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Toddler parasympathetic regulation and fear: Links to maternal appraisal and behavior

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Abstract

There is a growing recognition that parental socialization influences interact with young children's emerging capacity for physiological regulation and shape children's developmental trajectories. Nevertheless, the transactional processes linking parental socialization and physiological regulatory processes remain not well understood, particularly for fear-prone toddlers. To address this gap in the literature, the present study investigated the biopsychosocial processes that underlie toddlers' fear regulation by examining the relations among toddler parasympathetic regulation, maternal appraisal, and parenting behaviors. Participants included 124 mothers and their toddlers ($M_{\text{age}} = 24.43$ months), who participated in a longitudinal study of temperament and socio-emotional development. Toddlers' parasympathetic reactivity was found to moderate the links between maternal anticipatory appraisal of child fearfulness and (a) maternal provision of physical comfort and (b) preschool-age child inhibition. Additionally, maternal comforting behaviors during the low-threat task predicted preschool-age separation distress, specifically for toddlers demonstrating a low baseline RSA.

Keywords

respiratory sinus arrhythmia; parental socialization; fear regulation

1 | INTRODUCTION

During the first 3 years of life, caregivers witness a marked change in children's ability to engage in self-regulatory behaviors (Fox, 1989; Kopp, 1982; Rothbart, Ziaie, & O'Boyle, 1992). The developments that occur in children's capacity for self-regulation between infancy and the preschool period demand the caregivers to adapt to children's growing needs for autonomy while maintaining an appropriate degree of control and support (Teti & Huang, 2005). The importance of the caregivers' ability to provide an optimal degree of regulatory support during early childhood has been particularly evident in research documenting the impact of parental regulatory support on the developmental trajectories of fear-prone children (Kiel & Buss, 2012; Mount, Crockenberg, Bárrig J6, & Wagar, 2010).

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CONFLICTS OF INTEREST

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Although research has highlighted the developmental significance of parental adaptation to toddlers' emerging capacity for behavioral (Kiel & Buss, 2010) and physiological regulation of fear (Hastings, Kahle, & Nuselovici, 2014), relatively little is understood about how parents appraise toddlers' need for regulatory support in novel situations. In particular, the potential contribution of toddlers' physiological capacity to caregiver appraisal and behaviors has not been extensively investigated.

To address the above gap in the literature, the present study took a biopsychosocial approach to explore the relations among maternal appraisal, parental regulatory support, and toddlers' parasympathetic regulation, as indexed by respiratory sinus arrhythmia (RSA). RSA has received attention as an index of parasympathetic regulation of emotion (Beauchaine, Gatzke-Kopp, & Mead, 2007; Porges, 2011), for children who are fear-prone (Talge, Donzella, & Gunnar, 2008) or clinically anxious (Kossowsky, Wilhelm, Roth, & Schneider, 2012; Schmitz, Krämer, Tuschen-Caffier, Heinrichs, & Blechert, 2011). Although prior research has documented the links between fearful children's parasympathetic regulation and parenting (e.g., Hastings, Sullivan, et al., 2008; Kennedy, Rubin, Hastings, & Maisel, 2004), much remains to be understood about the nature of the relationships among fear-prone toddlers' parasympathetic regulation and distinct aspects of parental socialization. As such, the present study aimed to replicate and extend previous research by examining toddlers' parasympathetic regulation, maternal appraisal of toddlers' fear, and their joint relations with maternal parenting behavior and children's fear behaviors.

1.1 | Transactional processes linking parental appraisal and behaviors

Parenting behaviors characterized by extreme levels of warmth and control (e.g., overprotection, oversolicitousness, intrusiveness) have been identified as factors that maintain children's fearful and anxious traits (Rapee, 1997, 2002; Rubin & Mills, 1991). Given the potential etiological role of parenting behaviors, there has been a growing interest in identifying the determinants of specific "anxiety-enhancing" parenting behaviors (Ginsburg & Schlossberg, 2002). Although available evidence indicates that such anxiety-enhancing parenting behaviors are multiply determined by various factors (e.g., parental anxiety, child fearfulness/anxiousness), the importance of parental appraisal of children's capacity for coping has been consistently documented in the extant literature. More specifically, parental appraisal of children's fearfulness (Kiel & Buss, 2010), shyness (Rubin, Nelson, Hastings, & Asendorpf, 1999), and anxiety (Creswell, Apetroaia, Murray, & Cooper, 2013; Hudson & Rapee, 2005) have been shown to predict specific parenting behaviors that reinforce fearful and dependent behaviors in children. These findings lend support to theories which suggest that parental perception of children's regulatory capacity motivates behaviors (e.g., comforting, protectiveness) aimed at minimizing children's experience of distress (Rapee, 1997). Inadvertently, such behaviors lead children to overestimate the threat present in the environment and to rely on parental support for coping with challenges. Parental overprotective behaviors may thus limit children's opportunities to acquire independent self-regulatory skills (Rapee, 1997; Thompson, 2001). Indeed, parental overprotectiveness (Kiel & Buss, 2011; Mount et al., 2010; Spokas & Heimberg, 2009) and intrusiveness (e.g., infantilizing, offering unnecessary support) have been frequently linked

to children's anxiety-spectrum behaviors, including symptoms of separation anxiety (Wood, 2006).

