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Developmental origins of infant stress reactivity profiles: A multi-system approach

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Abstract

Background: This study tested the hypothesis that maternal physiological and psychological variables during pregnancy discriminate between theoretically informed infant stress reactivity profiles.

Methods: The sample comprised 254 women and their infants. Maternal mood, salivary cortisol, respiratory sinus arrhythmia (RSA), and salivary α-amylase (sAA) were assessed at 15 and 32 weeks gestational age. Infant salivary cortisol, RSA, and sAA reactivity were assessed in response to a structured laboratory frustration task at 6-months of age. Infant responses were used to classify them into stress reactivity profiles using three different classification schemes: HPA-axis, autonomic, and multi-system. Discriminant function analyses evaluated the prenatal variables that best discriminated infant reactivity profiles within each classification scheme.

Results: Maternal stress biomarkers, along with self-reported psychological distress during pregnancy discriminated between infant stress reactivity profiles.

Conclusions: These results suggest that maternal psychological and physiological states during pregnancy have broad effects on the development of the infant stress response systems.

Key Words: Fetal programming; stress reactivity; salivary α-amylase; salivary cortisol; respiratory sinus arrhythmia; psychological distress

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Introduction

The developmental origins hypothesis postulates that fetal adaptations consequent to in utero exposures, such as maternal stress, circulating hormones, or nutrition, confer later risk or resilience for health and disease (Barker, 1998). Fetal programming of the stress response systems (SRS) is thought to be a central mechanism by which prenatal exposures influence human development with potential for lifelong effects. The SRS filters and mediates the relationship between the individual and the environment and because of this, understanding developmental processes that lead to different patterns of stress responsivity has become a major focus in neuroscience, psychology, and medicine (e.g., Del Giudice, Hinnant, Ellis, & El-Sheikh, 2012; Ellis, Jackson, & Boyce, 2006; Gunnar, Frenn, Wewerka, & Van Ryzin, 2009). For heuristic purposes, individual differences in the physiological profiles within and across the components of the SRS can be described using a limited number of patterns that reflect plausible forms of coordination among physiological systems and that are supported by observed associations with psychological function, social relations, and the development of mental and physical disorders (Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011; El-Sheikh, Arsiwalla, Hinnant, & Erath, 2011; El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008; Keller & El-Sheikh, 2009; Nederhof, Marceau, Shirtcliff, Hastings, & Oldehinkel, 2014; Stifter, Dollar, & Cipriano, 2011). In the present investigation we used patterns of infant physiological responses to classify infants using previously described typologies for the autonomic and HPA axis components of the stress response, as well as a multi-system typology that integrates the major systems within the SRS. Our objective was to identify stress-relevant in utero exposures

that may contribute to the developmental origins of individual differences in these a-priori defined infant stress responses.

The Stress Response System

Two of the major physiological systems that are often examined in response to stress include an efficient and highly conserved set of integrated neuroendocrine systems: the autonomic nervous system (ANS), which consists of sympathetic (SNS) and parasympathetic (PNS) divisions, and the hypothalamic-pituitary-adrenal (HPA) axis. The ANS is a rapid response system that offers a first layer of response to stressful or challenging situations. Within the ANS, the PNS and SNS are anatomically distinct divisions which nevertheless innervate almost all of the same tissues and organs (McCorry, 2007). The PNS innervates internal organs via cholinergic fibres, predominates during quiet resting conditions, and tends to reduce physiological arousal. The SNS innervates internal organs via adrenergic fibres, stimulates synthesis of catecholamine's (e.g., epinephrine and norepinephrine), and predominates during emergency reactions. The SNS mediates physiological arousal through two separately controlled pathways – a fast acting neural pathway via noradrenergic innervation of visceral organs, and a slower adreno-medullary-hormonal pathway (Folkow, 2000). The direct neural innervations of the PNS and SNS allows for precise and rapid adjustments of end organ states within seconds.

The HPA-axis provides a second layer of response to stress that is most likely to produce measurable responses when a stressor is overwhelming, a situation is unpredictable, or the individual has little perceived control (Dickerson & Kemeny, 2004; Koolhaas et al., 2011). From a developmental perspective, the HPA-axis is highly responsive to mild stressors in neonates, including maternal separation and parental conflict, and becomes increasing less responsive over time (Gunnar, Talge, & Herrera, 2009). Stressors stimulate neurons in the

paraventricular nucleus of the hypothalamus to secrete corticotrophin-releasing hormone and arginine vasopressin into the anterior pituitary that stimulates the secretion of hormones, including adrenocorticotropic hormone (ACTH) and β-endorphin (Vedder, 2007). Through systemic circulation, ACTH reaches the adrenal cortex and stimulates biosynthesis of corticosteroids. Cortisol, one output hormone of this axis, mobilizes physiological and psychological resources to deal with the stressor (Flinn, 2006; Roozendaal, 2000; Sapolsky, Romero, & Munck, 2000), and suppresses further secretion of corticotrophin releasing hormone, regulating the HPA-axis through negative feedback. The HPA-axis is a slow-response system with a minimum latency of about 5 min for cortisol increases with peak levels achieved between 10 and 30-minutes after an acute stressor (Kirschbaum, Pirke, & Hellhammer, 1993). The preponderance of evidence suggests that high cortisol reactivity is associated with more internalizing problems whereas low cortisol reactivity is associated with more externalizing problems (Dickerson & Kemeny, 2004).

The doctrine of autonomic space

The doctrine of autonomic space is a quantitative theoretical model that describes the simultaneous action of the PNS and SNS on target organs that are dually innervated by both branches of the ANS (Berntson, Cacioppo, & Quigley, 1991; Berntson, Cacioppo, Quigley, & Fabro, 1994). The PNS and SNS are generally under reciprocal central control, with increasing activity in one division resulting in decreasing activity in the other division. Reciprocal control promotes strong unidirectional change in the target organ or system. For example, simultaneous PNS withdrawal and SNS engagement on the heart will result in an increase in heart rate and force of myocardial contraction. While usually under reciprocal control, the PNS and SNS independently innervate tissues and organs with little or no cross talk (Jänig & McLachlan,

1992), allowing for instances when the PNS and SNS act in a non-reciprocal fashion. Co-activation entails conjoint activation of the PNS and SNS whereas co-inhibition entails decreased activation of the two divisions. Patterns of non-reciprocal ANS activation yield less reliable responses in target organs with the actual effect highly dependent on the relative dominance of the PNS and SNS.

Evidence suggests that reciprocal SNS activation and PNS inhibition is normative in response to stress (Alkon, Boyce, Davis, & Eskenazi, 2011; Salomon, Matthews, & Allen, 2000) and is generally associated with desirable outcomes. For example, an investigation of ANS function among 62 children reported that reciprocal sympathetic activation during a stressor was associated with better emotion regulation during a disappointment task (Stifter et al., 2011). While this pattern of reciprocal PNS and SNS activation has been reported as a resilience factor (El-Sheikh et al., 2009), nonreciprocal PNS and SNS activation has been reported as a vulnerability factor for externalizing behaviour in children exposed to marital conflict (El-Sheikh & Erath, 2011; El-Sheikh et al., 2009; Gordis, Feres, Olezeski, Rabkin, & Trickett, 2009), and children with behaviour problems often exhibit co-inhibition in response to laboratory stress challenges (Beauchaine, Gatzke-Kopp, & Mead, 2007; Boyce et al., 2001).

The need for multi-system approaches

Although reactivity patterns within a single SRS component (e.g., PNS, SNS, HPA-axis) have been individually linked to psychosocial and health outcomes, a better understanding of the links between SRS and developmental outcomes may be obtained by measuring concurrent activity within the three systems (Bauer, Quas, & Boyce, 2002). Given that the SRS is an integrated set of physiological systems, there is a need to better understand the organization and coordination of stress responses across the PNS, SNS, and HPA-axis. Moreover, components of

the SRS may compensate for one another and responses in one SRS component may be obscured by responses in another component. For example, glucocorticoids produced by the HPA-axis can act to augment or suppress SNS-mediated changes in cardiovascular function, metabolism, and immune function (Sapolsky et al., 2000). Established and emerging models of the SRS, such as the Allostatic Load (McEwen, 1998) and Adaptive Calibration models (Del Giudice, Ellis, & Shirtcliff, 2011) call for multi-system research paradigms.

Coordination among the components of the stress response system

The ANS and HPA-axis are mediated by largely overlapping circuits in the limbic forebrain, hypothalamus, and brainstem (see Ulrich-Lai & Herman, 2009 for a review) that act to coordinate responses to stress and adversity (Del Giudice et al., 2011; Ellis et al., 2006; Schlotz et al., 2008). The PNS and the HPA-axis response to stress and challenge tend to be reciprocally coordinated (Doussard-Roosevelt, Montgomery, & Porges, 2003) whereas SNS and HPA-axis responses to stress tend to be directly coordinated (Goldstein & Kopin, 2008).

The coordinated action of SRS components is often conceptualized in terms of symmetric or asymmetric responses to stressors and Bauer et al. (2002) proposed testable interactive models for understanding the nature of ANS and HPA-axis coordination. The HPA-axis and ANS are proposed to serve complimentary roles in responding to stress and dissociations or asymmetry in responses reflect ineffective or poor coordination in physiological systems that may be associated with poor functioning, and emotional and behavioral difficulties.

The coordinated, counterbalancing roles of the PNS, SNS, and HPA-axis in the integrated stress response can be indexed using a variety of accessible physiological measures. Proximal measures of PNS influence can be measured non-invasively through an electrocardiographic recording using respiratory sinus arrhythmia (RSA) - the neural regulation of heart rate (HR) by

parasympathetic influence emanating from the vagus nerve (Berntson et al., 1997). ANS influence can be approximated non-invasively in saliva using the surrogate marker salivary α-amylase (sAA; Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Nater & Rohleder, 2009) - an enzyme produced by the saliva gland that is thought to reflect the direct-neural adrenergic component of the stress response and plasma catecholamines (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996), albeit not in a 1:1 manner (Nater & Rohleder, 2009). Data suggests that sAA is more sensitive to mild mental stress than are parameters such as blood pressure and heart rate (van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Lastly, the assessment of cortisol in saliva is a valid and reliable reflection of unbound cortisol in blood and reflects HPA-axis activity (Kirschbaum & Hellhammer, 1994).

