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Dysregulated Fear, Social Inhibition and RSA: A Replication and Extension

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Abstract

Behavioral inhibition indicates increased risk for development of social anxiety. Recent work has identified a pattern of dysregulated fear (DF), characterized by high fear in low threat situations, that provides a more precise marker of developmental risk through early childhood. This study tested a new longitudinal sample of children ($n = 124$) from age 24 to 48 months. Replicating prior findings, at 24 months we identified a pattern of fearful behavior across contexts marked by higher fear to putatively low-threat situations. DF was associated with higher parental report of social inhibition at 24, 36 and 48 months. Extending prior findings, we observed differences in cardiac physiology during fear-eliciting situations suggesting that the neurobiological underpinnings of DF relate to difficulty with regulation.

Keywords

dysregulated fear; RSA; social inhibition

The literature is replete with studies examining how differences in early temperament may canalize development. Which children are at greatest risk? What processes place them at higher risk? The prediction from early fearful temperament to later anxiety symptoms has been a major focus in the developmental psychopathology literature. Several longitudinal studies have examined the trajectories of anxiety risk for behaviorally inhibited children (e.g., Chronis-Tuscano et al., 2009). Recently, we have examined which fearful children are at greatest risk ([blinded for review]) by identifying a distinct pattern of dysregulated fearful behavior that may indicate increased risk for development of social withdrawal and social anxiety symptoms better than traditional markers of behavioral inhibition. In the present study, we replicated and extended these findings. Given that many developmental processes are thought to have neurodevelopmental underpinnings (e.g., anxiety; Cicchetti & Gunnar, 2008; Pine, 1999) we extend the replication by explicitly taking a biopsychosocial approach and additionally examining whether dysregulated/normative fear behavior may be driven by

differences in how children's parasympathetic systems contribute to regulation in fear-eliciting situations.

Fearful Temperament and Early Risk for Social Withdrawal and Anxiety

Social anxiety disorders are among the most common disorders in children and adolescents. Prevalence rates in the general population range from 6 to 9%, and when considering subclinical levels of symptoms up to nearly 20% (Angold, Costello, Farmer, Burns, & Erkanli, 1999; Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). Extreme fearful temperament, most often studied as *behavioral inhibition*, is an early emerging individual difference characterized by avoidance, withdrawal and distress to novel situations and people (Garcia-Coll, Kagan, & Reznick, 1984; Kagan, Reznick, Clarke, Snidman, & Garcia-Coll, 1984), across normative (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001) and disordered populations (Hirshfeld-Becker et al., 2008), behavioral inhibition has consistently been identified as an early predictor of social anxiety disorder (SAD; Chronis-Tuscano et al., 2009; Hirshfeld-Becker et al., 2007). However, not all behaviorally inhibited children remain socially withdrawn or develop anxiety symptoms. We have argued that the noted discontinuity between early behavioral inhibition and later anxiety may be because the children being identified as behaviorally inhibited are actually quite heterogeneous – a mix of at-risk and not-at-risk children ([blinded for review]). The general problem is that the *extreme fearful temperament* and *behavioral inhibition* categorizations are too broad in that they lump together multiple patterns of fearfulness – only some of which place children at elevated risk for later social anxiety symptoms (Oler et al., 2012; Shackman et al., 2013b). Indeed, the several longitudinal studies that have examined the trajectories of anxiety risk for behaviorally inhibited children are mixed, with only some of the behaviorally inhibited toddlers developing social anxiety (Chronis-Tuscano et al., 2009; Degnan et al., 2014; Penela, Walker, Degnan, Fox, & Henderson, 2015).

Context Inappropriate Fear

Implicit in models of extreme fearful temperament, and most notably in models of behavioral inhibition, is an assumption that all fearful behavior is maladaptive. Following this assumption, a common approach in the temperament literature is to aggregate across situations in order to measure the typical level of fear behavior. These approaches are most often focused on the average intensity of fear behavior whereby children above some cutoff are identified as, for example, behaviorally inhibited (Garcia-Coll et al., 1984; Kagan et al., 1984). Alternatively, more differentiated patterns of fear behavior can be identified by considering the pattern of behavior across situations. Fear provokes action readiness – and, in high threat situations is adaptive. Only when mismatched to the situation, either by being overly extreme in intensity or surfacing in situations that are not threatening, is fear maladaptive (Buss, Davidson, Kalin, & Goldsmith, 2004; Goldsmith & Davidson, 2004). The implication is that the identification of fearful temperament or behavioral inhibition should take into account the situational specificity of children's fear behavior. Indeed, primate studies examining interplay of context and fear behavior (Kalin, 1993; Kalin, Shelton, Rickman, & Davidson, 1998; Oler et al., 2012; Shackman et al., 2013a) highlight the critical identifier of anxious temperament as *context inappropriate* fear behavior.

Following this line of reasoning, children at greatest risk for development of social anxiety disorders may be those who display a specific pattern of behavior, namely *high fear in low threat situations*.

To examine this possibility, Buss (2011) assessed fear behavior of $N = 111$ toddlers across novel situations (e.g., modified from the Lab-TAB) that ranged from putatively low-threat contexts that pulled for engagement and pleasure (e.g., Puppet Show) to putatively high-threat contexts that pulled for withdrawal and displeasure (e.g., Spider). The pattern of fear was modeled as both a continuous variable, representing the slope of fear ‘intensity’ across the tasks ordered from low to high threat where a flatter slope represented greater dysregulation of fear; and as latent profiles where two distinct patterns of fear behavior emerged. Most toddlers (90% of sample) exhibited a normative fear profile, characterized by context appropriate fear behavior – low levels of fear behavior in low-threat situations and higher levels of fear behavior in high-threat situations. In contrast, a small group of toddlers (10% of sample) exhibited a *dysregulated fear* profile characterized by context inappropriate fear behavior – high levels of fear in both the low-threat and high-threat situations. Tracked for four years, dysregulated fear was associated with greater social withdrawal (Buss, 2011) throughout the preschool years and kindergarten, as well as social anxiety disorder symptoms (Buss et al., 2013) at 6 years, compared to a more normative fear profile. Social situations and social outcomes seem to be particularly salient for dysregulated and other types of fearful children (e.g., behaviorally inhibited) (Hirshfeld-Becker et al., 2008). In the Buss (2011) paradigm, most of the situations were social in nature and a parallel comparison of social to non-social (i.e., object) situations revealed the same pattern of findings.