Although parental appraisal of children's vulnerability may directly elicit specific parenting behaviors, parental appraisal does not invariably lead to anxiety-enhancing parenting behaviors. More specifically, emerging evidence suggests that contextual influences (e.g., degree or type of stressor) interact with the characteristics of the children (e.g., behavioral regulation of fear) or those of the parents (e.g., parental anxiety) to evoke anxiety-enhancing parenting behaviors (Kiel & Buss, 2012; Murray et al., 2012). Therefore, findings underscore the need to examine the specific conditions under which parental appraisal may directly predict anxiety-enhancing parenting behaviors. Recent developments in biopsychosocial models of anxious behaviors suggest that parental socialization influences interact with children's emerging capacity for physiological regulation to influence the developmental trajectories of fear-prone children (Hastings et al., 2014; Hastings, Sullivan, et al., 2008). In particular, children's parasympathetic regulation has received attention as a physiological index of parasympathetic regulation that may operate jointly with social regulatory processes to influence children's regulation of fear.

1.2 | RSA as a physiological index of regulatory competence

Temperamental differences in fearfulness and inhibition have been attributed to individual variation in arousal threshold and to children's capacity to regulate behavioral and physiological responses to novel situations (Kagan, 1997). At the behavioral level, regulatory competence in the context of potential threat is demonstrated by one's flexible use of strategies that promote situationally appropriate engagement or self-soothing behaviors. In contrast, dysregulated fear responses to potential threat have been characterized by extreme behavioral response to fear (e.g., freezing) (Buss, Davidson, Kalin, & Goldsmith, 2004) or excessive dependence on caregivers' regulatory support (Kiel & Buss, 2011), particularly in low-threat contexts that do not warrant heightened fear responses (Buss, 2011).

At the physiological level, children's parasympathetic regulation of cardiac arousal has received growing interest as a physiological index of regulatory capacity (Beauchaine et al., 2007). Specifically, RSA, a measure of the heart rate variability that occurs at the frequency of respiration, is commonly used as a marker of parasympathetic influences on the heart rate variability that is mediated by the 10th cranial (vagus) nerve. In the presence of environmental challenge, decreases in parasympathetic influence—indexed by greater RSA suppression response—are thought to underlie physiological states (i.e., increased heart rate and metabolic output) that facilitate mobilization of physiological resources, task engagement, and active coping (Beauchaine et al., 2007; Porges, 2007). Indeed, there is evidence to suggest that RSA suppression response is associated with behavioral indicators of adaptive regulatory response, including longer attention span and soothability (Huffman et al., 1998), effortful control (Sulik, Eisenberg, Spinrad, & Silva, 2015), less negativity, fewer behavioral problems, and better social skills (Calkins & Keane, 2004). In contrast, less RSA suppression response to environmental challenge has been related to a diminished behavioral regulatory capacity. Specifically, direct links were documented between less RSA

suppression response to challenge and fearfulness (Liew et al., 2011), anxious solitude (Gazelle & Druhen, 2009), social anxiety disorder (Schmitz et al., 2011), and selective mutism (Heilman et al., 2012). Nevertheless, RSA suppression response has not been consistently found to relate to fear, inhibition (e.g., Marshall & Stevenson-Hinde, 1998) or internalizing problems in the expected directions (see Graziano & Derefinko, 2013 for a recent review).

Whereas RSA reactivity is thought to reflect the ability to modulate one's internal states to respond flexibly to the changing environmental demands, resting RSA has been theorized to be an index of stress vulnerability that reflects the ability to maintain homeostasis in the absence of external challenge (Porges, 2011). Low baseline RSA has been linked to difficulties with regulation of negative affect (Stifter & Fox, 1990), reticence (Henderson, Marshall, Fox, & Rubin, 2004), and anxiety symptoms during adolescence (Greaves-Lord et al., 2010). However, findings have also been mixed about the associations between low baseline RSA and measures of fear (Schmidt, Fox, SchulkIn, & Gold, 1999; Stevenson-Hinde & Marshall, 1999; Talge et al., 2008), anxious behaviors (Monk et al., 2001), or internalizing problems (Hastings, Nuselovici, et al., 2008). Regarding the inconsistent findings linking RSA and children's fear-related behaviors, it has been suggested that the association between parasympathetic regulation and fear-related behaviors may be moderated by other factors, such as caregiver behaviors (e.g., Hastings et al., 2014; Hastings, Sullivan, et al., 2008). Taken together, available evidence indicates that low baseline RSA (Porges, 2011) and less RSA suppression response to challenge are indicative of children's poor physiological regulatory capacity (Calkins & Keane, 2004). Accumulating evidence further indicates that children's poor parasympathetic regulation increases their susceptibility to the anxiety-enhancing effects of parental behaviors such as overprotectiveness (Hastings et al., 2014; Hastings, Sullivan, et al., 2008). As such, in the context of poor physiological regulation and specific socialization experiences, fear-prone children may be at a greater risk for fear dysregulation and anxiety-related outcomes.

1.3 | Interplay of parental socialization processes and physiological regulation

Beginning in early infancy, various aspects of parental behaviors have been shown to interact with children's physiological response to stress (Moore et al., 2009). In particular, maternal physical touch has been identified as a factor that directly influences children's response to and recovery from stress (Feldman, Singer, & Zagoory, 2010). Maternal physical contact provided under a stressful context (i.e., still-face paradigm) has been linked to less RSA suppression, fewer behavioral displays of stress, and greater positive affect in infants (Feldman et al., 2010). For preschool-aged children, parental behaviors that are generally construed as positive (e.g., supportiveness) or risk-enhancing (e.g., protective overcontrol) were related to children's internalizing problems in expected directions, only for children demonstrating a physiological vulnerability in the form of less RSA suppression (Hastings, Sullivan, et al., 2008). More specifically, fathers' supportive parenting was inversely related to internalizing problems, only for children showing less RSA suppression during a difficult puzzle task. Similarly, paternal protective overcontrol was positively related to internalizing problems for children showing a less RSA suppression response. Consistent with these findings, a longitudinal study by Hastings and colleagues (2014) provided evidence that

maternal overprotective parenting predicts anxiety problems only for socially wary children showing poor parasympathetic control (i.e., low baseline RSA).

