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Fearful Temperament and Stress Reactivity Among Preschool-Aged Children

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Abstract

In this study, we examined the relation between physiological stress-reactivity and temperamental fearfulness in 162 preschool-aged children. Both the autonomic and neuroendocrine arms of the mammalian stress system were examined. Larger stress responses were defined as greater sympathetic activation, parasympathetic withdrawal and cortisol increases to stressor tasks. Fearful temperament was examined using parent report and behavior in response to fear-evocative laboratory tasks. There was little evidence that larger sympathetic activation or parasympathetic withdrawal was associated with fearful temperament. Greater cortisol reactivity, however, was associated with fearful temperament. Additional analyses examined those children who were consistently fearful across all measures, and the results remained largely the same. However, there was some suggestion that consistently fearful compared to non-fearful children might be more likely to exhibit sympathetic activation to the fear-evocative stimuli. These findings provide support for the argument that fearful temperament is associated with greater stress reactivity in young children. Nonetheless the size of the associations was small and future studies will need to determine whether reactivity of stress-sensitive physiological systems contributes to the development of individual differences in fearful temperament or merely reflects these differences.

Keywords

temperament; behavioral inhibition; cortisol; vagal tone; pre-ejection period; children

The role of temperamental fearfulness in individual differences in stress reactivity and regulation is of growing interest in developmental neuroscience (e.g., Buss et al., 2003; Kagan, Reznick, & Snidman, 1988) and biological psychiatry (Bakshi & Kalin, 2000; Smoller et al., 2005). This interest reflects an enhanced understanding of the neurobiology of both conditioned (LeDoux & Phelps, 2000) and unconditioned fear (Davis, Walker, & Lee, 1997) and belief that the distributed neural systems underlying acute expressions of fear also support more stable, temperamental variations in fearfulness (Rothbart, 1989). The distributed neural circuits that orchestrate fear behavior have outflows to both arms of the mammalian stress system (Charmandari, Tsigos, & Chrousos, 2005). Specifically, they activate both the autonomic nervous system (ANS) to support fight/flight reactions to threat and the hypothalamic-pituitary-adrenocortical (HPA) system, a counter-regulatory system that has multiple roles in adaptation.

One role of the HPA system is to shape future behavioral and physiological responses to threat through regulating gene expression in fear-orchestrating brain regions such as the amygdala (Sapolsky, Romero, & Munck, 2000). Accordingly, if individuals who are

temperamentally disposed to respond with fear to strange, novel or threatening events produce larger and more prolonged responses of the HPA system, then these responses themselves may operate to lower thresholds for fearful, inhibited responses to subsequent threats, creating a developmental cascade that increasingly stabilizes individual differences in fearfulness (Rosen & Schulkin, 1998). Activity of the HPA axis has been identified as one mechanism through which extremely fearful or inhibited children might become pathologically anxious adults (Schulkin, McEwen, & Gold, 1994).

Discussed as behavioral inhibition, extremes in temperamental fearfulness have been the focus of considerable developmental research in the last few decades (e.g., Biederman et al., 1993; Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Kagan, 2001). Despite this, relations between fearful temperament and stress reactivity are still unclear, particularly with regards to reactivity of the HPA system in young and middle childhood (Gunnar, 2001). While fearful or inhibited children have been found to have higher home baseline cortisol levels compared to extremely uninhibited children (de Haan, Gunnar, Tout, Hart, & Stansbury, 1998; Kagan, Reznick, & Snidman, 1987; Schmidt et al., 1997), they do not necessarily exhibit larger or more prolonged HPA responses to novel or strange people or situations (see for review, Gunnar, 2001). Further, a recent meta-analysis of temperament in children showed a slight bias toward greater fearfulness in girls (Else-Quest, Hyde, Goldsmith, & Van Hulle, 2006); however, studies have not found consistent sex effects in children's HPA responses (Gunnar & Vazquez, 2006). In searching for possible reasons for these discrepancies, some have argued that hyper-responsivity of the HPA axis is more closely tied to freezing or extreme expressions of inhibition (Buss, Davidson, Kalin, & Goldsmith, 2004). However, others have suggested that inhibiting approach to novel events effectively reduces the child's contact with those events, and this buffers the child from activations of the HPA axis (Bruce, Davis, & Gunnar, 2002). This latter argument is based on evidence that elevations in cortisol are most likely to be exhibited under conditions of threat in which the individual lacks control (Dickerson & Kemeny, 2004).