Why are children with a dysregulated fear profile the ones that are at elevated risk for later social anxiety? Generally, (in)sensitivity of emotional responses across contexts is viewed as an important predictor of psychopathology (Bonanno et al., 2007; Chaplin & Aldao, 2012; Coifman & Bonanno, 2010; Shackman et al., 2016). Indeed, contextually inappropriate emotional behavior is implicated in the development of a range of maladaptive behavior patterns (Buss, 2011; Buss et al., 2013; Diaz & Bell, 2012; Locke, Davidson, Kalin, & Goldsmith, 2009; Locke, Miller, Seifer, & Heinze, 2015; Luebke, Kiel, & Buss, 2011; McDermott, Watkins, Rovine, & Rikoon, 2014; Shackman et al., 2013a). The evidence that this pattern of dysregulated fear may be an early indicator of a developmental trajectory into social anxiety is intriguing (Buss, 2011; Buss et al., 2013). However, this evidence was obtained using a low risk, community sample where only about 10% of toddlers exhibited the dysregulated fear pattern. Thus, replication is critical to provide further evidence that showing a pattern of dysregulated fear provides a more homogeneous assessment of fearfulness that strongly predicts the development of social anxiety disorder symptoms. The present study was formulated to obtain a larger sample of children who may display dysregulated fear and examine the robustness of the pattern of fearfulness and its projections toward social anxiety symptoms.

Respiratory Sinus Arrhythmia as an Index of Dysregulation

The dysregulated fear label implies that these children have difficulty in regulating their fear response in specific contexts. Physiologically, this dysregulation may be marked by action of

the parasympathetic branch of the autonomic nervous system, which plays an important role in physiological regulation of stress (Porges, 1995; Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996). With increasing task demands, parasympathetic control over the heart decreases, releasing a brake and facilitating behaviors needed to engage with the demands of the task or environment (Porges, 1995; Porges, 2007). This process is marked by respiratory sinus arrhythmia (RSA), which measures heart rate variability that corresponds to the respiratory cycle. When parasympathetic control is strong, RSA is high, and when parasympathetic control is low, RSA is low. Thus, according to the Polyvagal Theory (Porges, 1995; 2007) when faced with threat (and successfully responding to that threat), RSA should be lower.

Several studies over the past two decades have supported this general hypothesis. Greater suppression of RSA has been associated with fewer behavior problems and better emotion regulation in preschoolers (Calkins & Dedmon, 2000; Hastings et al., 2008; Porges, 1996), sustained attention in school-aged children (Suess, Porges, & Plude, 1994), and a decreased risk of behavior problems (both internalizing and externalizing) for children with high environmental risk (El-Sheikh, 2001). Generally, the evidence suggests that children whose physiological systems are responding appropriately to task demands do better. In complement, there is evidence that children whose physiological systems are dysregulated – mismatched to task demands – do worse. For example, low baseline RSA (Forbes, Fox, Cohn, Galles, & Kovacs, 2006; Shannon, Beauchaine, Brenner, Neuhaus, & Gatzke-Kopp, 2007) and less pronounced RSA suppression have been associated with internalizing problems (Calkins, Graziano, & Keane, 2007; Gentzler, Santucci, Kovacs, & Fox, 2009), poor emotional regulation, and extreme emotional response (Buss, Goldsmith, & Davidson, 2005; El-Sheikh, Harger, & Whitson, 2001; Friedman & Thayer, 1998).

Following the evidence that lowering of RSA in response to task demands (i.e., RSA suppression) is a marker of adaptive functioning, we would expect that children who display a dysregulated fear pattern of behavior would have higher RSA during potentially fear-eliciting tasks (less pronounced suppression or even augmentation) compared to children who display a more normative fear behavior pattern. This general hypothesis, however, does not consider the dynamics of regulation. Both Porges (2007) and Beauchaine (2001) have argued that adaptive regulation of RSA to challenge is a marker of emotion regulation and, more broadly self-regulation (Beauchaine, 2001; Porges, 2007). Suppression of RSA in response to a threatening situation is adaptive – facilitating behaviors needed to engage with the demands of the task or environment. However, failure to suppress RSA from baseline to a demanding task, or even increases (i.e., augmentation) of RSA may be a marker of dysregulation. From this perspective, children who display a dysregulated fear behavior pattern would be more likely to *fail to suppress RSA to task demands than children who display a more normative fear behavior pattern*. This difference in fearfulness will be particularly observable in the low-threat, social situations.

The Present Study

The present study was designed to replicate and extend our understanding of dysregulated fear in childhood (Buss, 2011; Buss et al., 2013). There were three goals. The first was to

prospectively follow a new sample of children to see if we could again identify a *dysregulated fear* pattern of behavior across contexts. In the original sample, the dysregulated pattern of fearful behavior, characterized mainly by high fear in low-threat contexts, was observed in less than 10% of the sample. Although this proportion is consistent with other unselected samples in the behavioral inhibition literature (Garcia-Coll et al., 1984), the relatively small number of children showing a dysregulated fear pattern limited the kinds of analyses we could conduct to explore this phenotype fully. Here, we extended the previous work by oversampling for children at risk for developing social anxiety. We hypothesized a pattern of increased fear behavior as the putative level of threat increased across the six episodes. Variation in this typical pattern would reflect greater dysregulation, specifically a pattern showing high fear across all tasks, including the low-threat tasks. Our second goal was to demonstrate that dysregulated fear, identified in toddlerhood, is related to development of social inhibition symptoms concurrently, 1, and 2 years later. We hypothesized that, in line with previous findings, dysregulated fear would be associated with elevated social inhibition during preschool.

The pattern of fearful behavior that characterizes dysregulation in children highlights that the development of social anxiety disorders may be rooted in how individuals regulate their emotions and behavior. Thus, we were interested in uncovering mechanisms of adaptive regulation to clarify why these children are at heightened risk. That is, what regulatory processes underlie the dysregulated fear behavior? RSA reactivity to challenge is a psychophysiological process that has been consistently linked with regulation (Beauchaine, 2001; Porges, 2007), but is increasingly being recognized as highly context-dependent (Hastings et al., 2014). Thus, the third goal was to examine how RSA, often thought of as a marker of regulation, is associated with a pattern of dysregulated fear behavior. We hypothesized that a pattern of dysregulated fear will be associated with an RSA pattern across multiple laboratory episodes that would be consistent with physiological dysregulation. Specifically, we predicted that dysregulated fear would be associated with higher RSA during the fear episodes which is consistent with less suppression (or augmentation of RSA) to challenge. Furthermore, we hypothesized that this dysregulated pattern of RSA would also be associated with social inhibition symptoms at 36 and 48 months and moderate the association between dysregulated fear and social inhibition.

