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## Identifying Profiles of Multisystem Physiological Activity Across Early Childhood: Examining Developmental Shifts and Associations with Stress and Internalizing Problems

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### Abstract

Physiological regulation is an important predictor of health across the lifespan. Regulation occurs across multiple collaborative systems, yet few empirical studies explore multisystem activity and how this collaborative regulation develops early in life. The current study used latent profile analysis to evaluate multisystem regulation in the autonomic nervous system and hypothalamic pituitary adrenal (HPA) axis in 150 racially/ethnically diverse, low-income children at 18- and 36-months. At both timepoints, profiles of generally moderate activity (*Moderate Arousal*) and heightened baseline activity (*Anticipatory Arousal*) emerged. A profile of typically adaptive patterns across all systems (*Active Copers*) emerged at 18-months and a profile of heightened HPA Axis activity (*HPA-axis Responders*) emerged at 36-months. Persistent membership in the *Anticipatory Arousal* profile across time was associated with exposure to greater maternal stress at 18-months and child internalizing problems at 36-months. These findings highlight early multisystem profile development and suggest associations with stress and later behavior problems.

### Keywords

Autonomic Nervous System; hypothalamic pituitary adrenal axis; multisystem; regulation; development

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The authors have no conflicts of interest to disclose

The human stress response encompasses a complex organization of multiple biological systems that work together to mount mobilizations to and recovery from life's challenges. Although physiological research has garnered increased attention as an important tool for understanding individual differences in psychological outcomes, questions still remain about how these systems work together, the development of these collaborative systems, and what early environmental factors influence such development. The first few years of life are characterized by pronounced developmental plasticity, and examinations of the organization and stability, as well as antecedents and consequences, of multisystem functioning in early childhood are particularly important for understanding the development of pathological processes. Two main physiological systems involved in regulation are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. The ANS is comprised of two inputs, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The HPA axis is responsible for the neuroendocrine component of the stress response, which releases a cascade of hormonal signals culminating in the release of cortisol from the adrenal glands (Gunnar & Quevedo, 2007; Sapolsky et al., 2000). When examined singularly, research harnessing physiological indicators from each of these stress response systems has observed associations with a host of adaptive and maladaptive outcomes in childhood including behavior problems, attachment, and psychopathology (Bubier et al., 2009; Bush & Boyce, 2016).

The ANS and HPA axis develop rapidly throughout the first few years of life (Gunnar & Quevedo, 2007), and research suggests that stress during this time is a salient predictor of individual differences in physiological reactivity (Boyce et al., 2012; Engel & Gunnar, 2020). Further, physiological regulation across these systems may act as a mechanism linking early life stress to later behavior problems (Boyce & Ellis 2005; Cummings et al., 2009). For example, models of early life stress predicted blunted and diminished cortisol responsivity across time, which was associated with concurrent variations in mental health symptom severity (Essex et al., 2011). Similarly, relations between positive parenting quality and externalizing may be mediated by lower respiratory sinus arrhythmia reactivity, a reflection of PNS functioning (Zhang et al., 2020). However, studies using single system investigations sometimes show opposing results, suggesting that other unmeasured factors may be influencing these associations (e.g., high baseline RSA predicts both lower and higher levels of behavioral problems; Gordis et al., 2009; Treadwell et al., 2010). Given that the stress response is comprised of multiple collaborative systems, simultaneous examination of functioning across multiple physiological systems may more fully elucidate stress response processes and resolve such conflicting findings. Given the pronounced developmental plasticity of stress-sensitive physiological systems during the early years of life, examinations of multisystem functioning and its antecedents and consequences in early childhood are particularly important. Therefore, the current study sought to explore children's multisystem regulation at 18- and 36-months and relations among early stress, multisystem profiles, and behavioral outcomes.

As understanding of individual physiological systems has increased and analytic techniques have advanced, researchers have begun to call for examinations of the synergistic (or antagonistic) nature of stress responsivity across multiple system (Bauer et al., 2002; Hostinar & Gunnar, 2013). The *doctrine of autonomic space* posits that the sympathetic and

parasympathetic systems function along two dimensions with varying degrees of reciprocity (reciprocal to non-reciprocal) and coactivity (coactivation to coinhibition) to regulate responses (Berntson et al., 1994). The Adaptive Calibration Model (ACM) extended this theory by theorizing four patterns of rest and reactivity across the SNS, PNS, and HPA axis: *Sensitive* (high PNS rest and reactivity, moderate SNS rest and reactivity, and moderate HPA rest and high reactivity), *Buffered* (moderate-to-high PNS rest and reactivity, low-to-moderate SNS rest and reactivity, moderate HPA rest and reactivity), *Vigilant* (low PNS rest and low-to-moderate PNS reactivity, high SNS rest and reactivity, and moderate-to-high HPA rest and reactivity), and *Unemotional* (low rest and reactivity across all systems) (Del Giudice et al., 2011). Studies exploring multisystem regulation among young children are limited, however extant research has found evidence of patterns of multisystem reactivity that differentially predicted occurrence of behavior problems in kindergarten children (Ellis et al., 2017; Roubinov et al., 2020) and adolescents (Ellis et al., 2017).

