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Latent profiles of children’s autonomic nervous system reactivity early in life predict later externalizing problems

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Abstract

Prior researchers have observed relations between children’s autonomic nervous system reactivity and externalizing behavior problems, but rarely considers the role of developmentally-regulated changes in children’s stress response systems. Using growth mixture modeling, the present study derived profiles of parasympathetic nervous system reactivity (as indicated by respiratory sinus arrhythmia (RSA)) and sympathetic nervous system reactivity (as indicated by pre-ejection period (PEP)) from low income, primarily Mexican American children measured repeatedly from infancy through age 5 ($N = 383$) and investigated whether profiles were associated with externalizing problems at age 7. Analyses identified two profiles of RSA reactivity (*reactive-decreasing* and *U-shaped reactivity*) and three profiles of PEP reactivity (*blunted/anticipatory reactivity, reactive-decreasing, non-reactive increasing)*. Compared to children with an RSA profile of *reactive-decreasing*, those with an RSA profile of *U-shaped reactivity* had marginally higher externalizing problems, however this difference was not statistically significant. Children who demonstrated a profile of *blunted/anticipatory* PEP reactivity had significantly higher externalizing problems compared to those with a profile of *non-reactive increasing,* likely related to the predominantly male composition of the former profile and predominantly female composition of the latter profile.

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Author Contributions:

Danielle S. Roubinov: Dr. Roubinov refined the research question, interpreted the data, drafted the original manuscript, and made substantive revisions to the manuscript.

Jenn-Yun Tein: Dr. Tein conducted data analyses, interpreted the data, contributed to the writing and editing of the manuscript, and provided final approval of the manuscript.

Katherine Kogut: Ms. Kogut oversaw all aspects of data collection for the study, contributed to the editing of the manuscript, and approved the final version of the manuscript.

Robert Gunier: Dr. Gunier advised and assisted with data analyses, interpreted the data, contributed to the editing of the manuscript, and provided final approval of the manuscript.

Brenda Eskenazi: Dr. Eskenazi conceptualized and designed the study, made substantial contributions to the acquisition of the data, contributed to the editing of the manuscript, and provided final approval of the manuscript.

Abbey Alkon: Dr. Alkon designed the autonomic nervous system (ANS) protocol and ANS data collection procedures, trained staff on the acquisition of ANS data, regularly reviewed the quality of the ANS data collection, and analyzed and interpreted the results. She also contributed to the writing and editing of the manuscript and approved the final version of the manuscript.

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Findings contribute to our understanding of developmental trajectories of ANS reactivity and highlight the utility of a longitudinal framework for understanding the effects of physiological risk factors on later behavior problems.

Keywords

Autonomic nervous system; physiological reactivity; externalizing problems; growth mixture modeling; early childhood

It is widely accepted that children vary in their psychophysiological responses to stress, with important implications for the development of psychopathology (Shonkoff, 2003). In particular, individual differences in early patterns of reactivity across the parasympathetic and sympathetic branches of the autonomic nervous system (ANS) have been implicated not only as biomarkers or predictors of psychopathology (Boyce et al., 2001) but also as indicators of children's differential susceptibility to the effects of the environment (Rudd, Alkon, & Yates, 2017). Despite considerable advances in our knowledge of children's psychophysiology and mental health outcomes, scarce progress has been made in understanding these associations within the context of a robust developmental lens. Among the hindrances to such work are the lack of longitudinal studies with repeated measures of ANS activity and limited consideration of within- and between-individual variability in physiology. In the current paper, we leverage the power of growth mixture modeling (GMM) to uncover unique, heterogeneous profiles of children's parasympathetic and sympathetic nervous system functioning from 6 months to 5 years of age. We then examine relations between the GMM-derived longitudinal profiles and externalizing behavior problems when children were 7 years old. We examine these relations in a sample of high-risk, Mexican-American children residing in a low-income agricultural community.

Autonomic nervous system reactivity: Developmental and contextual considerations

Autonomic nervous system (ANS) reactivity is a particularly rich and informative source of individual differences in physiology that may underlie the development of psychopathology (Zisner & Beauchaine, 2016). Briefly, the ANS is comprised of two branches, the parasympathetic nervous system (PNS) and sympathetic nervous system (SNS). The PNS is characterized in terms of its "rest and digest" functionality that opposes or is co-linear with the SNS (i.e., the PNS reduces physiological arousal and promotes restorative activity; Berntson, Norman, Hawkley, & Cacioppo, 2008). PNS activity is often measured by respiratory sinus arrhythmia (RSA), the natural variation in heart rate that occurs during the respiratory cycle or inspiration and expiration. In response to challenge, the PNS may withdraw to facilitate an adaptive stress response (Beauchaine, Gatzke-Kopp, & Mead, 2007; Porges, 2007). The degree to which this disengagement occurs reflects RSA reactivity. Lower levels of RSA during a challenging condition in relation to a resting state are indicative of heightened reactivity (greater PNS withdrawal) while higher RSA indicates lower reactivity (greater PNS engagement) (Allen & Matthews, 1997). In contrast, the SNS is responsive to "fight and flight" in conditions of danger, fear, or challenge. SNS activity on the heart is frequently measured by pre-ejection period (PEP), which is the time interval in milliseconds between the onset of the left ventricular ejection and the opening of the aortic valve (Uchino, Cacioppo, Malarkey, & Glaser, 1995). The change in PEP during challenging conditions compared to baseline or a resting condition is defined as PEP reactivity and akin to RSA measures, lower levels indicate higher reactivity (greater PEP shortening) and higher levels indicate lower reactivity (lesser PEP shortening).