Method

Participants

The present study made use of data obtained from 124 children (61 girls, $M_{age} = 24.43$ months, $SD_{age} = .47$) who participated in a larger prospective longitudinal investigation of temperament and socio-emotional development after being screened for fear and anxiety (described below). Participants were drawn from a primarily rural region of the Northeastern United States. The screening procedures took place between 2006 and 2008, and the three waves of longitudinal data described in this investigation were collected between 2006 and 2010. Most children resided in married two-parent households (97%), and were predominantly non-Hispanic, European American (90.4%), followed by Asian/Asian American (6.4%), American Indian (1.6%), and Hispanic and African American (0.8%).

Family income ranged from <15,000 (3%) to >\$60,000 (49%) with most families (90%) earning more than \$30,000. Mothers' education ranged from 10 to 20 years ($M = 15.62$, $SD = 2.41$) and fathers' ranged from 12 to 20 years ($M = 16.22$, $SD = 2.32$).

Procedure

18-month screening—Using local birth announcements and a university recruitment database of families interested in research participation, we recruited and received a screening instrument from 481 parents of full-term, healthy birth children between 18 and 20 months of age. The goal of this initial screening was to oversample for toddlers elevated in fear, inhibition and anxiety for inclusion in the prospective longitudinal study. Screening was based on two parent-report questionnaires. First, using the Infant and Toddler Social and Emotional Assessment (ITSEA; details described below) (Carter, Briggs-Gowan, Jones, & Little, 2003) we identified children who scored at least one standard deviation (SD) above age- and gender-based norms on at least one of three fear-related scales (inhibition to novelty, separation distress, and anxiety/worry). Second, using six questions designed to capture parents' perceptions of their child's fear in situations that are novel, but fun and engaging (*Toddler Wariness Questionnaire*) we identified children who scored higher than 1 SD above the mean (established on the first 100 screened cases) on items concerning "out of context" or exaggerated fear responses to situations that are novel, yet meant to be engaging. Items include: "My child is wary in situations where most children are not."; "My child is wary in situations that are typically fun for children (e.g., meeting a mascot)."; "My child is wary in new situations even when I am there for support."; "My child is afraid of many different types of things/situations (e.g., meeting new people, animals)."; "My child actively avoids meeting new people or playing with new things."; "In new situations (e.g., meeting a new person), my child tends to become less active (e.g., stops playing) than s/he is normally and remains inactive for more than a few minutes."

In order to be identified as a "fear" target for enrollment, toddlers had to meet the following criteria: 1 SD above the mean at least two of the scales from the ITSEA scales and on the *TWQ*. In total, 121 of the screened children were identified as potentially high fear ($M = 1.25$, $SD = .41$ for ITSEA Inhibition to Novelty; $M = 1.04$, $SD = .37$ for ITSEA Separation Distress; $M = .26$, $SD = .21$ for ITSEA Anxiety/Worry; $M = 3.98$, $SD = 1.02$ for *TWQ*) and were invited to participate. Of these, $n = 62$ joined the study, and the other $n = 62$ participants were randomly selected from the remaining pool of screened, non-high fear children.

24-month laboratory visit—Children and one parent (98% mothers) visited the laboratory for a 2-hour visit within a month of the child's second birthday, wherein they completed a series of structured tasks. After obtaining parental consent, providing the parent with an overview of the entire visit, and completing an initial episode with the child (*Risk Room*), children and parents were outfitted for ambulatory wireless electrocardiograph (ECG) recording. Framed as a game, the child chose some stickers for themselves, their parent, and the experimenter to wear on their skin. The selected stickers were secured to three disposable, pre-gelled electrodes that were placed on the distal right collarbone, lower left rib, and lower right rib. With the electrodes in place, and the ambulatory monitoring

equipment secured in a small backpack, the child (and parent) proceeded into the heart of the visit. In brief, following a cardiac baseline (child and experimenter sat together to read books), the 6 novel episodes were (in fixed order): *Puppet Show* (3 min, object fear; Exuberant puppets put on a show and engaged child in games), *Stranger Approach* (1.5 min, social fear; Male stranger entered the room, talked to and tried to play with the child), *Robot* (1 min, object fear; Remote-controlled robot moved around, lit up and made noises in the corner of the room), *Clown* (3 min, social fear; Friendly clown attempted to engage the child in a series of games), *Stranger Working* (2 min, social fear; Female stranger entered the room and worked in the corner), *Spider* (1 min, object fear; Large, remote-controlled spider approached child). Detailed descriptions of each of these structured tasks can be found in Buss (2011). After the final episode, children (and parent) returned to the initial room to remove the ambulatory ECG recording equipment. Children were given a small prize for participation, parents were thanked and families departed.

36- and 48-month questionnaire packets—Approximately 1–2 weeks before children turned 36 months and 48 months of age, parents were mailed a packet of questionnaires to complete. Parents provided written consent at that time for their participation in each of those assessments.

