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Regular Article

Variation in coupling across neural and cardiac systems of regulation is linked to markers of anxiety risk in preschool

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Abstract

Both cortical and parasympathetic systems are believed to regulate emotional arousal in the service of healthy development. Systemic coordination, or coupling, between putative regulatory functions begins in early childhood. Yet the degree of coupling between cortical and parasympathetic systems in young children remains unclear, particularly in relation to the development of typical or atypical emotion function. We tested whether cortical (ERN) and parasympathetic (respiratory sinus arrhythmia [RSA]) markers of regulation were coupled during cognitive challenge in preschoolers ($N = 121$). We found no main effect of RSA predicting ERN. We then tested children's typical and atypical emotion behavior (context-appropriate/context-inappropriate fear, anxiety symptoms, neuroendocrine reactivity) as moderators of early coupling in an effort to link patterns of coupling to adaptive emotional development. Negative coupling (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression) at age 3 was associated with greater atypical and less typical emotion behaviors, indicative of greater risk. Negative age 3 coupling was also visible for children who had greater Generalized Anxiety Disorder symptoms and blunted cortisol reactivity at age 5. Results suggest that negative coupling may reflect a maladaptive pattern across regulatory systems that is identifiable during the preschool years.

Keywords: childhood anxiety risk; EEG/ERP; ERN; RSA; temperamental fear

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The development of self-regulation is a primary task of early childhood (Calkins & Williford, 2009; Kopp, 1982). During the preschool period, roughly ages 3–5 years, self-regulation of negative emotions shows marked gains (Kopp, 1989). Because biological systems are believed to support the self-regulation of emotion (Levenson, 2003, 2014; Thayer & Lane, 2000), empirical efforts to understand the early development of self-regulation frequently focus on associations between neurophysiological function and adaptive or maladaptive emotion propensities. Among the most frequently targeted are neural markers of self-monitoring, or the ability to effectively monitor one's own behavior in order to make necessary adjustments (Brooker et al., 2019; Brooker & Buss, 2014; Meyer et al., 2018), and parasympathetic control, or the ability to effectively modulate metabolic output to meet shifting contextual demands (Porges, 2003; Thayer & Lane, 2009). While systems of self-monitoring and parasympathetic control have overlapping neuroanatomical roots, we know surprisingly little about the degree to which they work together to support the development of self-regulation. That is, it is unknown whether these early systems of self-regulation work together or in a compensatory fashion to support the self-regulation of negative emotion in preschoolers. Understanding the nature of this association is important given that persistent psychophysiological dysregulation is linked to highly prevalent negative outcomes in young children, such as anxiety risk (Buss & McDoniel, 2016; Kessler et al., 2005; Van Hulle et al., 2017). Thus, understanding associations between cortical and parasympathetic regulatory systems may be useful for more clearly elucidating dynamic psychophysiological correlates of self-regulation and identifying mechanistically-driven intervention targets for children at risk for negative outcomes.

Self-monitoring can be reliably indexed at the neural level in children as young as 3 years of age using ERN (Brooker et al., 2019; Brooker, 2018; Grammer et al., 2014). ERN appears as a negative deflection in averaged EEG recordings approximately 50-100 ms following error commission (Falkenstein et al., 1991). ERN is maximal at frontocentral electrode sites and is thought to be generated by the Anterior Cingulate Cortex (ACC; Dehaene et al., 1994; Van Veen & Carter, 2002). The enhancement of ERN across development is associated with more adaptive learning and flexible response modulation (Tamnes et al., 2013). Notably, individual differences in ERN are linked to risk for anxiety problems in the form of fearful behavior in children. For example, both high and moderate (but not low) fear at age 2 predict frontal ERN activity at age 4.5 (Brooker & Buss, 2014) and fearfulness, but not shyness, is associated with reduced ERN in 6 year olds (Meyer & Klein, 2018). While a larger ERN is typically associated with elevations in generalized relative to specific (i.e., social) anxiety presentations in older samples (Meyer et al., 2018), the nature of this relation appears reversed before age 9 (Meyer et al., 2018), whereby a

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smaller ERN predicts later anxiety and related disorders. Nonetheless, across ages, the ERN shows robust and reliable associations with anxiety risk (Moser, 2017) and symptoms in young children (Meyer, 2017).

Another reliable marker of self-regulation in children is cardiac vagal flexibility, or the modulation of cardiac activity via the vagus nerve to fit a dynamic range of challenges (Porges, 2009; Thayer & Lane, 2009; Wessel et al., 2011). Cardiac vagal flexibility can be indexed through Respiratory Sinus Arrhythmia (RSA). Suppression of RSA involves the withdrawal of parasympathetic control over autonomic activity to mobilize threat responsivity. Increased RSA suppression, linked to increased heart rate, facilitates efficient responses to real or perceived threats. Given that unique contexts call for different behaviors, there is not only one adaptive pattern of change in RSA (Hastings et al., 2014; Shakiba et al., 2020). However, RSA suppression regularly predicts adaptive self-regulation in early life. In young children (2–4.5 years of age), reduced RSA suppression (or RSA augmentation) is associated with poorer self-regulation (Brooker et al., 2013; Brooker & Buss, 2010), increased fear and negative emotionality, and worse adjustment (Calkins & Keane, 2004). These social, emotional, and behavioral difficulties represent notable risk factors for the development and maintenance of anxiety and related disorders across the lifespan (Beauchaine, 2015; Cho & Buss, 2017). However, some emotion-specific effects exist in the association between RSA and adaptation (Gatzke-Kopp et al., 2015), underscoring the importance of interpreting effects in light of the incentives of the eliciting context.

According to the Neurovisceral Integration Model (Thayer & Lane, 2000), emotion–cognition interactions influence parasympathetic activity in order to facilitate goal-directed behavior. Therefore, the adaptive effects of cardiac vagal flexibility reflect the functional integrity of both neural and parasympathetic activity implicated in self-regulation. Polyvagal Theory (Porges, 2003, 2009) suggests that the vagus nerve specifically evolved to facilitate this brain-body feedback loop linking cortical with parasympathetic activity. In this way, individuals may adjust their physiological arousal to flexibly meet the demands of their environment, particularly social environments.

Expectations for coupling between cortical and parasympathetic systems in the service of self-regulation are grounded largely in theory, though a limited amount of empirical work with adults supports the plausibility of coupled systems. For example, consistent with both the Neurovisceral Integration and Polyvagal approaches, older samples evidence links between activation of the prefrontal cortex (PFC) and parasympathetic activation via bidirectional pathways mediated by the anterior cingulate cortex (ACC; Beauchaine, 2015; Thayer & Lane, 2009; Wessel et al., 2011). Further, both ERN and RSA suppression are directly associated with initial ACC activation and frontal brain activity (Wessel, 2012), suggesting a functional association between these cortical and cardiac indices might exist. Yet such a link has scarcely been tested directly, particularly in early childhood when coordinated regulatory processes are being calibrated (Hostinar & Gunnar, 2013; Kopp, 1982) and critical brain development facilitates cognitive involvement in regulatory processes (Cachia et al., 2014; Fjell et al., 2012). Indeed, developmental changes in the ACC during the preschool period are linked to concurrent age-related growth in observed behavioral monitoring (Tamnes et al., 2013) and cognitive control efficiency (Cachia et al., 2014; Fjell et al., 2012); these associations are not present in late childhood or adolescence.