Among the few studies to explore multisystem development in children, we recently employed Latent Profile Analysis (LPA) to assess multisystem rest and reactivity in a community sample of children assessed at the beginning and end of the kindergarten year (Roubinov et al., 2020). We documented three distinct regulatory profiles of multisystem activity derived from measures of rest and reactivity across the ANS (via heart rate [HR], respiratory sinus arrhythmia [RSA], and pre-ejection period [PEP]), and HPA axis (via cortisol). The first profile, termed *HPA Axis Responders*, reflected children who evidenced higher-than-average HPA axis reactivity. The second profile, *Active Copers/Mobilizers*, captured children with low ANS activation as rest, and moderate reactivity across systems. The third profile, *Anticipatory Arousal/ANS Responders*, was characterized by heightened activation prior to the stress protocol, as well as high ANS reactivity. Importantly, these three profiles replicated across both timepoints and also bore some similarity to those hypothesized in the ACM. Specifically, the *Active Copers/Mobilizers* profile was similar to the hypothesized *Buffered* profile, and although there were some striking differences in the HPA response, the *Anticipatory Arousal/ANS Responders* was similar to the *Sensitive* ACM profile. Our prior study also documented an association between persistent membership in the *HPA Axis Responders* profile across time and higher child externalizing problems. Further, although some children transitioned into a different profile, the majority of children remained in the same physiological profile across timepoints. This may reflect the hypothesis that physiological reactivity begins to stabilize around this age, however, no studies have examined multisystem organization and stability at earlier ages.

To test whether novel profiles hypothesized by the ACM and found in kindergarten-aged children (Roubinov et al., 2020) are present in a younger, more disadvantaged sample, the current study employed LPA to derive profiles of HR, RSA, PEP, and cortisol during periods of rest and reactivity in children at 18- and 36-months of age. In addition to exploring associations in younger children when physiological systems may be especially sensitive to environmental inputs, the current sample is also diverse with respect to socioeconomic status, race/ethnicity, and stress exposure (see Table 1). This may extend generalizability of observed findings beyond the previously measured population. Second, we explored the similarity of profiles across time and the extent to which children remained in the same profile or shifted to a different profile from 18- to 36-months. In line with our previous

study, we hypothesized that we would identify three distinct profile characterizations which bore similarity to those hypothesized in the ACM (e.g., *Sensitive* and *Buffered* profile). However, given the significant developmental differences across samples, we hypothesized that there would be differences in the shifts across time, with younger children potentially having more flexible responding. Third, we assessed whether higher reports of maternal stress at 18-months predicted physiologic profile membership across time (i.e., relation to stability/shifts in profile membership from 18 to 36 months). Finally, informed by previous physiology-behavior associations reported by Roubinov and colleagues (2020), we explored whether stability and/or shifts across profiles was associated with internalizing and externalizing behavior problems at 36-months.

## Method

### Participants

Participants included 150 mother-infant dyads who were part of a longitudinal study to explore the effects of environmental stress, maternal weight, and health on child health and development (see Bush et al., 2016 for details). Inclusion criteria for the women were: 1) English speaking, 2) between 18–45 years of age, 3) 8–23 weeks pregnant with singleton, 4) have a pre-pregnancy BMI of 25 – 40 kg/m<sup>2</sup>, and 6) incomes less than 500% of the Federal Poverty Level (i.e., \$73,550 for a family of 2), which is considered low-income given the high cost of living in the geographic area from which participants were drawn (US Department of Health and Human Services, 2011). Women were excluded if they 1) had medical conditions that were known to interfere with baseline body composition or gestational weight gain, 2) were currently taking medications related to weight loss, diabetes, antidepressants, antipsychotics, opiate drugs, or corticosteroids, or 3) have received gastric bypass surgery. Institutional review board approved all study protocols and written informed consent was collected from the women before initiation of any data collection with mother or child.

The current study sample included only dyads who participated in data collection at the 18-month ( $N = 137$ ;  $M = 19.11$  months,  $SD = 1.59$ ) or 36-month ( $N = 115$ ;  $M = 38.59$  months,  $SD = 3.31$ ) assessments. The sample included 51.3% female children and was racially and ethnically diverse, including 32.69% African American, 27.88% mixed race, 24.03% Latinx, 7.69% White, and 4.81% Asian American, 2.88% other. Approximately two-thirds (68%) of mothers were married or partnered, 54% were multiparous, and family income ranged from \$0 to \$98,000 (Median = \$19,000). Approximately 25.9% completed high school or less, 50.0% had some college or vocational training, and 19.2% earned a college degree.

### Procedures

Procedures for the stress inducing protocol have been detailed in prior publications (Bush et al., 2016; Stephens et al., 2020). At both assessments, children completed an age-specific Developmental Challenges Protocol (DCP) which includes challenges across cognitive, sensory, and emotional domains. Prior to the initiation of the DCP protocol and placement of electrodes for ANS data collection, researchers collected the first saliva sample from the children. At the start of the protocol, resting ANS cardiac measures were collected during a

2-minute neutral, pre-recorded audio lullaby or story at 18- and 36-months, respectively. At 18-months, challenges included a jack-in-the-box startle task (cognitive), a sour lemon juice taste (sensory), and listening to a recording of a sick infant crying (emotional). At 36-months, challenges included a picture naming task (cognitive), a sour lemon juice taste (sensory), and watching a scary video clip (emotional). Following the completion of the protocol, electrodes were removed, and the final saliva sample was collected at approximately 30 minutes after the DCP protocol in order to capture peak cortisol stress reactivity (Granger et al., 2008).

## Measures

**Demographics.**—Gestational age and child sex were obtained via labor and delivery medical records. At 18-months, mothers reported on their race/ethnicity and that of the child, as well as total household income and household size, which were used to calculate a continuous score of U.S. federal poverty level based on income adjusted for household size (Sebelius, 2011).

**Maternal Perceived Stress.**—Mothers reported on their perceived stress using the 10-item Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983) when children were 18-months. The PSS is a widely-used, highly reliable, and valid, self-report questionnaire that assesses an individual's perceptions of his or her generalized stress and coping over the previous month rather than reactions to specific events. A mean PSS score was computed across all 10 items as long as greater than 75% of the items were answered. Internal consistency was high ( $\alpha = 0.85$ ).