Developmental Origins of SRS

Evidence suggests that exposures to adversity during sensitive periods in utero or early postnatal development may lead to altered neuronal structure and function, predisposing the individual to later psychosocial and health problems (Barker, 1998; Gluckman & Hanson, 2004; Seckl & Holmes, 2007). For example, prenatal maternal psychological distress (i.e., an amalgamation of comorbid subjective stress, anxiety, and depressed mood) is associated with neonatal methylation of infant glucocorticoid receptors and subsequent elevated infant HPA-axis reactivity (Glover, Miles, Matta, Modi & Stevenson, 2005; Oberlander et al., 2008) and elevated maternal prenatal cortisol is associated with exaggerated infant PNS reactivity (Rash, Campbell, Letourneau, & Giesbrecht, 2015). Further, human and animal evidence suggests that exposure to relatively high levels of cortisol and maternal psychological distress alters the function of the fetal HPA-axis in a manner that varies with the type of stressor and time of gestation (Braun,

Challis, Newnham, & Sloboda, 2013; Kajantie, 2006; Kapoor & Matthews, 2008; Seckl & Holmes, 2007).

One central tenant of the developmental origins hypothesis is that the maternal SRS, and specifically cortisol, influences the development of fetal/infant SRS. Peptide and steroid hormones of less than 0.7-1.2 kDa, such as cortisol and catecholamines, readily cross the placental barrier (Fisher, 1998) and exert a direct influence on fetal development. Although the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) regulates fetal exposure by converting cortisol to inactive cortisone, human studies have indicated that maternal psychological distress is associated with increased neonatal cortisol levels (Field et al., 2004) and a decreased efficiency of the placenta at converting cortisol to cortisone (Oberlander et al., 2008) that may lead to elevated cortisol exposure in utero. Given that cortisol receptors are highly expressed in the developing brain (Sánchez, Young, Plotsky, & Insel, 2000) and are important for the developing central nervous system (Kapoor & Matthews, 2008), prenatal exposure to glucocorticoids may impair the development of feedback mechanisms in the fetal HPA-axis, resulting in altered basal functioning and responsiveness (Seckl & Holmes, 2007). Similarly, approximately 5-10% of maternal catecholamines are transferred to the developing fetus (Merlot, Couret, & Otten, 2008) and can lead to constriction of placental blood vessels, decreases in fetal supply of glucose, activation of the fetal HPA-axis (Challis, Matthews, Gibb, & Lye, 2000), and catecholamine release (Gu & Jones, 1986). Taken together, evidence suggests that maternal stress, along with HPA-axis and ANS function during pregnancy have the potential to alter fetal/infant SRS function.

The Present Investigation

The present study evaluated whether components of the maternal SRS along with subjective reports of psychological distress reliably discriminate amongst discrete patterns of theoretically informed and a-priori defined infant ANS and HPA-axis reactivity patterns, as described above. Discriminant function analyses (DFAs) were run to test the hypotheses that theoretically informed infant autonomic, HPA-axis, and multi-system stress reactivity profiles are associated with maternal prenatal physiological and psychological variables. Data for this study were obtained using a prospective longitudinal cohort design in which we collected measures of psychological distress, diurnal salivary cortisol and salivary α-amylase (sAA), heart rate, and RSA from pregnant women in early and late gestation and assessed infant RSA, sAA, and salivary cortisol during rest and frustration tasks at 6 months of age.

Methods

Participants

Participants were women and infants enrolled in a prospective longitudinal study of nutrition during pregnancy (see Kaplan et al., 2014 for further details). Women were excluded if they reported: (a) non-singleton pregnancy, (b) using steroid medication, including perinatal betamethasone, (c) smoking, (d) consuming alcohol or illicit drugs, or (e) known pregnancy or fetal complications (e.g., preeclampsia, fetal genetic anomalies) at time of enrollment. Data collection was scheduled to avoid recent dental work (which may result in oral bleeding) and illness during data collection (e.g., fever), both of which may interfere with accurate assessment of salivary cortisol (Kivlighan et al., 2004). Because gestational age at birth and birthweight are associated with infant HPA axis function (Buss et al., 2012; Glover, Miles, Matta, Modi, & Stevenson, 2005), we made an a priori decision to exclude from current analysis infants with low birthweight (< 2500 g) or preterm birth (< 37 weeks gestation). Further, infants with congenital

heart defects (e.g., arrhythmias), as determined by chart review, were excluded because these conditions can severely affect cardiac vagal control (Finley, Nugent, Hellenbrand, Craig, & Gillis, 1989). The sample was well-educated (89.7% had post-secondary education), primarily White (77.3%), financially stable (mean household annual income $70,000 - $99,999 CAD), mature (mean age = 31.4 years, *SD* = 3.8), and primiparious (50%). The study protocol was approved by the University of Calgary Health Research Ethics Board and participants provided informed consent prior to each procedure.

Procedures

Pregnant women collected diurnal suites of saliva (for cortisol and sAA assay), wore an ambulatory heart rate monitoring device, and responded to standardized questionnaires assessing psychological distress on two consecutive days in both early pregnancy (T1) at ~15 (*SD* = 3.6) weeks gestational age, and late pregnancy (T2) at ~32 (*SD* = 0.97) weeks gestational age. Participants attended an individualized training session at T1 during which they received instructions about the saliva collection device and the personal digital assistant (PDA) data collection device. The PDA was programmed to ring as a reminder for participants to collect saliva samples, administer the psychological distress questionnaire, and record exact timing of data collection. Participants also kept a paper diary of sample collection and any discrepancies between the PDA and paper diary were resolved by contacting the participant. To facilitate adherence to the study protocol, the PDA was programmed to allow a 20-minute response window following the initial signal, after which the reminder and questionnaire were no longer available to the participant.

Infants were brought to the laboratory at approximately 6-months of age (*M* = 24.69 weeks, *SD* = 2.69). Figure 1 is a graphical depiction of the infant laboratory assessment. Upon

arrival at the laboratory, infant negative affect and behavioral state was rated by a trained research assistant. Infant negative affect was assessed via the negative affect scale of the Behavior Rating Scales of the Bayley Scales of Infant Development 2nd edition (Bayley, 1993), which assesses the amount and intensity of infant fussing/crying on a 5-point scale from "no negative affect displayed" to "three or more intense, heightened, or prolonged displays of negative affect". Behavioral state was assessed using the Neonatal Behavior Assessment Scale (Brazelton & Nugent, 1995) which assesses infant arousal on a 7-point scale from 1 "quiet sleep" to 7 "crying." A baseline saliva sample was collected midway through a 10-minute laboratory acclimation period. Infants were then outfitted with an ambulatory heart rate monitor (described in more detail below). The heart rate monitor remained connected to the infant during the laboratory session and for 24-hour ambulatory monitoring (data from ambulatory monitoring not presented here).

Following the acclimation period, infants completed components of the Laboratory Temperament Assessment Battery (Lab-TAB prelocomotor version 3.1; Goldsmith & Rothbart, 1996), which consisted of several phases completed in the following order:

Baseline. Infants were seated on their mothers' lap during a 3-minute baseline recording and mothers were asked to keep movement and interaction with their infant at a minimum.

Attention. Visual attention was assessed using a series of colorful images presented on a 30 X 38 cm computer screen. Data from this task are not included in the present analysis.

Frustration. A series of frustration tasks were presented. Previous research has shown that the selected tasks reliably elicit a stress response in infants (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007). The infant was seated in a highchair at the end of a table such that the infant could easily reach objects placed on the table. The infant's mother was seated to the

child's left and the experimenter to the child's right. Frustration tasks were discontinued if the experimenter deemed that the infant was too distressed to continue (as per standard procedure for the Lab-TAB we used as a guiding rule 20 s of hard crying) or if the parent elected to stop.

The frustration series began with a toy retraction task. The child chose and then played with a novel toy for 15 s after which time the mother gently removed the toy outside of the infant's reach and left it there for 30 s. The toy was then returned to the child for 15 s prior to administration of trials 2 and 3. At the end of the 3rd trial the infant was allowed to play with the toy during setup for the next task.

The second frustration task involved an attractive toy placed behind a plexiglass barrier. The infant was first allowed to engage with a stuffed rabbit. After 15 s of play, the experimenter placed a plexiglass barrier approximately 9 cm in front of the infant, and asked the parent to remove the toy from the infant and place it directly behind the barrier. The toy remained behind the barrier for 30 s, after which the child was allowed to play with it for 15 s. This procedure was repeated 2 more times after which the infant was allowed to play with the toy during setup for the next task.

The final frustration task was a gentle arm restraint task. The mother was asked to stand behind the infant and gently grasp the infant's forearms and firmly hold them to the infant's side for 30 seconds while an attractive toy was placed directly in front of the infant. A 15 second recovery period was allowed prior to a 2nd trial during which the infant played with the toy or was comforted by the parent. At completion of the arm restraint task the parent and infant were invited to play with a box of toys for approximately 15 minutes, at the end of which a post-stress saliva sample was collected to mark the end of the laboratory assessment.

Measures

***Maternal cortisol and* sAA** during pregnancy were collected using whole saliva obtained from under the tongue with the Salimetrics Oral Swab (Salimetrics, State College, PA). Saliva collection was completed at home over 2 consecutive days (excluding weekends) at both T1 and T2. Four samples per day were obtained on the following schedule: upon waking, 30 min after waking, at 1130h and 2100h.

Participants were instructed to turn on the PDA upon awakening, record their waking time, and collect a saliva sample. The PDA was programmed to ring 30 minutes later at which time participants collected their second saliva sample of the day. This procedure was used to assess the cortisol and sAA awakening responses (CAR and sAAAR). Each sampling event generated a unique code on the PDA corresponding to a prelabeled saliva tube and provided a precise time stamp for each saliva sample.