Slow frontal brain maturation in young children may facilitate central autonomic nervous system based response learning in childhood by allowing lower levels to "tune" priors (internal predictions based on experience) appropriately before top-down influences come fully online (Harteveld et al., 2021; Porges & Furman, 2011; Smith et al., 2017). Consistent with this possibility, individual differences in RSA would more likely stabilize before individual differences in ERN. However, efforts to establish normative developmental trajectories for both ERN (i.e., increases in amplitude [Meyer et al., 2018] and variability [Brooker, 2018] with age) and RSA suppression (i.e., increases from 2 months to 5 years, then decreases with age; Dollar et al., 2020), suggest considerable individual variability in both measures in early childhood. Such variability may reflect dynamic codevelopment across systems that could be leveraged for a more useful understanding of adaptive vs. maladaptive development of regulatory systems and individual differences in outcomes. For example, smaller childhood ERN in and of itself may be associated variably with maladaptive outcomes because it is only one of multiple influences on complex emotion behaviors. Thus, there is added utility in understanding the implications of a smaller ERN coupled with compensatory parasympathetic influence (i.e., greater RSA suppression) relative to less parasympathetic influence (i.e., less RSA suppression or even RSA augmentation) for more sensitively determining individual risk for negative outcomes. Therefore, one aim of this work was to identify the normative presence or absence of coupling in ERN and RSA during the preschool years.

Beyond normative function, a critical challenge in investigations of neurophysiological systems of regulation, including cross-system coupling, is that of interpreting adaptive and maladaptive patterns of function. Particularly in studies of childhood risk, before disorder manifests and in years when self-reports can be unreliable (Grills & Ollendick, 2003), the implications of individual differences in physiological function can be unclear. However, consistent with the Developmental Psychopathology perspective emphasizing multiple levels of analysis (Cicchetti & Dawson, 2002), longitudinal work that also includes observations of child behavior allows for the interpretation of individual differences in patterns of physiology alongside typical and atypical behavioral responses and/or childhood outcomes. For example, coupling between regulatory systems may be interpreted as adaptive if it occurs in the presence of typical emotion behaviors, and this argument is strengthened if it is also associated with more adaptive long-term function. In addition, it may be useful to interpret patterns of coupling in relation to other established biological correlates of regulation or dysregulation, embedding it into an overall picture of individual propensities for adaptive or maladaptive function.

The typical (versus atypical) nature of children's regulatory behaviors is perhaps most interpretable in light of the eliciting context. Consistent with a functionalist perspective on emotion (Campos, 1996), context-appropriate emotion behaviors suggest a response that is well-matched to the incentive properties of the eliciting context (i.e., displays of fear in a fear-eliciting context), while context-inappropriate emotion behaviors suggest a mismatch between the eliciting context and children's emotion responses (i.e., displays of fear in a positivity-eliciting context). Context-inappropriate fear responses, in particular, are linked to dysregulated emotion tendencies (Brooker et al., 2016; Buss, 2011) and enhanced risk for the development of anxiety problems (Buss

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et al., 2013). In contrast, context-appropriate fear responses such as stranger fear are evolutionary in origin, putatively adaptive, and common in 2-3 year olds (Brooker et al., 2013; Marks, 1987). Thus, patterns of coupling across biological systems in children with propensities for context-inappropriate fear responses may be more likely to reflect atypical regulation than patterns of coupling linked to context-appropriate emotion responses.

Similar to contextual affect, neuroendocrine reactivity has established associations with early emotion behaviors and maladaptive outcomes (Brooker et al., 2013; Hostinar & Gunnar, 2013; Koss & Gunnar, 2018; Van Hulle et al., 2017). Neuroendocrine reactivity reflects activation of the hypothalamic–pituitary–adrenal (HPA) axis, the human end product of which is cortisol. Like other regulatory systems, the HPA axis functions to maintain the biological resources available for responding to environmental challenge, although it works on a much slower time scale relative to systems like RSA (Gunnar & Quevedo, 2007). Even so, blunting of the HPA axis (i.e., hypo-cortisolism) is accordingly linked to chronic stress and threat reactivity in preschoolers (Badanes et al., 2011; Doom et al., 2018; Gunnar & Vazquez, 2001), and potential chronic dysregulation across the lifespan. Indeed, cortisol output shares unique associations with fear expression and emerging internalizing behaviors in the preschool period (Buss et al., 2011). Therefore, activity in well-explicated systems like the HPA axis can help to interpret coupled ERN and RSA activity in relation to emotion propensities and anxiety risk in young children.

In sum, although supported by overlapping neurovisceral mechanisms, indices of multi-systemic regulatory function are not well understood, both broadly and in early childhood when self-regulatory abilities undergo critical development (Beauchaine, 2015; Bornstein & Suess, 2000; Dawson et al., 1992; Hostinar & Gunnar, 2013; Koenig, 2020; Kopp, 1982, 1989). Understanding how regulatory systems develop in relation to each other and in relation to emotion behaviors in early childhood is important for elucidating dynamic psychophysiological correlates of self-regulation and identifying mechanistically-driven correlates of adaptive relative to maladaptive developmental outcomes. We tested for distinct patterns of coupling between cortical (i.e., ERN) and parasympathetic (i.e., RSA) regulatory systems in the preschool period. We used concurrent and longitudinal observations of children's emotion behaviors linked to child anxiety risk along with neuroendocrine reactivity (i.e., cortisol output) to characterize significant patterns of coupling.

We hypothesized that significant coupling, or correlation, would exist when predicting ERN from RSA during a cognitively challenging Go/No-go task (GNG; Brooker & Buss, 2014; Meyer, 2017). Specifically, we predicted a larger ERN would be coupled with more RSA suppression. We chose a standard GNG task given its effectiveness in eliciting ERN in children as young as age 3 (Brooker, 2018), and also because there is an established pattern of RSA response to cognitive challenge (Zeytinoglu et al., 2020). Thus, we could be confident that null effects, if found, were not the result of an inability to elicit sufficient variability in either ERN or RSA response. However, we do not assert that any effect would be specific to a GNG challenge.

In line with both the Neurovisceral Integration Model and Polyvagal Theory, significant ERN-RSA coupling may be most expected when accounting for emotion behavior (Porges, 2009; Thayer & Lane, 2009). Therefore, we further hypothesized that patterns of coupling characterized by a smaller ERN and less RSA suppression (or RSA augmentation) would be most visible in relation to markers of anxiety risk such as greater atypical fear (high fear in a low-threat context), lower typical fear (low fear in a high threat context), greater anxiety symptoms, and blunted neuroendocrine reactivity. In contrast, we expected coupling characterized by a larger ERN and more RSA suppression, reflecting coordinated regulation, to be most related to adaptive child outcomes such as less atypical fear, greater typical fear, less anxiety symptoms, and more neuroendocrine reactivity. Separate models were employed to allow for a conceptual replication of effects across different levels of analysis related to anxiety risk.