While studies using cortisol, the hormone produced by the HPA axis, have been less consistent in finding associations with temperamental fearfulness or inhibition, significant associations have frequently been noted in studies examining associations with autonomic activity. Many of these studies have relied on measures of heart rate and heart rate variability (e.g. Kagan, Reznick, Clarke, Snidman, & Garcia-Coll, 1984). With some consistency, extremely fearful, behaviorally-inhibited children compared to uninhibited children, have been noted to exhibit high and unvarying heart rates when they are being confronted with the stimuli that elicit their fearful, inhibited behavior. Kagan (e.g., 1994) has argued that the explanation for their high and unvarying heart rates and their extremely inhibited behavior lies in a lower threshold for activation of amygdala-centered fear circuits which, in turn, activate the sympathetic nervous system, producing strong sympathetic drive on the heart (Snidman, 1989). Notably, estimates of associations between behavioral inhibition and heart rate activity are generally larger in samples of children selected for the extremes of inhibition (Reznick, Gibbons, Johnson, & McDonough, 1989), as other studies exploring variation within a typically-developing population of children have failed to observe such effects (Buss et al., 2004).

Although others have pointed to sympathetic activity as the basis for the high and unvarying heart rates of extremely fearful or inhibited children, Porges (1991; 1992) has argued that most of the variation in heart rate observed under the mild threat conditions imposed on children in the laboratory likely reflects variations in vagal mediation of heart rate. In addition, he has suggested that stress and fear vulnerability is expressed through low vagal or parasympathetic input to both the central nervous system and the cardiac system via the nucleus ambiguus arm of the vagus. Accordingly, researchers studying fearful or

behaviorally-inhibited temperament have sought to distinguish vagal, or parasympathetic, contributions to heart rate regulation under conditions of mildly threatening stimuli from those of the sympathetic arm of the autonomic nervous system.

Until recently, differentiating sympathetic and parasympathetic contributions to heart rate variability has been difficult (Berntson, Cacioppo, & Quigley, 1994). Methods using spectral analysis have provided some differentiation of sympathetic and parasympathetic associations with fearful temperament (Marshall & Stevenson-Hinde, 1998), but this method is not as specific as those using cardiac impedance to measure pre-ejection period (PEP; sympathetic) and measures of respiratory sinus arrhythmia to index vagal tone (VT; parasympathetic) (Berntson et al., 1994). Nonetheless, the few studies reporting associations between PEP and fearful temperament in young children have not yielded strong associations. Thus, Buss et al. (2004) found that shorter PEP (i.e., greater sympathetic activation) measured under baseline conditions was only marginally associated with “dysregulated” fear responses, or the display of fearfulness in non-threatening contexts, in a sample of toddlers. Buss, Goldsmith, & Davidson (2005) provided a partial replication of these findings in that greater levels of observed negative affectivity (i.e., fear and sadness) were again marginally associated with shorter PEP. However, these associations were even more tenuous when fearfulness and PEP were measured under conditions of challenge. Perhaps this was because PEP measures did not change significantly in response to the stressor tasks used in these studies of young children: stranger approach (Buss et al., 2005) or watching fear-eliciting video clips (Talge, Donzella, & Gunnar, under review). Although it can be argued that such paradigms were not challenging enough to elicit sympathetic activation, there is some indication that this branch of the nervous system may become increasingly responsive with development (Buss et al., 2005; Finley & Nugent, 1995). It was also the case that in studies of fearfulness and PEP, the children were not selected to reflect extremes in temperamental fearfulness; thus, as in the studies of heart rate and temperamental fear, associations may be attenuated when extreme groups are not the focus of the research.

In the following study, we examined associations between temperamental fearfulness and reactivity of both the autonomic and neuroendocrine arms of the mammalian stress system in preschool-aged children. There has been considerable controversy in the literature over whether researchers should use only direct observations of behavior to assess children's emotional dispositions or whether parent-report provides a useful adjunct to laboratory observations (see for discussion, Rothbart & Bates, 1998). Based on Rothbart's arguments that parent reports are useful, we chose to base our assessments of fearful or behaviorally-inhibited temperament on both structured laboratory tasks derived from the Goldsmith Laboratory Temperament Assessment Battery (LabTAB, Goldsmith, Reilly, Lemery, Longley, & Prescott, 1999) and the Children's Behavior Questionnaire (CBQ, Putnam & Rothbart, 2006). Further, as laboratory tasks to elicit fearful temperament have used novel objects (Kagan et al., 1987; Buss et al., 2004) and unfamiliar people (Rubin & Coplan, 1998; Schmidt, et al., 1997; Buss et al., 2004), we included both types of tasks in our battery.