Pregnant women were asked to refrain from consuming food, caffeine, citric drinks and dairy, and to avoid vigorous exercise or brushing their teeth within 30 minutes prior to saliva collection and to report adherence to these guidelines (Granger, Cicchetti, et al., 2007). Saliva samples were stored at -80°C until they were shipped frozen to Salimetrics, State College, PA.

Samples were assayed for salivary cortisol using an enzyme immunoassay that has a lower limit of sensitivity of 0.007 μg/dL, standard curve range from 0.012 to 3.0 μg/dL, and average intra- and inter-assay coefficients of variation 3.5% and 5.1%, respectively. Method accuracy, determined by serial dilution was 100.8% and 91.7%. A random 10% of samples were assayed in duplicate to confirm reliability; the intra-assay coefficient of variation between the duplicate tests was 3.5%. Mean values from duplicate samples were used for analysis.

The sAA assay uses a chromogenic substrate, 2-chloro-ρ-nitrophenol linked to maltotriose. The enzymatic action of sAA yields 2-chloro-ρ-nitrophenol, which can be

spectrophotometrically measured at 405 nm using a laboratory plate reader. The amount of sAA activity present in the sample is directly proportional to the increase (over a 2-min period) in absorbance at 405 nm. Results were computed in U/mL of sAA. The assay requires 10 μL of saliva. Intra-assay coefficient of variation was 5.5%. Inter-assay variation was 4.7%. Given that sAA concentrations are affected by both salivary flow rate (mediated by the PNS) and protein secretion (mediated by the SNS) (Bosch, Veerman, de Geus, & Proctor, 2011), we controlled for parasympathetic influence on sAA by adjusting for flow rate using a previously described method (Beltzer et al., 2010). In brief, raw sAA concentration (U/mL) was multiplied by flow rate where flow rate was computed by dividing the sample volume (in mL) by the collection time (in minutes). This method was used to compute an adjusted sAA concentration (U/min).

Infant salivary biomarkers were assessed at 6 months using a synthetic swab (Salimetrics Children's Swab, Salimetrics, State College, PA) which has been validated for the collection of cortisol and sAA (Bright, Frick, Out, & Granger, 2014).

Maternal and Infant RSA was measured using continuous heart period recordings of R-R intervals sampled at a rate of 1000Hz. Pregnant mothers and infants wore a Firstbeat Bodyguard recording device (Firstbeat Technologies, Oy Jyvaskyla, Finland) connected to two leads by pre-gelled (Ag/AgCl) disposable electrocardiograph (ECG) electrodes attached beneath the right clavicle and to the left ribcage.

Infant birth-weight percentile was calculated using birth-weight obtained from birth records. Birth-weight percentiles were adjusted for sex and gestational age at birth, according to growth charts derived from the 1999-2000 US Natality Datasets (Oken, Kleinman, Rich-Edwards, & Gillman, 2003).

Maternal Prenatal Psychological Distress was measured at the T1 and T2 assessments using items from the Profile of Mood States (POMS; McNair & Heuchert, 2007), a multidimensional measure of mood with strong psychometric properties (e.g., coefficient alpha values range between .79 and .93; Bourgeois, LeUnes, & Meyers, 2010). Using a procedure adapted from Cranford et al. (2006), we selected 14-items from anger, anxiety, depression, fatigue, and vigor/positive affect scales that could be administered using the personal digital assistant (PDA). Participants rated their experience on each item on a 5-point Likert scale from "not at all" to "extremely," based on their feelings during the previous 30-minutes. The 30-minute window was chosen to account for the delay in HPA-axis response to psychological experience (Kirschbaum & Hellhammer, 1989). As per standard scoring procedures for the POMS, a psychological distress score was derived for each sampling moment by subtracting the vigor items from the sum of the remaining items. Reliability of this 14-item scale was adequate, Cronbach's $\alpha = .88$.

Pregnancy Anxiety was assessed at the T1 and T2 assessments using a 10-item self-report measure (Rini, Dunkel-Schetter, Wadhwa, & Sandman, 1999). Pregnancy anxiety evaluates the extent to which pregnant women worry about their health, the health of their baby, labor and delivery, and caring for their baby. This reliable measure, Cronbach's $\alpha = .81$ (Rini, Schetter, Hobel, Glynn, & Sandman, 2006), was specifically developed for use in pregnancy research and has been used in research examining the effects of maternal distress on offspring outcomes (Buss, Davis, Hobel, & Sandman, 2011). Scores range from 0 to 30 with higher scores indicating greater pregnancy anxiety.

Prenatal maternal depressed mood was assessed at the T1 and T2 assessments using the *Edinburgh Depression Scale (EDS*; Cox, Holden, & Sagovsky, 1987). The EDS is a reliable,

valid, and sensitive 10-item self-report measure of depression symptoms in the perinatal period. High scores (≥ 12) are strongly correlated with physician diagnosis of Major Depressive Disorder (Jomeen & Martin, 2007). While originally designed to measure symptoms of postnatal depression, the EDS has been shown to be a reliable and valid measure of depression symptoms among pregnant women with adequate sensitivity (79%) and specificity (85%) (Jomeen & Martin, 2007).

Data Reduction

Maternal cortisol during pregnancy was used to calculate three summary measures of HPA axis function: 1) the cortisol awakening response (CAR; calculated using the trapezoid method for area under the curve increase), as a measure of the morning increase in cortisol (Fries, Dettenborn, & Kirschbaum, 2009), 2) the area under the curve from ground (AUCg) as a measure of total cortisol secretion (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003), and 3) the regression slope fitted through the raw cortisol data to describe the pattern of cortisol decline over the course of the day (Fekedulegn et al., 2007). These same indicators were calculated for ***maternal sAA*** – note that whereas cortisol has a positive awakening response and negative slope during the day sAA has a negative awakening response and positive slope during the day. To ensure valid assessment of the awakening responses, samples used to calculate the CAR and sAA awakening response (sAAAR) were excluded if they were taken more than 15 minutes after waking for the waking sample (n = 101; 9.2%) and more than 50 minutes after waking for the 2nd sample (n = 121; 11.1%) (Okun et al., 2010). Given that the distributions of raw cortisol and sAA values are typically skewed and the normal diurnal profiles may be approximated by an exponential curve, raw cortisol and sAA values were transformed using the natural logarithm before calculations were performed. The AUCg calculations were performed

using the trapezoidal method and included the waking, 1130h and 2100h samples. Because individuals had different amounts of time between waking and the 2100h sample, AUCg calculations were standardized to reflect 850-minutes of total hormonal output, which was the average duration between waking and 2100h. Calculation of cortisol and sAA slopes were performed by regressing (ln) cortisol and sAA values on time after waking using the waking, 1130h and 2100h samples. The individual *b*-coefficients that result from these regressions reflect the duration of time (in minutes) that it would take to achieve a one standard deviation change in the natural logarithm of cortisol or sAA. For example, a *b*-coefficient of -2000 for cortisol is interpreted as 2000 minutes to achieve a one unit decrease in the natural logarithm of cortisol. Values closer to zero reflect steeper slopes (less time to achieve a one unit decrease) while values further from zero reflect flatter slopes. The waking+30 minute sample was excluded from calculations of daytime slopes because it is a discreet and distinct component of the cortisol circadian cycle (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010).

Nevrokard advanced heart rate variability (HRV) software (Medistar, Izola, Slovenia) was used to calculate ***Maternal RSA*** using three 5-minute segments sampled from a 24-hour continuous recording of R-R intervals sampled on the following schedule: 30 min after waking, at 1130h and 2100h. These times were chosen to correspond with the collection of salivary analytes. This sampling paradigm resulted in 12 samples of RSA for each participant (six in early pregnancy, six in late pregnancy). Five minutes is a standard interval for short-term recordings of HRV (Task Force, 1996). Each five-minute segment was screened for artifacts according to recommendations (Berntson, Quigley, Jang, & Boysen, 1990). An algorithm was set to detect ectopic beats, defined as interbeat intervals below 200 ms, above 2000 ms, and those that differed from the previous and subsequent 50 interbeat intervals by a value greater than

20%. Interpolation was used to correct ectopic and missing beats. After scanning for artifacts, HRV was quantified using Fast Fourier Transformations (FFT). Each segment was linearly detrended and subjected to a Hamming window. Power spectral density functions were then assembled and estimates of power were adjusted to account for the attenuation produced by the Hamming window. Maternal RSA was quantified as the average power spectral density of R-R fluctuations occurring in the respiratory band (0.15 – 0.40 Hz) (Berntson et al., 1997). RSA for early and late pregnancy were calculated as the mean RSA value occurring across the six 5-minute segments at each time point.

Infant change in ***cortisol*** and ***sAA*** from baseline to stress task were calculated as area under the curve increase from baseline to post-stress. ***Infant RSA*** was quantified in a manner similar to that used for quantifying maternal RSA with the following differences. R-R intervals during each laboratory challenge were re-sampled and partitioned into 45-second segments (3 segments occurring during each task). Infant RSA was quantified as the average power spectral density of R-R fluctuations occurring in the respiratory band (.24 – 1.04 Hz) recommended for use with infants (Bar-Haim, Marshall, & Fox, 2000). RSA for each task was calculated as the mean RSA value occurring across the three 45 second segments composing each task. The variable $RSA_{Frustration}$ was created as the average value (ms^2/Hz) for $RSA_{Toy\ Retraction}$, $RSA_{Toy\ Barrier}$, and $RSA_{Restraint}$. $\Delta RSA_{Frustration}$ was calculated by subtracting $RSA_{Frustration}$ from $RSA_{Baseline}$. Using this formula, higher values reflect greater decrease in RSA from baseline to frustration.