Methods

Participants

This research was approved by the Institutional Review Board at Montana State University [RB070213-FC]. The sample included 121 preschoolers (58% female) assessed longitudinally at ages 3 ($M = 3.59$, $SD = 0.15$, range = 3.13–3.99 years) and 5 ($M = 5.52$, $SD = 0.12$, range = 5.28–5.98 years) as part of a larger study between Spring 2014 and Winter 2017. Children were recruited from the general community via flyers, news advertisements, mailings based on local birth records, and word-of-mouth. Parents were compensated after each study visit. Sample characteristics are summarized in Table 1.

Procedures

Parents enrolled children in the study by contacting research staff. Upon arrival at the laboratory, parents were informed of study procedures and provided written consent for their child's participation. At age 3, children participated in a modified Go/No-Go (GNG) task while continuous EEG and ECG data were collected. Children then participated in two standardized behavioral paradigms intended to elicit either positive (watching a puppet show) or negative (meeting a stranger) emotion (Buss & Goldsmith, 2000). At age 5, the primary caregiver completed the Anxiety and Related Disorders Interview Schedule for DSM-5 (ADIS-5 PV; Albano & Silverman, 2016) and salivary cortisol was collected from children at three time points: upon arrival, 30 minutes following peak arousal to the EEG procedure, and at the end of the visit.

Measures

ERN

ERN was elicited from children using a modified GNG task (Brooker, 2018) during which either a spaceship (no-go stimulus) or an asteroid (go stimulus) was presented vertically in the center of a 23 computer screen using Presentation stimulus delivery software (Neurobehavioral Systems, Inc.). Children were instructed to push the response button as quickly and accurately as possible to destroy the asteroids but be careful not to destroy other spaceships. Stimuli were presented for 1200 ms and were preceded (200 ms) and followed by (300–800 ms) a gray fixation cross. The full task comprised 2 blocks of 40 trials presented pseudo randomly (at least 60% go trials) for a maximum of 80 trials per child. Prior to the task, children practiced with a research assistant using laminated pictures and completed two computerized practice blocks of 10 trials each. Children received a sticker after completing each block. To equate task difficulty across participants, an error rate of roughly 50% was maintained through an automated procedure that decreased stimulus presentation time by 50 ms following 2 consecutive correct responses and increased

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Table 1. Participant characteristics for children and their biological parent

	Children ($n = 121$)	Mothers ($n = 114$)	Fathers ($n = 93$)
Mean Age (years)	3.59 ($SD = .15$)	34.53 ($SD = 5.16$)	36.14 ($SD = 5.77$)
Ethnicity			
Hispanic/Latino		2 (1.8%)	4 (4.3%)
Not Hispanic/Latino		100 (87.7%)	79 (84.9%)
Unspecified		12 (10.5%)	10 (10.8%)
Race			
Caucasian		97 (85.1%)	80 (86.0%)
African American/Black		0	0
Asian American		2 (1.8%)	2 (2.2%)
American Indian/Alaska Native		3 (2.6%)	3 (3.2%)
Native Hawaiian/Pacific Islander		0	0
Other		0	0
Unspecified		12 (10.5%)	8 (8.6%)
Education (years)			
Some High School (9–11)		2 (1.7%)	0
High School Graduate (12)		5 (4.4%)	3 (3.2%)
Trade or Some College (13–15)		27 (23.7%)	9 (9.7%)
College Graduate (16)		27 (23.7%)	11 (11.8%)
Master's degree (17–19)		23 (20.2%)	4 (4.3%)
Doctoral Degree (20+)		3 (2.6%)	0
Unspecified		27 (23.7%)	66 (71.0%)
Income			
less than $15,000		3 (3.4%)	2 (2.9%)
$15,001–$20,000		5 (5.6%)	3 (4.4%)
$20,001–$30,000		7 (7.9%)	4 (5.9%)
$30,001–$40,000		6 (6.7%)	3 (4.4%)
$40,001–$50,000		7 (7.9%)	2 (2.9%)
$50,001–$60,000		12 (13.5%)	9 (13.2%)
$60,001–$70,000		6 (6.7%)	10 (14.7%)
$70,001–$80,000		10 (11.2%)	6 (8.8%)
$80,001–$90,000		9 (10.1%)	9 (13.2%)
$90,000 or greater		24 (27.0%)	20 (29.4%)

Note. Data for those mothers ($n = 89$) and fathers ($n = 68$) that reported gross annual income are presented.

stimulus presentation time by 50 ms following 2 consecutive incorrect responses.

Continuous EEG data were acquired during GNG using a BioSemi Active 2 recording system and a 64-channel cap with Ag-AgCl-tipped electrodes arranged according to the 10–20 labeling system. To collect eye movements, electrodes were also placed at the outer canthi of the left and right eye and at the supra and infra orbital sites of the left eye. Two electrodes were also placed on the mastoids for later re-referencing. During recording, data were referenced to the Common Mode Sense and Driven Right Leg electrodes and sampled at a rate of 2048Hz.

Data were processed offline using Brain Vision Analyzer Version 2.1. All electrodes were re-referenced to the average of the right and left mastoids, high-pass filtered at 0.1 Hz, and corrected for eye movement or blinks (Gratton et al., 1983). Correct and incorrect trials were segmented (−200 – 600 ms) and baseline corrected for 200 ms prior to the response. Artifacts were marked in segmented data when one of the following criteria were met: a voltage step of more than 75 μV between data points, a difference of 150 μV within 200ms, amplitudes below 0.5 μV within a 50ms period, and activity that exceeded + 100μV or − 100 μV. Remaining segments were visually inspected for artifacts. Clean segments were averaged and low-pass filtered at 30 Hz. For participants with at least six trials of usable data (Olvet & Hajcak, 2009; Pontifex et al., 2010), peak negative amplitudes between 0 and 100 ms were marked as ERN at electrodes Cz and Fz, which were selected based on previously-reported ERN in these data (Canen & Brooker, 2017); see Brooker (2018) for waveforms. Split-half reliabilities for ERN were moderate at age 3 ($r = .62$; ICC = .64). To isolate error-specific activity, a linear regression was run predicting incorrect trial amplitudes from correct trial amplitudes (Meyer & Klein, 2018). Unstandardized residuals from this analysis were saved, with more negative values indicating larger ERN and more positive values indicating smaller ERN (independent of correct trial activity).

RSA

Measures of cardiac output were collected during GNG using the same BioSemi Active 2 recording system by placing 2 exogenous electrodes on each wrist (i.e., either side of the midline). The ECG signal was sampled at a rate of 500 Hz and bandpass filtered at 40 and 250 Hz to reduce low (e.g., signal drift) and high-frequency artifacts (e.g., changes in muscle tension) that can distort the signal and reduce data quality (Pérez-Riera et al., 2018).