**Behavior Problems.**—Mothers reported on internalizing and externalizing symptomatology using the Child Behavior Checklist for ages 1.5 – 5 when children were 36 months old (CBCL; Achenbach & Rescorla, 2000). The CBCL is one of the most widely-used measures for assessing children's behavior problems with well-established reliability and validity (Warnick et al., 2008) and has been further validated for use in socioeconomically and ethnically diverse samples (Gross et al., 2006). Cronbach's alpha on the internalizing and externalizing subscales of the CBCL was 0.87 and 0.92, respectively. Children's symptoms were modeled continuously to capture associations across the continuum of symptoms, with higher scores representing greater symptomatology.

**Autonomic Nervous System (ANS).**—ANS regulation was collected continuously from toddlers throughout the DCP using BioNex hardware and BioLab acquisition software version 3.0. The full ANS collection and scoring methods protocol has been previously described (Stephens et al., 2020). After familiarizing children with the equipment, four disposable spot electrodes were placed on the children's neck and chest to collect impedance and respiratory measures and three spot electrodes were placed on the right clavicle, lower left rib, and right abdomen for ECG measures (Bush et al., 2016). Data were monitored on the computer for signal and noise and data were stored offline for later analysis. Data were filtered, extracted, and then scored in 30-second intervals using Mindware software (HRV 3.1.0F and IMP 3.1.0H, Mindware Technologies, Ltd., [www.mindwaretech.com](http://www.mindwaretech.com)). Data cleaning procedures involved examining for artifacts, checking all outliers ( $>3SD$ ), and

deleting individual data files for infants if more than 25% of the 30-second epochs were unscorable.

**Resting RSA and RSA reactivity.**—RSA is the naturally occurring variation in heart rate that occurs as a function of respiration. RSA was estimated as the natural logarithm of the variance of heart period within the high-frequency bandwidth associated with respiration at this age, 0.15 to 1.04 Hz (De Rogalski Landrot et al., 2007; Bar-Haim, Marshall, & Fox, 2000; Rudolph, Rudolph, Hostetter, Lister, & Siegel, 2003). Across both assessments, measures of resting RSA were derived by calculating the mean RSA magnitude during the 2-minute pre-challenge resting period. Mean magnitude of RSA was also calculated for each challenge task and then averaged to create an overall mean challenge RSA across all tasks for 18- and 36-months separately. To calculate RSA reactivity, standardized residual scores were created by regressing RSA values during the challenge tasks on RSA values during the pre-challenge rest. Negative residual scores are indicative of greater RSA reactivity (a decrease in RSA; parasympathetic withdrawal) while positive residual scores are indicative of lower RSA reactivity (an increase in RSA; parasympathetic activation).

**Resting PEP and PEP reactivity.**—PEP is a systolic time interval representing the elapsed duration from the beginning of electrical stimulation until the ejection of blood from the left ventricle. PEP data were extracted and scored using impedance technologies where the ECG and impedance waveforms were used to obtain PEP measures quantified as the time interval in milliseconds from the onset of the ECG Q-wave to the B point of the dZ/dt wave (Berntson et al., 2004). Measures of resting PEP and PEP reactivity were derived in the manner described above for RSA. Negative residual PEP scores are indicative of greater PEP reactivity (a decrease in PEP; PEP shortening or sympathetic activation) while positive residual scores are indicative of lower PEP reactivity (an increase in PEP; PEP lengthening or sympathetic deactivation).

**Resting HR and HR reactivity.**—HR is influenced by dynamic interactions among the parasympathetic, sympathetic, and other cardiovascular reflexes (Porges & Furman, 2011), and thus is not a ‘pure’ measure of parasympathetic or sympathetic activity. Once RSA and PEP data were cleaned and scored, HR was calculated as the number of R-peaks in each 30-second epoch. Measures of resting HR and HR reactivity were derived in the manner described above for RSA and PEP. Positive HR residual scores are indicative of HR acceleration (i.e., a stress reactivity response) to the challenge protocol.

**Cortisol.**—Saliva samples were collected using the Salimetrics Children’s Swab (Salimetrics Inc., State College, PA, USA) which was placed in the children’s mouth for approximately 30-seconds until saturated and then placed in a swab storage tube. Samples were then stored at  $-20^{\circ}\text{C}$  until they were ready to be sent by courier on dry ice to a laboratory at the University of Trier, Germany to be assayed for cortisol. Assays were conducted in duplicate using a time-resolved fluorescence immunoassay (DELFI; Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). After thawing, samples were mixed and centrifuged, and cortisol was assayed using a commercial immunoassay with chemiluminescence detection (Cortisol Luminescence Immunoassay: IBL-Hamburg,

Hamburg, Germany; detection limit of 0.179 nmol/l). Cortisol values were normalized using natural log transformation prior to conducting data analyses (McCarthy et al., 2009). Cortisol rest was defined as the cortisol value during the first collection before the start of the protocol. To measure cortisol reactivity, a standardized residual score was calculated by regressing the post-protocol cortisol value on the pre-protocol value, adjusting for time of day at the first sample. A positive residual score indicates heightened cortisol reactivity during the challenge protocol.