The first five years of life are a period of heightened plasticity of physiological stress response systems, and the development of PNS and SNS functioning may be particularly sensitive to qualities of childhood environmental contexts during this time (Del Giudice, Ellis, & Shirtcliff, 2011). In light of this, longitudinal research that captures the trajectories of RSA and PEP reactivity may offer a richer and more comprehensive understanding of children's psychobiological functioning than cross-sectional studies. Likely due to the cost and time-intensive nature of repeated assessments of physiology, longitudinal studies of ANS reactivity remain scarce, particularly those with more than two time points. As a result, there remain many unanswered questions regarding the development of children's ANS reactivity, particularly early in life. Such equivocal issues include the extent to which ANS reactivity varies *within and between children across development*, which helps elucidate the overall developmental continuity (or discontinuity) in ANS reactivity over time (i.e., whether ANS reactivity increases, decreases, or stays the same as children develop; Hinnant, Philbrook, Erath, & El-Sheikh, 2018). In a sample of children assessed at two and four years old, measures of RSA reactivity were only moderately correlated, suggesting the presence of developmentally-regulated changes within individuals (Calkins & Keane, 2004). Other longitudinal studies during toddlerhood (Wilkinson & Howse, 2003), early childhood (Doussard-Roosevelt, Montgomery, & Porges, 2003), and middle childhood (Hinnant et al., 2018) have reported similarly low levels of stability in RSA reactivity. Within young children, evidence of both linear (Perry et al., 2013) and curvilinear (Conradt et al., 2014) change in RSA reactivity has been observed across time. Notably, prior studies have observed significant between-individual variability, suggesting that RSA reactivity did not change in the same way for all children, however their analytic approach did not allow for a more nuanced examination of heterogeneity. Compared to RSA, PEP reactivity has received even less attention and has been hypothesized to be particularly sensitive to the qualities of the laboratory context in which it is measured, rendering it difficult to draw strong conclusion about stability or change (Zisner & Beauchaine, 2016). In a prior examination of the present study's sample of children, PEP reactivity decreased on average and varied within individuals over time, however between-individual variability was not tested (Alkon, Boyce, Davis, & Eskenazi, 2011).

Children's ANS reactivity has also been associated with childhood poverty and sex. Children living in poverty during early childhood have shown dysregulated physiological patterns of hypo- or hyper-responsivity (Evans & Kim, 2013; Obradović, 2012). It is hypothesized that the multiple, chronic stressors associated with chronic poverty exposure place excessive demands on children's physiologic systems such that they may have a reduced efficiency of the ANS system (Evans & Kim, 2013). Although the majority of ANS studies of children under 6 years of age do not show sex differences (e.g., Alkon et al., 2003; Giuliano, Roos, Farrar, & Skowron, 2018; Perry et al., 2014), a study of infants found lower

PEP during the episodes of the Still Face Paradigm among boys compared to girls (Suurland et al., 2017).

Although the aforementioned longitudinal studies have helped inform our understanding of ANS development, most have been limited by conventional growth modeling approaches which cannot assess individual variability in growth trajectories (Jung & Wickrama, 2008). Rather than assume that all individuals are drawn from a homogenous population with uniform patterns of physiologic development, it may be the case that there are varied subgroups that exhibit different growth trajectories of RSA and PEP reactivity over time. Such between-individual differences are ideally addressed by growth mixture modeling (GMM), a statistical approach that identifies heterogeneity in the population and creates discrete profiles of individuals who are classified together based on their similar response patterns (Jung & Wickrama, 2008). In the present study, we used GMM to test the hypothesis that there would be at least two subgroups of different developmental trajectories of PEP and RSA reactivity in a sample of children assessed repeatedly during the first 5 years of life. Importantly, we examined these relations in a sample of children from low-income Mexican-origin families who may be disproportionately affected by conditions that adversely affect physiological development. Hispanic children are one of the largest and most rapidly growing racial/ethnic minority groups in the United States, currently comprising approximately one-quarter of the population and projected to be nearly one-third of the population by 2050 (U.S. Census Bureau, 2017). However, they remain grossly underrepresented in current studies of children’s psychophysiology and development.

Associations between children’s autonomic nervous system functioning and externalizing problems

ANS reactivity may relate to the onset and development of children’s socioemotional and behavioral health problems, including externalizing problems. PNS activity is a key component of children’s regulatory capacities in challenging or stressful environmental circumstances (Porges, 2007) and empirical literature consistently associates greater RSA reactivity with more sustained attention, heightened engagement with the environment, and improved emotion regulation and coping skills (Porges & Furman, 2011). In contrast, both lower levels of RSA reactivity (Keller & El-Sheikh, 2009; Hinnant & El-Sheikh, 2009) and excessive RSA reactivity (particularly among clinical samples; Beauchaine, Gatzke-Kopp, & Mead, 2007) may be associated with greater externalizing problems, perhaps suggesting a U-shaped curvilinear relation whereby symptomatology is greatest among those with lower and higher reactivity and lowest among those with moderate reactivity. Given the potential for dynamic changes in RSA reactivity during the early developmental years, examining non-linear relations in a longitudinal framework is an important area of research that remains largely unexamined.

In regards to SNS reactivity, it has been suggested that blunted activity in response to stress (as reflected by a lack of PEP reactivity) may indicate physiological underarousal that engenders impulsivity, risk-taking, and other sensation-seeking behaviors that are encompassed within the broader category of externalizing behavior (Zisner & Beauchaine, 2016). Consistent with such a theory of underarousal, empirical research has observed associations between lower levels of PEP reactivity (or no reactivity) and externalizing behavior among preschool age children (Beauchaine et al., 2013) through young adults (for review, see Beauchaine & Gatzke-Kopp, 2012).

Importantly, RSA and PEP reactivity captured at a single time point may be limited in their ability to elucidate associations with externalizing behaviors. Rather, developmentally-regulated changes in children's stress reactivity may influence externalizing symptoms. For example, El-Sheikh, Keiley, and Hinnant (2010) found that children with greater externalizing behavior problems exhibited decreasing levels of SNS reactivity across middle childhood as measured using skin conductance, while SNS trajectories were stable among those with lower symptoms. Similarly, children with a blunted pattern of RSA reactivity during early childhood exhibited greater behavior problems, an association not observed among those with increasing levels of RSA reactivity (Conradt et al., 2014). Although it is well-established that boys exhibit higher levels of externalizing behavior problems compared to girls (Martel, 2013), the extent to which physiological risk factors for externalizing behavior may differ by sex is less well understood and results have been inconsistent across the literature (Eisenberg et al., 2012; El-Sheikh, 2005; Hastings et al., 2008, Obradović et al., 2010). Some research has more readily observed psychophysiological correlates of externalizing behavior among males compared to females, suggesting that externalizing behavior among females is more strongly associated with social/environmental (rather than biological) factors (Beauchaine, Hong, & Marsh, 2008).