Measures

Fear behavior (24-months)—Children’s fearful behavior was measured via second-by-second micro-coding of specific behaviors observed during each of the six structured tasks designed to elicit object or social fear (*Puppet Show*, *Clown*, *Stranger Approach*, *Stranger Working*, *Robot*, *Spider*). Because comprehensive details about the entire coding scheme are available elsewhere (e.g., Buss, 2011; Buss et al., 2013), we describe only our assessment of fear behavior here. Specifically, observed fear behavior included facial expressions of fear, bodily expressions of fear, freezing behavior, and close proximity to parent. Independent teams, blind to study hypotheses and trained to a minimum of 90% interrater agreement (with $\kappa = 0.70$ for each individual behavior), coded behavior for each second from videotapes. *Facial expressions* of fear in each context were coded using the AFFEX coding system (Izard, Dougherty, & Hembree, 1983), which differentiates among emotion expressions using three different facial regions. Facial fear was coded as present in any one-second epoch in which the child’s brows were straight or normal but slightly raised and drawn together, the eyelids were raised or tense, and/or the mouth was open with corners pulled straight back (agreement based on at least 15% of cases per episode = 94%, $\kappa = .81$). Presence and duration of *bodily expressions* of fear were coded by identifying instances when the child exhibited specific behaviors like diminished play (agreement = 96%, $\kappa = .87$). *Freezing* was identified as present if the child became still or rigid in response to stimuli for more than 2s (e.g., limbs unmoving and arranged awkwardly), to keep this code distinct from a briefer orienting response to changes in the activity (agreement = 94%, $\kappa = .83$). *Proximity to parent* was assessed for each second as whether the child was or was not in close proximity to the parent (close proximity was defined as touching the parent or within one arm’s length of the parent; agreement = 98%, $\kappa = .97$). We also computed a measure of children’s *latency to freeze* by calculating the number of seconds between the beginning of each task and the first observation of freezing behavior.

Each child's second-by-second time-series for each structured task episode was then summarized using the durations of these five behaviors, following the same procedures used in the prior study (Buss, 2011): latency to freeze (reverse scored), duration of facial fear, duration of bodily fear, duration of freezing, and duration of close proximity to the parent. Because there were acceptable levels of consistency across the codes (Cronbach's $\alpha = .61$ to $.73$), the five duration scores were averaged and adjusted for each individual's length of episode to derive a total *Percentage of Episode Spent Engaging in Fearful Behavior* score for *Puppet Show* ($M = 35.72$, $SD = 23.65$), *Clown* ($M = 33.45$, $SD = 22.83$), *Stranger Approach* ($M = 27.16$, $SD = 17.40$), *Stranger Working* ($M = 26.31$, $SD = 17.02$), *Robot* ($M = 59.66$, $SD = 28.36$), and *Spider* ($M = 62.26$, $SD = 21.89$). As will be described below, the percentage of time children exhibited fearful behavior in each of the six structured tasks was then used to derive behavior profile groups.

Respiratory sinus arrhythmia (RSA; 24-months)—Children's ECG was continuously recorded during 13 task episodes using Mindware Wi-Fi ACQ software, Version 1.0 (Mindware Technologies, LTD, Westerville, OH). The ECG signal was sampled at a rate of 500ms and band-pass filtered at 40 and 250 Hz. RSA was calculated from the ECG signal by detrending data using a first-order polynomial to remove the mean and any linear trends, cosine tapering, and submitting to Fast Fourier Transform. RSA was defined as the natural log integral of the high frequency 0.24 to 1.04 Hz power band and calculated in 30s epochs. Each 30s epoch of ECG data was visually inspected by trained research assistants for artifact identification using Mindware Heart Rate Variability (HRV) version 2.51. The software identifies inter-beat intervals (IBIs) and flags physiologically implausible intervals for manual inspection using an established algorithm (Berntson, Quigley, Jang, & Boysen, 1990). All data were inspected and edited by three trained scorers. Interrater reliability was calculated on ~25% of the cases (624 epochs) and was high (86%). Note that to be construed as a reliable match, final RSA values for any 30s epoch from two scorers had to differ by less than 0.1 (Buss, Davis, & Kiel, 2011).

Inhibition to Novelty/Social Inhibition—Parents reported on their children's *inhibition to novelty* at the 24-month laboratory visit, and again when their child was 36-months old (via mailed survey) using the Infant and Toddler Social and Emotional Assessment (ITSEA; (Carter et al., 2003), a 166-item questionnaire asking about socioemotional and behavioral problems exhibited by the child in the past month. Inhibition to novelty scores were calculated as the average response (on a 3-point scale: 0 = *not true/rarely*, 1 = *somewhat or sometimes true*, 2 = *very true or often true*) to the 5 items from the inhibition to novelty subscale (Cronbach's $\alpha = 0.85$ and 0.82 at age 24- and 36-months, respectively). Parents generally reported their children as somewhat inhibited at both 24-months ($M = 1.02$, $SD = 0.54$) and 36-months ($M = 0.88$, $SD = 0.52$). Parents reported on their children's *social inhibition* at the 48-month laboratory visit using the MacArthur Health Behavior Questionnaire (HBQ; (Armstrong & Goldstein, 2003; Essex et al., 2002), a 172-item questionnaire about their children's behavior during the past six months. Social inhibition was measured as the average response (on a 3-point scale: 0 = *rarely applies*, 1 = *somewhat applies*, or 2 = *certainly applies*) to 3 items (Cronbach's $\alpha = 0.80$) from the social withdrawal subscale that were specific to social wariness (e.g., "shy with other children";

“shy with unfamiliar adults”; “is afraid of strangers”), and distinct from social disinterest (e.g., “prefers to play alone”; “is a solitary child”; which is not typically associated with social anxiety, see (Coplan, Rubin, Fox, Calkins, & Stewart, 1994; Gazelle, 2010). Parents generally rated their children as somewhat socially inhibited ($M = .72$, $SD = .43$) at 48-months.

Data Analysis

We employed a variety of multivariate analytic methods to (a) identify patterns of fear behavior that reflect fear dysregulation, and to (b) examine how these fear patterns and physiological reactivity related to children’s social inhibition at age 24, 36, and 48 months.

We first sought to characterize observed fearful behavior across the six threat episodes. Consistent with how dysregulated fear has been identified in prior studies (Buss, 2011; Buss et al., 2013), we standardized the *Percentage of Episode Spent Engaging in Fearful Behavior* scores from each of the six novel, fear contexts (1. *Clown*, 2. *Puppet Show*, 3. *Stranger Working*, 4. *Stranger Approach*, 5. *Robot*, 6. *Spider*). Individual propensity to exhibit fear across the six threat episodes was derived using a measurement model based on a nonlinear growth model (Ram & Grimm, 2015). Specifically, each child’s six fearful behavior scores, $fear_{it}$, were modeled as a nonlinear function of the putative level of situational threat, $threatlevel_t$, given by the ordinal ranking (1 to 6) shown above. Specifically, patterning of fear behavior across tasks was modeled using a hyperbolic tangent function, \tanh (see e.g., Liebovitch, Vallacher, & Michaels, 2010). Specifically,

$$fear_{it} = \beta_0 + \beta_1 [\tanh (threatlevel_t - \lambda_i)] + e_{it} \quad (1)$$

where $fear_{it}$ across tasks is modeled as a sigmoidal trajectory defined (by fixing $\beta_0 = \beta_1 = 50$) to travel from observation of 0% fear behavior at very low level of threat to 100% fear behavior at very high level of threat with inflection point given by λ_i . The person-specific inflection points, λ_i , thus locate the specific level of threat at which each child would, based upon their behavior in all six tasks, display fear for 50% of task time. Here, the λ_i parameter estimates (subsequently referred to as *fear sensitivity*) provide a measure of a child’s propensity to display fear relative to the threat/intensity of the tasks (normalized to the sample and specific tasks to which the child was exposed in this study). Models were estimated separately for each child in SAS 9.2 (proc nlin), with the child-specific fear sensitivity scores collected into a new variable for use in subsequent analyses.