### Analytic Plan

All latent profile analyses (LPA) were performed in Mplus Version 8 (Muthén & Muthén, 2017). Separate LPAs were conducted at 18- and 36-months to examine similarities and differences in profiles across these ages. All LPA models included eight physiological indicators (controlling for child age and sex): baseline HR, RSA, PEP, cortisol and HR, RSA, PEP, and cortisol reactivity. Model building procedures assessed varied latent structures ranging from two to five class solutions (i.e., allowing regulatory indicators to cluster in two to five distinct profiles). Model building and parameter constraint procedures directly followed those that were conducted in the PAWS analyses (for more detail, see (Roubinov et al., 2020). The Bayesian Information Criterion (BIC; Schwarz, 1978) was used evaluate competing models, with lower BIC represents better fit. This strategy enables relative contrasts among both nested and non-nested models (West et al., 2012). Consistent with recommended procedures (Masyn, 2013), we also assessed profile solutions on the basis of substantive interpretation, the proportion of the sample within each profile, the Akaike Information Criterion (AIC; Shibata, 1977), and model entropy (Ram & Grimm, 2009). Given high entropy (.80), which indicates that individuals were classified with confidence and there is adequate separation between the latent classes (Ram & Grimm, 2009), each child was assigned to the most likely profile based on the estimated posterior probabilities for each profile. Using the posterior classifications, we then conducted multivariate analysis of variance (MANOVA) to compare the profiles on demographic characteristics (child sex, racial/ethnic minority status, gestational age, and family socioeconomic status), an environmental exposure that is potentially associated with profile membership (maternal perceived stress) and socioemotional outcomes at 36-months (internalizing symptoms and externalizing symptoms). Although numerous comparisons were possible, our approach was guided by efforts to replicate our previously observed associations between membership in the *HPA Axis Responders* profile and greater behavior problems at age 6 (Roubinov et al., 2020). Thus, we chose to compare associations for children who shifted into the *HPA Axis Responders* profile at 36 months relative to those who remained in the *Moderate Arousal* or *Anticipatory Arousal* profiles over time. All follow-up comparisons across profiles were conducted in SPSS Version 26.<sup>1</sup>

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<sup>1</sup>Latent transition analysis (LTA) was originally planned to explore stability or shifts in profile membership across time. However, differences in the patterning of indicator means within the profiles across timepoints (i.e., lack of measurement invariance over time) and non-convergence issues due to model complexity precluded the use of LTA.



## Results

### Model Building for Latent Profile Analysis

Sample demographics for the current study, as well as for those in the previous study of kindergarten children (for comparison), are presented in Table 1. Notably, the present sample consists of participants that has a larger representation of people of color and socioeconomically disadvantaged families compared to our prior sample. In both the 18- and 36-month analyses, our model building contrasts generally revealed better fit for models specifying two- or three- profile solutions as compared to models specifying four or five solutions, which had much larger BIC values, log likelihood replication problems, and a very small number of participants in one or more profiles. Therefore, we conducted an in-depth inspection of the results from the two- and three-profile solutions to select the best fitting model (see Table 2). BIC of the two- and three-profile solutions at both 18- and 36-months were comparable, with the two-profile being negligibly smaller at both ages (Wasserman, 2000). Smaller AIC values and higher entropy supported three-profile solutions over two-profile solutions. From a conceptual standpoint, the three-profile models also had more meaningful and substantively interpretable patterns of indicator means compared to the two-profile models. Based on these considerations, we retained the three-profile solution for both the 18- and 36-month assessments.

### Characteristics of the Latent Profiles

Characteristics for each latent profile across both 18- and 36-months are presented in Table 3 and Figure 2. Profile characterizations were determined by comparing the mean magnitude of each physiological indicator across profiles. Those with non-overlapping confidence intervals denoted that the magnitude of that indicator was significantly different than the other profiles. These comparisons yielded the sample-specific patterning of the profiles described below. Evaluation of the models across both timepoints revealed that two of the three profiles were similar across time in overall pattern of responding, with only small variations in magnitude from 18- to 36-months. However, at both 18- and 36-months, a profile emerged that was unique to that timepoint (i.e., one profile was specific to 18 months and one profile was specific to 36 months).

**18-month Profile Characteristics.**—Among the three profiles that emerged in the final 18-month model solution, one profile included children whose multisystem functioning was characterized by the lowest resting HR, highest resting RSA, low HR, RSA, and PEP reactivity, and low cortisol rest and reactivity. Given these typically adaptive patterns of physiological functioning at rest, and the degree to which the sample-relative patterning bore similarity to the sample-relative patterning of the *Active Copers/Mobilizers* profile previously observed among children in PAWS (see Figure 1), the term *Active Copers* was used to describe children in this profile. Notably, the current sample did not evidence the same moderate ANS reactivity response as the PAWS sample, so the term mobilizers was not included for this profile. Children in a second profile exhibited moderate resting HR, RSA, and PEP and the lowest resting cortisol. Further, reactivity in this profile was characterized by generally moderate HR, PEP, and RSA reactivity, and very little cortisol reactivity. Therefore, we refer to this profile as *Moderate Arousal*. As depicted in Figures 1

and 2, the Moderate Arousal profile did not appear to have a direct parallel to any of the profiles that emerged in our prior study of kindergarten children. Finally, the third 18-month profile was characterized by the lowest levels of resting PEP, resting RSA, and highest resting HR across all profiles, which reflects heightened sympathetic activation and greater parasympathetic withdrawal prior to the initiation of the stress protocol. Resting cortisol levels among children in this profile were also the highest compare to other profiles, however, cortisol reactivity was moderate and similar in magnitude to the *Active Copers* profile. Reflecting the heightened levels of arousal at rest, and similarities with the sample-relative patterning of the *Anticipatory Arousal/ANS Responders* profile that emerged in our prior study (see Figure 1), this final profile was termed *Anticipatory Arousal*. This profile did not have the same ANS dominant reactivity response (i.e., negative mean RSA reactivity scores) that was characteristic of the *Anticipatory Arousal/ANS Responders* profile in the PAWS study, therefore we did not include that distinction in the naming of this profile.