RSA analyses were performed offline using the Mindware editing program Mindware HRV, Version 2.51 (Mindware Technologies, Ltd, Westerville, OH) and a validated algorithm to identify IBIs and detect physiologically improbable intervals based on the overall distribution (Berntson et al., 1990). All data were visually inspected for artifact identification and editing. Data were detrended using a first-order polynomial to remove the mean and any linear trends, cosine tapered, and submitted to fast Fourier transform. Child RSA was defined as the natural log integral of the .24–1.04 Hz power band and calculated in 30 sec epochs (minimum of 20 sec for final epochs). To protect against artificial changes in RSA due to changes in respiration (Grossman et al., 1990), children who cried or had hiccups ($n = 3$) were not scored. Thus, the final data set contained 92 children with complete heart rate data during GNG.

Similar to previous work (Brooker & Buss, 2010), estimates of RSA in response to challenge were extracted from two-level mixed growth curve models (Singer, 1998). Data were centered at the beginning of the tasks (i.e., the first 30 s epoch). Then, growth curve models in which linear time predicted RSA values were used to estimate linear changes in RSA across the GNG task. Individual scores of linear change were extracted for each child using the SAS PROC REG procedure. Calculated in this way, positive and negative slope values reflect linear changes in RSA over time, resulting in positive values when RSA increases across the task (less suppression/more augmentation) and negative values when RSA decreases across the task (more suppression).

Context appropriate and inappropriate fear

Fear at age 3 was observed during two standardized behavioral paradigms derived from the preschool version of the Laboratory

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Temperament Assessment Battery (Lab-TAB- Buss & Goldsmith, 2000): *Context-appropriate fear* was assessed during a Stranger Approach episode designed to elicit fear and wariness. For this, an experimenter led the parent and child into a room where parents were asked to sit quietly and remain uninvolved. The child was given a set of age-appropriate toys to play with. After the child had played with the toys for 30 sec, a male experimenter entered the room. The stranger approached the child slowly and watched them play for up to 2 min while he asked several questions (e.g., "Are you having fun today?") before leaving the room.

Context-inappropriate fear was assessed during a Puppet Show episode designed to elicit positive affect. For this, an experimenter instructed the parent to sit with their preschooler on their lab opposite a puppet theater approximately 10 feet away. Two puppets played a series of three games together (catch, fishing, sticker), continually inviting the toddler to join them. Each game lasted 1 minute, after which the puppets declared the show over and said goodbye.

Trained coders scored both Stranger Approach and Puppet Show fear behaviors offline using video recordings of the episodes. Coders were required to achieve a minimum reliability of $\kappa = 0.70$ with a master coder before coding independently. The following behaviors were coded for across each episode: intensity of facial fear, bodily fear, distress vocalizations, verbal hesitancy, gaze aversion, and escape behaviors. Codes for fear behaviors were assigned every 5–10 sec, per Lab-TAB coding instructions. Roughly 20% of episodes were double coded to prevent coding drift (Stranger Approach mean $\kappa = 0.80$; Puppet Show mean $\kappa = 0.78$). Codes were averaged across the episode for each behavior. Average scores were then standardized within behavior and mean composited to create a single measure of fearfulness for each episode.

Anxiety risk

GAD symptoms

Generalized Anxiety Disorder (GAD) symptoms were evaluated when children were 5 years old. Parents completed the ADIS-5 (Albano & Silverman, 2016), a semi-structured interview derived from DSM-5 criterion for anxiety and other related conditions in children. Interviewers were psychology graduate students who established training reliability on administering and scoring the interview with a Master-level clinical psychologist. Interviews were tape recorded and double coded on 7% of cases and reliability was high (>90% agreement across all scales). For each DSM-5 diagnosis, interviewers rated the degree of distress and lifestyle impairment associated with characteristics of each disorder (0 = *none*, 8 = *very severely disturbing/disabling*). Interviewers inquired about each of the 4 worry behaviors proposed for GAD (i.e., avoidance, over-preparation, procrastination, reassurance seeking). After recording a description of the worry behavior, the behavior was rated on a 0–8 scale of frequency/severity (0 = *never*, 8 = *always/very severe*). Symptoms endorsed correspond to 6 criteria across the 4 worry behaviors. Therefore, possible sum scores range from 0-6, with 6 indicating clinical threshold. Three participants met the criteria for GAD disorder at age 5.

Neuroendocrine reactivity

To assess cortisol levels across the lab visit, three saliva samples were collected from each preschooler; a pre-task baseline collected upon arrival, a sample 30 minutes following peak arousal to the EEG procedure, and a sample at the end of the visit. Saliva samples were collected by holding a dental swab (Salimetrics, LLC part 5501.20) under the child's tongue or in the corner of the mouth for approximately 90 s.

All samples were stored at −80°C until they were thawed and centrifuged. All samples were assayed in duplicate using a highly sensitive enzyme immunoassay with a detection range from 0.012μg/dL to 3μg/dL. Samples were included in analyses only if the coefficient of variation (CV) was less than 20%. Mean CV values for included data were 7.18 ($SD = 5.15$). Average intra- and inter-assay coefficients of variation were < 7%. Raw cortisol samples were regressed on time since arriving to the lab. The distributions of lab cortisol measures were skewed and so were log10 transformed. Samples were excluded from analyses if the child was ill, taking antibiotics at the time of the sample, and/or if the sample was taken within 1 hour of eating. A reactivity score indexing total cortisol output across the lab visit was calculated as the area under the curve with respect to ground using precise time of sample collection (Pruessner et al., 2003); higher values reflect greater neuroendocrine reactivity across the lab visit (Gunnar, 1992).

Data analysis

All analyses were performed using Mplus software package version 8.4 (Muthén & Muthen, 2017). Following preliminary analyses to examine data distributions and bivariate associations to exclude potential demographic covariates, the primary study aims were addressed. Electrode locations Cz and Fz were tested in separate models based on previous work suggesting ERN and child fear relations may show some electrode specificity (Brooker & Buss, 2014). Considering existing literature suggesting common sex-effects across measures of RSA (Koenig et al., 2017; Rudd et al., 2017), ERN (Grammer et al., 2014; Moser et al., 2016), and anxious behaviors (Zhou et al., 2022) in early childhood, sex and age were controlled for in primary analyses.

First, to assess whether coupling was present between ERN and RSA, we tested simple linear associations between these measures. Then, to assess whether the relation between ERN and RSA may be linked to concurrent and longitudinal risk for anxiety problems, we tested context-appropriate fear (concurrent), context-inappropriate fear (concurrent), GAD symptoms (longitudinal), and cortisol reactivity (longitudinal) in separate models as moderators of the linear association reflecting coupling (Hayes, 2022). The rationale for including longitudinal measures as moderators of earlier associations was to test whether children's early patterns of coupling might be explained as adaptive or maladaptive based on observed outcomes.