Finally, we examined if children’s fear sensitivity and RSA at 24 months were, after controlling for 24-month social inhibition, related to later (36 and 48 months) social inhibition, and if that association was moderated by RSA. Continuous predictors were sample centered and analyses were conducted using the PROCESS macro in SPSS (Version 22).

Missing Data and Imputation—We had complete fear behavior and maternal reported inhibition data at 24 months. Most children wore the backpack and electrodes without

difficulty, but a few children refused the whole procedure ($n = 15$) or discontinued wearing the electrodes before the end of the visit ($n = 7$). Partial data were lost due to technical difficulties ($n = 1$) and/or noisy data ($n = 8$). There were no systematic differences between children with complete versus missing data on the composite fear behaviors, fear profiles, or maternal report of inhibition at 24-, 36- or 48-months ($ps > .05$). By 48 months we had parent-reported *Social Inhibition* for 93 children. The children with missing 36- and 48-month data did not differ at age 24-months on any of the key study variables (observed fear, RSA, or ITSEA Inhibition; $ps > .15$). Little's MCAR test ($\chi^2 = 4486.15$, $df = 11632$, $p = 1.0$) suggested that data were missing at random. Thus, missing data were imputed using expectation maximization methods (using Mplus 6.2; Muthen & Muthen, 2012).

Results

Results are organized into three sections. First, we start with a description of the fear sensitivity models. Second, we examine the bivariate relations among the study variables to explore hypothesized associations. Third, regressions were used to examine developmental associations between earlier fear sensitivity and RSA and later social inhibition. Of note, demographics were unrelated to key study variables and there were no gender differences ($ps > .06$). Thus, these variables were not examined further.

Fear Sensitivity Calculation

Our first task was to derive the fear sensitivity scores as outlined in the data analysis section above (Equation 1). The collection of nonlinear curves indicating each child's sensitivity to exhibit fear across the episodes are shown in Figure 1. As can be seen children's fear, as hypothesized, increased across tasks with the individual differences in where their specific trajectory crosses 50% captured by the *fear sensitivity* (λ_i) score. The prototypical child's inflection point (50% fear behavior) was located at 4.82 (threat level between the stranger approach and robot situation), but with substantial between-child differences in the location of this inflection point ($SD = 1.15$; range = 0.19 to 8.16).

Fear Sensitivity and Social Inhibition

To confirm that fear sensitivity was associated with greater social inhibition, we examined the correlations between fear sensitivity and parent-reported inhibition to novelty obtained at 24- and 36- months, and social inhibition obtained at 48-months. Results are summarized in Table 1. At all three ages, fear sensitivity was associated with inhibition ratings such that greater dysregulation in fear was associated with higher scores on parent-reported inhibition.

Fear Sensitivity and Physiological Reactivity

We examined the general pattern of relations among task-specific fear behavior, task-specific RSA, fear sensitivity, and parent-reported inhibition – correlations given in Table 1. First looking at task-specific associations, higher RSA was associated with greater fear behavior in the *Puppet Show*, *Stranger Approach*, and *Robot* tasks. Fear sensitivity was also associated with higher RSA during the *Clown*, *Stranger Approach*, *Stranger Working*, and *Robot* tasks, suggesting that fearful and inhibited children may fail to suppress RSA during threat tasks. Finally, the general pattern of positive associations between RSA and parent-

reported inhibition at 24- and 36-months suggests that lower RSA in these tasks is a marker of adaptive function.

Predicting Social Inhibition from Fear Sensitivity and RSA

Finally, we examined the prediction to parent-reported inhibition from fear sensitivity, RSA and the interaction between fear sensitivity and RSA. We controlled for 24-month inhibition in the age 36-months and age 48-months models. Given the pattern of strong correlations across tasks for RSA, we averaged RSA across tasks for these analyses. Regression results are presented in Table 2. All three models were significant, but only at Age 36-months were all the parameters significantly different than zero. Consistent with the bivariate associations, greater DF and higher RSA was associated with higher parent-reported social inhibition. However, these effects were subsumed under a significant interaction. Probing the interaction (-1 *SD* mean, 1 *SD* on RSA) revealed, as shown in Figure 2, that the association between fear sensitivity and social inhibition was only significant at high RSA, $b = -.15$ (.05), $t = -.3.09$, $p = .003$; approached significance at mean RSA, $b = -.07$ (.04), $t = -.1.64$, $p = .10$; but not at low RSA, $b = .02$ (.06), $t = .25$, $p = .80$.

Discussion

The goals of the present study included (1) replication of identifying a pattern of dysregulated fear in toddlers (Buss, 2011) in a new sample; (2) replication of the prediction from toddler dysregulated fear to elevated social inhibition (Buss, 2011; Buss et al., 2013); and (3) extending our understanding of the implications of dysregulated fear for children's development by linking this pattern of high fearfulness in low-threat contexts to physiological reactivity as a putative psychophysiological marker of dysregulated emotion processes in toddlerhood. Results were largely consistent with expectations—we were able to replicate the identification of children with dysregulated fear in this new sample, showed that this pattern of fear responding predicted inhibition across early childhood, and identified modest evidence for the idea that children who manifest a dysregulated fear pattern of behavior would also show a distinct pattern of physiological responding to laboratory challenges.