**36-month Profile Characteristics.**—Among the three profiles in the 36-month model solution, there was a profile of children who exhibited the highest cortisol reactivity and lowest resting cortisol across all profiles. Resting HR, PEP, RSA, and RSA reactivity were moderate and PEP reactivity was higher than both other profiles. Thus, given these patterns of heightened HPA axis reactivity and the similarity of this profile with sample-relative patterning of the *HPA Axis Responders* profile in the kindergarten study (see Figure 1), the term, *HPA Axis Responders* was also used to describe children in this profile. Of note, this profile was unique to 36-months and did not have a parallel at 18-months. A second profile was characterized by the lowest resting HR, highest resting RSA and average resting cortisol and PEP. Further, reactivity in this second profile was characterized by moderate HR, PEP, and RSA reactivity, and negative cortisol reactivity indicating that pre-task cortisol was slightly higher than post-task cortisol. Based on these patterns and similarities with the *Moderate Arousal* profile at 18-months, the same nomenclature was used for this profile. The third profile was characterized by the lowest levels of resting RSA and highest resting HR across all profiles, which reflects heightened activation prior to the initiation of stress protocol. Resting cortisol levels among children in this profile were similar to children in those in the *Moderate Arousal* profile. However, children in this profile had the lowest HR, RSA, and PEP reactivity, and moderate cortisol reactivity. This final profile was labeled, *Anticipatory Arousal*, reflecting its similarity to the profile of the same name that was observed at 18 months.

### Exploration of Relations to Profile Membership

**Demographics and profile proportions across time.**—Profile membership at 18-months did not differ on the basis of sex, ethnic minority versus non-minority status, family SES, or gestational age of the child ( $ps > .11$ ). Analysis of the 36-month profiles also indicated no significant differences in sex, ethnic minority versus non-minority status, family SES, or gestational age of the child across profiles ( $ps > .41$ ).

Next, we examined the stability of profile membership across time. Among the 104 children who had physiological data at both assessments, 42 (41.4%) remained in the same profile and 61 (58.6%) shifted into a different profile across time (see Table 4). Only 1 child shifted

from *Active Copers* at 18-months to *HPA Axis Responders* at 36-months. Twenty-eight children remained stable in *Moderate Arousal* profile and 14 children remained stable in the *Anticipatory Arousal* profile across time. The emergence of the *HPA Axis Responders* profile at 36-months was equally propagated by those who were in the *Moderate Arousal* and *Anticipatory Arousal* profiles, with 5 children shifting from each.

**Associations with Maternal Stress.**—MANOVA was used to examine potential differences among the profiles of multisystem functioning based on exposure to maternal stress when children were 18 months of age. Profile comparisons included three patterns across time, those who shifted into the *HPA Axis Responders* profile at 36 months relative to those who remained in the *Moderate Arousal* or *Anticipatory Arousal* profiles over time (within a subset of the total sample,  $N = 52$ )<sup>2</sup>. There were significant differences in mothers' perceived stress at 18 months ( $F_{(2,53)} = 5.815$ ,  $p = .006$ ), with Bonferroni-adjusted post hoc comparisons ( $p = .010$ ) indicating that children who remained in the *Anticipatory Arousal* profile had mothers who reported greater stress ( $M = 19.59$ ,  $SD = 5.14$ ) compared to children who shifted into the *HPA Axis Responders* profile at 36-months ( $M = 11.80$ ,  $SD = 5.12$ ) and those who remained in the *Moderate Arousal* profile ( $M = 14.57$ ,  $SD = 5.35$ ). There was no significant difference in maternal stress at 18-months between children who shifted into the *HPA Axis Responders* and those who remained in the *Moderate Arousal* profile from 18- to 36 months.

**Associations with Socioemotional Behavior.**—Similarly, MANOVA was used to examine potential differences among the profiles of multisystem functioning based on children's behavior problems at 36 months of age. There were statistically significant profile differences in internalizing behavior ( $F_{(2,53)} = 3.28$ ,  $p = .040$ ) and marginally significant profile differences in externalizing behavior ( $F_{(2,53)} = 3.007$ ,  $p = .056$ ). Bonferroni-adjusted post hoc comparisons of internalizing problems ( $p = .043$ ) indicated that children who remained in the *Anticipatory Arousal* profile exhibited greater internalizing problems at 36-months ( $M = 53.00$ ,  $SD = 6.39$ ) than children who shifted into the *HPA Axis Responders* profile ( $M = 40.37$ ,  $SD = 8.35$ ). There was no significant difference in internalizing problems between the *HPA Axis Responders* and the *Moderate Arousal* profiles. Although analysis of externalizing problems differences was only marginally significant, findings followed similar patterns where the *Anticipatory Arousal* profile was at greater risk for exhibiting externalizing problems ( $M = 52.23$ ,  $SD = 9.09$ ) than those who shifted into the *HPA Axis Responders* profile ( $M = 40.38$ ,  $SD = 9.45$ ). Children who shifted into the *HPA Axis Responders* and those who remained in the *Moderate Arousal* profile did not significantly differ in externalizing problems.

## Discussion

The current study provides innovative information about the developmental organization and shifts of children's multisystem physiological regulation during a period when physiological systems may be especially sensitive to environmental inputs. Further, the findings represent

<sup>2</sup>Those included in the follow-up analysis did not significantly differ from those who were excluded on reports of maternal stress ( $t_{99} = -.576$ ,  $p = .566$ ), internalizing ( $t_{96} = -1.128$ ,  $p = .262$ ), or externalizing ( $t_{96} = -.892$ ,  $p = .376$ ).

an important conceptual replication of previously observed physiological profiles (see Roubinov et al., 2020), which is a notable contribution given increased attention to the need for replication in psychological science (Maxwell et al., 2015). Compared to our prior work, the current study also represents a larger proportion of racial/ethnically minority and socioeconomically disadvantaged families (see Table 1), which increases the generalizability of the multisystem profile characterizations.