Although the findings reviewed above highlight the interaction between parasympathetic regulation and parenting behaviors, some evidence suggests that children's physiological regulation directly predicts parental behaviors. For instance, baseline RSA assessed early in infancy predicted maternal postpartum behaviors for infants who were preterm. Specifically, infants' low baseline RSA predicted fewer engagement behaviors such as maternal display of positive affect or affectionate touch (Feldman & Eidelman, 2007). Similarly, a longitudinal study of children's baseline RSA and parenting by Kennedy and colleagues (2004) reported that maternal parenting practices are directly predicted by toddlers' baseline RSA. Whereas baseline RSA at age two positively predicted maternal supportive parenting two years later, lower levels of baseline RSA at age 2 predicted higher levels of maternal restrictive parenting practices at age 4. (Kennedy et al., 2004). It is possible that the predictive relation between toddler baseline RSA and subsequent parenting reflect mothers' adaptation to their own appraisal of children's fearfulness or emotional dysregulation. To better understand the nature of relation between toddlers' RSA and maternal appraisal of toddler fear-regulation, the present study examined the interrelations among toddler RSA, maternal appraisal of children's fear-related behaviors, and maternal comforting behaviors.

1.4 | The present study

Previous research has demonstrated both direct and interactive relations among children's parasympathetic regulation and parenting behaviors that increase children's risk for anxiety (Kennedy et al., 2004; Hastings et al., 2014). The present study aimed to extend the extant literature by examining the transactional relationship between toddlers' capacity for physiological regulation and caregiver behaviors that enhance toddlers' risk for anxiety-related problems. To this end, two questions were addressed: First, building upon prior work suggesting that toddlers' behavioral fear moderates the link between mothers' anticipatory appraisal of toddler fear and parenting behaviors (Kiel & Buss, 2012), the present study examined whether and how toddlers' parasympathetic regulation may also function as a moderator of the association between maternal appraisal and parenting behaviors. Specifically, it was hypothesized that maternal appraisal of toddlers' fear *prior to* the Puppet Show episode would predict mothers' comforting behaviors *during* the actual task, particularly for toddlers showing less RSA suppression *during* the task.

Second, toddlers' parasympathetic regulation and maternal care-giving influences were examined as joint predictors of preschool-age fear related outcomes. In examining maternal appraisal of toddler fear behaviors, two dimensions of maternal appraisal were used. The two dimensions included maternal anticipation of toddlers' behavioral expressions of fear and wariness ("*fear anticipation*") and toddlers' dependent and clingy behaviors ("*dependency anticipation*"). Additionally, the present study focused on mothers' comforting behavior—characterized by physical soothing and affectionate behaviors—in light of research documenting its direct link to children's parasympathetic regulation (Feldman et al., 2010). In particular, affectionate physical comfort offered in situations that do not warrant such parental behaviors has also been characterized as a component of parental

overprotectiveness (Kiel & Buss, 2011; Rubin, Burgess, & Hastings, 2002), a construct which has been shown to increase fear-prone children's risk for anxiety-related outcomes (Hastings et al., 2014). The relationships between toddlers' parasympathetic regulation and maternal socialization variables were examined in the context of an interactive puppet show task. This task was specifically chosen based on previous research which established the Puppet Show task as a "low-threat" laboratory episode (Buss, 2011; Kiel & Buss, 2012). It was hypothesized maternal comforting behaviors offered during the Puppet Show would predict preschool-age fear behaviors, only for toddlers who demonstrate poor parasympathetic regulation. More specifically, a positive relation between maternal comforting and preschool-age inhibition/separation distress was expected for toddlers showing low baseline RSA and/or less RSA suppression during the Puppet Show.

2 | METHOD

2.1 | Participants

The present study examined a sample of 124 mothers and toddlers (62 female) who participated in a larger study of temperament and socio-emotional development. Participants were identified through the birth records published in a semi-rural northeastern city and its surrounding communities. Recruitment letters were mailed to eligible families with toddlers between the ages of 18 and 20 months. Families that provided consent to participate in the study were asked to complete initial screening questionnaires designed to obtain an over-sample of fearful toddlers. The Infant Toddler Social Emotional Assessment (ITSEA; Carter, Briggs-Gowan, Jones, & Little, 2003) and a brief questionnaire about children's fear responses to novel situations were used for screening. An initial sample ($n = 125$) included a subgroup of fearful toddlers ($n = 63$) scoring at least one standard deviation above the mean on parent rating of fearfulness and wariness. The remainder of the sample ($n = 62$) was unselected for other temperamental traits. Family income assessed during age 2 assessment ranged from \$15,000 or less to more than \$60,000, with a majority of the families identified as middle class ($M = 49.72$, $SD = 10.70$ on the Hollingshead index). Approximately 50% of families reported income above \$60,000. The sample was primarily Caucasian ($n = 113$; 90.4% of the sample), followed by Asian American ($n = 8$; 6.4%), American Indian ($n = 2$; 1.6%), African American ($n = 1$; 0.8%) and Hispanic ($n = 1$; 0.8%).

2.2 | Procedures

This study examined behavioral, cardiovascular, and questionnaire data that were obtained during the age 2 and three assessments. During the age 2 laboratory visit ($M_{\text{age}} = 24.43$, $SD = 0.47$), toddlers and their mothers participated in a series of standard and modified laboratory episodes designed to assess children's inhibition, fear, or distress. These included the baseline episode and the toddler version of the Laboratory Temperament Assessment Battery (Lab-TAB; Buss & Goldsmith, 2000). Mothers remained in the same room with the children throughout the visit and were provided with standardized instructions about their involvement in different tasks. Following the laboratory visit, a packet of questionnaires including assessments of socio-emotional functioning was mailed when children were approximately 3 years of age.