The predicted pattern of children's fear behavior across the six, fear-eliciting tasks emerged. The six contexts ranged from low to high threat and were designed to elicit varying amounts of withdrawal and approach behaviors. In our previous work ([blinded for review]), for most 24-month-olds there was a predictable linear increase in fear and avoidance as the level of threat increased which was modeled as a slope. In fact, most toddlers find the low-threat situations to be fun and engaging and, accordingly, show very little fear or avoidance. Consistent with the previous study ([blinded for review]) we found evidence that this gradual increase in fear responding was characteristic of most toddlers. This normative pattern was characterized by moderate to low fear sensitivity scores such that reaching the threshold of fear responding occurred in the relatively higher threat tasks (especially *Robot* and *Spider*). As predicted, we replicated previous findings and found a pattern of fear sensitivity consistent with higher levels of fear behavior across all the episodes reflected in lower fear sensitivity scores. Our replication of this dysregulated fear pattern, characterized by high

fear in low threat, in a second cohort of children follows other two-cohort developmental studies of behavioral inhibition (Garcia-Coll et al., 1984; Kagan et al., 1987; Calkins et al., 1996; Fox et al., 2001).

One notable difference between the patterns identified here and those from our previous work is important to address. In the previous cohort ([blinded for review]), the normative pattern was characterized by a *linear* slope of increasing fear as level of threat increased. Inspection of the fear means across tasks revealed that a linear slope would not fit the data well. In contrast, the model used here provided for nonlinearity in the pattern of fear across tasks. In particular, we saw that the average fear elicited in *Stranger Working* and *Stranger Approach* was as low (if not lower) than the hypothesized low-threat tasks (*Puppet Show* and *Clown*). One possible reason for this difference is that in addition to the differences across tasks in putative level of threat, the tasks can also be differentiated on the basis of social threat versus object threat. However, as we discuss in our previous study ([blinded for review]) there is no way to disentangle the level of threat from the type of threat in this design because we do not have high-threat social tasks. Nevertheless, the distinction between social and non-social tasks is evident, as is also highlighted and discussed below for the RSA results.

The second goal of the study was to replicate the findings of increased risk for social inhibition in children with a temperamental pattern of dysregulated fear. Consistent with our previous studies ([blinded for review]) and the hypotheses of the current study, a pattern of dysregulated fear was associated with parent-reports of higher inhibition (inhibition to novelty and social inhibition) concurrently and at 1 and 2 years after the laboratory visit.

Psychophysiological Markers of Dysregulated Fear and Risk for Social Inhibition

The third goal of the present study was to examine RSA as a putative biomarker of dysregulated fear. To this end, we examined the patterns of RSA across the six fear tasks at the 24-month, laboratory visit. We demonstrated that higher task RSA was associated with more task-specific fear for most of the threat episodes suggesting that this pattern of RSA maybe associated with difficulty regulating fear. The fear sensitivity score was also associated with RSA, especially during the tasks consistent with social threat. Additional analyses revealed a significant association between task-level RSA and social inhibition.

Our final set of analyses examined how RSA and fearful behavior were associated with inhibition to novelty and social inhibition, concurrently and across a 2-year period. In the preliminary set of correlations, there was evidence that higher task RSA was associated with higher parent-reported inhibition at 36 months. Consistent with predictions, fear sensitivity and RSA interacted to predict social inhibition at 36 months. Specifically, greater fear sensitivity was associated with greater inhibition (after controlling for 24-month inhibition) when average RSA was high. A similar pattern of associations emerged at 48 months, but did not reach significance (perhaps due to use of a different measure of social inhibition at this age).

Consistent with Polyvagal theory (Porges, 1995; 2007) and models of RSA (Beauchaine, 2001) as regulatory, RSA decreases in order to engage the “fight-flight” system and to

respond to the task demands. We argue that this pattern of RSA also is a marker of dysregulated physiological regulation. It is more consistent with a failure to suppress RSA enough to engage with the task. Of course, we know from our behavioral results that children with a more dysregulated fear profile are far less likely to ever engage with the task. These children are more likely to freeze and tend to not interact or approach. It could be that these children are attempting to regulate their behavioral distress by keeping the parasympathetic “brake” on, rather than releasing the brake and engaging with the environment as the children with a more normative fear profile. Porges’ theory would suggest that this freezing response could reflect the more phylogenetically primitive vagal system that promotes avoidance and immobilization (Porges, 2007).

Another possibility is that these fearful children are engaging the parasympathetic nervous system (PNS) to regulate their distress. We hypothesize, based on previous work (Buss et al., 2004), that these children would also show elevated sympathetic (SNS) reactivity (e.g., cardiac pre-ejection period). Activation of the SNS and stress-reactivity is consistent with theory and research in inhibited children (Kagan, 1994). Thus, future work could examine whether co-activation of the PNS and SNS is characteristic of extremely fearful and dysregulated children. Taken together, the RSA results demonstrate that a pattern of high task RSA, reflecting either augmentation or simply a failure to adequately suppress RSA, is associated with more fearful behavior and a pattern of dysregulated fear and can serve as a biomarker for this type of behavior. Of note, and a question that should drive further research, is why we mainly observed these RSA differences in the social fear tasks. Although this is consistent with the behavioral literature linking early fearful temperament to social inhibition and anxiety (Buss, 2001; Buss et al., 2013; Chronis-Tuscano et al., 2009), this is the first study to demonstrate the possible specificity of this biomarker when measured in social fear contexts. As we have argued before, in relation to behavior, context matters in determining at-risk behavior ([blinded for review]). Therefore, the same may be true when considering biomarkers of these at-risk behaviors (Buss, Morales, Cho, & Philbrook, 2015).

Implications and Future Directions

Across three samples, we have demonstrated the importance of taking into account the eliciting context when identifying patterns of maladaptive fearful behavior ([blinded for review]). In the previous studies, we have demonstrated that this dysregulated pattern of high fear in low-threat situations is associated with a different pattern of physiological stress responding ([blinded for review]) and increased risk for social inhibition and anxiety symptoms ([blinded for review]). As we have argued before, this work has implications for identifying which fearful children we should be worried about in terms of the development of anxiety symptoms ([blinded for review]). This model is consistent with other research and models of psychopathology in adults and children that highlight the role of context in identifying maladaptive emotional responses (Bonanno et al., 2007; Cole, Michel, & O'Donnell Teti, 1994; Gruber & Keltner, 2007; Rottenberg & Vaughan, 2008). Of course, to date we have only followed these children into early childhood, so future work is needed to determine the continued risk for social inhibition and anxiety.