There were a number of notable similarities in the multisystem patterning between the study of kindergarten children and the current investigation of younger children. First, the *Active Copers* profile present at the 18-month assessment was similar to the *Active Copers/Mobilizers* profile in the kindergarten study. However, the current *Active Copers* profile did not have an activating ANS or HPA reactivity response (as reflected by positive RSA and PEP reactivity values and negative cortisol reactivity; Table 3) that was characteristic of the *Active Copers/Mobilizers* in the kindergarten study. Research suggests that reactivity responses may increase across the first years of life (Alkon et al., 2014), and this difference between studies may point to the development of reactivity responses as children get older. That said, this profile did not replicate at 36-months, which is somewhat contrary to this interpretation of linear progression in reactivity across time and introduces more complexity into understanding these developmental patterns. A unique profile, *HPA Axis Responders*, also emerged at 36-months, characterized by heightened HPA axis reactivity. Although this was the least populated profile at 36-months, it was similar to the *HPA Axis Responders* found in the kindergarten study. Early characterizations of the timing of HPA axis development suggest that cortisol reactivity may not fully “come online” until later in childhood (Granger et al., 2007; Gunnar & Donzella, 2002). The current findings, in conjunction with the prior kindergarten study, highlight consistency in this profile at later ages, and may indicate 18-to-36-months as a critical period for the development of a more pronounced cortisol response. The *Anticipatory Arousal* profile also replicated at both timepoints in the current study and was conceptually similar to the *Anticipatory Arousal/ANS Responders* profile that was observed in the study of kindergarten children. Although the current sample did not have the same robust reactivity response as the kindergarten sample, this may be expected given prior evidence of stable baseline measures and more variable reactivity before the age of 5 (Alkon et al., 2014).

Interestingly, although the *Moderate Arousal* profile was observed at both 18- and 36-months, this profile differed from any found in the kindergarten study. This difference may point toward important developmental patterns and periods of reorganization in multisystem regulatory development. Specifically, as reactivity increases with age, these moderate responses observed in 3-year-olds may become more defined, particularly as children transition into kindergarten, an important developmental milestone. As compared to our prior study of kindergarten children, a smaller percent of children remained stable in the same profile, which aligns with preliminary research suggesting that physiological systems may be more flexible across early years of time and begin to stabilize after age five (Calkins & Keane, 2004; Gunnar & Donzella, 2002). Importantly, the high-risk nature of the current sample, differences in child demographics between samples (e.g., child age, racial/ethnic diversity, and SES), as well as the variations in tasks due to the selection of developmentally

appropriate challenges, may also account for the discrepancies in the profile characterizations noted in this sample and those of the previous kindergarten study.

In addition to replicating previously observed physiological profiles, the current study documented environmental exposures and socioemotional outcomes associated with profile membership over time. Greater maternal perceived stress at 18-months was associated with children's persistent membership in the *Anticipatory Arousal* profile across time. This profile is marked by high activation of physiological systems while not under challenge, which may be indicative of maladaptive responses to stress. Early experiences of heightened stress exposure have been linked to elevated autonomic activation at rest, and while elevations may be adaptive in the short term to navigate stressful environments, sustained elevations may be associated with negative behavioral outcomes (El-Sheikh et al., 2013; Propper & Holochwost, 2013). Indeed, children's sustained classification in the *Anticipatory Arousal* profile was also associated with the highest levels of internalizing problems at 36-months. This relative activation at rest is indicative of hypervigilance, and relates to theoretical assertions that multisystem physiological patterns of "vigilance" develop in early contexts of high stress (Del Giudice et al., 2011; Ellis et al., 2017). Remaining in heightened states of arousal when no challenge is present may be an adaptive process in order to navigate an adverse home environment, however, it may come at a cost to socioemotional well-being, and eventuate in internalizing symptomatology.

We anticipated that children in the *HPA Axis Responders* profile would exhibit heightened behavior problems given previously observed relations with externalizing among kindergarten children who demonstrated this pattern of physiological functioning. Interestingly, the *HPA Axis Responder* profile was not associated with children's socioemotional functioning in the current study. The emergence of a strong cortisol response in toddlerhood may reflect the developmental course of the HPA axis, which generally reaches full potential during childhood (Gunnar & Donzella, 2002). Thus, the children who demonstrated this profile at 36 months (recall that *this* profile was not observed at 18 months) may be exhibiting normative (and typically adaptive) physiological changes (Gunnar & Quevedo, 2007). In contrast, sustained levels of HPA axis reactivity at older ages may confer the risk for externalizing problems (Essex et al., 2002; Miller et al., 2007; Roubinov et al., 2020). Further examinations of this early period may be particularly important for elucidating such explanations and identifying at-risk physiological profile organizations in order to develop targeted early interventions before problems manifest.

### Strengths and Limitations

The present study includes a number of unique strengths, including a longitudinal cohort of racially and ethnically diverse young children and the assessment of physiological measures across multiple stress response systems. This makes the replication of physiological profiles that were observed in a sample of older, kindergarten children particularly informative for understanding developmental shifts in multisystem regulation and extends the generalizability of our results. Despite these strengths, a number of limitations should be considered in interpreting these results. First, although our sample size is considerable given the depth of physiological measurements in a difficult to assess age group, its size is modest

given the complexity of the models we assessed. In particular, given the small sample and the relatively small proportion of children in the HPA Axis Responder profile in the current investigation, the non-significant findings with externalizing symptoms may also have been due to a lack of power to detect significant associations. Future studies with larger samples are needed to replicate these findings and evaluate formal transition probabilities. Second, we relied on LPA, a data-driven approach, to determine distinct and meaningful profiles of physiological activity. This approach is well suited to characterize the variability of children's physiological response patterns, however conclusions about the number of different profiles are based upon both objective statistical parameters and *subjective* interpretations (Masyn, 2013). Finally, our examination of environmental factors that may influence multisystem physiology was limited to maternal stress, and future studies exploring multi-level environmental exposures early in life are needed.