Current study

To address gaps in extant literature, the present study was conducted with the following aims: (1) To explore heterogeneity in RSA and PEP reactivity trajectories from 6 months to 5 years of age using GMM and (2) To examine relations between the GMM-derived trajectory profiles and children's externalizing behavior problems at age 7. We hypothesized the presence of at least two distinct profiles of both RSA and PEP reactivity and anticipated that significant linear and/or curvilinear change in reactivity would emerge. Externalizing problems were expected to be higher among children who demonstrated a low and/or decreasing trajectory of RSA or PEP reactivity over time.

Methods

Participants

Participants in the present study were drawn from a larger longitudinal birth cohort study of the potential health effects of pesticides and other environmental exposures in a population of pregnant women and their children living in an agricultural community in California (Eskenazi et al., 2003). The parent project, Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), enrolled 601 pregnant women over a 1-year period of time (October 1999 to October 2000). Women were eligible if they were ≥18 years of age, <20 weeks gestational age, eligible for California's low-income health care program (MediCal), spoke English or Spanish, and were planning to deliver at the county hospital. We followed the women through the delivery of 537 live born children. Data used in the present study were collected during study visits that took place during pregnancy and when children were 6 months, 1 year, 3.5 years, 5 years, and 7 years of age. Women were interviewed twice during pregnancy (n = 601), once after delivery (n = 536; 89%), and when children were ages 6 months (n = 433; 72%), 1 year (n = 418; 70%), 2 years (n = 414; 69%), 3.5 years (n = 364; 61%), 5 years (n = 350; 58%), and 7 years (n = 353; 59%). During all visits, mothers provided information about family demographics, psychosocial exposures, and child mental health. Children's ANS activity at rest and in response to challenge was collected using a standardized protocol (described below) that was administered at 6 months, 1 year, 3.5 years, and 5 years of age, though budgetary constraints reduced the sample size by about 50% at the 6 months, 1 year, and 3.5 year assessments. We excluded children with medical conditions that could affect their ANS activity (n = 6). Written informed consent was obtained from all women and oral assent from all children at age 7; all research was approved by the University of California, Berkeley and University of California, San Francisco, Committees for the Protection of Human Subjects.

Measures

Autonomic nervous system (ANS) reactivity.—The ANS protocol was modified from a previously standardized, valid, and reliable protocol (Alkon et al., 2003). Challenges were presented across social, cognitive, physical, and emotional domains that represented normative, common stressors to which children are exposed. At 6 months and 1 year of age, the infants were administered a standardized 7-minute ANS protocol with three 1-minute challenges (jack-in-the-box, vibrator applied to leg, and listening to a sick baby cry) preceded and followed by a resting condition of a 2-minute lullaby (Alkon et al., 2006). The protocol was administered with the infant sitting in the mother's lap and facing the experimenter. At 3.5 and 5 years of age, the standardized protocol included four 2-minute challenges (social interview, number recall, concentrated lemon juice applied to tongue, and emotion-evoking video) preceded and followed by a resting condition of 2-minute stories read aloud (Alkon et al., 2003). During the 3.5 and 5 year assessments, the child's parent was not present.

All protocols were administered by bilingual, bicultural staff in private rooms located in a research office next to the local hospital or in a recreational vehicle redesigned as a research laboratory and parked in front of the participant's home. The protocol was administered in the language spoken at home during infancy and in the language of choice at 3.5 and 5 years, either Spanish or English. Data quality and the fidelity of the protocol were assessed by observing the protocol administration and electrode placement through onsite observations or videotape reviews of the reactivity protocol by two research staff.

Band electrodes were used to collect the ANS data at 6 months and 1 year (Alkon et al., 2006) and spot electrodes at 3.5 and 5 years of age (Alkon et al., 2003). The tetrapolar configuration of electrodes included placement of 2 bands/spots on the neck and 2 bands/spots on the trunk to collect impedance, electrocardiograph, and respiratory measures. After the bands/spots electrodes were in place, the experimenter checked the electrocardiography (ECG) and impedance signals to verify quality cardiac signals during which time no data were collected. Data were acquired at 6 months and 1 year of age using the Minnesota

Impedance Cardiograph HIC-2000 and at 3.5 and 5 years of age using the Biopac MP150, as the HIC-2000 was no longer available. Continuous measures of heart rate (HR), ECG, *Z*0 (basal impedance), and *dZ/dt* (first derivative of the impedance signal) were collected during the protocol. A 4-mA AC current at 100 Hz was passed through the 2 outer, current electrode bands/spots, and *Z*0 and *dZ/dt* signals were acquired from the 2 inner voltage-recording bands/spots. RSA indices were calculated using the interbeat intervals on the ECG reading, respiratory rates derived from the impedance (e.g., *dZ/dt*) signal, and a bandwidth range of 0.24 to 1.04 Hz at 6 months and 1 year of age and 0.15 to 0.80 Hz at 3.5 and 5 years of age (Bar-Haim, Marshall, & Fox, 2000; Bazhenova, Plonskaia, & Porges, 2001). PEP was measured in milliseconds as the interval between the ECG's Q wave and the B notch on the *dZ/dt* signal.

The data were filtered, extracted, and then scored using the ANSsuite software at 6 months and 1 year (Alkon et al., 2003; Cacioppo, Uchino, & Berntson, 1994) and Mindware (https://mindwaretech.com/) at 3.5 and 5 years. Data cleaning procedures were similar to those of other researchers (Calkins, Graziano, Berdan, Keane, & Degnan, 2008; Salomon, 2005) and included checking all outliers (>3 SD) minute by-minute and calculating summary scores. Individual data were visually checked within each protocol for outliers relative to adjacent data. Data were deleted if 25% of the minutes in the protocol were not scoreable. The percent of children who completed the ANS protocol and had scored and usable data increased by age: 6 months (83%), 1 year (86%), 3.5 years (93%), and 5 years (95%). Missing data were due to child or parent refusals, equipment failure, or noisy data due to child movement or electrode displacement.

Mean resting[1] and mean challenge summary scores were calculated for RSA and PEP measures. Cronbach alpha coefficients conducted for RSA and PEP challenge measures were strong (alphas ranged from 0.88–0.99), indicating high reliability across the measures and supporting the use of mean challenge scores to create the reactivity scores. Reactivity or difference scores were calculated as the mean challenge minus mean resting conditions (Allen & Matthews, 1997). Negative reactivity scores were indicative of greater RSA reactivity (a decrease in RSA or RSA withdrawal) or greater PEP reactivity (a decrease in PEP or PEP shortening), while positive scores were indicative of lower RSA reactivity (an increase in RSA or RSA augmentation) or lower PEP reactivity (an increase in PEP or PEP lengthening).