This early-identified pattern of behavior only represents one risk factor. As such, not all dysregulated fear children will have maladaptive outcomes so understanding the processes that are associated with this pattern of behavior are critical to identifying maladaptive trajectories. We proposed that the underlying regulatory processes may be one mechanism that accounts for this fearful pattern 1) across contexts (i.e., failure to down-regulate fear in the moment) and 2) longitudinally by influencing the development of executive processes that develop across early childhood ([blinded for review]). In the present study, we examined a putative biomarker of regulation, cardiac RSA, and demonstrated that dysregulated fear is associated with a unique pattern of RSA characterized by elevated RSA across tasks which together predicted increased inhibition one year later. The hypothesis that this pattern of fearful behavior represents a failure of regulation rather than just being about increased reactivity is consistent with a recent study of an adult follow-up of a sample of behaviorally inhibited children. Jarcho and colleagues demonstrated that adults with a history of childhood behavioral inhibition had a unique pattern of neural activity associated with cognitive control during specific trial types (i.e., contexts), but not behavioral reactivity, compared to adults without a history of behavioral inhibition (Jarcho et al., 2013). In a subsample of children from the current sample, we found differences in neural activity for dysregulated children consistent with this regulatory hypothesis as well as consistent with the broader anxiety literature (Brooker & Buss, 2014; Phelps, Brooker, & Buss, 2015). There is growing evidence that risk for adjustment or maladjustment anxiety in fearful children is linked to individual differences in self-regulation (Penela et al., 2015) –either in terms of under-control or over-control. It could be that dysregulated fear is the consequence of a child’s inability or difficulty with down-regulation of fearful reactivity (Buss & Goldsmith, 1998; Thompson, 1994) and once triggered, even in low-threat situations, regulating fearful responses is difficult resulting in extreme fearfulness across a variety of situations. Evidence for this type of dysregulation comes from work that we, and others, have done linking neural, sympathetic and neuroendocrine biomarkers to both behavioral inhibition (Fox et al., 2001; Schmidt et al., 1997) and dysregulated fear (Buss et al., 2004; Davis & Buss, 2012). In addition to a pattern consistent with under-control, extreme fearful behavior has also been linked to a pattern of over-control, increased vigilance to the environment which constrains behavioral approach such that there is an increased readiness to respond to threat even when threat is not present. For instance, we found that a pattern of neural activity consistent with over-control (e.g., EEG delta-beta coupling; ([blinded for review]) and vigilance (e.g., error-related negativity; [blinded for review]) for fearful children compared to non-fearful children.

Limitations

Results should be interpreted in the context of some sampling and measurement limitations. Our sample was largely homogeneous with respect to race/ethnicity and SES. Although this homogeneity is consistent with the majority of other studies in this literature, care should be taken when generalizing to more diverse populations. Our ambulatory measurement of ECG prioritized assessment of RSA. However, regulatory and reactive processes exist at the interplay between parasympathetic (RSA) and sympathetic nervous system activity. Additional work is needed to understand how the sympathetic system contributes to the neurobiological underpinnings of children’s fear behavior. Further investigation of

differences in “timing” of physiological reactivity and regulation will require new methods for tracking second-to-second changes in RSA. Additional precision in RSA change will propel identification and understanding of how the moment-to-moment function of children’s physiology contributes to long-term development. Although we followed these children for two years, we are unable to make definitive statements about how dysregulated fear contributes to long-term developmental risks, in part because different measures were used to index inhibition at different ages. Robust understanding of the developmental sequelae will require larger scale longitudinal and/or measurement burst studies that track behavior and physiological data periodically through childhood, adolescence, and into adulthood.

Conclusion

One barrier to progress in the field of childhood anxiety is a limited understanding of the mechanisms and processes through which anxiety disorders in children develop. Questions that need to be addressed, and that this study contributes new data for, include who is at risk for developing anxiety, under what conditions (i.e., how can we identify these children), and what neurodevelopmental processes are involved. The present study, which replicates and extends our previous work ([blinded for review]), contributes to this literature by demonstrating robustness in this pattern of fear as a marker of risk for anxiety-related problems. We demonstrated, in a second cohort of children, that a dysregulated pattern of fearful behavior - characterized by high fear in low threat situations – is associated with social inhibition across a 2-year period and is also associated with a different pattern of cardiac reactivity. These findings suggest that the fearful behavior that characterized these dysregulated children may reflect underlying regulatory processes, specifically, difficulty with regulation of fear arousal.

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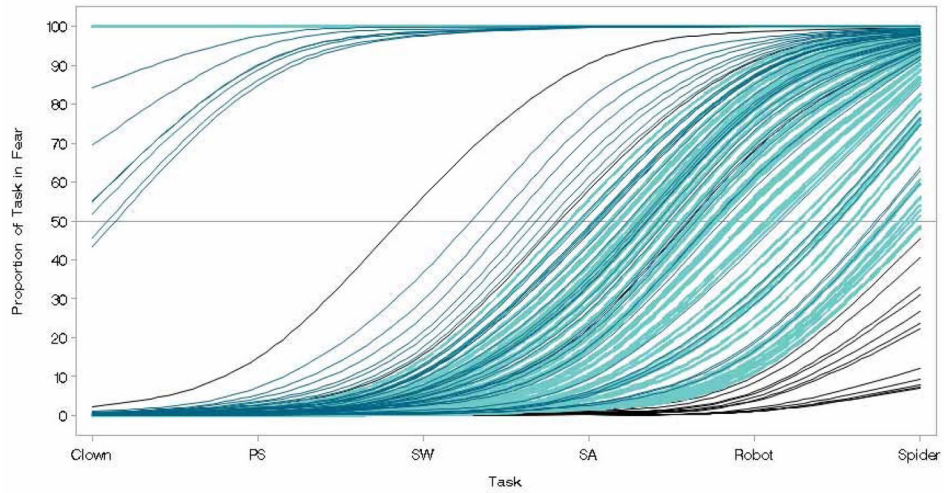


Figure 1.

Plot of Expected Fear Behavior Derived from Nonlinear Growth Model with a *Fear Sensitivity* Parameter

Note: Curves represent individual's expected display of fear across tasks with successively greater levels of threat. Individual differences in fear sensitivity derived from the λ_j parameters of Equation estimates. PS = Puppet Show, SA = Stranger Approach, SW = Stranger Working.