## Conclusion

In summary, we conducted a novel, methodologically and statistically rigorous examination of multisystem stress responsivity across 18- and 36-months in a racially and ethnically diverse sample. Three distinct profiles were identified at each timepoint, several of which were consistent across time and replicated previously observed profiles in a sample of slightly older, kindergarten children. We also established important associations between an identified multisystem profile and maternal stress and preschool-age internalizing problems. This study provides a foundation for future multisystem exploration of the ways in which multisystem physiology may moderate and/or mediate associations between early adversity and later behavior problems. Such examinations have particular values for informing multilevel, multidomain Research Domain Criteria (RDoC)-informed studies that examine the neural underpinnings of developmental psychopathology. Advancing the identification of children who are at early risk for behavioral problems will support appropriate holistic interventions to increase their overall health and well-being.

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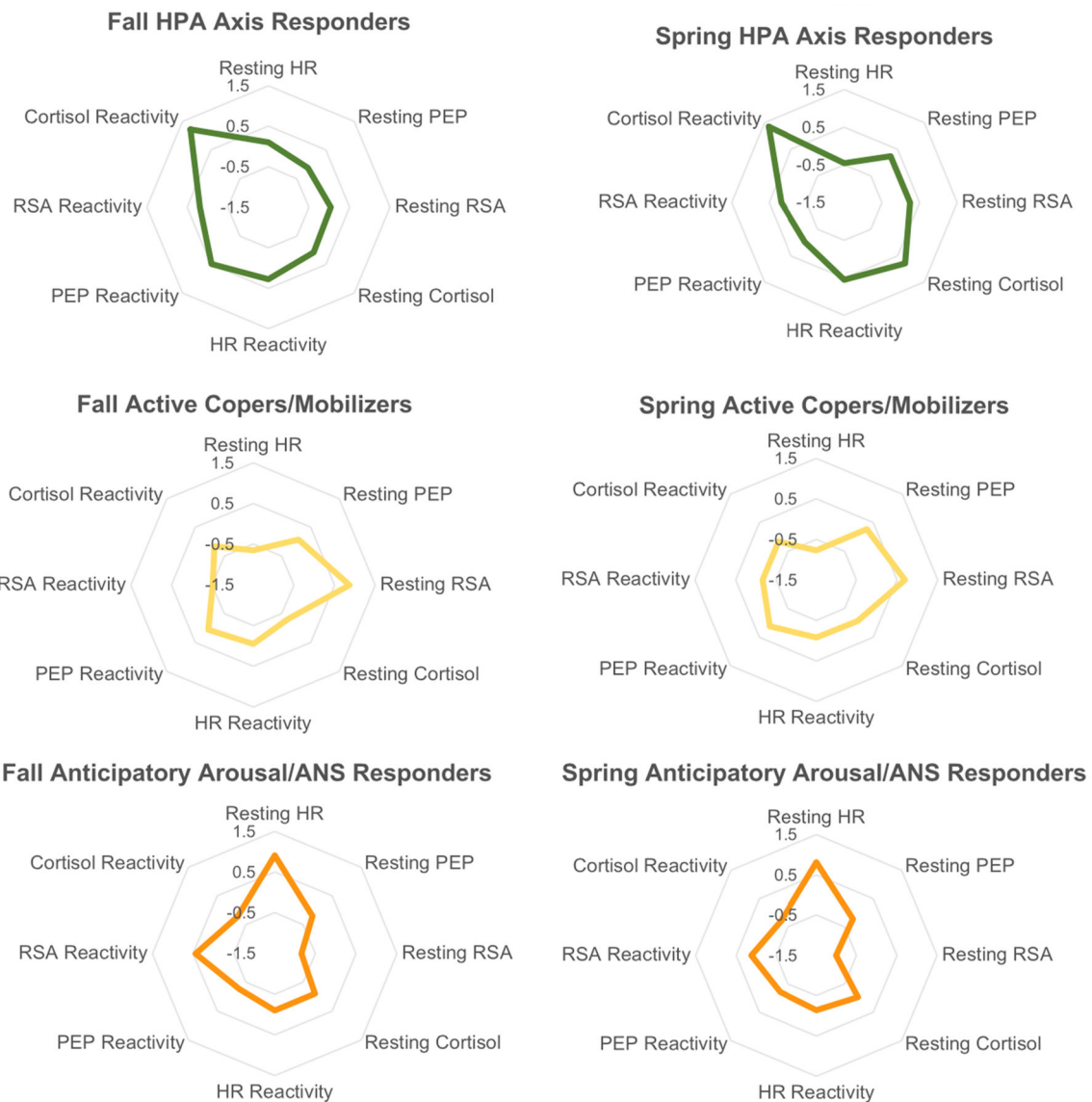
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### Highlights

- Early life experience is important for multisystem physiological regulation development
- Profiles replicate with theorized patterns and an independent sample of older children
- 18- to 36-months is an important window for HPA axis reactivity development
- Early maternal stress was related to profile development
- Stability in “risky” profiles was associated with internalizing problems



**Figure 1.** Three latent profiles of multisystem physiology derived by LPA as reported in Roubinov et al., 2020.

Note: Figure is from Roubinov DS, Boyce WT, Lee MR, Bush NR. Evidence for discrete profiles of children’s physiological activity across three neurobiological system and their transitions over time. *Developmental Science*. 2020:e12989, included here to demonstrate similarity of multisystem profiles across the two studies (see Figure 2). This figure is pending re-print approval from *Developmental Science*.



**Figure 2.** Graphical representation of 3 class latent profile analysis models at 18- and 36-months using standardized averages of latent indicator means. Note: Resting values are graphed such that zero (the center line) is sample average values. Reactivity is graphed such that farther from zero (the center line) indicates greater reactivity. Positive values for HR and cortisol represent greater reactivity, while greater reactivity for RSA and PEP are represented by negative values.