Thus, two aspects of temperamental fearfulness were examined: inhibition to novel events (nonsocial fear) and inhibition to novel social stimuli (social fear or shyness). The CBQ provides scales for fear and shyness, and the LabTAB has structured tasks that involve both nonsocial (Risk Room) and social (Stranger Approach) stimuli. While children may differ in their responses to these different fear-eliciting conditions, there is no reason to believe that once the neural circuitry of fear is activated by either of these types of fear-eliciting stimuli, outflow to either the sympathetic or HPA arms of the mammalian stress system would differ. Thus, to provide a cumulative index of fearful temperament we used all the available

sources of information. However, to allow a more specific examination of fear-stress associations in this age group, we also performed follow-up analyses examining each measure separately.

To provide clean indices of sympathetic and parasympathetic activation in response to threat, we used emotion-eliciting video clips to produce changes in cardiac activity. This allowed us to keep the children seated during the assessment, thus reducing movement artifact and confounds introduced by variations in children's motor activity levels that are, by definition, associated with inhibition of approach to the unknown. To assess activity of the HPA axis, we used sequential measures of salivary cortisol. Based on pilot work using a similar experimental paradigm (Talge, Eibs, Bruce, & Donzella, 2003), baseline measures of cortisol were obtained approximately 25 min after the child arrived at the laboratory, and stress response measures were computed using baseline corrected area under the curve computed on the last 4 samples taken during the laboratory assessment.

Three questions were addressed from this paradigm: 1) using a community sample of preschool-aged children, would fearful children show larger and more prolonged cortisol responses to the challenges they were exposed to in the laboratory, and would these differences in stress-reactivity also be observed in measures of the autonomic arm of the mammalian stress system; 2) would these associations be specific to the type of fear-eliciting stimulus whether social or nonsocial; and 3) would associations between temperamental fearfulness and stress-reactivity only emerge when children were selected to be more extreme in temperamental fearfulness? For this last question, we identified fearful and non-fearful children by whether they were consistently fearful (above the median) on each of our measures of fearful temperament we employed. In addition to these questions, because there are relatively few studies of young children employing PEP as a measure of sympathetic regulation of the heart, we also examined whether variations in heart rate to the fear-eliciting videos reflected significant sympathetic input or, instead, reflected vagal regulation of heart rate. If the latter was the case, we would not expect to obtain evidence of significant association between fearful temperament and sympathetic activity (PEP) using the paradigm we employed, although we might obtain associations between fearful temperament and activity of the parasympathetic nervous system as indexed using vagal tone.

Method

Participants

The participants were 162 children (78 boys; 84 girls) between the ages of 3.2 to 5.0 years ($M = 3.8$ years). Treatment of participants was consistent with the ethical guidelines of the American Psychological Association and was approved by the university's Institutional Review Board. The sample was predominantly white (88%), and 72% of families had at least one parent with a college degree. Families were recruited as part of a larger study of child care, and all children were enrolled in home-based child care for 30 hours or more per week.

Procedure

Children were accompanied to the laboratory by a parent, usually the mother (91%). Upon arrival, researchers reviewed procedures with the parent and obtained informed consent, after which the first cortisol sample was taken. The Risk Room paradigm (LabTAB non-social fear; approximately 7 minutes) was then implemented. At the end of this assessment, the researcher read the child a story about Curious George™ as an astronaut who wore sensors and went to space. The researcher then invited the family to a room across the hall to

play “astronaut” with Curious George™, a 1' stuffed toy. As part of the astronaut game, the experimenter placed sensors on the child for autonomic assessment. EMG sensors for startle response were also placed; those data will not be presented here. As soon as the sensors were placed, the parent departed and a second cortisol sample was collected. A behavioral measure of effortful control (Kochanska's Dinky Toys task; Kochanska, Murray, Jacques, Koenig, & Vandegeest, 1996), that will not be discussed in this paper, was then performed and then the child was shown emotion-evoking video clips for 15 minutes. Between each video clip, the experimenter spoke briefly to the child, reassured if necessary, and encouraged him/her to continue to sit quietly. After the final video, a third cortisol sample was collected. After this cortisol sample, the child participated in the LabTAB Empty Box vignette (not discussed further in this report), prior to the LabTAB Stranger Approach vignette.