Children's externalizing behavior.—Mothers reported on children's externalizing symptoms at 7 years old using the Behavior Assessment Systems for Children 2 (BASC-2; Reynolds & Kamphaus, 2004). The BASC-2 is a 160-item inventory that assesses the frequency of certain behaviors exhibited by children in the home setting. Mothers were directed to report on children's behavior *"recently (in the last several months)"* using a four-point Likert scale (0 = *Never*, 1 = *Sometimes*, 2 = *Often*, 3 = *Almost Always*). The externalizing scale includes items that assess aggression, hyperactivity, oppositional behavior, and conduct problems. Summed raw scores were converted into standardized *T*

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[1] Mean scores were calculated for the second resting conditions, but the resting scores for minutes 1 and 2 were significantly lower than the last 2 minutes. Therefore, subsequent resting scores refer to minutes 1 and 2.

scores for interpretation with higher scores representing more externalizing behaviors (Cronbach’s alpha = 0.89).

Covariates/confounds.—Predictors and confounds were included in our analyses on the basis of relations observed in prior empirical research and because these variables are necessary for correct specification of latent profiles (Muthén, 2004). First, child sex and family poverty level (living at or below the poverty level versus above the poverty level) were included as predictors in growth mixture models given prior research suggesting such demographic factors may distinguish varied patterns of ANS reactivity (Alkon et al., 2011; Salomon et al., 2000). Second, children’s gestational age has been shown to relate to ANS functioning (Chatow, Davidson, Reichman, & Akselrod, 1995), thus we controlled for gestational age (in weeks, extracted from medical records) when predicting later externalizing problems from RSA or PEP reactivity profiles. The larger study targeted children’s exposure to organophosphate pesticides, however there were no consistent relationships between prenatal-and child-level organophosphate exposures and ANS measures during early childhood (Quirós-Alcalá et al., 2011). Therefore, we do not control for pesticide exposure in this manuscript.

Analytic Plan

We conducted our analysis plan in phases. All analyses were conducted in Mplus 8 (Muthén & Muthén, 1998-2017). We fit a series of growth mixture models (GMM) to identify profiles of reactivity over time. GMM explored unobserved (or latent) profiles of children with similar trajectory patterns of the scores on RSA and PEP reactivity over the four assessment points and estimated the posterior probability of each child being a member of each profile. Child sex and family poverty levels were included as the predictor variables of the latent profiles and growth factors (i.e., intercept and slope). Weighted effect coding was used to define sex and poverty levels so the resulting regression coefficients for the latent classes and growth factors (i.e., growth trajectory) within each profile reflected the sample mean of sex and poverty level. Note that the model fit and class solutions are not affected by the coding scheme of the categorical variables, but the regression coefficients vary depending on what the zero value represents for the coded variable, which is the sample mean for the weighted effect coding. If sex was found to have significant effects on the growth factors, we probed the sex differences on the growth patterns separately for boys versus girls. Given that all families lived below the poverty threshold, we did not probe poverty level differences.

Mplus default settings for conducting GMMs leverage all available data, thus children with at least one timepoint of ANS data were included in the growth models. We started with a 1-profile model and successively increased the number of profiles by one until model fit indices leveled off or we encountered convergence issues. Without substantive theory about the forms of the trajectories (i.e., linear vs. quadratic model), we also compared the results of linear growth models to quadratic growth models. The residual variances were sometimes constrained to zero for the growth factors to avoid convergence problems (Hox, 2002). Thus, our approach essentially paralleled that of latent class growth analysis (Nagin, 1999, 2005). To avoid getting local maximum solutions, we repeated models with multiple sets of start values (Muthén & Muthén, 1998-2017) and ensured that the best log-likelihood value was replicated (Muthén, 2004). We determined the optimal number of profiles based on several fit indices and likelihood ratio tests (Tein, Coxe, & Cham, 2013): Bayesian information criterion (Schwarz, 1978), sample-size adjusted Bayesian information criterion (SABIC; (Sclove, 1987), Vuong-Lo-Mendell-Rubin likelihood ratio test (Lo, Mendell, & Rubin, 2001), and parameter bootstrapped likelihood ratio test (Peel & McLachlan, 2000). For models with similar relative fit indices, we relied on entropy to gauge whether the latent profiles were highly discriminating (Nylund, Asparouhov, & Muthén, 2007; Ram & Grimm, 2009), as well as substantive interpretations, trajectory patters, and proportion of the sample within each latent growth profile into account. Finally, to determine whether a linear or quadratic model provided optimal fit, we examined whether the quadratic functions were significant and/or whether the quadratic functions were significantly related to the predictors. 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 multiple regression models in Mplus to test whether ANS profiles (as identified by the aforementioned GMMs) were associated with children's externalizing behavior problems at 7 years of age and whether there were significant interactions between the ANS profiles and sex in the prediction of externalizing problems. We controlled for gestational age in the regression models.

Results

Sample characteristics and preliminary analyses

The present study included children with data from 6 months to 7 years of age. For the GMM, we included children with data on ANS trajectory profiles collected at 6 months, 1 year, 3.5 years, and 5 years ($n = 383$ for RSA models, $n = 377$ for PEP models). For the subsequent regression analyses, we included children with ANS trajectory profiles, gestational age and externalizing behavior problems collected at 7 years of age ($n = 383$, with FIML to handle missing data). We examined whether children with (n = 308) and without behavioral data (n = 75) differed on their RSA or PEP profile. There was no difference in RSA classification for children with and without externalizing behavior data. Children with missing data on externalizing symptoms at 7 years of age were more likely to be classified in the PEP Profile 2 than the other two PEP profiles (described below), χ^2 (2) = 6.08, p = 0.048. As indicated by t-tests and chi-square analyses, this subsample was otherwise representative of the larger sample on all other key study variables. Average externalizing behavior problems were higher among male children compared to female children (male $M = 46.40$, $SD = 10.27$, female $M = 43.48$, $SD = 7.25$; t(df) = 2.92 (306), $p < .01$). See Table 1 for demographics and descriptive statistics in the present study.