Missing data

ERN was missing for 64 participants; RSA was missing for 27 participants; observed fear was missing for 25 (Puppet Show) and 28 (Stranger Approach) participants; anxiety symptom data were missing for 39 participants; cortisol data were missing for 51 participants. An analysis of patterns of missing data suggested that data were missing at random (Little's MCAR $\chi^2(119) = 98.752$, $p = .912$), and identified 11 cases missing all variables. Missing data were handled using a Full-Information Maximum Likelihood procedure that uses all available data to produce unbiased parameter estimates, thus allowing one to take advantage of the full sample from which at least some data are available (Enders, 2010). Of the original sample ($N = 121$), the final analytic sample comprised 110 children.

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Table 2. Descriptive statistics and bivariate correlations for primary study variables

	n	*M*	*SD*	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. Child sex	109	.580	.496		*−1.225* *d* = −.238	*−1.027* *d* = −.278	*−.225* *d* = −.061	*.891* *d* = .189	*−.641* *d* = −.133	*.019* *d* = .004	*1.019* *d* = .238	*−.284* *d* = −.089
2. Child age at enrollment	108	3.585	.149			.038	−.104	−.062	−.234*	−.329**	−.057	−.211
3. ERN–Cz (age 3)	58	.000	9.691				.710***	−.241*	.016	−.088	.040	−.189
4. ERN–Fz (age 3)	58	.000	7.362					−.223*	.033	−.009	.064	−.369*
5. RSA (age 3)	92	−.062	.490						−.051	−.121	.115	−.199
6. CI fear (age 3)	97	.000	.885							.082	−.012	−.133
7. CA fear (age 3)	94	.000	.439								−.097	.351*
8. GAD symptoms (age 5)	83	.830	1.673									−.260
9. Neuroendocrine reactivity (age 5)	51	44.186	14.544									

Note. RSA slope, GAD symptoms, and cortisol output were mean-centered prior to analyses. For child sex, male = 0 and female = 1. *** $p < .001$, ** $p < .01$, * $p < .05$; results presented in italics are *t*-tests with effect sizes measured using Cohen's *d*.

Results

Descriptive statistics, *t*-tests, and bivariate correlations are reported in Table 2. Younger children showed more context-appropriate ($r[109] = -.329$, $p < .001$) and context-inappropriate fear ($r[109] = -.234$, $p = .015$). ERN amplitudes were highly correlated across electrodes ($r[109] = .710$, $p < .001$). Cortisol output at age 5 was negatively correlated with frontal ERN ($r[109] = -.369$, $p = .034$) and positively correlated with context-appropriate fear ($r[109] = .351$, $p = .029$) at age 3.

ERN-RSA coupling

The bivariate relation between ERN and RSA at age 3 was significant at both electrode Cz ($r[109] = -.241$, $p = .028$) and Fz ($r[109] = -.223$, $p = .050$), whereby smaller ERN was associated with more RSA suppression *or* larger ERN was associated with less RSA suppression (hereafter "negative coupling"). We then tested whether coupling between cortical and parasympathetic activity exists when predicting ERN from RSA, controlling for child age and sex, using multiple linear regression. Results indicated no significant relation between ERN and RSA at either electrode Cz ($B = -4.257$, $SE[B] = 2.213$, 95% *CI* $[-8.595, 0.081]$, $p = .054$) or Fz ($B = -3.156$, $SE[B] = 1.732$, 95% *CI* $[-6.550, 0.238]$, $p = .068$).

ERN-RSA coupling and anxiety risk

Context-appropriate fear

Context-appropriate fear at age 3 moderated the relation between RSA and ERN at Cz ($B = 16.010$, $p = .013$) but not Fz ($B = 1.976$, $p = .710$) (Table 3; Model 1, 2). Probing this effect at high (+1 *SD*) and low (−1 *SD*; Aiken & West, 1991) levels of context-appropriate fear revealed no significant coupling at high levels of context-appropriate fear ($B = 5.249$, $p = .247$). However, at low levels of context-appropriate fear, preschoolers showed more negative coupling (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression; $B = -8.803$, $p = .001$) (Fig. 1).

Context-inappropriate fear

Context-inappropriate fear at age 3 moderated the relation between RSA and ERN at Fz ($B = -5.815$, $p = .031$) but not Cz ($B = -3.222$, $p = .373$) (Table 3; Model 3, 4). Probing the interaction in the same manner as previously noted revealed that,

Table 3. Age 3 anxiety risk as a moderator of ERN – RSA coupling

		95% CI for *B*		
Variable	*B*	*LL*	*UL*	SE(*B*)
Model 1: Cz				
Constant	19.042			27.745
Child sex	1.305	− 3.399	6.008	2.400
Child age	− 5.302	− 20.344	9.740	7.675
RSA	− 1.777	− 6.551	2.997	2.436
CA fear	− 1.615	− 7.235	4.005	2.867
RSA × CA fear	16.010*	3.412	28.608	6.428*
Model 2: Fz				
Constant	29.556			22.344
Child sex	.022	− 3.754	3.798	1.927
Child age	− 8.142	− 20.254	3.969	6.179
RSA	− 3.107	− 7.057	.843	2.015
CA fear	− 1.569	− 6.142	3.003	2.333
RSA × CA fear	1.976	− 8.450	12.403	5.320
Model 3: Cz				
Constant	− 4.769			28.979
Child sex	2.325	− 2.605	7.256	2.516
Child age	1.008	− 14.754	16.770	8.042
RSA	− 4.632*	− 9.003	− .261	2.230*
CI fear	.658	− 2.208	3.524	1.462
RSA × CI fear	− 3.222	− 10.318	3.873	3.620
Model 4: Fz				
Constant	17.831			21.346
Child sex	.473	− 3.165	4.110	1.856
Child age	− 4.968	− 16.578	6.642	5.923
RSA	− 3.858*	− 7.148	− 0.569	1.678*
CI fear	.687	− 1.392	2.765	1.061
RSA × CI fear	− 5.815*	− 11.087	− .542	2.690*

Note. CI = confidence interval; LL = lower limit; UL = upper limit. Dependent Variable: Error–Related Negativity (ERN). *$p < .05$, **$p < .01$, ***$p < .001$.

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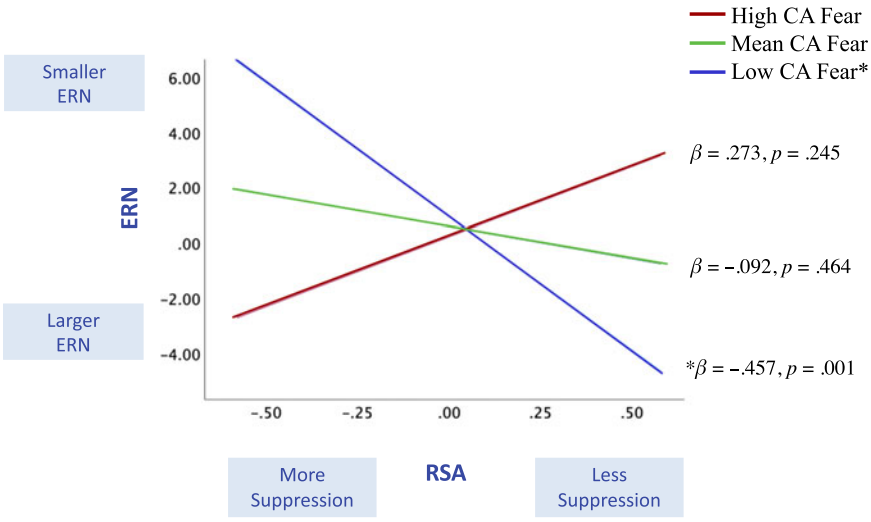

Figure 1. Age 3 context-inappropriate fear as a moderator of ERN-RSA coupling.