Immediately after Stranger Approach, the child was reunited with the parent for a 30 minute parent-child interaction period (not described further in this report), after which the fourth cortisol sample was taken. Twenty more minutes of testing involving tasks not analyzed in this report were then performed (Pointing Stroop from Berger, Jones, Rothbart & Posner, 2000, LabTAB Transparent Box, and Kochanska's No-Peek Gift task, Kochanska et al., 1996), prior to the final cortisol sample. During the laboratory session, the parents completed the Children's Behavior Questionnaire. The behavioral and physiological measures assessing temperamental fearfulness and stress reactivity are the ones included in this report (see below). The other assessments mentioned have not yet been fully coded for analysis.

Stimuli and Measures

Risk Room Layout and Equipment—The Risk Room was 10' 5" × 12' 8" and equipped with novel objects as described in Goldsmith's LabTAB (Goldsmith, et al., 1999). Following procedures in the LabTAB manual, children were given 5 minutes to explore the Risk Room (i.e., Phase I), after which the researcher entered and prompted the child to touch each object (i.e., Phase II). Parents were present in the room at all times, although they were asked to interact minimally with their children. Each session was videotaped for later coding. Coding was completed using the LabTAB instructions, and two variables (tentativeness of play and total play) were examined in this report. Tentativeness of play reflected hesitancy to approach or engage in play with the novel objects in the Risk Room and was scored on a 0–3 scale every 20 seconds. Total play reflected the duration of play (in seconds) with the objects in the Risk Room. These variables were chosen because they were normally distributed, and together, reflected the core of the concept of fearfulness or inhibition to the unknown. Additionally, because experimenter's attempts to engage the child in play might have elicited social fear, these variables were selected from Phase I of the task, where the experimenter was not present. Based on 10% of the sessions, the intraclass correlations for coder agreement were .95 and .98 for tentativeness of play and total play respectively. These variables were negatively correlated, $r(162) = -.70, p < .001$; therefore, they were standardized, total play was reverse scored, and the two measures were averaged to yield a measure of *Risk Room Fear*.

Stranger Approach—Following procedures in the LabTAB manual, an adult previously unseen by the child served as Stranger. The Stranger engaged in a scripted interaction with the child, purporting to seek paperwork from the now-absent experimenter. The Stranger adopted a neutral expression, queried the child on mundane facts, e.g., “Have you been here before?”, and engaged in 10–20 second periods of silence following each question. The episode was videotaped for later behavioral coding, and coding was completed using the LabTAB instructions. Two variables were examined in this report, which were scored on a

0–3 scale during the interval between each prompt, and then averaged across the intervals: “hesitancy” or conversational non-responsiveness to the stranger, and “activity decrease” or extent to which the child displayed decreased gross motor activity. These variables were chosen because they best captured behavioral freezing or stilling in response to the stranger, a profile that has been associated with reactivity of stress-sensitive neurobiological systems in both developing children and animals (Buss et al., 2004). Based on 15% of the sessions, the intraclass correlations for coder agreement were .98 and .99 respectively. Data were not available for 6 children, due to either technical difficulty, inability to separate from parent, or only a partial session was completed. Hesitancy and activity decrease were positively correlated, $r(156) = 0.56, p < .001$; therefore, they were standardized and averaged to yield *Stranger Approach Fear*.

Children's Behavior Questionnaire (CBQ)—Parents completed the short form CBQ (Putnam & Rothbart, 2006) while their children completed the tasks describe above. Two scales were examined in the present study: CBQ Shyness and CBQ Fear. Sample items from each scale include “[child] sometimes prefers to watch rather than join other children playing” and “[child] is afraid of loud noises.” For full details of the CBQ, see <http://www.bowdoin.edu/~sputnam/rothbart-temperament-questionnaires>. The Cronbach's alphas for the two scales were .85 and .72 respectively.

Emotion-Evoking Videos—A series of four video clips (3 min each) were presented to children in a standard order (adjustment, neutral, fear, positive), while cardiac data were collected. To be consistent with the astronaut theme, a clip was selected from an educational video that showed NASA space footage. This neutral clip was intended to give children a brief adjustment period to the collection procedure, and physiology collected was not examined. The emotion clips were selected from G-rated cartoons marketed to children. The neutral clip was from Raymond Briggs' *Snowman*, and the fear clip was a dinosaur chase scene from the *Land Before Time*. The positive clip was a scene from *Winnie the Pooh and the Honey Tree*, and was used primarily to restore the children to a happy state following the fear video. Parents reported on the child's experience with each of these clips. Most (89%) of the children had not seen the neutral video clip, while most (73%) had seen the fear clip. Children who had never seen the fear clip did not differ on any of measures of fearful temperament from those who had seen it at least once, $t's < 1.0, ns$. However, children who saw the fear clip for the first time tended to show a larger decrease in vagal tone than did children who had seen it at least once, $t(74.8) = 1.95, p = .055$, but they did not differ in PEP or cortisol response, $t's < 1.0, ns$. Therefore, we examined associations between VT response and fearful temperament with and without the children who had never seen the video clip.