2.2.1 | Age 2 laboratory visit—The age 2 laboratory visit began with a 5 min baseline task, during which toddlers remained seated in a small table and engaged in quiet, sedentary activities (e.g., coloring, reading a book) with an experimenter. Collection of toddlers' cardiovascular data began during this baseline assessment. While children engaged in the baseline tasks, mothers were provided with brief descriptions of the upcoming episodes and were asked to answer interview questions ("Pre-episode interview") regarding their toddlers' expected reaction to each episode. The present study focused on the Puppet Show (PS) episode, which was designed as an interactive episode that may elicit a range of responses including pleasure, approach wariness, and object fear. The PS was the first Lab-TAB episode administered during the age 2 visit and it was preceded by a short break that lasted a few minutes in duration. As soon as mothers and their toddlers sat on a chair located across the room from the puppet stand, a female experimenter hiding behind the stand began the show by asking the toddler to introduce oneself to the two puppets. Next, the puppets invited the toddlers to participate in games (playing catch, fishing) that required the toddlers to approach the puppet stand and to comply with specific requests (e.g., picking up a ball, taking turns playing a fishing game, grabbing a sticker). Of the six episodes that originated from the Lab-TAB, the PS has been shown to elicit comparatively fewer expressions of fear and greater behavioral engagement relative to other episodes (e.g., Spider, Robot); hence, it was designated as a "low-threat" task in previous research (Buss, 2011). Toddlers who demonstrate heightened fear response during this task were found to be at an elevated risk for developing anxiety symptoms (Buss, 2011; Buss et al., 2013).

2.3 | Measures

2.3.1 | Maternal anticipation of toddler fear and dependency (age 2)—Prior to the laboratory episodes, maternal anticipation of toddlers' fear and dependency was assessed using a version of the pre-episode interview (Goldsmith & Rothbart, 1991) that was adapted to incorporate the specific episodes used in this study. Mothers were provided with brief descriptions of the upcoming laboratory tasks and were asked to provide a rating of the likelihood that their toddler might engage in a number of specific behaviors using the following 4-point rating scale: 1 = *definitely yes*, 2 = *probably yes*, 3 = *probably no*, and 4 = *definitely no*. Responses were reverse coded to maintain consistency with the response format of other study variables. For the present study, mothers' anticipatory rating of (a) toddlers' wary/fearful response to puppets (i.e., fear) and (b) toddlers' desire to be held by or to stay near the mother (i.e., dependency) were examined.

2.3.2 | Comforting behaviors (age 2)—Mothers' use of comforting behaviors during the PS was examined as a behavior that may have a proximal influence on toddlers' physiological regulation during a novel context (Feldman et al., 2010) and as a component of parental protectiveness that has implications for children's risk for anxiety (Kiel & Buss, 2010; Rubin et al., 2002). A behavioral coding system that was designed to capture varying levels of maternal comforting behaviors was used (Kiel & Buss, 2010). Defined as affectionate and soothing physical contact with the child, comforting behaviors were coded on a 0–3 scale for every 10 s epoch of the PS. The coding ranged from: 0 = *absence of physical comforting behaviors* to 3 = *hugging or embracing*. Three trained coders achieved a

minimum kappa of 0.7 and interrater reliability of 0.9 with the master coder. All cases were double-coded.

2.3.3 | Respiratory sinus arrhythmia (age 2)—Cardiac activity was recorded during the 5 min baseline and the PS episode using the Mindware Wi-Fi ACQ software, Version 1.0 (Mindware Technologies, LTD, Westerville, OH). Offline analyses of the obtained data were performed using the Mindware Heart Rate Variability version 2.51, with ECG signal sampled at a rate of 500 ms and band-pass filtered at 40 and 250 Hz. Interbeat intervals (IBI) and improbable intervals were identified given the overall distribution using the Mindware Heart Rate Variability editing software. Data were detrended using a first-order polynomial and were submitted to fast Fourier Transformation. RSA was operationalized as the natural log integral of the very high frequency 0.24 to 1.04 Hz power band and was calculated in 30 s epochs. Visual inspection and editing of artifacts in the ECG data were completed by three scorers who achieved good interrater reliability (agreement = 86%) on 25% of the files. RSA during the baseline task and the PS was computed by averaging across the 30 s epochs. Change in RSA from baseline to the PS was computed by creating residualized change scores to account for the resting RSA value obtained during the baseline.

2.3.4 | Maternal rating of inhibition and separation distress (age 3)—As part of the preschool-age assessment, maternal ratings of children's inhibition and separation distress were obtained using the Infant-Toddler Social and Emotional Assessment—Revised (ITSEA; Carter & Briggs-Gowan, 2000). The ITSEA was designed to assess 12–36 month old children's socio-emotional and behavioral problems and competence. The internalizing domain includes subscales that measure inhibited temperamental traits and distinct clusters of anxiety symptoms. Good psychometric properties were documented for the instrument (Carter et al., 2003). To examine preschool-age inhibition, the Inhibition to Novelty scale was used (five items, $\alpha = 0.77$; e.g., “Takes a while to feel comfortable in new places [10 min or more].”). The Separation Distress scale (six items, $\alpha = 0.68$; e.g., “Hangs on to you or wants to be in your lap when with other people.”) was used to assess children's separation related distress. All items were rated on a 3-point scale (0 = *not true/rarely*, 1 = *somewhat true/sometimes*, 2 = *very true/often*).