Analytic Strategy

Our data analytic strategy represented the combination of a theory-driven approach to infant classification with a data-driven approach to evaluate which maternal prenatal predictors best discriminate between infant stress response profiles. First, infants were categorized into

theoretically meaningful and a-priori defined stress reactivity profiles (described below). Theory (Bauer et al., 2002; Berntson et al., 1991; Del Giudice et al., 2011) and previous research (Alkon et al., 2011; Alkon et al., 2006; El-Sheikh & Erath, 2011; El-Sheikh et al., 2009; Kroenke et al., 2011; Salomon et al., 2000) support the use of such stress reactivity profiles. Next, discriminant function analysis (DFA) was used as a data-driven approach to determine the prenatal maternal characteristics that best discriminated between infant stress reactivity profiles.

Infant Stress Response Profiles

HPA-Axis Stress Response Profiles

Infant HPA-axis profiles were constructed using positive (> 0) and negative (< 0) difference scores (change from baseline) for cortisol, indicating responders and none-responders, respectively.

Autonomic Stress Response Profiles

Infants were categorized into well validated autonomic reactivity profiles (Alkon et al., 2006; Berntson, Cacioppo, & Quigley, 1993) from the cross classification of positive (> 0) and negative (< 0) difference scores (change from baseline) for sAA and RSA, refer to Table 1. Infants were classified as showing co-activation (sAA > 0, RSA > 0), co-inhibition (sAA < 0, RSA < 0), or reciprocal activation (sAA > 0, RSA < 0; or sAA < 0, RSA > 0) (Berntson et al., 1993).

Multi-System Stress Response Profiles

Consistent with the interactive hypothesis (Bauer et al., 2002) that symmetrical responding across multiple stress systems reflects coordination among the SRS, infants were categorized into multi-system reactivity profiles that reflect multi-system symmetry (sAA > 0, RSA < 0, cortisol > 0; or sAA < 0, RSA > 0, cortisol < 0), ANS symmetry coupled with HPA-

axis asymmetry (sAA > 0, RSA < 0, cortisol < 0; or sAA < 0, RSA > 0, cortisol > 0), multi-system co-activation (sAA > 0, RSA > 0, cortisol > 0), multi-system co-inhibition (sAA < 0, RSA < 0, cortisol < 0), or multi-system asymmetry (sAA > 0, RSA > 0, cortisol < 0; or sAA < 0, RSA < 0, cortisol > 0), refer to Table 1.

Statistical Procedures.

Data Screening. Data from 255 mother-infant dyads were screened for potential outliers. Univariate outliers were identified as values that exceeded a z-score of 3.29 and adjusted according to recommendations (Tabachnick & Fidell, 2012). No more than four values (1.5%) were adjusted for any variable. There was no indication that data was missing in a non-random fashion, *Little's MCAR* $\chi^2(4681) = 3616$, $p = 1.0$. Missing data was estimated using maximum likelihood estimation with 25 iterations. Multivariate outliers were assessed using Mahalanobis distances. Multi-collinearity among prenatal predictors was assessed through tolerance and variance components. No prenatal predictor had a tolerance below .40 and only two predictors had a variance component above .56. Maternal prenatal HR T1 and HR T2 loaded onto the same dimension with variance components of .84 and .72, respectively, indicating some degree of multi-collinearity. To deal with multi-collinearity, mean HR during pregnancy was calculated as the average of HR T1 and HR T2, and was used in subsequent analyses.

Discriminant function analysis of infant reactivity profiles. DFAs were performed through SPSS multivariate analysis of covariance (MANCOVA) using the DISCRIM command to determine what combination of maternal prenatal T1 and T2 variables best discriminated between infant reactivity profiles. Our use of DFA was focused on its ability to identify the set of prenatal predictors that maximally discriminate between infant stress reactivity profiles (Tabachnick & Fidell, 2012). Three separate DFAs were performed, one each with infant HPA-

axis reactivity profiles, autonomic reactivity profiles, and multi-system reactivity profiles entered as the grouping variable. Maternal CAR, sAAAR, cort-AUCg, sAA-AUCg, cortisol slope, sAA slope, HR, RSA, psychological distress, depressed mood, and anxious mood during T1 and T2 were entered as potential predictors, along with infant birth-weight percentile, infant baseline cortisol, baseline sAA and baseline RSA as covariates. A stepwise entry method was selected with *p*-in = .10 and *p*-out = .20. Only maternal predictor variables that significantly discriminated between infant reactivity profiles were included in the final DFAs reported below.

Results

The initial sample of 294 pregnant mothers and their infants was reduced to a final sample of 254. Eighteen infants were born preterm (< 37 weeks) or low birth weight (< 2500g) and excluded from the analysis. Twenty-one infants were missing values on two out of three SRS components and were excluded from analyses. Further, one case was identified as a multivariate outlier with a value that exceeded $\chi^2(22)_{critical}$ of 48.27 and was excluded from analyses. Descriptive statistics for maternal and infant variables are presented in Table 2.

Adherence to ambulatory saliva collection

Adherence to ambulatory saliva collection was excellent. Women returned 97.9 percent (2302 of out of a possible 2,352) of salivary samples during early pregnancy and 86.2 percent (2,028 out of a possible 2,352) during late pregnancy.

Manipulation check for infant stress responses

There was a reliable decrease in infant RSA, M_{diff} = -55.14 ms^2/Hz, $t(253) = 2.27$, SE = 24.32, $p < .05$, from baseline to stress, and reliable increases in cortisol, M_{diff} = 0.65 ln(μg/dL), $t(253) = 2.00$, $SE = 0.33$, $p < .05$, and sAA, M_{diff} = 3.48 ln(U/mL), $t(253) = 5.38$, $SE = 0.71$, $p <$

.01. These results suggest that the stress paradigm successfully generated reliable stress responses, refer to Table 3.

Classification of infants into reactivity profiles

Table 3 reports final classification of infants into reactivity profiles for the HPA-axis, ANS, and multi-system classifications. Although a majority of infants (54%) demonstrated a cortisol increase following the stressor, the remaining 46% had no increase or decreased. Within the ANS, 51% of infants demonstrated reciprocal ANS activation, whereas 30% exhibited co-activation and 19% co-inhibition. The frequency of each infant stress reactivity profile was similar across sex. Within the multi-system classification, the distribution of infants was fairly equal across the five profiles. The largest group (27%) had ANS symmetry combined with cortisol asymmetry. The remaining groups were multi-system symmetry (25%), multi-system co-activation (19%), multi-system asymmetry (19%), and multi-system co-inhibition (10%).

To determine whether factors related to travel or arrival at the laboratory or infant behavior in the baseline were related to these profiles we conducted a series of univariate ANOVAs for the following potential covariates: time of arrival, negative affect during the period from arrival to start of stressor, behavioral state at time of arrival, and behavioral state during baseline recording. None of the effects were significant (all p values > .25) suggesting that arrival and baseline characteristics of the infant were not related to reactivity profiles.

We conducted univariate ANOVAs to determine whether baseline characteristics of the infant SRS were related to their classification into stress response profiles. HPA-axis stress reactivity profiles were not associated with infant baseline cortisol, sAA, or RSA, all p's > .60. In contrast, ANS stress reactivity profiles were associated with infant baseline ln(RSA), $F(2,251) = 22.26$, $SE = 0.11$, $p < .01$, and baseline ln(sAA), $F(2, 251) = 3.98$, $SE = .77$, $p < .05$, but not

with baseline cortisol, $F(2, 251) = 2.01$, $SE = 0.01$, $p = .14$. Given that baseline values may have contributed to infant classification, at least for the ANS classification, baseline values for infant cortisol, sAA, and RSA were included as covariates in the DFA models.

Prenatal predictors of infant HPA-Axis stress reactivity profiles

The combination of covariates (i.e., birth-weight percentile and infant baseline cortisol) did not significantly influence the relationship between prenatal predictors and HPA-axis stress reactivity profiles, *Pillais* = .01, $F(6, 498) = 0.38$, $p = .89$. As shown in Table 4, cortisol slope T1, RSA T1, and sAAAR T1 separated the two infant HPA-axis stress reactivity profiles along one discriminant function, $F(3, 248) = 5.36$, $p < .01$, accounting for 6.4% of the variance in cortisol reactivity profiles, Canonical $R^2 = .064$. Infants whose cortisol increased during stress had mothers with a steeper daytime cortisol slope T1, $M = -2425.56$ (min/ln(µg/dL)), $SD = 1199.73$, lower basal RSA T1, $M = 423.50$ ms^2/Hz, $SD = 364.67$, and a larger sAAART1, $M = -12.24$, $SD = 13.21$, while infants whose cortisol decreased during stress had mothers with a flatter daytime cortisol slope T1, $M = -2830.49$ (min/ln(µg/dL)), $SD = 1468.95$, higher basal RSA T1, $M = 560.16$ ms^2/Hz, $SD = 486.81$, and a smaller sAAART1, $M = -9.19$, $SD = 12.66$. Characteristics of prenatal predictors associated with reactivity profiles are summarized in Table 3 and descriptive statistics for each group are reported in Supplemental Table 1.

Prenatal predictors of infant autonomic stress reactivity profiles

The combination of covariates (i.e., birth-weight percentile, and infant baseline sAA and RSA) did not significantly influence the relationship between prenatal predictors and ANS stress reactivity profiles, *Pillais* = .02, $F(6, 496) = 0.59$, $p = .80$. Two discriminant functions significantly separated the three infant autonomic stress reactivity profiles, with a combined $F(4, 496) = 5.74$, $p < .01$, accounting for 12.8% of the total relationship between predictors and

groups, Canonical R^2 = .128, refer to Table 4. The first function accounted for 88.3% of between group variance. After removal of the first function, the association between ANS reactivity profiles and prenatal predictors was not significant, $F(1, 247) = 2.09$, $p = .13$, indicating that the second discriminant function only discriminated between ANS profiles when used in conjunction with function one.

sAA slope T1 loaded positively on function 1, psychological distress T2 loaded negatively onto function 2, and RSAT2 was a complex variable loading negatively onto both functions. As shown in Figure 2, the combination of a decreasing diurnal sAA slope T1 and relatively high psychological distress T2 maximally discriminated infants with co-inhibition, $b_{\text{sAA slope T1}}$ = -184.65 (min/ln(U/min)), SD = 433.73, $M_{\text{psychological distress T2}}$ = 1.48, SD = 0.41, from infants with reciprocal activation, $b_{\text{sAA slope T1}}$ = 45.28 (min/ln(U/min)), SD = 507.71, $M_{\text{psychological distress T2}}$ = 1.32, SD = 0.28, and co-activation, $b_{\text{sAA slope T1}}$ = 169.23 (min/ln(U/min)), SD = 423.27, $M_{\text{psychological distress T2}}$ = 1.31, SD = 0.29. RSA T2 was a complex variable, with weak loadings on both functions. Relatively low RSA T2 could be used to discriminate infants with co-activation, M = 226.72 ms^2/Hz, SD = 207.80 from infants with reciprocal activation, M = 292.12 ms^2/Hz, SD = 257.51.