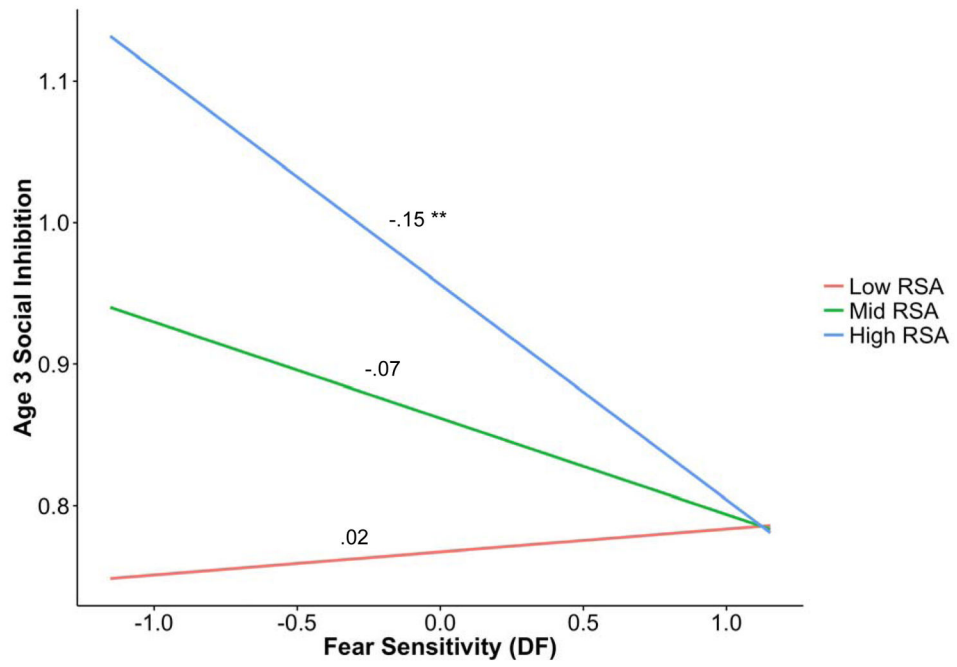


Figure 2.
Interaction Plot Between Fear Sensitivity and RSA
Note: RSA from average of all 6 tasks. Interaction probed at the mean and \pm 1 S.D.

Table 1

Correlations Among Study Variables.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|-----------------------------|---------|---------|---------|---------|-------|---------|--------|--------|--------|--------|--------|------|---------|--------|--------|----|
| 1. Clown Fear | - | | | | | | | | | | | | | | | |
| 2. Puppet Show Fear | .43*** | - | | | | | | | | | | | | | | |
| 3. Stranger Working Fear | .43*** | .39*** | - | | | | | | | | | | | | | |
| 4. Stranger Approach Fear | .33*** | .22** | .20** | - | | | | | | | | | | | | |
| 5. Robot Fear | -.06 | 0.05 | -.06 | 0.16 | - | | | | | | | | | | | |
| 6. Spider Fear | .27** | 0.1 | 0.11 | 0.09 | -.11 | - | | | | | | | | | | |
| 7. Clown RSA | 0.16 | 0.07 | 0.14 | 0.17 | 0.13 | 0.07 | - | | | | | | | | | |
| 8. Puppet Show RSA | .22* | .29*** | .22* | 0.11 | 0.1 | 0.06 | .72*** | - | | | | | | | | |
| 9. Stranger Working RSA | .22* | 0.11 | 0.14 | 0.08 | .21* | 0.003 | .67*** | .62*** | - | | | | | | | |
| 10. Stranger Approach RSA | .24** | 0.17 | 0.17 | .30** | .23* | 0.04 | .78*** | .65*** | .56*** | - | | | | | | |
| 11. Robot RSA | 0.14 | 0.05 | 0.101 | .20* | .20** | 0.07 | .68*** | .56*** | .50*** | .68*** | - | | | | | |
| 12. Spider RSA | 0.09 | 0.07 | -0.07 | 0.03 | 0.14 | 0.03 | .64*** | .59*** | .50*** | .62*** | .62*** | - | | | | |
| 13. Fear Sensitivity | -.41*** | -.33*** | -.35*** | -.46*** | 0.04 | -.57*** | -.24** | -.14 | -.019* | -.022* | -.022* | -.02 | - | | | |
| 14. Age 2 Social Inhibition | .27** | .22** | .23** | 0.12 | -.06 | .20* | .18* | 0.07 | .18* | 0.14 | 0.14 | 0.09 | -.37*** | - | | |
| 15. Age 3 Social Inhibition | .23** | 0.15 | .21* | 0.13 | 0.001 | 0.01 | .19* | 0.09 | 0.16 | .21* | 0.13 | 0.16 | -.24** | .74*** | - | |
| 16. Age 4 Social Inhibition | .25** | .25** | .19* | -.04 | -.05 | 0.01 | 0.03 | 0.09 | 0.14 | 0.09 | -.01 | 0.06 | -.19* | .44*** | .50*** | - |

Note. N= 124.

* p < .05,

** p < .01,

*** p < .001.

Table 2

Regressions predicting Parent-Reported Social Inhibition from DF and RSA

| | 24-Month Inhibition | 36-Month Inhibition | 48-Month Inhibition |
|------------------------|---------------------|---------------------|---------------------|
| | Estimate (SE) | Estimate (SE) | Estimate (SE) |
| 24-Month Inhibition | | .72 *** (.06) | .44 *** (.09) |
| RSA | .14 (.22) | 0.34* (.15) | .09 (.22) |
| Fear Sensitivity | -.07 (.22) | .31* (.15) | .06 (.21) |
| Fear Sensitivity x RSA | -.02 (.03) | -.06* (.05) | -.02 (.04) |
| <i>F</i> Statistic | 6.51 ** | 39.94 *** | 7.39 ** |
| <i>R</i> ² | .14 | .57 | .20 |

Note. *N* = 124. Unstandardized estimates. SE = Standard Error.

* *p* < .05,

** *p* < .01,

*** *p* < .001. RSA was averaged across all tasks. Age 36-month and Age 48-month model parameters after controlling for Age 24-month Inhibition.