**Table 1.**

Comparison of descriptive statistics across the current (SEED) and kindergarten (PAWS) samples

Dichotomous Variable	Total SEED Sample (N = 104)		Total PAWS sample (N = 338)	
	N	%	N	%
Child Sex female	52	50.00	163	48
Race/Ethnicity				
: Black/African American	34	32.69	64	19
: Multiracial	29	27.88	74	22
: Latinx	25	24.03	13	4
: White	8	7.69	145	43
: Asian	5	4.81	37	11
: Other	3	2.88	7	2
Maternal Education				
: High school or less	27	25.9	26	8
: Some college or vocational training	52	50.0	55	17
: College degree	20	19.2	57	18
: Some graduate or professional school beyond college	5	4.8	39	12
: Professional or graduate degree	--	--	145	45
<b>Continuous Variables</b>	<b>Mean</b>	<b>SD</b>	<b>Mean</b>	<b>SD</b>
Child Age at latest timepoint (months)	38.55	3.18	63.84	3.84
Maternal Reported Household Income (\$)	37,694	36,633	\$60,000–\$70,000	---
Maternal Stress	15.02	6.59	---	---
Internalizing	46.55	10.76	0.40	0.44
Externalizing	46.84	11.31	0.33	0.32

Note: The PAWS sample reported on salary ranges, therefore standard deviations are not available. Similarly, reports of Internalizing and Externalizing behaviors in the PAWS sample were reported using a different measure and are not directly comparable to the current sample (range of scores: 0 – 2).

**Table 2.**

Model fit indices for LPAs with one to five profile solutions

	Profile Counts (n)					Entropy	AIC	BIC
	1	2	3	4	5			
<b>18-months</b>								
Two-profile	52	79	---	---	---	.69	4278.88	4398.02
<b>Three-profile</b>	<b>23</b>	<b>68</b>	<b>40</b>	---	---	<b>.82</b>	<b>4239.21</b>	<b>4400.97</b>
Four-profile	21	21	58	31	---	.82	4219.72	4441.11
Five-profile	15	27	21	12	56	.89	4224.64	4500.66 <sup>τ</sup>
<b>36-months</b>								
Two-profile	55	60	---	---	---	.67	3509.040	3605.11
<b>Three-profile</b>	<b>11</b>	<b>59</b>	<b>45</b>	---	---	<b>.80</b>	<b>3456.97</b>	<b>3605.19</b>
Four-profile	10	47	21	37	---	.81	3422.44	3622.82
Five-profile	11	8	38	58	0	.87	3476.26	3728.79 <sup>τ</sup>

Note: Bold indicates final retained profile solutions. AIC = Akaike Information Criterion, BIC = Bayesian Information Criterion.

<sup>τ</sup> = models where LL was not replicated despite utilizing maximum iterations.

**Table 3.**

Profile membership patterns over time (N = 104)

18 month profiles	36 month profiles		
	HPA Axis Responders	Moderate Arousal	Anticipatory Arousal
Active Copers	1 (0.96%)	10 (9.61%)	5 (4.80%)
Moderate Arousal	5 (4.80%)	28 (26.92%)	13 (12.50%)
Anticipatory Arousal	5 (4.80%)	23 (22.11%)	14 (13.46%)

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**Table 4.**

Profile-specific ns, latent-indicator means, and confidence intervals of latent-indicator means for final 3-class models

	18-months	36-months
	Active Copers N = 20	HPA Axis Responders N = 11
Resting HR	112.13 (108.53, 115.74)	103.11 (100.43, 105.79)
Resting RSA	6.27 (5.96, 6.59)	6.71 (6.48, 6.94)
Resting PEP	81.74 (80.48, 83.00)	89.62 (87.35, 91.88)
Resting Cortisol	0.04 (-0.13, 0.22)	-2.18 (-2.83, -1.54)
HR Reactivity	-3.45 (-4.90, -2.00)	4.89 (3.35, 6.43)
RSA Reactivity	0.49 (0.36, 0.63)	-0.90 (-1.18, -0.62)
PEP Reactivity	0.81 (0.24, 1.37)	-5.48 (-7.37, -3.60)
Cortisol Reactivity	-0.05 (-0.20, 0.11)	2.15 (1.47, 2.84)
	<b>Moderate Arousal N = 50</b>	<b>Moderate Arousal N = 45</b>
Resting HR	117.48 (116.04, 118.91)	97.87 (96.61, 99.13)
Resting RSA	5.12 (4.98, 5.26)	7.21 (7.07, 7.34)
Resting PEP	81.98 (81.04, 82.92)	86.79 (85.79, 87.79)
Resting Cortisol	-0.07 (-0.20, 0.06)	0.27 (0.13, 0.41)
HR Reactivity	1.94 (1.11, 2.77)	6.12 (5.43, 6.80)
RSA Reactivity	-0.05 (-0.14, 0.05)	-0.68 (-0.78, -0.59)
PEP Reactivity	-0.12 (-0.44, 0.19)	-0.97 (-1.26, -0.69)
Cortisol Reactivity	0.02 (-0.10, 0.14)	-0.20 (-0.33, -0.07)
	<b>Anticipatory Arousal N = 67</b>	<b>Anticipatory Arousal N = 59</b>
Resting HR	137.20 (133.45, 140.96)	111.42 (109.90, 112.94)
Resting RSA	3.98 (3.75, 4.20)	5.65 (5.48, 5.83)
Resting PEP	77.90 (76.77, 79.03)	87.30 (86.01, 88.59)
Resting Cortisol	0.12 (0.00, 0.24)	0.21 (0.04, 0.37)
HR Reactivity	-4.76 (-7.00, -2.51)	1.67 (0.98, 2.36)
RSA Reactivity	0.55 (0.42, 0.69)	-0.19 (-0.27, -0.10)
PEP Reactivity	1.42 (0.93, 1.90)	0.95 (0.50, 1.39)
Cortisol Reactivity	-0.06 (-0.21, 0.08)	-0.28 (-0.45, -0.11)

*Note.* A lack of overlap in the confidence intervals for a given indicator among profiles suggests the profiles differ significantly on that indicator.