Discriminant function analysis of multi-system (ANS and HPA axis) stress reactivity profiles

The combination of covariates (i.e., birth-weight percentile, and infant baseline cortisol, sAA and RSA) significantly influenced the relationship between prenatal predictors and multi-system stress reactivity profiles, *Pillais* = .11, $F(9, 738) = 3.01$, $p < .01$. After adjustment of covariates, infant multi-system stress reactivity profiles were separated by three discriminant functions, with a combined $F(12, 738) = 3.50$, $p < .001$, accounting for 16.1% of the total

relationship between predictors and groups, Canonical R^2 = .161, refer to Table 4. After removal of the first function, there was a significant association between groups and predictors, $F(6, 490) = 2.71$, $p < .05$, indicating that the prenatal predictors associated with the second discriminant function are related to multi-system profiles independent of the prenatal predictors in the first discriminant function. The second function accounted for 5% of the relationship between groups and predictors. After removing the first two functions the association between multi-system reactivity profiles and prenatal predictors was not significant, $F(2, 246) = 2.07$, $p = .13$, indicating that function 3 only discriminated between multi-system stress response profiles when used in conjunction with the first two functions.

The first function was characterized by RSA T2, the second by daytime cortisol slope T1, and the third by sAAAR T1. As shown in Figure 3, multi-system symmetry was maximally discriminated from multi-system co-inhibition using sAAR T1. Multi-system symmetry was characterized by a relatively small sAAAR T1, M = -7.59, SD = 12.00 while multi-system co-inhibition was characterized by a relatively large sAAAR T1, M = -14.54, SD = 11.02. Multi-system co-activation was maximally discriminated from all other profiles by a relatively steep cortisol slope T1, M = -2062.16 (min/ln(µg/dL)), SD = 1117.72. Finally, a small RSA T2 maximally separated infants with multi-system symmetry, M = 208.37 ms^2/Hz, SD = 204.39, and multi-system asymmetry, M = 221.64 ms^2/Hz, SD = 176.72, from infants with multi-system co-inhibition, M = 345.92 ms^2/Hz, SD = 332.79, or ANS symmetry coupled with HPA-axis asymmetry, M = 370.96 ms^2/Hz, SD = 278.16.

Discussion

We prospectively investigated whether prenatal maternal psychological distress and physiological characteristics could discriminate between biologically-based infant stress

reactivity profiles within and across physiological systems (i.e., ANS and HPA-axis). Markers of maternal parasympathetic, sympathetic-adrenal-medullary, and HPA-axis activity, along with self-reported psychological distress during pregnancy discriminated between infants with different autonomic, HPA-axis, and multi-system stress response profiles. For the HPA axis, it was daytime cortisol slope, sAA awakening response, and basal RSA in early pregnancy that distinguished between responsive and non-responsive profiles. Autonomic response profiles were distinguished by daytime sAA slope during early pregnancy, and psychological distress and RSA measures from late pregnancy. The multi-system profiles were distinguished by daytime cortisol slope and the sAA awakening response in early pregnancy along with basal RSA in late pregnancy. These effects held after adjusting for baseline characteristics and relevant covariates. Taken in the context of other studies suggesting that maternal stress during pregnancy has profound and enduring effects on the fetal/infant ANS and HPA axis, these results suggest that multiple aspects of maternal psychological and physiological states during pregnancy may contribute to the organization of the infant SRS.

Infant Stress Reactivity Profiles

Consistent with previous research, there was significant individual variability in infant PNS, SNS, and HPA-axis responses to frustration tasks despite the expected overall decreases in RSA and increases in cortisol and sAA. This variability allowed for classification of infants into stress response profiles created with the guidance of two prominent theories - the doctrine of autonomic space (Berntson et al., 1991) and the interactive model of stress responding (Bauer et al., 2002).

Within the HPA-axis profiles, infants who displayed cortisol increases had mothers with relatively steep daytime cortisol slopes, larger sAA awakening responses, and lower basal RSA

during early pregnancy. Opposite association with maternal prenatal physiology were observed for infants who did not mount an HPA-axis response to the stressor. In the context of previous research suggesting that HPA-axis non-response to a relevant stressor is associated with externalizing problems (Dickerson & Kemeny, 2004), our findings suggest that in-utero exposure to the combination of flattened maternal cortisol slope, blunted sAA awakening response and higher RSA may be a risk factor for dysregulation of child HPA-axis and psychopathology. There is evidence to suggest that flattened daytime cortisol slopes in adults and blunted or absent sAA awakening responses reflect dysregulation of the HPA-axis and SNS (Thoma, Joksimovic, Kirschbaum, Wolf, & Rohleder, 2012), and therefore the association of these maternal parameters with infant HPA-axis non-response is in agreement with current understanding of the developmental origins hypothesis. The findings for higher maternal RSA associated with infant HPA-axis non-response seem prima facie counter-intuitive. In general, higher basal RSA is thought to act as a protective factor against negative physical and mental health outcomes whereas reduced RSA is associated with increased risk of physical disease (Thayer, Yamamoto, & Brosschot, 2010) and psychopathology (Beauchaine, 2015). In the context of pregnancy, however, the correlates of RSA are poorly defined and dramatic pregnancy-related adaptations within the ANS may fundamentally alter the implications of RSA for maternal and infant health. For example, pregnancy itself is characterized by significantly reduced RSA (Stein et al., 1999), but mothers who exhibit conditions such as gestational hypertension show significantly elevated RSA (Ekholm, Tahvanainen, & Metsälä, 1997). In the context of our findings, relatively lower RSA may indicate appropriate PNS adaptation to pregnancy. Further research into typical and atypical patterns of change in basal RSA during pregnancy is needed to elucidate our observed results.

Within the ANS profiles, mothers with decreasing daytime sAA slopes during early pregnancy and relatively greater psychological distress during late pregnancy were more likely to have infants who exhibited co-inhibition of SNS and PNS during stress at 6-months of age. Given that daytime sAA slopes are normatively positive, decreasing sAA slopes may indicate SNS dysregulation. The results suggest that dysregulation of the maternal SNS and psychological distress may play a role in the formation of infant ANS co-inhibition, which in children is a known risk factor for behaviour problems (Beauchaine et al., 2007; Boyce et al., 2001). Maternal RSA during late pregnancy was a relatively weak discriminator but did separate infants with co-activation from other infants. Infants with co-activation, which is thought to be a vulnerability factor for externalizing behaviour in children (El-Sheikh & Erath, 2011; El-Sheikh et al., 2009; Gordis et al., 2009) had mothers with relatively less RSA during late pregnancy.

Within the multi-system profiles, infants who exhibited symmetrical PNS, SNS, and HPA-axis responses had mothers with relatively blunted sAA awakening responses during early pregnancy coupled with low basal RSA during late pregnancy. Given that PNS and the HPA-axis response to stress and challenge tend to be reciprocally coordinated (Doussard-Roosevelt et al., 2003) and that SNS and HPA-axis responses to stress tend to be directly coordinated (Goldstein & Kopin, 2008), this multi-system symmetry profile is theorized to reflect effective coordination of stress response systems and should be associated with salutary physical and mental health outcomes. Nevertheless, this profile was associated with aspects of maternal physiology during pregnancy that are known risk factors for later health and behaviour problems. Infants who exhibited PNS, SNS, and HPA-axis co-activation were also characterized by mothers with relatively blunted sAA awakening responses during early pregnancy and low basal RSA during late pregnancy but this profile was unique in that it was the only profile associated

with a relatively steep maternal cortisol slope during early pregnancy. Infants within the profiles that exhibit co-inhibition and asymmetry between ANS and HPA-axis were unique in that they were associated with mothers who had relatively large sAA awakening responses during early pregnancy combined with relatively high basal RSA during late pregnancy. The overall set of results is somewhat surprising in that none of the multi-system profiles had a pattern of associations with maternal prenatal physiology that could be considered protective or salutary. We note, however, that one of the ambiguities inherent within the multi-system classification is that multiple patterns of infant physiology are sometimes grouped within the same profile (see Table 1) and this may limit the specificity of the associations between prenatal exposures and SRS function.

Composition of the prenatal predictors

The fact that aspects of the maternal PNS, SNS, HPA-axis and psychological distress during pregnancy reliably discriminated among infant stress response profiles was not surprising (it is implicitly predicted by the developmental origins hypothesis), but the overall pattern of associations between the maternal predictors and infant SRS may yield further insight into the ways that maternal experience and physiology affects fetal and infant development. Three observations are particularly noteworthy. First, all three components of the maternal stress physiology contributed to discriminating between infant stress reactivity profiles, suggesting that all three components of the maternal stress physiology work together to fine-tune the SRS of the developing fetus. This observation highlights the importance of adopting a multi-system perspective.