Two types of measures were abstracted from the cardiac data: Pre-Ejection Period (PEP) as an index of sympathetic activity and Vagal Tone (VT) as an index of parasympathetic activity. *PEP*: Impedance cardiography was collected using the SORBA CIC-1000 (SORBA Medical Systems, Inc., Milwaukee, WI). A 500 μA signal at 50KHz was applied to two electrodes placed behind the right ear and on the left iliac crest; change in impedance was recorded from two electrodes placed on the left side base of the neck and left mid-axillary line at the level of the xiphoid. Trained scorers examined the raw data for artifact due to movement or misdetection of ECG landmarks. 14% of cases were reliability scored, with 86% agreement. An average of 15% of trials contained some artifact, though nearly 30% of sessions required only one or no edits. Artifact-free ECG and impedance signals were ensemble averaged over 20 second samples. PEP was calculated as the time (msec) from the beginning of electrical systole (the Q wave of the ECG) to the beginning of the mechanical contraction (dZ/dt , or change in impedance over change in time). *VT*: Measures of VT were acquired using the Vagal Tone Monitor-II (VTM-II, Delta-Biometrics, Inc., Bethesda, MD). Raw ECG data were digitized at 1 KHz and bandpass filtered at 5 Hz and 200 Hz, with a 3

dB cutoff. A computer algorithm detected R-spikes from the raw wave, and computed interbeat intervals (IBI's). MXedit software was used to visually display the heart period data, to edit outliers, and to quantify mean IBI and the Vagal Tone index for each video condition. The primary coder was trained to reliability standards by the Porges Lab. An average of 4 edits was made per session of approximately 975 datapoints; thus, less than 1% of data required edits. PEP and VT scores from the neutral clip served as baseline, and response measures were computed as the difference during the Fear Video and Neutral Video. These measures were calculated such that higher scores indicated greater sympathetic activation (i.e. larger PEP decreases) and larger parasympathetic withdrawal (i.e. larger VT decreases).

Salivary Cortisol—Saliva was obtained for cortisol determination by having the children dip a 1.5" cotton dental roll into approximately .025 g of cherry flavored Kool-Aid™ mix and mouth the cotton to obtain the sweet taste. This small amount of Kool-Aid™ has not been found to significantly affect the cortisol assay (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005). Once the cotton roll was saturated, it was placed in a needless syringe and the saliva was expressed into a 1.5 ml Eppendorf Safe-Lock microtube, sealed and frozen at -20°C until assaying. Five saliva samples were taken in the laboratory with the timing of sampling recorded. Timed from arrival, and with standard deviations of 5 min, these samples were taken on average at 0, 24, 47, 87, 109 min after the child's arrival in the laboratory. To control for time of day, sessions were blocked into morning ($n = 112$; Start Time = 10:30 AM), and afternoon sessions ($n = 50$; Start Time = 3:30 PM). The sample obtained at arrival in the laboratory was used to examine whether time-of-day affected cortisol levels. No effect was obtained, nor did morning or afternoon sampling affect any of the four cortisol samples examined in the present report, $F(1, 141) = .02, ns$. Thus we combined children tested in the morning and afternoon for the following analyses. Samples were assayed in duplicate for cortisol using a time-resolved fluorescence immunoassay (DELFA). Intra- and inter- assay coefficients of variation were at or less than 6.7% and 9.0%, respectively, and duplicates correlated highly, $r = .997, p < .001$. Values were not positively skewed, as is sometimes the case, thus no log-transformations were applied. Area under the curve (Cort_{AUC}) was estimated using the trapezoidal method with correction for baseline (25 min sample) for children with at least 3 of the 4 saliva samples needed for its computation, such that higher values reflect greater cortisol production over the course of the lab visit. Because the cortisol values were highly correlated (r 's ranged from .53 to .82), missing samples were estimated by computing and averaging the Z-scores for their available data and assigning them a missing sample value that was commensurate. Additionally, using boxplots, data from 8 children were identified as extreme outliers on Cort_{AUC} , 4 in each direction. These data were Winsorized for analysis; that is, the extreme values were replaced with observed values. Findings did not differ between raw and Winsorized values.

