sample 5 to 6 ($t[112] = 2.71$, $p = .008$, $d = .51$; and $t[116] = 2.70$, $p = .008$, $d = .50$). Thus, all physiological stress markers showed post-task recovery, though at different times. Table 2 shows descriptive statistics for raw (untransformed) stress marker values across samples.

HLM Models

The models reported below were all fit using HLM 6. As described above, piecewise growth models were fit to determine how each salivary marker responded to the stress task (see Table 2 for baseline model results). Cortisol showed a significant recovery slope, and only sAA showed both significant reactivity and recovery slopes. DHEA-S and sCRP slopes did not reach significance. At the same time, significant between-person variability in each of these parameters, $\chi^2(114) = 173.23 – 485.97$, p's < .001, meant that individual participants' response trajectories could differ markedly from these overall patterns.

Next, models were fit to test associations between cortisol and other stress markers both at the level of within-person alignment of response trajectories across the session and at the level of between-person concordance in activation levels (see Table 4, top panel). Cortisol related significantly and positively to DHEA-S and sAA at both within- and between-person levels, and to sCRP at the within-person level only. This means that rises and falls in cortisol across the session tended to parallel those in DHEA-S, sAA, and sCRP, and that people with higher overall levels of cortisol also tended to have higher levels of DHEA-S and sAA. Each of these models yielded a significant improvement in fit compared to the baseline model, according to change in the deviance statistic, $\chi^2(4) = 25.73–61.92$, p's < .001.

Significant between-person variability in the alignment terms, $\chi^2(114) = 151.19 – 175.04$, p's < .01, suggested that cortisol could be more or less closely related to other stress markers, depending on characteristics of the participant. To begin to explain these individual differences, participants' post-task stress appraisals and affect were entered as predictors of alignment terms (see Table 4, lower panel). Secondary appraisals predicted greater cortisol alignment with sAA, whereas negative affect (and primary appraisals at a trend level) predicted greater alignment with sCRP.[4] This means that participants experiencing more positive states—i.e., more competent to manage the stressor, less negative emotion—showed a stronger cortisol association with sAA trajectories, but greater dissociation from sCRP trajectories. These models explained 22.2% and 12.0% of the variance in cortisol-sAA and cortisol sCRP associations, respectively. Although there were no significant predictors of cortisol alignment with DHEA-S, a marginally significant inverse association with positive affect was detected.

Discussion

This study adds to an emerging view of how multiple biological systems coordinate to respond to stress, and how coordination among systems (or lack thereof) relates to subjective stress. In particular, we showed that cortisol responses to social evaluative threat typically

[4]Tests of participant sex x subjective stress interactions revealed that while post-stress negative affect related to more positive cortisol-sCRP coordination for both men and women, the effect was stronger for women. No other sex-moderated effects were found.

align with adrenal androgen (DHEA-S), autonomic (sAA), and immune/inflammatory (sCRP) responses across the same time points, but that the degree of alignment with autonomic and inflammatory responses depends on stress appraisals and affect. By examining not one stress response system in isolation, but the concert of energy mobilization and defense/repair processes represented by multiple systems, we may come closer to understanding how individual differences in sensitivity to environmental stress contribute to human health and well-being.

The present findings converge with previous evidence that the cortisol response is typically aligned with that of other stress systems—both a protective HPA axis component represented by DHEA-S and an autonomic mobilization component represented by sAA. The observed multilevel coordination between cortisol and DHEA-S is consistent with prior evidence of an association between the two adrenal outputs, and may help to understand beneficial impacts of DHEA-S during stress (i.e., as a buffer/repair mechanism protecting against cortisol impacts). Similarly, the observed associations of cortisol with sAA at both within- and between-person levels of analysis support the idea that HPA axis and ANS are often (though not always) invoked together to respond to environmental challenges. While these markers showed differing profiles of absolute change related to stress, we found congruence between mean cortisol, DHEA-S, and sAA levels across people, and between relative changes in these systems observed within people. In addition, we add to an emerging literature regarding an immune/inflammatory component of the stress response by showing dynamic alignment of cortisol with sCRP (though not concordance in absolute activation levels). These multiple links across stress-responsive systems help to define the contours of a normative multisystem response, in which components with different response kinetics and physiological functions combine to allow a person to engage with and recover from a stressor. At the same time, between-person variability in the degree of coordination across systems suggested that response alignment does not characterize everyone equally, and individual differences in coordination could help to understand differential effects of stress.

Differences in cross-system alignment indicated that cortisol activity accompanied by sAA characterized more positive stress experiences (i.e., higher secondary stress appraisals). This fits with prior evidence that cortisol-sAA coordination during stress is adaptive (Laurent et al., 2011; 2013). As proposed in these previous papers, simultaneous activation of HPA axis and autonomic systems may enable more effective coping with acute stress than activation of the former alone. The present results further imply that such co-activation may depend on a person’s sense of competence to manage stressors.