Second, and consistent with previous studies suggesting that the effects of prenatal exposures on organ or tissue development are sensitive to the timing of the exposure in-utero, the

pattern of physiological predictors associated with infant response profiles suggest that early-mid pregnancy exposures were more strongly related to the organization and function of the infant SRS, relative to exposures in later pregnancy. This was particularly true with regard to the maternal SNS and HPA-axis, both of which were related to infant stress response profiles only through early-mid pregnancy exposures. Early-mid pregnancy is a time characterized by the primary formation and largest growth of the human fetal adrenal cortex (Ishimoto & Jaffe, 2010), thus exposures in early-mid pregnancy potentially have greater organizational influence over the infant SRS. The exception to the overall pattern of early gestation exposures was the maternal PNS, which was associated with the infant ANS and multisystem profiles via late gestation exposures. We speculate that because of the importance of vagus nerve function to our measures of maternal and infant PNS function, and because late gestation is a time characterized by neural maturation and myelination (Moore, Persaud, Torchia, & Persaud, 2008), the associations between late pregnancy maternal PNS function and infant PNS function may result from a greater potential to influence PNS function in late gestation.

Third, while there is value in this work, adopting a multi-system approach can result in a high level of complexity, particularly when interpreting profiles. In the present investigation we observed prenatal maternal predictors that crossed various stress response profiles and others that were unique to one profile. Self-reported psychological distress was a significant predictor only for ANS profiles suggesting that psychological distress has specific and unique effects on the ANS that are not captured by the physiological markers we included. This is consistent with previous research reporting a positive association between prenatal psychological distress during late pregnancy and fetal HRV (DiPietro et al., 2010). Moreover, Monk and colleagues reported greater increases in heart rate among fetuses of women with comorbid psychiatric status

undergoing the stroop task at 36 to 38 weeks gestational age relative to healthy controls and these effects were independent of prenatal cortisol (Monk et al., 2011). Interestingly, maternal psychological distress was not associated with infant HPA-axis response profiles in the context of predictors from the maternal HPA-axis, SNS and PNS. In keeping with tenants of the Developmental Origins hypothesis, this finding is consistent with the proposal that the effects of prenatal psychological distress on the infant SRS are mediated by the maternal stress response systems. Also noteworthy is that fact that dynamic measure of stress system function (i.e., the awakening response and daytime slope) were associated with all infant SRS profiles but summary measures of total system output (i.e., AUCg) were not related to any of the infant SRS profiles.

Although it is known that maternal hormones, such as catecholamines and cortisol, cross the placenta (Fisher, 1998; Seckl & Holmes, 2007), the pathophysiological mechanisms through which the maternal ANS and HPA-axis are associated with infant stress reactivity profiles are not well understood. One possibility is through hormone mediated maturation of neural and structural regions involved in mounting a response to a stressor. A highly integrated set of neural structures referred to as the central autonomic network (CAN) has been described (Benarroch, 1993) and elucidated (Thayer, Hansen, Psychol, & Johnsen, 2009). The CAN includes overlapping structures that are involved in the regulation of the PNS, SNS, and HPA-axis (e.g., the hypothalamus, amygdala, and brainstem). Glucocorticoids are involved in the maturation of the CAN (see Braun et al., 2013; Harris & Seckl, 2011). Exposure to glucocorticoids in utero has been reported to reduce cerebral myelination in animals (Antonow-Schlorke et al., 2009) and cortical thickness in adolescents (Davis, Sandman, Buss, Wing, & Head, 2013). Further, elevated levels of catecholamines in maternal blood lead to constriction of

placental blood vessels, decreased fetal supply of glucose, activation of the fetal HPA-axis (Challis et al., 2000), and catecholamine release (Gu & Jones, 1986). Taken together, these observations suggest that the maternal stress response systems may contribute to the development of infant stress response profiles through a variety of hormonal and epigenetic pathways.

Importance of postnatal environment

Our results indicate that aspects of prenatal maternal stress physiology and psychological distress account for 6.4% to 16.1% of variability in infant stress reactivity profiles which constitute small to medium effect sizes (Cohen, 1992). The magnitude of these effects are similar to those reported for the association between child sexual abuse on various developmental outcomes (Paolucci, Genuis, & Violato, 2001). It is important to note that a large degree of variance was left unaccounted for and this is perhaps due to the impact of important postnatal factors that are also involved in shaping stress reactivity in early infancy. Parents play a vital role in the social regulation of infant stress reactivity through their responses to infant distress (Gunnar & Donzella, 2002). For instance, lower maternal sensitivity is associated with greater infant sympathetic activation, greater HPA reactivity, and lower parasympathetic activation during a stressor (Enlow et al., 2014). Infant stress reactivity has also been associated with other postnatal environmental factors including family functioning (e.g., marital conflict) (El-Sheikh & Erath, 2011; Towe-Goodman, Stifter, Mills-Koonce, & Granger, 2012), socioeconomic status (Thayer & Kuzawa, 2014), and parental mental health (Dierckx et al., 2009; Feldman et al., 2009). Thus, it is important to acknowledge that the postnatal environment can further modify infant stress reactivity development.

Implications for developmental psychopathology

To our knowledge this is the first investigation of prenatal influences of infant stress response profiles. This work may have implications for developmental psychopathology given recent studies implicating interactions between PNS, SNS, and HPA-axis function and behavioural outcomes in children. For example, consistent with theories of hypoarousal (Phillips, 2011), asymmetrical cortisol increases in conjunction with low ANS reactivity (measured using heart rate) to a social stress task was associated with externalizing disorders among 215 adolescents (Hastings et al., 2011). Similarly, asymmetrical cortisol decreases coupled with PNS withdrawal and SNS activation to a social stress task was associated with externalizing problems in a sample of 715 Dutch adolescents (Nederhof et al., 2014). Moreover, asymmetric low cortisol reactivity coupled with high sAA reactivity in response to viewing conflict vignettes was associated with emotional insecurity and maladjustment among 195 8-year-old children, albeit this association was moderated by marital discord (Koss et al., 2014). In the context of these other studies, our findings may suggest that stress response profiles in children mediate the effects of stress-relevant exposures during gestation on children's health and development.

Although our study highlights the relevance and utility of assessing multiple maternal stress response systems in regard to development of the infant SRS, it also highlights the need for both theoretical and methodological advances that can overcome some of the difficulties inherent in this approach. Whereas a great deal of research has highlighted the importance of adopting multi-system perspectives (Allwood et al., 2011; Bauer et al., 2002; Del Giudice et al., 2011; Gordis, Granger, Susman, & Trickett, 2006; Hill-Soderlund et al., 2008; Keller & El-Sheikh, 2009; McEwen, 1998) there has been considerably less guidance on how to operationalize and integrate information from stress-relevant systems. Because each component

of the fetal SRS forms and becomes functional in the context of the other components, multi-system approaches increase ecological validity, but they do so at the cost of parsimony. This trade-off remains a significant challenge to the field. Existing solutions include calculating ratios between systems to reflect relative activation (Ali & Pruessner, 2012), calculating interaction terms to reflect cross-system coordination (Chen, Raine, & Granger, 2015; El-Sheikh et al., 2011; Erath & El-Sheikh, 2015), or using cut-off criteria to create categorically distinct reactivity profiles (Alkon et al., 2006; Kroenke et al., 2011). There are strengths and limitations to each of these approaches. The ratio and interaction methods preserve information on the magnitude of stress responses but are less amenable to the simultaneous assessment of more than two components of the SRS and become difficult to implement as complexity increases. Although using categorical cut-offs ignores potentially meaningful differences between small and large stress responses and does not adequately account for the variability within infant stress responses, it offer a parsimonious approach to modeling three or more components of the SRS. None of the current approaches seem entirely satisfactory and there is therefore a need for both theoretical and methodological advances to guide future multi-system work. As noted by one anonymous reviewer, what may be needed is a composite variable of infant stress responses across multiple systems that allows for a continuous index of the magnitude and integration of these responses within each individual.

Limitations

There are several limitations to the present investigation. First, whereas research using animal models is suggestive of prenatal programming, our data are observational and do not provide evidence that the prenatal exposures we assessed directly influence infant stress response systems. Second, our laboratory sampling paradigm for the infant saliva was optimized for

cortisol, which usually peaks 20-30 minutes post-stressor, and not for sAA, which usually peaks within 5-10 minutes post stressor (Nater & Rohleder, 2009). Third, categorizing infant stress responses into theoretically meaningful and well-validated stress response profiles ignores information about the magnitude of stress response and equates infants who display large and small stress responses in the same direction. Fourth, indices of PNS and SNS activity were taken from discrete end organs (i.e., cardiac for PNS and salivary glands for SNS) and may not reflect the level of symmetrical coordination observed in an end organ dually innervated by the PNS and SNS. These findings should be replicated using pre-ejection period as a marker of SNS output. Fifth, we cannot rule out the possibility that genetic heritability plays a role in the associations between maternal physiology and infant stress reactivity profile. Finally, participants in this study were primarily White, middle to upper class, married women and caution should be taken when generalizing these results to low SES and ethnically diverse populations.

Conclusions

This prospective longitudinal investigation identified prenatal psychological and physiological markers associated with infant stress response profiles. The results suggest that individual differences in infant stress responses might be meaningfully related to stress-relevant exposures during gestation. The organization of infant stress response systems may have enduring impacts on the children's adaptation to their environments as well as health outcomes (Barker, 2002; Glover, 2011; Van Den Bergh, 2011).