GMM of RSA and PEP

Table 2 presents the results of the systematic GMM model fitting processes and the percentage of children classified in each latent class. By considering multiple fit indices, interpretability, and sample proportions, it was concluded that the 2-profile quadratic growth model for RSA and the 3-profile linear growth model for PEP provided the most parsimonious and optimal solutions. In regards to profiles of RSA reactivity, the fit statistics and classifications between the 2-profile linear model and 2-profile quadratic model for RSA were similar, however the 2-profile quadratic model emerged as a better fitting model overall in terms of fit statistics and entropy. Moreover, some of the quadratic growth factors were significantly related to gender and/or family poverty levels. Additional RSA profiles beyond two (i.e., 3-profile or 4-profile models) resulted in worsening model fit and lower entropy. The two solid lines in Figure 1a illustrates the growth patterns of the two profiles for RSA at the sample mean of sex and poverty levels. Table 3 provides the parameter estimates of the growth factors (i.e., intercept, slope). RSA Profile 1 ($n = 150$; 39%), labeled *reactive-decreasing*, represented children who evidenced heightened RSA reactivity during infancy and then shifted towards lower reactivity by 5 years of age. Child sex was not significantly associated with Profile 1 (male $n = 68$, female $n = 82$). Children in Profile 2 ($n = 233$; 61%), labeled *U-shaped reactivity,* were not reactive at 6 months, shifted to reactive at approximately 1 through 3.5 years, and returned to a state of non-reactivity at 5 years. Sex was significantly associated with the growth factors in this RSA Profile 2 (male $n = 118$, female $n = 115$). As shown by the dashed (for males) and dotted (for females) lines in Figure 1b, females had a steeper curve than males but both groups started and ended in similar states of non-reactivity.

Analyses of PEP profiles indicated that the 2-profile and 3-profile linear models had acceptable model fit without any improper solutions (see Table 2). Although some of the fit statistics of the 2-profile linear model were slightly better than the 3-profile linear model, one of the profiles in the former solution was very small (3% of the sample). The 2-profile quadratic model and 3-profile linear model also had somewhat comparable fit indices, however the latter had better entropy. Additionally, the 3-profile linear model yielded three distinct growth trajectories and a reasonable distribution of children across the profiles, providing a more robust and substantively interpretable solution. Model fit indices for the 3-profile quadratic model and 4-profile linear and quadratic models worsened and the entropy values were beyond acceptable range. Figure 2a illustrates the trajectories of the three linear profiles for PEP at the sample of mean sex (see also Table 3). Children in PEP Profile 1 ($n = 120$; 32%), termed *blunted/anticipatory reactivity,* generally demonstrated a pattern of blunted reactivity across the first 5 years of life (see Figure 2). The very positive PEP scores in Profile 1 suggest an anticipatory response; in other words, children in profile were highly activated prior to the initiation of the stressful protocols and had reactivity levels that declined only minimally across the duration of the protocol. Although sex was significantly associated with the growth factors, almost all of the children in this profile were males (n = 114; 95%). Children in the PEP Profile 2 ($n = 71$; 19%; Figure 2), labeled *reactive-decreasing*, demonstrated very high PEP reactivity in infancy and a relatively sharp linear decrease to lower reactivity through the preschool period. Again, though sex was significantly associated with the slope, almost all of the children in this profile were males (n = 64; 90%). The majority of children were classified in PEP Profile 3 ($n = 186$; 49%; Figure 2d), *non-reactive increasing,* demonstrating a lack of reactivity in infancy and increasing reactivity over time. In contrast to PEP Profiles 1 and 2, almost all of the children in this profile were females (n = 182; 98%)

Effects of ANS profile on children's externalizing behavior problems at age 7

The association of RSA profiles with children's externalizing behavior problems at 7 years of age did not differ by sex (the profile*sex interaction terms were nonsignificant, $p > .84$). There was a marginally significant relation between RSA profile and children's externalizing behavior problems, controlling for gestational age ($B = -1.804$, $SE = 1.025$, $t = -1.759$, $p = 0.078$). Results indicated that children in RSA Profile 1 (*reactive-decreasing*) from infancy to 5 years of age had lower levels of externalizing behavior problems than children in RSA profile 2 (*U-shaped reactivity)*, though this difference did not reach statistical significance. Given that the PEP profiles were highly confounded by sex, testing sex as a moderator was not informative. PEP Profiles 1 and 2 were primarily male (95% and 90%, respectively) and PEP Profile 3 was primarily female (98%). There was a significant relation between PEP profile and children's externalizing behavior problems such that children within PEP Profile 1 (*blunted/anticipatory reactivity)* had higher externalizing behavior problems compared to children in PEP Profile 3 (*non-reactive increasing*; $B = 2.530$, $SE = 1.154$, $t = 2.193$, $p = 0.028$). Children in PEP Profile 2 (*reactive-decreasing*) had marginally significantly higher externalizing behavior problems compared to children in PEP Profile 3 (*non-reactive increasing;* $B = 2.402$, $SE = 1.394$, $t = 1.723$, $p = 0.085$). There was no significant difference in externalizing behavior between children in PEP Profile 1 and in PEP Profile 2 (*blunted/anticipatory reactivity versus reactive-decreasing;* $p = 0.887$).

Discussion

To address scarce research that has explored developmental trajectories of ANS reactivity, the present study utilized an advanced statistical modeling technique to derive longitudinal profiles of RSA and PEP reactivity from 6 months through 5 years of age and evaluated associations between reactivity profile membership and later externalizing behavior problems. We examined these relations in a sample of low-income Mexican American children who resided in an agricultural community. Such contextual factors may increase risk for compromised physiological and psychological functioning, however are underrepresented in empirical research.

In order to capture children's developmental trajectories of ANS activity – which we posit to be more relevant to understanding relations with externalizing behavior problems than a single timepoint of ANS activity – we modeled patterns of RSA and PEP reactivity from 6 months to 5 years of age using GMM. Given the scarcity of longitudinal investigations of children's ANS activity, the present study's findings of heterogeneous profiles of RSA and PEP reactivity represent a significant contribution to the literature. We found that a two-profile quadratic model ideally described our RSA reactivity data. Children classified in RSA Profile 1, *reactive-decreasing,* demonstrated higher RSA reactivity during infancy and became less reactive by five years of age. In contrast, the children in RSA Profile 2, *U-shaped reactivity,* demonstrated low reactivity during infancy and became progressively more reactive over the course of the next two years before decreasing in reactivity again. By five years of age, children in RSA Profile 2 demonstrated low reactivity again, thus demonstrating a curvilinear pattern. PEP reactivity in the present study was described by a three-profile linear model. Children in the PEP Profile 1, *blunted/anticipatory reactivity,* were not reactive in infancy and remained non-reactive through age five. Moreover, the positive PEP scores among children in this profile suggest an *anticipatory* response of heightened reactivity prior to the start of the stressful protocol. PEP Profile 2, *reactive-decreasing,* included children who demonstrated high levels of PEP reactivity in infancy that declined in a sharp, linear fashion over the next 5 years of life. Finally, PEP Profile 3, *non-reactive increasing,* reflected children were non-reactive in infancy and became more reactive by age 5.