This study also adds new information about alignment with sCRP; not only did cortisol levels tend to rise and fall with changes in sCRP, but this link was strongest in participants who with higher negative affect following the stressor. Known negative health consequences of chronically high activation/inadequate post-stress recovery in the HPA axis and inflammatory systems may be especially pronounced when they occur together. This state may in turn be promoted by (and/or promote) negative emotionality. Taken together with the above, these findings suggest that health interventions should aim to reinforce the synchronization of HPA axis and autonomic responses to stress while uncoupling the HPA axis from inflammatory reactions. These ideas require further exploration of causal chains

between cognitions/emotions and physiology across a stress episode, as well as associations with stress-related health conditions not measured in this study.

Aside from within-person response coordination across systems, we found between-person concordance in mean levels of cortisol, DHEA-S, and sAA. This is consistent with a parallel "tuning" of output levels across these complementary systems. It is possible that differences in psychological adjustment arise when such normative balancing of stress-responsive systems is disrupted—i.e., when an overactive HPA axis drive is accompanied by downregulation of systems needed to repair stress-related damage and energize effective coping. Although we did not find relations between cortisol levels and current socioeconomic status, it is possible that earlier environmental conditions play a role in preserving or disrupting this balance of overall activation. Future research will be needed to understand how experience shapes the activation of each system, and the importance of concordance or discordance at this level.

More broadly, the current findings suggest certain positive cross-system linkages may be helpful for coping with stress (HPA axis with ANS), whereas others (HPA axis with inflammation) may be less so. As noted above, the present study design does not allow us to say whether cognitive appraisals and affective states give rise to or result from patterns of cross-system mis/alignment. It is likely that one or more third variables—for example, socialization experiences both within and outside the family, early conditions of relative deprivation or material/emotional insecurity—underlie both the subjective stress and physiological response patterns observed here. On the amelioration side, it is possible that training cognitive/emotion regulation skills in stress situations would strengthen more helpful cross-system alignments—i.e., autonomic support for the HPA axis, a braking influence of the HPA axis on inflammatory responses—resulting in downstream health benefits.

Limitations to the current study suggest areas to explore in future research. Distinctive characteristics of this study such as the all African American sample and cortisol and DHEA-S elevations at the beginning of the session may explain why continuous TSST reactivity slopes were not found for all stress markers. Few TSST studies have focused on African Americans (Hackman, Betancourt, Brodsky, Hurt, & Farah, 2012; Hackman et al., 2013), and there is some evidence that the task elicits lower physiological stress responses in African American compared to White participants (Mechlin et al., 2005). Further research using this and other stress paradigms in racially diverse samples is indicated. Relatedly, the small number of male participants precluded sex-specific analyses, though interaction tests suggested the link between negative affect and positive cortisol-sCRP coordination was stronger for women. Further exploration of possible sex differences in a more balanced sample represents an important next step.

While stress-related appraisals and affect offer a good initial yardstick for measuring the potential adaptive value of particular response constellations, other measures of successful coping and longer-term mental/physical health outcomes will be needed to confirm which profile/s can be considered optimal. In addition, this study relied on salivary measures of stress response systems; these have the advantage of allowing repeated, noninvasive

assessment that is unlikely to create additional reactivity, but it would be helpful to compare these measures with alternative stress system markers to see if the same patterns apply. This may be particularly relevant to salivary CRP, a newer and less well validated stress marker that has been shown to correlate significantly but only moderately with whole blood CRP (Out, Hall, Granger, Page, & Woods, 2012). The measurement and meaning of sCRP is not yet well understood, and the role of sCRP in stress reactivity research continues to be debated (Steptoe, Hamer, & Chida, 2007; Slavish et al., 2014). Thus, results pertaining to sCRP are best considered suggestive at this stage. Future research should consider both oral and blood markers of immune/inflammatory stress responses to better characterize how these responses relate to one another and to health outcomes. It should also be noted that while oral fluid measurement of sAA in response to psychosocial stress is well established, it is only a surrogate marker of autonomic activity, and the extent to which it reflects sympathetic versus parasympathetic activity is debated (Bosch, Veerman, de Geus, & Proctor, 2011).