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Table 1

Stress Response Patterns Comprising Stress Response Profiles

Stress Response System	Stress Response Profile	Response patterns		
		RSA	**Salivary α-Amylase**	**Cortisol**
ANS	Co-activation	> 0	> 0	
	Co-inhibition	< 0	< 0	
	Reciprocal activation	< 0	> 0	
		> 0	< 0	
HPA-Axis	Responders			> 0
	Non-Responders			< 0
Multi-system	Symmetry	< 0	> 0	> 0
		> 0	< 0	< 0
	ANS symmetry coupled with HPA-axis asymmetry	< 0	> 0	< 0
		> 0	< 0	> 0
	Co-activation	> 0	> 0	> 0
	Co-inhibition	< 0	< 0	< 0
	Asymmetry	< 0	> 0	< 0
		< 0	< 0	> 0

Note. ANS = Autonomic Nervous System; HPA-axis = Hypothalamic Pituitary Adrenal Axis

Table 2

Sample Characteristics and Descriptive Statistics

Maternal Variable	***M(SD)***	
Age (years)	31.67 (3.73)	
Pregnancy BMI (Kg/m^2)	24.62 (5.06)	
	T1 [*M(SD)*]	**T2 [*M(SD)*]**
CAR	0.92 (1.70)	1.58 (1.79)
sAAAR	-10.83 (13.02)	-9.52 (11.71)
CortAUCg	155.39 (39.01)	216.52 (44.64)
sAAAUCg	3201.22 (729.42)	3306.54 (720.12)
CortSlope (*b*) (min/ln(µg/dL))	-2604 (1306.79)	-2753.47 (1128.21)
sAASlope (*b*) (min/ln(U/min))	60.56 (407.27)	14.77 (453.20)
HR (BPM)	42.12 (483.81)	-3.21 (483.09)
RSA (ms^2/Hz)	485.34 (427.46)	272.55 (247.60)
Depressed mood	5.51 (4.16)	5.20 (3.94)
Pregnancy anxiety	0.75 (0.44)	0.65 (0.38)
Psychological Distress	1.35 (0.32)	1.35 (0.31)
Infant Variable	***M*(SD)**	
Gestational age at birth (weeks)	39.48 (1.16)	
Birth Weight Percentile	47.19 (28.89)	
Gestational age at T1 (weeks)	15.03 (3.60)	
Gestational age at T2 (weeks)	32.46 (0.97)	
Age at stress testing (weeks)	24.69 (2.70)	
Baseline RSA (ms^2/Hz)	495.31 (402.72)	
Baseline Cortisol (µg/dL)	0.19 (0.09)	
Baseline sAA ln(U/mL)	3.18 (0.89)	
ΔAUCi Cortisol	0.65 (5.35)	
ΔAUCi sAA	3.84 (11.39)	
ΔRSA (ms^2/Hz)	-55.14 (387.70)	

Note. N = 254 (134 male); CAR = Cortisol Awakening Response; sAAAR = Salivary α-Amylase awakening response; CortAUCg = Cortisol Area Under the Curve from Ground; sAAAUCg = Salivary α-Amylase Area Under the Curve from Ground; CortSlope = Individual Regression Slope for Cortisol; sAASlope = Individual Regression Slope for sAA; HR = Heart Rate; RSA = Respiratory Sinus Arrhythmia; T1 = Early Pregnancy Testing (~15 weeks gestation); T2 = Late Pregnancy Testing (~32 weeks gestation); ΔAUCi Area Under the Curve Increase from Baseline to Stressor; ΔRSA = Change in RSA from Baseline to Stressor.

Table 3

Infant Stress Response Profiles and Maternal Predictors that Discriminate Profiles

Profile	*N* (% male)	Maternal predictors
HPA-axis		
Responders	136 (59)	Steep Cortisol Slope T1; ↓RSA T1; Large sAAAR T1
Non-Responders	118 (46)	Flat Cortisol Slope T1; ↑RSA T1; Decreasing sAAAR T1
Autonomic		
Reciprocal activation	132 (52)	↓Psychological Distress T2; ↑RSA T2; ↑ daytime sAA slope T1
Co-Activation	77 (53)	↓Psychological Distress T2; ↓RSA T2; ↑ daytime sAA slope T1
Co-Inhibition	45 (53)	↑Psychological Distress T2; ↑RSA T2; ↓ daytime sAA slope T1
Multi-system		
Multi-Systems Symmetry	64 (55)	Small sAAART1; ↓RSA T2
ANS Symmetry – Cortisol asymmetry	68 (47)	↑RSA T2
Multi-Systems Co-activation	48 (56)	Steep Cortisol Slope T1
Multi-Systems Co-inhibition	26 (54)	Flat Cortisol Slope T1; ↑RSA T2; Large sAAART1
Multi-Systems Asymmetry	48 (48)	Large sAAART1

Note. N = 254 (134 male); ↓ = Low; ↑ = High; ANS = Autonomic Nervous System; RSA = Respiratory Sinus Arrhythmia; sAA = Salivary α-Amylase; sAAAR = Salivary α-Amylase Awakening Response; T1 = Early Pregnancy; T2 = Late Pregnancy.

Table 4

Discriminant Function Analyses of Infant Stress Response Profiles

Model	Predictor Variable	Correlation of Predictor Variables with Rotated Discriminant Functions 1	2	3	Univariate
					F(1, 250)
HPA-axis response profiles	CortSlope T1	.59			5.54*
	RSA T1	-.61			6.00*
	sAAART1	-.47			5.3*
	Canonical *R*	.25			
	Eigenvalue	.064			
Birth Weight Percentile and infant baseline cortisol served as covariates					
					F(2, 248)
Autonomic response profiles	sAA Slope T1	.99			7.53**
	Psychological Distress T2		.99		4.81*
	RSA T2	-.11	-.12		3.23*
	Canonical *R*	.34	.12		
	Eigenvalue	.13	.02		
Birth Weight Percentile, infant baseline sAA and infant baseline RSA served as covariates					
					F(4, 246)
Multi-system response profiles	RSA T2	.83			5.06**
	CortSlope T1		-.88		2.61*
	sAAAR T1			-.81	2.58*
	Canonical *R*	.31	.21	.13	
	Eigenvalue	.11	.05	.02	
Birth Weight Percentile, infant baseline sAA, infant baseline RSA, and infant baseline cortisol served as covariates					

Note. N = 254 (134 male); ANS = Autonomic Nervous System; RSA = Respiratory Sinus Arrhythmia; sAA = Salivary α-Amylase; sAAAR = Salivary α-Amylase Awakening Response; T1 = Early Pregnancy (~15 weeks gestation); T2 = Late pregnancy (~32 weeks gestation). With the exception of RSAT2 on the autonomic stress response profile, function loadings below .30 are not reported.