To our knowledge, this is the first application of growth mixture modeling to understand developmental trajectories of children's RSA and PEP reactivity. Consistent with the results of Conradt et al. (2014), we observed that children in RSA Profile 1 (*reactive-decreasing)* became less reactive over time, which may reflect the increasing regulatory capacities that develop with age (Conradt et al., 2016; Geisler, Kubiak, Siewert, & Weber, 2013). Importantly, not all children followed this trajectory of RSA change. In fact, a large proportion of the sample was classified within RSA Profile 2, *U-shaped reactivity*, in which children demonstrated a trajectory of increasing reactivity followed by decreasing reactivity such that levels of reactivity were generally comparable during infancy and by age 5. Prior researchers have observed increases in *resting* RSA during the first two years of life (Jewell, Suk, & Luecken, 2018). Similarly, we observed that children in RSA Profile 2 exhibited increases in RSA reactivity through the first two years of life, however results of the current study extend these findings to suggest that reactivity may then decrease between ages 2 and 5. Notably, research has been equivocal regarding developmental trends of PNS activity, with some studies suggesting that reactivity peaks in early childhood (Finley & Nugent, 1995) and other suggesting this does not occur until early adolescence (Massin & Von Bernuth, 1997). In fact, it may be the case that "one size does not fit all" and several profiles are needed to describe the full range of different developmental trajectories of RSA reactivity across the early years of life.

In regards to PEP, prior work during middle childhood and adolescence suggests SNS reactivity (as modeled using skin conductance level) is less stable than resting measures of SNS (Matthews, Salomon, Kenyon, & Allen, 2002). Given the scarcity of research on children's PEP reactivity (particularly in a growth mixture modeling framework), it is difficult to interpret the PEP profiles derived in the current study within the context of extant research. SNS reactivity may decline over time (El-Sheikh et al., 2010), a pattern that is consistent with what was observed in PEP Profile 2, *reactive-decreasing*. Although we are not aware of prior evidence of developmental patterns consistent with our other two observed PEP reactivity profiles (Profile 1: *blunted/anticipatory reactivity*, Profile 3: *non-reactive increasing*), previous empirical investigations have generally examined *overall sample PEP reactivity on average*, which may mask between-individual variability in trajectories. Importantly, patterns of stress responsivity are likely to be influenced by genetic inputs, environmental factors, and their interactions (Del Giudice et al., 2011). Future research is well-positioned to examine the extent to which such variables may be involved in distinguishing among the varied PEP profiles derived in the present study. Although male and female children were generally equally distributed across profiles of RSA reactivity, this was not the case for PEP reactivity profiles. Rather, PEP Profile 1 (*blunted/anticipatory reactivity*) and PEP Profile 2 (*reactive-decreasing*) were predominantly comprised of male children, while PEP Profile 3 (*non-reactive increasing)* was disproportionally female. Prior researchers have observed patterns lower resting PEP among males during infancy and the preschool period (Suurland, 2017). To our knowledge, the present study is the first to identify sex differences in PEP reactivity or trajectories.

In addition to modeling ANS reactivity profiles during the first five years of life, the present study examined relations between the profiles and children's externalizing behavior problems at 7 years of age. Children with heightened RSA reactivity in infancy that decreased over time (RSA Profile 1, *reactive-decreasing*) exhibited lower externalizing problems at age 7 compared to children who exhibited U-shaped RSA reactivity (RSA Profile 2), however this difference did not reach statistical significance. In light of the marginal p-value, we are cautious not to over-interpret our findings, however we note that prior research suggests higher levels of RSA reactivity may be protective against the development of externalizing problems by facilitating more adaptive behavioral and emotional regulatory processes (Graziano & Derefinko, 2013). Although children in both RSA Profile 1 and RSA Profile 2 exhibited periods of heightened RSA reactivity, children in the former profile are distinguished from the latter profile by reactivity that was heightened during the first year of life.

In addition to relations with RSA reactivity profiles, externalizing problems were found to be higher among children within PEP Profile 1 (*blunted/anticipatory reactivity*) compared to those in PEP Profile 3 (*non-reactive increasing*). Those in PEP Profile 2 (*reactive-decreasing)* also had marginally higher levels of externalizing problems than children in PEP Profile 3. As PEP Profiles 1 and 2 were disproportionally comprised of male children, well-known sex differences in externalizing behaviors (Shirtcliff & Essex, 2008) may help explain this pattern of findings. Moreover, prior researchers have observed that male children with higher levels of externalizing problems exhibit lower levels of PEP reactivity or PEP non-reactivity (Beauchaine & Gatzke-Kopp, 2012), supporting biological theories of underarousal that suggests that lower SNS reactivity may reflect temperamental fearlessness or sensation-seeking that contributes to aggressive and disruptive behavior (Ortiz & Raine, 2004). Interestingly, it appears as though the physiological risk of blunted or lower PEP reactivity for externalizing behaviors may be specific to male children; although both Profiles 1 and 3 depicted sympathetic underarousal during the first couple years of life, it was only the predominantly male Profile 1 that was associated with later externalizing behavior problems. The predominantly female PEP Profile 3 may also be distinguished from the predominantly male PEP Profile 1 by the fact that children in the former profile began to demonstrate some PEP reactivity after the first two years of childhood. In contrast, children in PEP Profile 1 remained non-reactive through age 5. This developmental shift toward greater PEP reactivity for the mostly female children in Profile 3 may have operated in a manner to protect against the development of later externalizing problems.