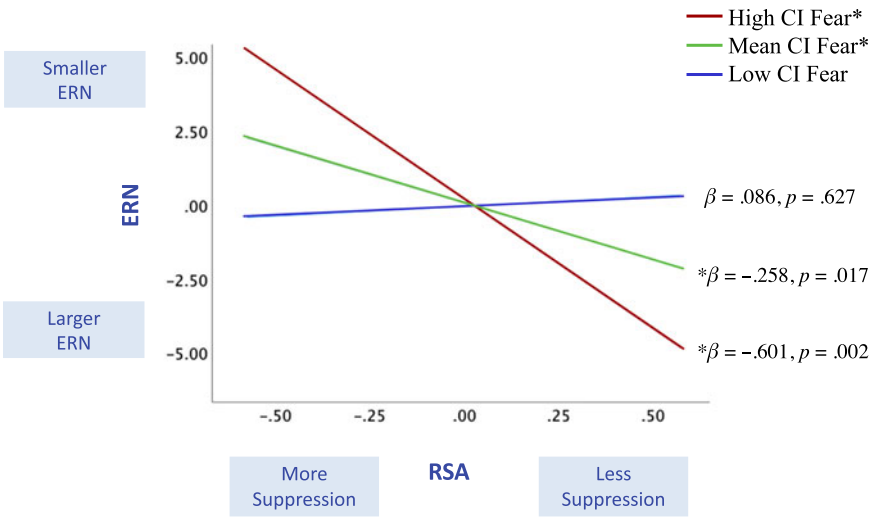

Figure 2. Age 3 context-appropriate fear as a moderator of ERN-RSA coupling.

when context-inappropriate fear was high, coupling was more negative (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression; $B = -9.004$, $p = .004$). There was no relation between RSA and ERN at low levels of context-inappropriate fear ($B = 1.288$, $p = .627$) (Fig. 2).

GAD symptoms

Anxiety symptoms at age 5 significantly moderated the association between RSA predicting ERN at Fz ($B = -7.710$, $p = .002$) but not Cz ($B = -5.459$, $p = .139$) (Table 4; Model 1, 2). When age 5 GAD symptoms were high, preschoolers showed more negative coupling (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression; $B = -11.290$, $p < .001$) at age 3. However, when GAD symptoms were low, preschoolers showed more positive coupling (i.e., smaller ERN, less RSA suppression *or* larger ERN, more RSA suppression; $B = 14.516$, $p = .014$) (Fig. 3).

Neuroendocrine reactivity

Neuroendocrine reactivity at age 5 significantly moderated the association between RSA and ERN at Fz ($B = 1.357$, $p < .001$) and Cz ($B = 1.811$, $p < .001$) (Table 4; Model 3, 4). When cortisol reactivity was high, preschoolers showed positive coupling (i.e., smaller ERN, less RSA suppression *or* larger ERN, more RSA suppression) at both Fz ($B = 11.855$, $p = .003$) and Cz ($B = 16.744$, $p = .010$). When cortisol reactivity was low, preschoolers showed more negative coupling (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression) at both Fz ($B = -22.171$, $p < .001$) and Cz ($B = -28.643$, $p < .001$) (Fig. 4).

Discussion

Overall, there was substantial ERN-RSA coupling in the preschool years, particularly when we accounted for children's levels of anxiety risk and neuroendocrine reactivity. Specifically, early patterns of negative coupling were discernible in association with concurrent atypical fear behaviors and poorer longitudinal outcomes. In contrast, positive coupling was found in association with better longitudinal outcomes. To our knowledge, this is the first study to identify relations between cortical and parasympathetic regulatory systems in early childhood and the first effort to interpret patterns of cortical-parasympathetic coupling using observed and longitudinal markers of anxiety risk across multiple levels of analysis.

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Table 4. Age 5 anxiety risk as a moderator of ERN – RSA coupling

Variable	*B*	95% CI for *B*		SE(*B*)
		LL	*UL*	
Model 1: Cz				
Constant	– 3.307			27.607
Child sex	2.339	– 2.599	7.278	2.520
Child age	.703	– 14.304	15.711	7.657
RSA	– 0.831	– 7.635	5.973	3.471
GAD symptoms	.507	– 1.732	2.746	1.142
RSA × GAD symptoms	– 5.459	– 12.685	1.767	3.687
Model 2: Fz				
Constant	19.025			20.055
Child sex	.402	– 3.184	3.989	1.830
Child age	– 5.135	– 16.045	5.776	5.567
RSA	1.613	– 3.206	6.432	2.459
GAD symptoms	.827	– .671	2.325	.764
RSA × GAD symptoms	– 7.710***	– 12.476	– 2.945	2.431***
Model 3: Cz				
Constant	62.166*			27.960*
Child sex	– 2.179	– 7.455	3.096	2.692
Child age	– 16.145*	– 31.063	– 1.228	7.611*
RSA	– 5.950*	– 10.629	– 1.270	2.388*
Neuroendocrine reactivity (AUCg)	.089	– .311	.489	.204
RSA × neuroendocrine reactivity (AUC_g)	1.811***	.971	2.650	.428***
Model 4: Fz				
Constant	77.686***			20.589***
Child sex	– 2.882	– 6.532	.768	1.862
Child age	– 20.471***	– 31.450	– 9.493	5.601***
RSA	– 5.158***	– 8.696	– 1.620	1.805***
Neuroendocrine reactivity (AUCg)	– .035	– .262	.192	.116
RSA × neuroendocrine reactivity (AUC_g)	1.357***	.793	1.922	.288***

Note. CI = confidence interval; LL = lower limit; UL = upper limit. Dependent Variable: Error–Related Negativity (ERN). *$p < .05$, **$p < .01$, ***$p < .001$.

Negative ERN-RSA coupling, which may reflect either smaller ERN and more RSA suppression *or* larger ERN and less RSA suppression/more augmentation, varied as a function of emotion behaviors in children that have previously been associated with increased risk for anxiety problems (i.e., greater context-inappropriate fear, greater anxiety symptoms, blunted cortisol reactivity). As such, negative coupling may index an underlying risk for dysregulation during the early preschool period. However, it should be noted that due to the correlational nature of analyses, we cannot conclude whether smaller ERN and more RSA suppression *and/or* larger ERN and less RSA suppression/more augmentation is linked to maladaptive outcomes within negative patterns of coupling. Instead, these results more broadly suggest that negative ERN-RSA coupling reflects systemic offsetting, as opposed to convergence (i.e., positive coupling), underlying maladaptation. In other words, children that employ significantly disproportionate cognitive relative to parasympathetic regulation in the presence of challenge (i.e., GNG) may broadly be at a higher risk for concurrent and longitudinal maladaptive behavior. Such a pattern may further indicate dysregulation within one neurophysiological system impacts the functional development of other systems of self-regulation (Koss & Gunnar, 2018).