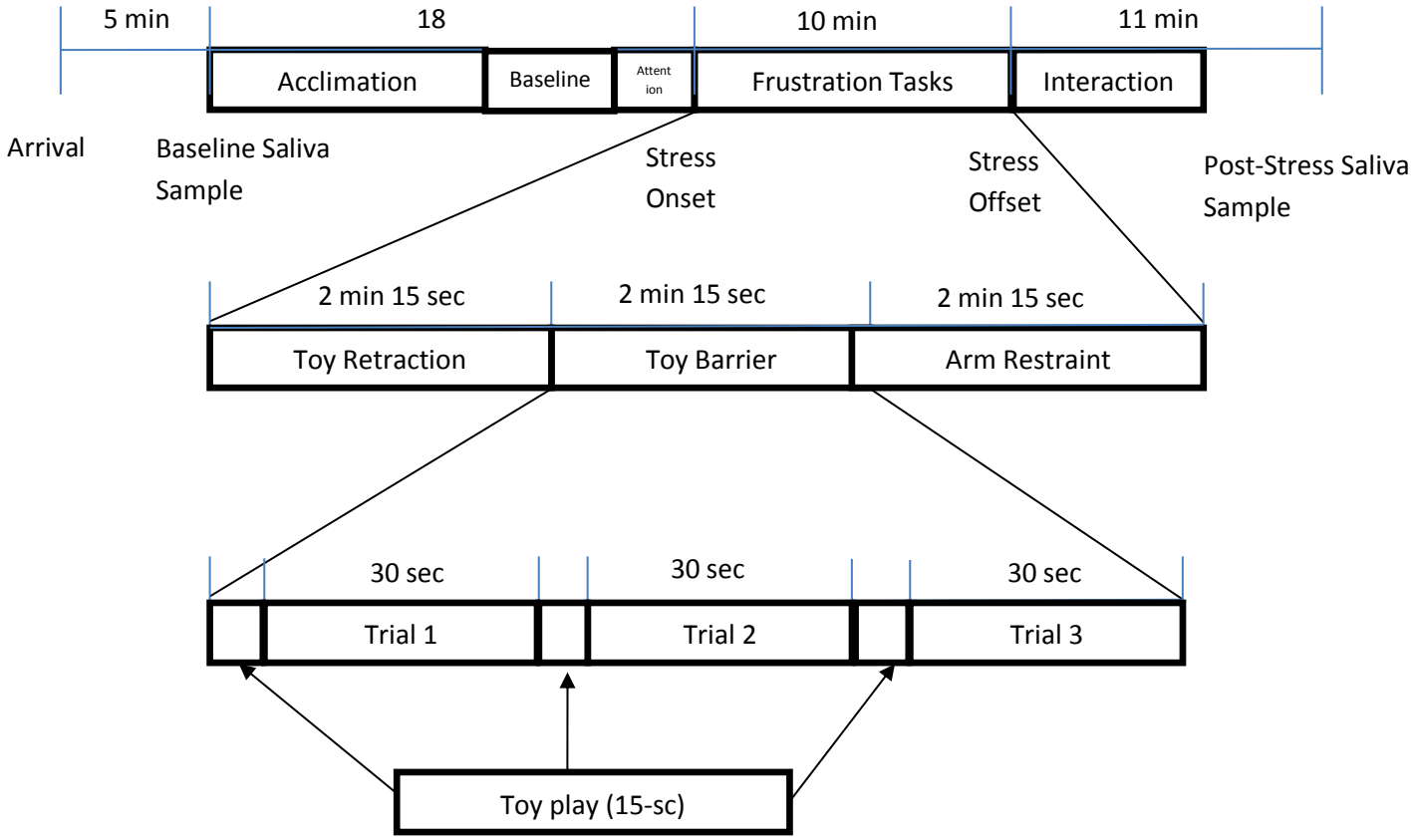

Figure 1

Graphical Depiction of Infant Laboratory Assessment

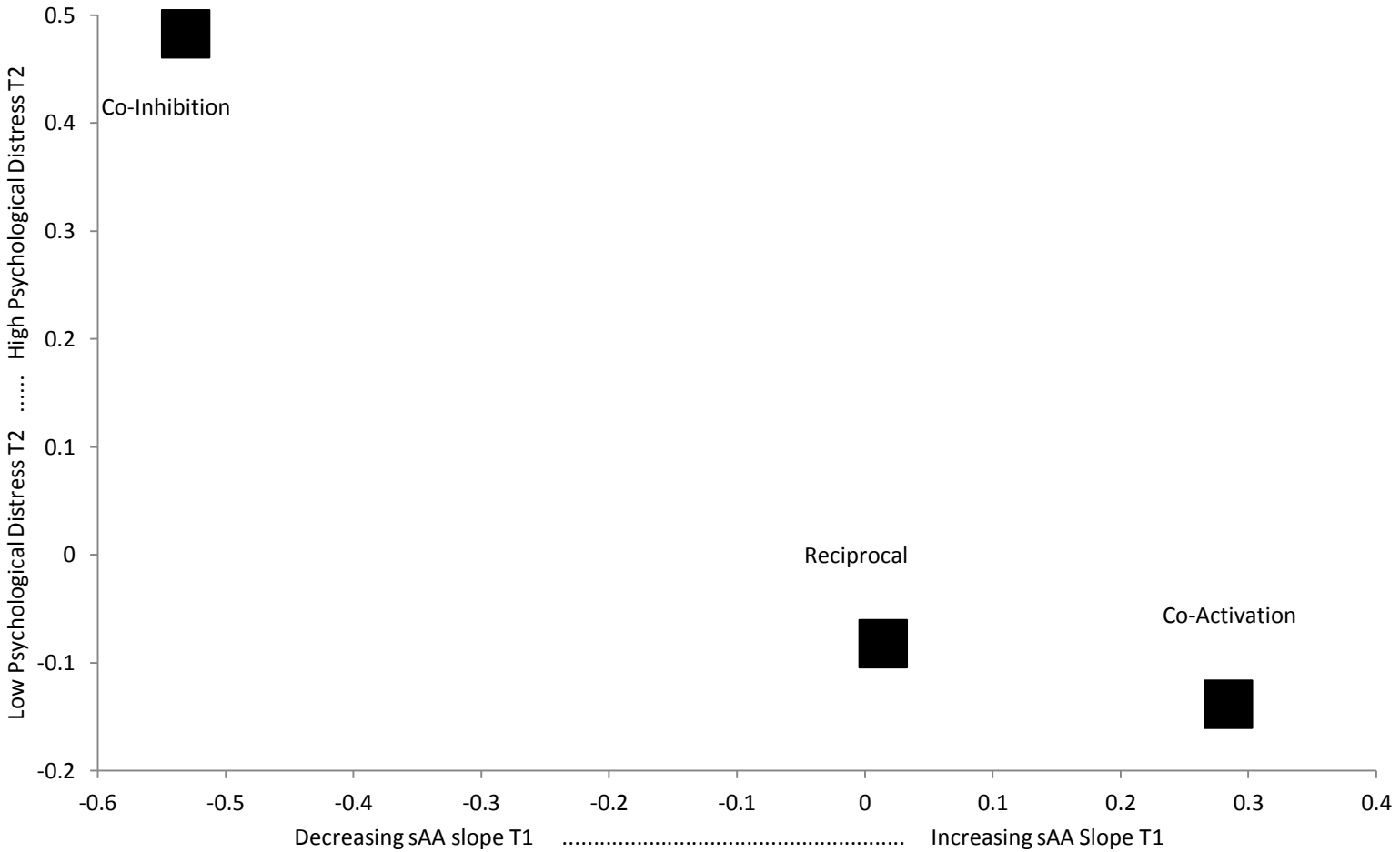

Figure 2. Discriminant function depicting the association between early pregnancy (T1) salivary α-amylase slope, late pregnancy (T2) psychological distress, and infant autonomic stress response profiles. The black squares depict centroids (i.e., multivariate means) for each group on each function. The numbers on the X and Y axes reflect the distances between each centroid on the linear combination of predictors that load onto each function. RSA T2 was not included in this graph due to low loadings on both functions.

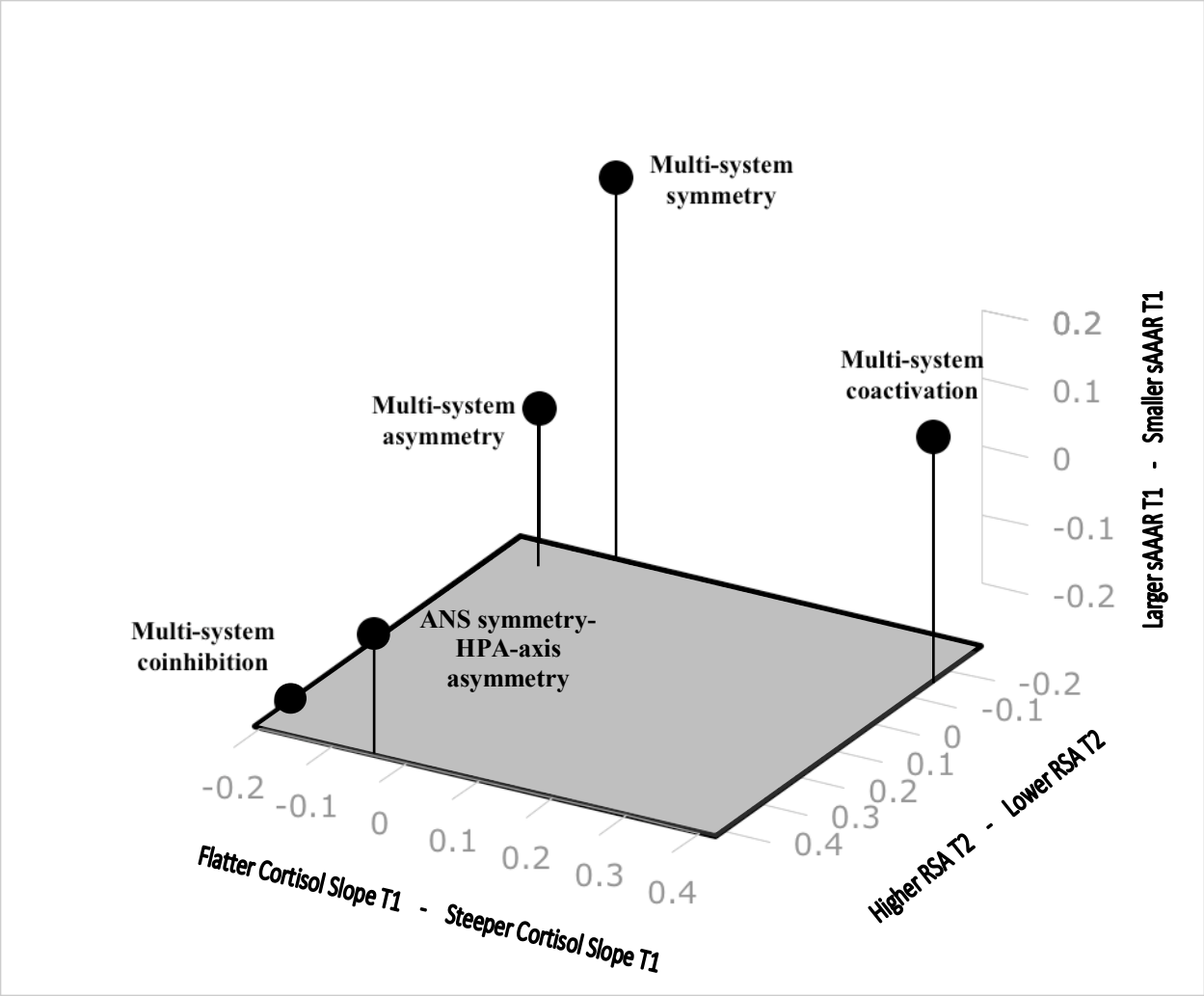

Figure 3. Discriminant function depicting the association between late pregnancy (T2) maternal respiratory sinus arrhythmia (RSA), early pregnancy (T1) salivary α-amylase awakening response (sAAAR), T1 daytime cortisol slope, and infant multi-systems stress response profiles. The shaded square depicts the floor of the 3-dimensional space. Positioning left or right, relative to the front of the space indicates discrimination by maternal cortisol T1. Positioning front or back, relative to the right side of the space indicates discrimination by maternal RSA T2. Height above the floor indicates discrimination by maternal sAAAR T2. The black circles depict centroids (i.e., multivariate means) for each group on each function. The numbers on the X, Y, and Z axes reflect the distances between each centroid on the linear combination of predictors that load onto each function.

Supplemental Table 1

	Cortisol Slope T1 (min/ln(µg/dL))	RSA T1 (ms^2/Hz)	sAAAR T1	sAA Slope T1 (min/ln(U/min)	Psychological Distress T2	RSA T2 (ms^2/Hz)	sAAAR T1
	Mean (SD)						
HPA-Axis							
Responders	-2425.56 (1199.73)	424.42 (362.48)	-12.24 (13.21)				
None-Responders	-2810.03 (1397.16)	555.57 (483.96)	-9.19 (12.66)				
Autonomic							
Reciprocal Activation				45.28 (507.71)	1.32 (0.28)	292.12 (257.51)	
Co-Activation				169.23 (423.27)	1.31 (0.29)	226.72 (207.81)	
Co-Inhibition				-184.65 (433.73)	1.48 (0.41)	293.53 (274.29)	
Multi-System							
Multi-system Symmetry	-2666.69 (1290.37)					208.37 (204.39)	- 7.59 (12.00)
ANS Symmetrical – HPA-axis asymmetrical	-2687.57 (1365.43)					370.95 (278.16)	- 12.11 (14.51)
Multi-systems Co-Activation	-2062.16 (1117.72)					229.87 (215.64)	- 11.53 (13.17)
Multi-systems Co-Inhibition	-2910.19 (1324.01)					345.91 (332.78)	-14.45 (11.02)
Multi-systems Asymmetry	-2779.52 (1312.46)					221.64 (176.72)	-10.60 (12.52)

Note. N = 254 (134 male); High; ANS = Autonomic Nervous System; RSA = Respiratory Sinus Arrhythmia; sAA = Salivary α-Amylase; sAAAR = Salivary α-Amylase Awakening Response; T1 = Early Pregnancy; T2 = Late Pregnancy.