2.3.5 | Toddler behavioral fear (age 2)—Previous research has demonstrated that toddlers' fearful behaviors moderate the association between maternal prediction of toddler behaviors and maternal protective behaviors (Kiel & Buss, 2011). To account for the effect of toddlers' behavioral expression of fear on maternal behaviors, observer rating of toddlers' fearful behaviors was included as a covariate. Trained coders completed a second-by-second microcoding of the toddlers' latency to freeze, duration of facial fear, duration of bodily fear, and duration of freezing during the PS (Buss, 2011). The fear composite was created by averaging the four facial and behavioral fear measures listed above. Interrater reliability for trained undergraduate coders was 0.73 (k) for the coding of facial expressions of fear and 0.81 (k) for behavioral measures of fear.

2.4 | Analytical approach

2.4.1 | Missing data—Examination of the data indicated that there was no systematic pattern of missingness (Little's MCAR: $\chi^2 = 45.89$, $p = 0.83$). Independent *t*-tests also did not indicate that toddlers without valid RSA data ($n = 28$) or age 3 questionnaire data ($n = 30$) differed on any of the demographic or study variables. Multivariate imputation by chained equations was used to impute and pool the results of the analyses (mice; van Buuren & Groothuis-Oudshoorn, 2011).

2.4.2 | Statistical analyses—A series of hierarchical regression analyses were completed to examine whether RSA moderated the relationship between maternal anticipation and comforting behaviors. Next, the moderating effect of toddlers' RSA on the longitudinal association between maternal comforting behavior and maternal rating of preschool-age inhibition and separation distress was also examined. Finally, additional models that included toddlers' age 2 behavioral fear were also tested. All of the predictor variables were mean-centered prior to creating the interaction term (Aiken & West, 1991). To examine the unique contribution of toddlers' parasympathetic regulation, observer rating of toddler behavioral fear during the PS episode was entered as a covariate in all of the models that did not include behavioral fear as an outcome variable. Child gender was not entered as a covariate, as independent *t*-tests did not indicate that any of the study variables differed as a function of gender.

3 | RESULTS

3.1 | Descriptive statistics

Means, standard deviations, and correlations among the study variables are presented in Table 1. Baseline RSA was not found to be significantly associated with behavioral fear, maternal appraisal, comforting behaviors, or preschool-age behavioral ratings. Greater RSA suppression was significantly associated with less maternal comforting behaviors ($r = .23$, $p = .03$) and was marginally associated with less toddler fear ($r = .19$, $p = .08$). Maternal fear anticipation was significantly correlated with toddler behavioral fear ($r = .29$, $p = .001$) but was only marginally associated with maternal comforting ($r = .15$, $p = .09$) and age 3 inhibition to novelty ($r = .17$, $p = .07$). In contrast, maternal dependency was significantly correlated with behavioral ratings of maternal comforting ($r = .31$, $p < .001$), toddler behavioral fear ($r = .31$, $p < .001$), and with age 3 inhibition ($r = .40$, $p < .001$) and separation distress ($r = .28$, $p = .003$). Fear and dependency appraisals were not directly associated with measures of RSA.

3.2 | Hierarchical regressions

3.2.1 | Relation to maternal comforting (age 2)—Maternal dependency anticipation prior to the PS was related to mothers' subsequent engagement in comforting behaviors during the PS ($b = 0.22$, $p = .01$) (Table 2). However, toddler baseline RSA was not related to maternal comforting behaviors; it also did not moderate the relationship between maternal dependency anticipation and comforting behaviors. Similarly, only a main effect of dependency anticipation ($b = 0.19$, $p = .03$) was found in a model that tested its potential interaction with RSA suppression.

One significant interaction effect between maternal fear anticipation and toddler RSA suppression was found to be related to maternal comforting behaviors ($b = 0.36, p < .001$). This significant interaction was probed by testing the simple slopes at low (one standard deviation below mean) and high (one standard deviation above mean) levels of RSA suppression. As hypothesized, test of simple slopes indicated that the relationship between maternal fear anticipation and comforting parenting behaviors was significant only for those toddlers who showed less RSA suppression during the PS ($t = 3.77, p < .001$) (Figure 1). Therefore, mothers who anticipated greater toddler fear were more likely to engage in greater comforting behaviors, only with their toddlers showing less RSA suppression response during the PS. By contrast, at low levels of maternal fear anticipation, mothers were significantly less likely to use comforting behaviors with toddlers showing less RSA suppression response. Maternal fear anticipation was not related to maternal comforting behaviors at high levels of toddler RSA suppression. Finally, toddler baseline RSA did not relate to maternal comforting behaviors independently or jointly with maternal anticipatory appraisal of toddler fear or dependency.

3.2.2 | Relation to behavioral fear (age 2)—Baseline RSA and RSA suppression were not significantly related to toddlers' behavioral fear independently or interactively with maternal appraisal or comforting behaviors (Table 3).

3.2.3 | Prediction of inhibition to novelty (age 3)—There was a significant main effect of maternal dependency anticipation on preschool-age inhibition to novelty (Table 3). Significant main effects of baseline RSA and RSA suppression, however, were not found. Consistent with the study hypothesis, a significant interaction effect between maternal fear anticipation and RSA suppression was found to predict age 3 inhibition ratings ($b = 0.38, p = .01$). Probing the simple slopes ($\pm 1 SD$) indicated that there was a positive relation between age 2 maternal fear anticipation and preschool-age inhibition, only for toddlers who demonstrated less RSA suppression during the PS ($t = 4.12, p < .001$) (Figure 2). Specifically, mothers who anticipated a greater toddler fear response prior to the PS were more likely to rate their children as being inhibited a year later, if their toddlers had shown less RSA suppression response during the PS. Conversely, mothers who did not anticipate toddlers' fear response were significantly less likely to rate their children as inhibited a year later, in the context of less age 2 RSA suppression. There was no significant relationship between maternal fear anticipation and preschool-age inhibition for toddlers who showed greater RSA suppression during the Puppet Show. Finally, fear anticipation did not interact with toddler baseline RSA to predict preschool-age rating of inhibition.