Results of the present study should be considered in light of several limitations. First, the protocol used to collect RSA and PEP reactivity required adaptations between the infancy and preschool ages; although such changes ensured that the tasks remained developmentally appropriate and challenging across assessments, the lack of consistency in the protocol may at least partly explain the changes in reactivity from infancy to the preschool ages. Second, it was not possible to collect ANS reactivity on all children across each of the timepoints, however standard missing data handling procedures for growth modeling with Mplus allowed models to retain children that provided any ANS data. Third, the unique sociodemographic characteristics of families in the current study may limit generalizability to other ethnic groups and non-agricultural, higher income families. Despite this limitation, we contend that the present study makes an important contribution given scarce research of longitudinal trajectories of physiology in diverse samples. Fourth, some prior research highlights the utility of simultaneous modeling of PNS and SNS reactivity consistent with the doctrine of autonomic space (Alkon, Boyce, Neilands, & Eskenazi, 2017; Berntson, Cacioppo, Quigley, & Fabro, 1994; Treadwell, Alkon, Quirolo, & Boyce, 2010). Future studies may build on the current research by deriving trajectories of concordant (or discordant) PNS and SNS responsivity over time. Fifth, our operational definition of ANS reactivity (challenge minus rest) was appropriate for the objectives of the present study, however there are other viable approaches to measuring psychophysiological reactivity (Burt & Obradović, 2013). Finally, we acknowledge that internalizing and externalizing symptoms are positively correlated during childhood (Essex, Armstrong, Burk, Goldsmith, & Boyce, 2011; Rudd, Alkon, & Yates, 2017). Given the particularly robust relations between ANS reactivity (particularly SNS reactivity) and early externalizing problems (Calkins, Blandon, Williford, & Keane, 2007; Snoek, Van Goozen, Matthys, Buitelaar, & Van Engeland, 2004; Utendale et al., 2014), we limited the scope of the present study to externalizing problems only, however future research is well-positioned to examine associations between RSA/PEP reactivity and other behavior outcomes.

In sum, results of the current study support the presence of discrete trajectory patterns of RSA and PEP reactivity from infancy through 5 years of age. Across both branches of the ANS, there were trajectories of change that suggested reactivity is not yet stable during early childhood. Within only the parasympathetic branch, a profile of U-shaped curvilinear reactivity emerged while patterns of sympathetic reactivity followed linear trajectories. Such PEP profiles appeared to distinguish among children’s behavior problems at age 7; externalizing behavior problems were significantly higher among those who demonstrated blunted/anticipatory PEP reactivity during (a profile comprised of mostly male children). A comprehensive understanding of early risk for psychopathology may be optimally achieved via longitudinal analyses of children’s trajectories of stress physiology in combination with ongoing study of the environmental context – beginning as early as infancy.

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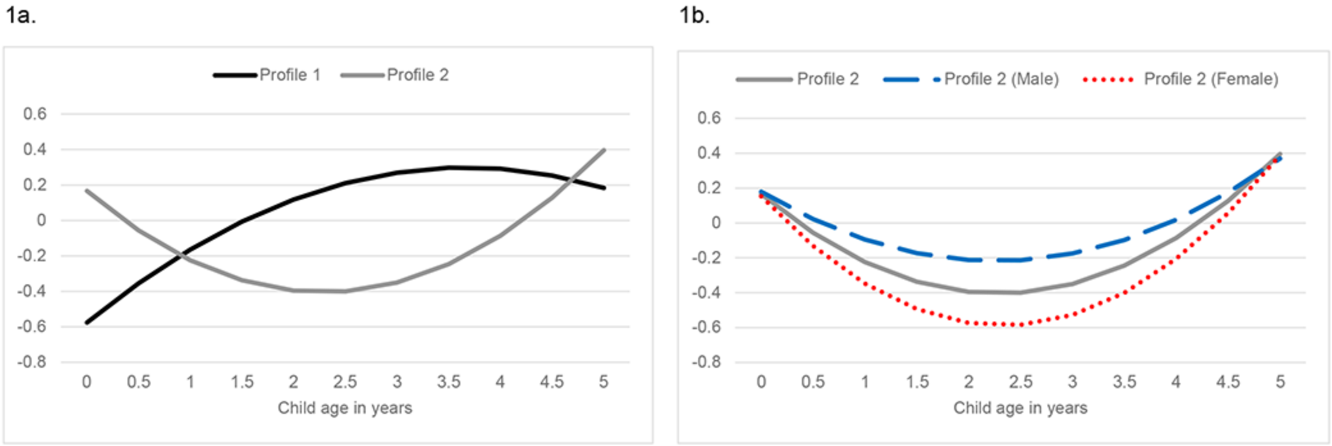

Figure 1.

Two profile quadratic model of RSA reactivity from 6 months to 5 years of age

Notes. More negative difference scores indicate higher RSA reactivity. Models adjusted for family poverty. Panel 1a graphs the trajectories at the sample mean of sex and poverty and panel 2a graphs the trajectories at the sample mean, as well as the sex-specific trajectories. There were no sex differences in class 1, thus males and females are graphed together.