We found negative ERN-RSA coupling when atypical fear responses (i.e., context-inappropriate fear) were increased and typical fear responses (i.e., context-appropriate fear) were decreased at age 3. This pattern of results is consistent with the Neurovisceral Integration Model (Thayer & Lane, 2000, 2009), which proposes that flexible behavioral expression across contexts is related to biological flexibility within the central and autonomic network. Specifically, our results support the notion that ERN-RSA coupling may reflect a functional pathway associated with appropriate, or adaptive, emotion and behavioral expression across specific contexts. Dysregulation among these systems, indexed here by negative patterns of activation, may be more related to maladaptive threat response. Heterogeneity in the respective relations between ERN and RSA and anxiety risk has been observed and frequently attributed to variations in the social context of tasks in which they are evaluated (Brooker et al., 2019; Cho & Buss, 2017; Dollar et al., 2020). The current study overlays these independent associations by identifying opposing patterns of putative dysregulation across codeveloping systems in relation to dysregulated fear expression across contexts.

Variation in ERN-RSA coupling at age 3 was observed at differing levels of children's anxiety symptoms at age 5. Specifically, negative ERN-RSA coupling (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression) was observed when GAD symptoms were high, while positive ERN-RSA coupling (i.e., smaller ERN, less RSA suppression *or* larger ERN, more RSA suppression) was observed when GAD symptoms were low. Although moderation of age 3 coupling by age 5 symptoms does not follow the typical pattern of moderation in longitudinal designs, it allowed us to directly ask whether patterns of coupling differed at age 3 in ways that were relevant for symptom levels 2 years later, making this line of inquiry critical for understanding the importance of the current work for child outcomes. That is, if patterns of coupling differed based on levels of age 3 fear but not age 5 symptoms, it may suggest that otherwise maladaptive patterns of early function self-correct over time. In contrast, different patterns of coupling at age 3 for high and low levels of age 5 symptoms suggest that patterns of dysregulation persist and have implications for the development of anxiety risk over time. Moreover, it suggests that individual differences in patterns of coupling at age 3 have predictive value over time. As such, these results further underscore an apparent functional significance of ERN-RSA coupling in adaptive emotional and behavioral expression in preschoolers, and the potential maladaptive effect of negative patterns of ERN-RSA regulation in early childhood.

Evidence in children suggests error may reflect a specific type of cognitive challenge, or even threat, related to cascading physiological reactivity consistent with defensive responding (Brooker & Buss, 2014; Meyer, 2017). As interpreted previously, negative ERN-RSA coupling likely reflects disproportionate cognitive and parasympathetic involvement during challenge, which may elicit neurophysiological responses similar to threat. In terms of task behavior, a negative pattern of coupling may reflect decreased behavioral monitoring coupled with increased parasympathetic

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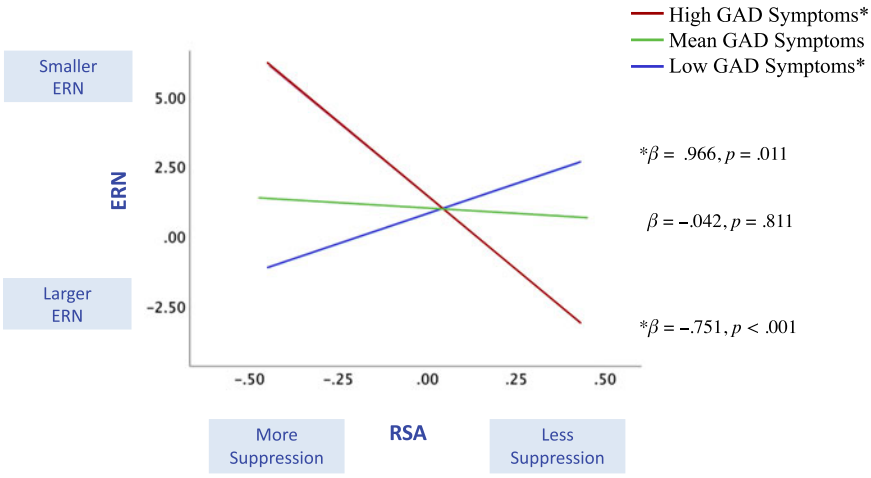

Figure 3. Age 5 GAD symptoms as a moderator of age 3 ERN-RSA coupling.

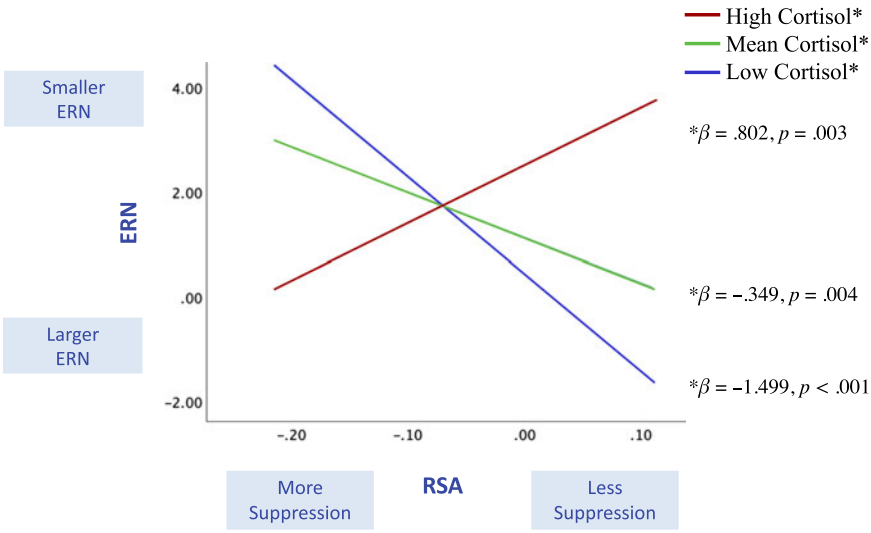

Figure 4. Age 5 cortisol as a moderator of age 3 ERN-RSA coupling.

response to challenge (smaller ERN, more RSA suppression) and/or increased behavioral monitoring coupled with decreased parasympathetic responsiveness to perceived challenge (larger ERN, less RSA suppression), both of which suggest a dysregulated threat response (e.g., over-generalized, over-regulated) which may increase risk for anxiety related disorder.