3.2.4 | Prediction of separation distress (age 3)—Dependency anticipation was uniquely related to mothers' report of preschool-age separation distress, after observer rating of toddler behavioral fear was controlled (Table 3). In contrast, fear anticipation did not predict maternal rating of preschool-age separation distress independently or interactively with toddler RSA.

One significant interaction effect emerged for maternal comforting behaviors and toddlers' baseline RSA in relation to age 3 separation distress ($b = -0.33, p = .003$). Test of the simple slopes indicated that maternal comforting during the PS predicted greater preschool-age

separation distress, only for toddlers who exhibited low baseline RSA ($t = 3.18, p = .002$) (Figure 3). More specifically, for toddlers with lower baseline RSA ($-1\ SD$), high maternal comforting ($+1\ SD$) during the PS predicted greater maternal rating of preschool-age separation distress. Low maternal comforting behavior ($-1\ SD$) predicted lower levels of preschool-age separation distress ratings for toddlers with low baseline RSA. No relationship between maternal comforting behaviors and preschool-age separation distress was found for toddlers who showed a higher baseline RSA.

4 | DISCUSSION

The present study investigated the biopsychosocial processes that underlie toddlers' fear regulation by examining the relations among toddlers' physiological and behavioral regulation of fear, maternal appraisal of toddler fear, and parenting behaviors. Findings suggest that toddlers' physiological regulation and maternal appraisal operate both independently and jointly as they relate to maternal comforting behaviors and to preschool-age inhibition. Interestingly, maternal appraisal of toddler fearfulness was found to interact with toddlers' parasympathetic regulation in a content-specific manner; toddlers' RSA reactivity moderated the link between maternal appraisal and comforting behaviors, only when mothers anticipated toddlers' fearful/wary—but not dependent—behaviors in response to a low-threat laboratory task. Furthermore, the above interaction was observed above and beyond the contribution of behavioral indicators of toddler fear regulation. Of note, there was no evidence that parasympathetic regulation and maternal influences interactively relate to toddlers' concurrent behavioral fear. Rather, there was a marginally significant main effect of RSA reactivity on behavioral fear (i.e., less RSA suppression predicting greater fear), a finding that was consistent with a previous study indicating that temperamentally fearful preschoolers demonstrate a marginally lower RSA suppression response during a fear-eliciting task (Talge et al., 2008). Thus, taken together, findings indicate that toddlers' capacity for physiological regulation may at times moderate the relations among social and behavioral indicators of risk for anxiety-related outcomes.

4.1 | Toddler parasympathetic regulation, maternal appraisal, and comforting behaviors

Consistent with the study hypothesis, maternal fear anticipation *prior to* the Puppet Show predicted the extent of regulatory support they offered *during* the actual task, only in the context of toddlers' poor physiological regulation (i.e., less RSA suppression). This finding highlights a specific condition under which maternal anticipation of child fear may lead to mothers' actual engagement in comforting behaviors. Notably, the interaction effect between maternal fear anticipation and RSA reactivity was present, while controlling for toddlers' behavioral expressions of fear. Therefore, it may be possible that physiological regulation and maternal appraisal operate independently of the overt signs of behavioral fearfulness to influence maternal behavioral responses in mildly fear-eliciting contexts. It should also be noted, however, that the direction of influence linking toddlers' parasympathetic regulation and maternal comforting behaviors could not be directly tested in the present study. As such, future research designed to disentangle the direction of influence linking the above factors may inform whether indices of physiological regulation may elicit specific parental behaviors aimed at minimizing child fear and distress.

4.2 | Toddler parasympathetic regulation, maternal appraisal, and preschool-age inhibition

As was hypothesized, toddlers' RSA reactivity was also found to interact with maternal fear anticipation to predict mothers' rating of children's inhibition to novelty a year later. That is, mothers who anticipated toddlers' fear responses prior to the Puppet Show were more likely to rate their children as being inhibited a year later, if their children had demonstrated less RSA suppression during the Puppet Show. Again, this interaction effect was present while accounting for the behavioral indicators of fear, suggesting that mothers who accurately anticipate toddlers' specific fear behaviors (Kiel & Buss, 2010) might also be more attuned to subtle signs of toddlers' dysregulated physiological responses. One possible interpretation of this finding may be that maternal appraisal of toddlers' physiologically dysregulated response during a mildly fear-eliciting task serves to reinforce mothers' enduring perception of children's fear-proneness. In this regard, this result may be in line with a previous study which indicated that maternal overcontrolling parenting orientation is stably maintained over time, particularly for toddlers' exhibiting a pattern of physiological vulnerability (i.e., low baseline RSA) (Kennedy et al., 2004).