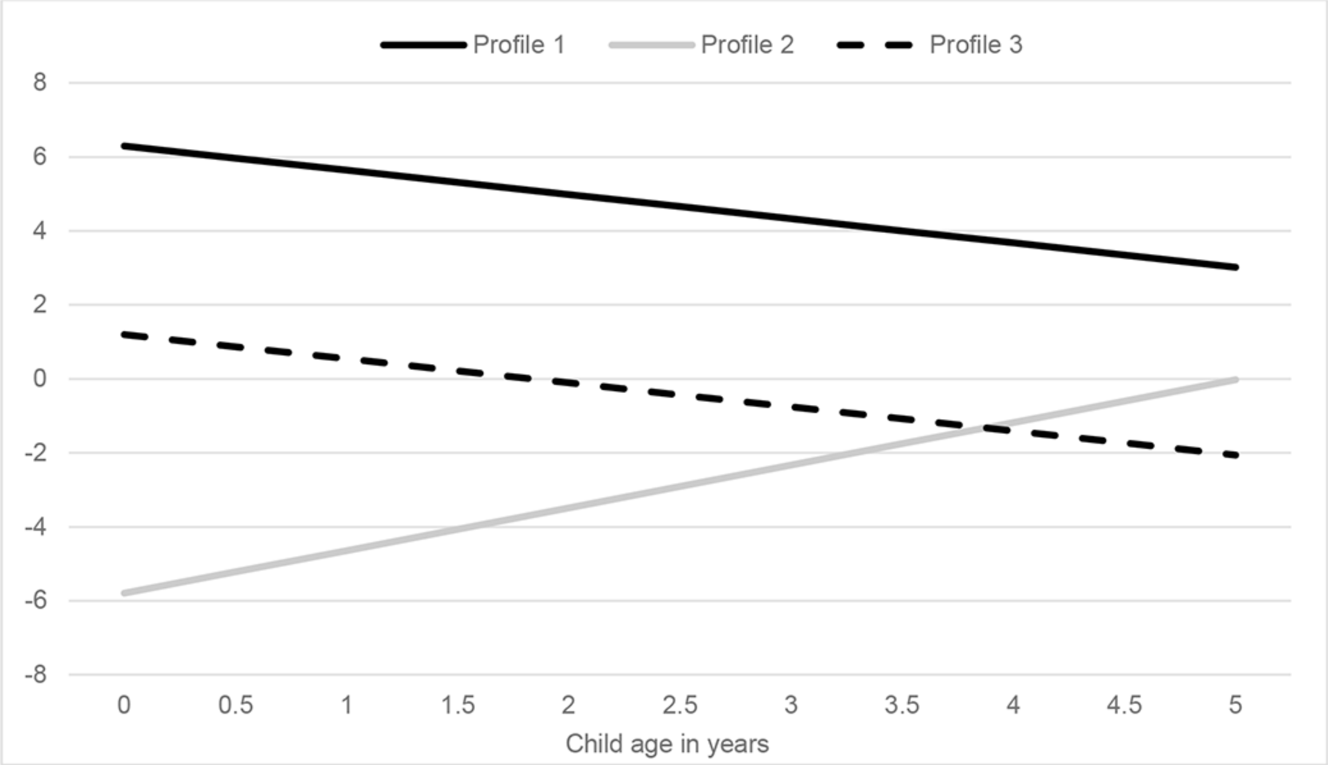

Figure 2.

Three profile linear model of PEP reactivity from 6 months to 5 years of age

Notes. More negative difference scores indicate higher PEP reactivity. Model adjusted for family poverty and graphed at the sample mean of sex and poverty. Profiles 1 and 2 were predominantly male and Profile 3 was predominantly female, so sex-specific trajectories are not depicted.

Table 1.

Demographic and descriptive statistics

Variable	n	Mean (SD) or n(%)	Min	Max
Child sex	383			
Female		197 (51%)		
Male		186 (49%)		
Socioeconomic status	383			
At or below 100% Federal Poverty Level (FPL)		239 (62%)		
At 200% FPL or above		144 (38%)		
Mother years living in the United States	383			
<= 5 year		182 (47%)		
6+ years		160 (42%)		
Entire life		41 (11%)		
Mother education level	383			
<= 6th grade		165 (43%)		
7-12th grade		141 (37%)		
>= High school graduate		77 (20%)		
Language spoken at home	383			
Mostly Spanish		343 (90%)		
Spanish and English equally		19 (5%)		
Mostly English		17 (4%)		
Other language		4 (1%)		
Parents' marital status	383			
Married/living as married		319 (83%)		
Not married		64 (17%)		
Gestational age	383	38.85 (1.78)	29	42
>=37 weeks				
< 37 weeks				
Externalizing behavior problem symptoms at 7 years	308	44.82 (8.87)	32	91

Table 2:

Model fit indices for GMM models with 1 to 4 latent profiles

Number of profiles	BIC	Adjusted BIC	VLMR	BLRT	Entropy	Proportion
Respiratory sinus arrhythmia (RSA)						
1 linear	1336.665	1304.937	N/A	N/A	N/A	
1 quadratic	1343.638	1302.391	N/A	N/A	N/A	
2 linear	1374.663	1311.206	0.137	0.089	0.810	.38|.62
2 quadratic	**1382.922**	**1300.428**	**0.033**	**<.001**	**0.836**	**.39|.61**
3 linear	1412.209	1323.369	0.513	0.667	0.765	.30|.30|.40
3 quadratic	1421.972	1304.576	0.400	0.235	0.703	.30|.31|.39
4 linear	1461.122	1343.726	0.562	0.600	0.731	.19|.25|.26|.30
4 quadratic	1474.023	1318.553	0.255	0.333	0.610	.04|.06|.07|.83
Pre-ejection period (PEP)						
1 linear	3331.901	3300.173	N/A	N/A	N/A	
1 quadratic	3346.658	3305.413	N/A	N/A	N/A	
2 linear	3347.307	3287.024	0.057	<.001	0.931	.03|.97
2 quadratic	3374.690	3295.371	0.290	<.001	0.747	.46|.54
3 linear	**3388.338**	**3299.500**	**0.009**	**0.286**	**0.933**	**.19|.32|.49**
3 quadratic	3418.524	3301.132	0.106	0.109	0.656	.07|.15|.78
4 linear	3434.891	3317.498	0.492	1.000	0.662	.05|.08|.21|.66
4 quadratic	3469.659	3314.193	0.479	0.208	0.623	.02|.03|.06|.89

Table 3:

Parameter estimates of growth factors and the effects of sex on the growth factors

	Intercept[a] M(SE)	Linear Slope[a] M(SE)	Quadratic Slope[a] M(SE)	Sex on Intercept[b] B(SE)	Sex on Linear Slope[b] B(SE)	Sex on Quadratic Slope[b] B(SE)
RSA Profiles						
Profile 1; *reactive-decreasing*	−0.576 (0.275) *	0.477 (0.248) *	−0.065 (0.043)	−0.044 (0.055)	0.072 (0.085)	−0.017 (0.016)
Profile 2; *U-shaped reactivity*	0.166 (0.104)	−0.499 (0.068) ***	0.109 (0.018) ***	−0.012 (0.044)	−0.138 (0.064) *	0.029 (0.012) *
PEP Profiles						
Profile 1; *blunted/ anticipatory reactivity*	6.298 (0.499) ***	−0.655 (0.172) ***	--	6.451 (0.507) ***	−0.994 *** (0.135)	--
Profile 2; *reactive-decreasing*	−5.796 (1.983) **	1.155 (0.465) *	--	0.701 (0.464)	−0.376 (0.122) **	--
Profile 3; *non-reactive increasing*	1.196 (0.636)	−0.651 (0.112) **	--	−0.799 (0.631)	0.511 (0.110) ***	--

*** $p \leq .001$

** $p \leq .01$;

* $p \leq .05$;

[a] Conditional mean of the growth factors after controlling for sex and poverty levels using weighted effect coding;

[b] Regression coefficients for the effects of sex on the growth factors after controlling for poverty levels, using weighted effect coding.