In contrast to negative coupling, positive ERN-RSA coupling, or relatively synchronous cognitive and parasympathetic regulation, may represent adaptive functioning at 3 years of age and may serve as a protective mechanism against the development of generalized anxiety symptoms by early childhood. For example, positive coupling may reflect decreased behavioral monitoring coupled with decreased parasympathetic response to perceived threat (smaller ERN, less RSA suppression) and/or increased behavioral monitoring and increased parasympathetic response to perceived threat (larger ERN, more RSA suppression), both of which suggest a relatively regulated threat response.

Finally, we found that negative ERN-RSA coupling (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression) was also longitudinally associated with blunted cortisol output. Blunted cortisol levels often cooccur with hyper-responsivity in other regulatory systems (Gunnar & Vazquez, 2001), so this result may further support the conjecture that negative ERN-RSA coupling reflects a dysregulated response that perpetuates regulatory difficulties over time. As age 5 begins a transition into middle childhood, when cortical and hormonal systems may be recalibrated to facilitate more flexible self-regulation (Hostinar & Gunnar, 2013), this result at this age may be developmentally meaningful. Specifically, the downregulation of cortisol output may reflect a biological "effort" to correct underlying psychophysiological dysregulation (Badanes et al., 2011; Brooker et al., 2013; Doom et al., 2018; Van Hulle et al., 2017). This interpretation is supported by the observation that positive ERN-RSA coupling (i.e., smaller ERN, less RSA suppression *or* larger ERN, more RSA suppression) was observed when cortisol output across the lab visit was increased, an adaptive and expected response in children (Gunnar et al., 2009; Gunnar, 1992). Of course, subsequent longitudinal studies across the critical transition to middle childhood will be needed to further elucidate the true cascade of developmental events impacting these codeveloping systems.

Taken together, these results provide novel corroboration of the assumptions outlined by the Neurovisceral Integration Model and Polyvagal Theory (Porges, 2003, 2009; Thayer & Lane, 2000, 2009). Specifically, ERN-RSA coupling may reflect a functional pathway which varies by concurrent and longitudinal emotion behavior. The use of discrete contexts allowed us to consider the adaptive or

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maladaptive nature of different patterns of coupling during the preschool period. Negative ERN-RSA coupling appears to be most closely linked to dysregulated threat reactivity. Our interpretation related to dysregulation stems from the specificity of negative coupling to dysregulated levels of fear across high and low-threat contexts. Because high levels of fear in a high threat context may be interpreted as normative, and even adaptive, a low fear response is most atypical in this setting. In contrast, high levels of fear in a low-threat context reflect a mismatch between the incentive properties of the environment and a child's responses. Critically, when one uses context in this way to interpret findings, there is consistency in the link between negative coupling and both concurrent and longitudinal propensities for dysregulation. However, if one were to consider only high vs. low levels of fear, the results would appear inconsistent across measures, underscoring the critical utility of context for developmental work.

Notably, our findings may be specific to the preschool years. A recent study reported null results when exploring functional coupling between prefrontal activation and cardiac regulation in infants aged 4–6 months (Nguyen et al., 2022), which may further underscore the importance of early childhood, including both toddlerhood and the preschool period, as a critical period for the initial organization and coordination of multi-systemic self-regulation underlying the development of adaptive, protective behavior (Calkins & Keane, 2004; Hostinar & Gunnar, 2013; Koenig, 2020; Kopp, 1982). Additionally, our findings differed somewhat by electrode location, which may represent a methodological limitation considering the poor spatial resolution of EEG. However, this pattern of results may also reflect the meaningfulness of this period for childhood brain development and the neural mechanisms that link ERN with RSA (i.e., ACC, PFC). In the current study, negative ERN-RSA coupling using frontal electrode sites was moderated by increased atypical fear at age 3, increased longitudinal GAD symptoms at age 5, and blunted neuroendocrine reactivity at age 5. In contrast, negative ERN-RSA coupling using central electrode sites was only moderated by decreased typical fear at age 3. Considering previous studies suggesting brain development occurs from posterior to anterior regions, and the Stress Acceleration model suggesting chronic dysregulation or stress activation may promote early frontal brain maturation (Callaghan & Tottenham, 2016), it is possible that frontal relative to central activity at age 3, indexed by ERN, may be relatively more important for delineating anxiety risk by age 5 in preschoolers and may to some extent index the structural integrity of ACC-mediated pathways modulating cortical influence on cardiac control.

Relative to older children, the current sample exhibited lower rates of anxiety symptoms and diagnoses. However, despite the low-risk nature of the sample, rates of anxiety symptoms and diagnoses are in line with prevalence in other normative preschool samples (Solmi et al., 2022; Whalen et al., 2017). Moreover, findings focused on clinical symptoms conceptually replicate the findings that are focused on variation in fear behaviors, which show considerable variation at this age. Such a pattern underscores the link between the two types of behaviors and strengthens the rationale for interpreting normative and potentially aberrant patterns of coupling. As such, our pattern of results across measures may reflect a relatively normative developmental process of linking physiological coupling to emerging risk for chronic anxiety in childhood.

While this study had several strengths including the use of a multi-method, longitudinal design and within subjects analyses, limitations may help to guide future research. For example, increasing the sample size and using more spatially sensitive neuroimaging measures to index brain maturation (i.e., ACC, PFC) may assist in characterizing the developmental trajectory of neural results and structures involved, as specificity of results across electrode locations and any functional implications (i.e., more frontal relative to central activity) represents a notable limitation. Additionally, adopting a statistical approach which elucidates within-person and directional relations among the current study variables would better inform understanding of developmental antecedents of childhood anxiety symptoms. For example, using methods such as cross-recurrence quantification analysis may be more effective in characterizing and quantifying potential nonlinear interrelationships between a pair of nonlinear time series such as RSA suppression (seconds) and ERN (milliseconds) to determine temporal order of activation and development. Finally, future research should assess generalizability of the current results across more diverse and clinical samples to better characterize child emotion behavior and anxiety risk.

In conclusion, the current study assessed coupling across cortical (ERN) and parasympathetic (RSA) regulatory systems during cognitive challenge in early childhood, then used observations of anxiety risk across multiple levels of analysis to characterize whether coupling between regulatory systems may denote risk for maladaptation across the preschool period. Negative ERN-RSA coupling (i.e., smaller ERN, more RSA suppression *or* larger ERN, less RSA suppression) was observed during cognitive challenge at age 3 when context-inappropriate fear was increased and context-appropriate fear was decreased at age 3, and when emerging anxiety symptoms were increased and cortisol output was decreased by age 5. In contrast, positive ERN-RSA coupling (i.e., smaller ERN, less RSA suppression *or* larger ERN, more RSA suppression) was observed at age 3 when anxiety symptoms were decreased and cortisol output was increased by age 5. Therefore, negative ERN-RSA coupling may reflect maladaptive threat response underlying and perpetuating behavioral dysregulation across development and may represent an early, clinically-relevant treatment mechanism informing prevention and intervention strategies for childhood anxiety and related disorders.

Data availability statement. The data and syntax that support the results reported in this manuscript are available through the Open Science Framework: https://osf.io/8y5zs/?view_only=2989caef2d04408f941f703754e06d86

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Competing interests. None.

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