Finally, differing response kinetics across physiological markers (with some showing ongoing reactivity or recovery slopes, some showing rapid alternations and/or more limited periods of reactivity or recovery) made it difficult to detect and describe consistent response components across markers. Conducting sessions relatively early in the day—i.e., mid-day rather than later afternoon—and the relatively short (10 minute) acclimation period prior to the first sample may have made it more difficult to detect a sample-wide increase in cortisol levels. Furthermore, participants were asked to give a dried bloodspot at the first sample, which may have elicited additional anticipatory stress at this first time point (see Girgis, Shea, & Husband, 1988). These methodological points likely restricted our ability to show continuous cortisol reactivity, resulting in a significant increase from the pre- to post-task samples, but not from study entry to post-task. In future studies of this type it would be helpful to conduct sessions later in the day (when cortisol levels are more stable) without the additional stress of blood collection and with a longer pre-stress acclimation period. As noted above, further investigation of possible race-specific determinants of reactivity is also warranted. The sampling time window may also have been too brief to detect sCRP responses, in particular, given indications that blood CRP peak response may not appear until 120 post-stressor (see Slavish et al., 2015). Closer study of response dynamics in each across a longer sampling period and across different stress context might reveal more about multisystem responses and alignment than could be demonstrated here, including predictors of cortisol-DHEA-S alignment.

The adaptive significance of a person’s response to environmental demands likely equals more than the sum of its parts, with coordination of response components during reactivity and recovery offering unique insights missed by single-system measurement. A multisystem approach to the study of individual differences in environmentally sensitive biological systems seems paramount to constructing meaningful models of adaptive stress responding. By broadening the lens through which we view biological reactivity and regulation, a more complete picture of stress adaptation may come into focus.

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Research Highlights

- We examine coordination of cortisol responses with other stress-responsive systems
- Cortisol coordinated with adrenal androgen, autonomic, and inflammatory responses
- Positive stress appraisal predicted stronger cortisol-autonomic response alignment
- Negative affect predicted stronger cortisol-inflammatory response alignment

Table 1

Sample Descriptive Information (n = 115)

Variable	Proportion
Income	
< $10,000	25.4%
$10,000–$24,999	28.9%
$25,000–$49,999	23.7%
$50,000–$99,999	19.3%
$100,000–$149,999	2.6%
Education	
High school or less	47.8%
Associate's degree or professional certificate	27.0%
Bachelor's degree	15.7%
Master's degree	9.6%
Employment	
Employed full-time	12.2%
Employed part-time	25.2%
Self-employed	6.1%
Student	32.2%
Unemployed	24.3%
Health Conditions	
Diabetes	2.6%
Thyroid	1.7%
Endocrine disorder	1.7%
Cancer	3.5%
Smoker	10.4%
Exercise	
Rarely or never	7.0%
1–2 days per week	17.4%
3–4 days per week	43.5%
5–6 days per week	12.2%
7 days per week	20.0%
Body Mass Index	
Normal (< 25.00)	46.9%
Overweight (25.00–29.99)	36.8%
Obese (>30.00)	16.3%

Table 2

Stress Marker Values by Sample Collection Time (n = 115)

Sample	Cortisol (μg/dl)	DHEA-S (pg/ml)	sAA (U/ml)	sCRP (pg/ml)
1	0.22(0.02)	1476.46(132.65)	37.58(2.72)	4737.76(872.00)
2	0.19(0.01)	1315.85(156.35)	60.61(5.83)	7665.56(2396.58)
3	0.23(0.02)	1511.56(176.99)	68.14(6.74)	8155.63(2623.55)
4	0.22(0.02)	1358.65(138.69)	51.23(4.95)	5776.15(1270.78)
5	0.18(0.01)	1231.88(106.57)	47.57(4.92)	6580.17(1797.58)
6	0.17(0.01)	1310.82(101.90)	49.80(5.20)	8220.07(2689.32)

Note. Raw (untransformed) scores shown. sAA and DHEA-S values are adjusted for salivary flow rate. Standard errors of the mean are given in parentheses.

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Table 3

Baseline Models of Physiological Stress Trajectories

	Reactivity Slope (Samples 1–3)		Recovery Slope (Samples 3–6)	
	β	***p***	***β***	***p***
Cortisol	.012	.898	**−.230**	**.001**
sAA	**.428**	**< .001**	**−.227**	**< .001**
DHEA-S	−.073	.174	−.018	.592
sCRP	.054	.180	.012	.779

Note. Significant effects highlighted in bold.

Table 4

Cortisol Coordination with Other Stress Markers

	DHEA-S		sAA		sCRP	
	β	***p***	***β***	***p***	***β***	***p***
Cortisol Intercept	.017	.796	.016	.818	.014	.842
Other stress marker mean (*average level coordination*)	**.16**	**.005**	**.15**	**.004**	−.050	.464
Other Stress Marker Covariate (*matched phase coordination*)	**.36**	**<.001**	**.28**	**.001**	**.18**	**.048**
Primary appraisal	.15	.143	−.094	.340	.16	.080
Secondary appraisal	.057	.487	**.14**	**.024**	.009	.886
Negative affect	−.11	.140	−.058	.397	**.15**	**.034**
Positive affect	−.19	.076	−.084	.341	−.0002	.999

Note. Significant effects highlighted in bold.