4.3 | Parasympathetic regulation, maternal comforting, and preschool-age separation distress

Toddlers' physiological vulnerability—as indexed by low baseline RSA—was found to interact with maternal comforting behaviors during the Puppet Show to predict greater preschool-age separation distress. This finding aligns with two distinct lines of previous research examining children's risk for anxiety-related outcomes. First, prior research has suggested that low baseline RSA and socialization influences jointly contribute to children's fear (Hastings, Sullivan, et al., 2008) or anxiety-related behavioral outcomes (Hastings et al., 2014). For instance, maternal protectiveness has been found to increase children's risk for anxiety, only for socially wary children with low baseline RSA (Hastings et al., 2014). Similarly, low baseline RSA and maternal protective behaviors were interactively related to children's socially fearful behaviors (Hastings, Sullivan, et al., 2008). As indicated by the above findings, results obtained in the present study also suggest that physiological vulnerability may enhance fear-prone toddlers' susceptibility to the potentially anxiety-enhancing effects of maternal parenting behaviors.

Second, prior research has indicated that maternal protective behaviors that are accompanied by affectionate comforting increase fearful toddlers' risk for inhibited behaviors, specifically in the context of low-threat situations (Kiel & Buss, 2012). Consistent with this finding, mothers' affectionate comforting behaviors offered in a low-threat puppet show predicted preschool-age separation distress, only for toddlers with low baseline RSA. Although physical soothing delivered in the context of a stressful situation may reflect a form of *sensitive parenting*, high levels of physical soothing and affectionate behaviors (e.g., embracing/hugging, kissing) offered in the context of a low-threat situation may represent *overprotective parenting* (Mount et al., 2010). As noted previously, parents who engage in high levels of overprotective behaviors—displays of physical affection, shielding children from stimuli with an embrace—may unwittingly limit critical opportunities for their physiologically vulnerable toddlers to develop autonomous self-regulatory skills (Hastings et al., 2014). In turn, such behaviors may promote toddlers' continued reliance on parental

regulatory support and contribute to a tendency to experience distress in the absence of external regulatory support. Taken together, findings of the present study lend support to previous research suggesting that children's physiological vulnerability and parenting behaviors jointly enhance children's susceptibility to inhibited or dependent patterns of behaviors.

4.4 | Limitations and conclusions

Interpretation of the present findings warrants a consideration of the specific features of the study. First, toddlers' fear-related behaviors and maternal parenting were examined in the context of a low-threat puppet show, based on previous research which indicated that elevated behavioral fear and maternal protective support offered during such low-threat task predicted children's risk for anxiety (Kiel & Buss, 2012). Additionally, the present study examined a community sample of toddlers who were oversampled for fearfulness. Although the present study was well-suited to detect the interplay between toddlers' fear regulation and maternal influence occurring within this low-threat context (Buss, Davis, & Kiel, 2011), it should be noted that this study was not designed to examine the influence of other potentially confounding variables (e.g., genetic risk for inhibition or anxiety, environmental risk such as family stress) on toddler fearfulness. Second, the present study exclusively examined maternal ratings of child behaviors (i.e. anticipatory ratings of toddler fear and preschool-age outcome), with a specific aim of examining the associations among maternal appraisal and behaviors. Findings revealed a differential pattern of relations among distinct dimensions of maternal rating (e.g., two dimensions of maternal anticipatory ratings), observer rating of parenting behaviors, and indices of physiological regulation. These results suggested that the effects are not likely to be entirely attributable to shared source variance. Finally, parenting behaviors and toddler RSA response were examined concurrently and thereby limited the interpretation regarding the direction of influence linking both constructs. Future investigations designed to disentangle the direction of effects among these constructs should examine the potentially evocative influences of toddlers' parasympathetic regulation on caregiver behaviors.

The findings of the present study highlighted the specific conditions under which biological and social factors may operate jointly to relate to parenting and adjustment outcomes in preschool-age children. Specifically, parental appraisal of toddlers' emerging capacity for fear regulation may have implications for the degree of regulatory support parents actually provide for their fear-prone toddlers. Excessive parental regulatory support, in turn, may reinforce children's fearful traits, particularly for children who demonstrate poor parasympathetic regulation as toddlers. In sum, findings indicate the potential value of further examining the etiological significance of different aspects of maternal cognition for toddlers presenting with temperamental and/or physiological vulnerability for fear dysregulation.

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FIGURE 1.
RSA suppression as a moderator of the association between maternal fear anticipation and
comforting behaviors

FIGURE 2.
RSA suppression as a moderator of the association between maternal fear anticipation and preschool-age inhibition

FIGURE 3.
Baseline RSA as a moderator of the association between maternal comforting behaviors and preschool-age separation distress

TABLE 1

Descriptive statistics and bivariate correlations

Variable	Mean	SD	1	2	3	4	5	6	7	8
1. Anticipated toddler fearfulness	2.16	0.67	—							
2. Anticipated toddler	2.68	0.58	.28**	—						
3. Dependency maternal	4.87	5.52	.15 [†]	.31***	—					
4. Comforting baseline RSA	4.46	1.07	.04	.08	-.01	—				
5. RSA	.00	0.99	-.01	.16	.23*	-.08	—			
6. ITSEA inhibition to novelty	0.88	0.51	.17 [†]	.40***	.11	.01	.11	—		
7. ITSEA separation distress	0.78	0.38	-.03	.28**	.08	.08	.06	.43***	—	
8. Behavioral fear	63.69	37.64	.29***	.31***	.40***	.15	.19 [†]	.11	.07	—

RSA = respiratory sinus arrhythmia; RSA = residualized change score of RSA from baseline to puppet show; ITSEA = infant toddler social emotional assessment.

[†] $p < .10$,

* $p < .05$,

** $p < .01$,

*** $p < .001$.

TABLE 2

Hierarchical regression predicting maternal comforting behavior

	Comforting behaviors			
	<i>R</i> ²	<i>F</i>	<i>b</i>	<i>p</i>
Step 1	.03	1.11	—	—
Fear	—	—	0.05	.60
BRSA	—	—	−0.07	.48
Fear × BRSA	—	—	0.05	.64
Step 2	.17	6.01 [*]	—	—
Behavioral fear	—	—	0.41	<.001
Step 1	.16	6.50 [*]	—	—
Fear	—	—	0.08	.36
RSA	—	—	0.11	.31
Fear × RSA	—	—	0.36	<.001
Step 2	.28	10.64 ^{**}	—	—
Behavioral fear	—	—	0.37	<.001
Step 1	.10	4.66 [*]	—	—
Dependency	—	—	0.22	.01
BRSA	—	—	−0.05	.64
Dependency × BRSA	—	—	0.10	.26
Step 2	.22	8.09 ^{**}	—	.004
Behavioral fear	—	—	0.36	<.001
Step 1	.15	6.44 [*]	—	—
Dependency	—	—	0.19	.03
RSA	—	—	0.16	.11
Dependency × RSA	—	—	0.15	.12
Step 2	.25	9.17 ^{**}	—	.002
Behavioral fear	—	—	0.33	<.001

Comforting = maternal comforting behaviors; Fear = maternal fear anticipation; Dependency = maternal dependency anticipation; RSA = respiratory sinus arrhythmia; BRSA = baseline RSA; RSA = RSA suppression during puppet show.

^{*}*p* < .05,

^{**}*p* < .01.

TABLE 3
Models predicting behavioral fear, preschool-age inhibition to novelty and separation distress

	Behavioral fear (age 2)				Inhibition to novelty (age 3)				Separation distress (age 3)			
	<i>R</i> ²	<i>F</i>	<i>b</i>	<i>p</i>	<i>R</i> ²	<i>F</i>	<i>b</i>	<i>p</i>	<i>R</i> ²	<i>F</i>	<i>b</i>	<i>p</i>
Step 1	.19**	8.92**	—	—	.03	1.18	—	—	.11*	4.34*	—	—
Comforting	—	—	0.40	<.001	—	—	0.08	.93	—	—	0.08	.46
BRSA	—	—	0.15	.12	—	—	−0.01	.43	—	—	0.07	.49
Comforting × BRSA	—	—	−0.05	.61	—	—	−0.13	.25	—	—	−0.33	.003
Step 2	—	—	—	—	.04	1.08	—	—	.11 [†]	3.37 [†]	—	—
Behavioral fear	—	—	—	—	—	—	0.07	.50	—	—	0.02	.88
Step 1	.13*	5.69*	—	—	.05	1.90	—	—	.05	2.09	—	—
Fear	—	—	0.27	.002	—	—	0.15	.13	—	—	−0.06	.59
BRSA	—	—	0.13	.65	—	—	−0.01	.88	—	—	0.07	.51
Fear × BRSA	—	—	−0.17	.10	—	—	−0.13	.27	—	—	−0.23	.05
Step 2	—	—	—	—	.05*	1.55*	0.21	—	.06	1.68	—	—
Behavioral fear	—	—	—	—	—	—	0.05	.63	—	—	0.04	.68
Step 1	.12*	5.42*	—	—	.15*	5.94*	—	—	.04	1.59	—	—
Fear	—	—	0.28	.002	—	—	0.19	.06	—	—	−0.04	.72
RSA	—	—	0.19	.05	—	—	0.04	.77	—	—	0.01	.94
Fear × RSA	—	—	−0.07	.55	—	—	0.38	.01	—	—	0.21	.12
Step 2	—	—	—	—	.15*	4.81*	—	—	.05	1.48	—	—

	Behavioral fear (age 2)				Inhibition to novelty (age 3)				Separation distress (age 3)			
	<i>R</i> ²	<i>F</i>	<i>b</i>	<i>p</i>	<i>R</i> ²	<i>F</i>	<i>b</i>	<i>p</i>	<i>R</i> ²	<i>F</i>	<i>b</i>	<i>p</i>
Behavioral fear	—	—	—	—	—	—	0.07	.48	—	—	0.09	.38
Step 1	.12 [*]	5.40 [*]	—	—	.20 ^{**}	8.50 ^{**}	—	—	.09 [†]	3.80 [†]	—	—
Dependency	—	—	0.29	.001	—	—	0.44	<.001	—	—	0.30	.004
BRSA	—	—	0.10	.33	—	—	0.03	.78	—	—	0.08	.44
Dependency × BRSA	—	—	−0.08	.47	—	—	0.20	.06	—	—	0.07	.47
Step 2	—	—	—	—	.21 [*]	6.71 [*]	—	.01	.09 [†]	2.94 [†]	—	—
Behavioral fear	—	—	—	—	—	—	0.01	.10	—	—	−0.02	.85
Step 1	.13 [*]	5.60 [*]	—	—	.18 ^{**}	7.89 ^{**}	—	—	.09 [†]	3.70 [†]	—	—
Dependency	—	—	0.28	.002	—	—	0.41	<.001	—	—	0.29	.01
RSA	—	—	0.13	.20	—	—	0.06	.61	—	—	0.01	.90
Dependency × RSA	—	—	−0.06	.57	—	—	0.11	.30	—	—	−0.01	.89
Step 2	—	—	—	—	.19 [*]	6.14 [*]	—	—	.09 [†]	2.84 [†]	—	—
Behavioral fear	—	—	—	—	—	—	−0.01	.88	—	—	−0.02	.84

Comforting = maternal comforting behaviors; Fear = maternal fear anticipation; Dependency = maternal dependency anticipation; RSA = respiratory sinus arrhythmia; BRSA = baseline RSA; RSA = RSA suppression during puppet show.

[†] $p < .10$,

^{*} $p < .05$,

^{**} $p < .01$.