Missing Data

Of the 162 children, 1 child was missing CBQ data, 6 were missing Stranger Approach data because of equipment malfunction or inability to separate from parent, 12 were missing PEP and VT data because of refusal to allow researchers to place the electrodes on them, an additional 3 were missing PEP data and 9 were missing VT data because of technical difficulties, while 11 refused all cortisol samples need for this report, and 8 did not supply sufficient samples to estimate the cortisol area under the curve. Overall, 126 of the 162 children had complete data on all measures. T-tests were computed comparing the scores of children with missing data from those with full data for the 7 key variables examined in this report. Only one measure, Risk Room Fear, yielded any evidence of a difference, $t(45.2) = 1.7, p = .08$, with children with missing data tending to be more fearful in the Risk Room ($M = .31, n = 36, SD = 1.2$) than children with complete data ($M = -.06, n = 126, SD = 0.83$).

However, this difference, which was only at the trend level, was non-significant when we corrected for the number of tests performed. Therefore, in the subsequent analyses, missing data were considered missing at random and Ns were allowed to vary.

Results

Construction of fearful temperament composite and groups

To construct a cumulative index of fearful temperament, we first examined the means and standard deviations of each of the 4 measures of fearful temperament (see Table 1a) and their inter-correlations (see Table 1b). CBQ Shyness was significantly correlated with Stranger Approach Fear, and less strongly to Risk Room Fear. None of the other measures were significantly associated. Despite this, to provide a cumulative index of fearful temperament reflecting fearfulness across situations, we standardized and combined all four measures, yielding one index of *Fearful Temperament*. This measure was normally distributed with a mean of 0.01 and *SD* of 0.60. However, because of the low to null associations among these various measures of fearfulness, we also retained each component scale to include in subsequent analyses between fearful behavior and stress reactivity. Furthermore, groups were formed to identify children who were consistently fearful or non-fearful across the four measures. Using median splits, we identified 28 children who were below the median on all the measures and 26 who were above the median. These children were labeled Consistently Non-Fearful and Consistently Fearful.

Gender Differences

Preliminary analyses were also computed to determine whether sex would need to be controlled in subsequent analyses. T-tests were conducted on the cumulative index of Fearful Temperament and on each of the components. The results indicated that girls ($M = .13$, $n = 84$, $SD = .50$) scored higher than boys ($M = -.10$, $n = 74$, $SD = .67$) on the cumulative index Fearful Temperament, $t(160) = -2.5$, $p < .01$, which reflected significant differences in CBQ Fear, $t(159) = -2.33$, $p < .05$, and Stranger Approach Fear, $t(154) = -2.6$, $p < .01$. In addition, 35% of the Consistently Non-Fearful and 69% of the Consistently Fearful children were girls, $\chi^2(1) = 6.07$, $p < .05$. Based on these data, it was decided that if significant sex differences in measures of stress-reactivity were also noted, sex would need to be controlled statistically in analyses of fear and stress-reactivity associations.

Preliminary Analyses of Physiological Measures

Descriptive data for the all the physiological measures are shown in Table 2, and intercorrelations among these measures are shown in Table 3. As indicated in the table, change scores for PEP did not differ from zero, $t(146) = -.04$, *ns*. Analysis of the individual values indicated that 55% of the children had PEP change scores that were positive, indicating great sympathetic activity during the fear video compared to neutral. VT responses tended to be positive (indicating parasympathetic withdrawal) with 87% of the children showing this pattern of response, $t(141) = 11.9$, $p < .001$. There was no association between changes in PEP and VT, $r(139) = -.10$, *ns*. An examination of the heart rate data used in computation of PEP and VT indicated that, on average, there was a 2.72 beat per minute increase in heart rate in responses to the fear video [$SD = 2.9$; paired $t(146) = 11.3$, $p < .001$], with 86.3% of the children showing this pattern. Children who showed greater parasympathetic withdrawal to the fear video showed larger heart rate increases, $r(139) = .60$, $p < .001$; while there was no association between changes in PEP and heart rate, $r(160) = -.10$, *ns*.

The measure of cortisol response ($Cort_{AUC}$) had a positive mean, although there was great variability in response, and a one-sample test of the area under the curve was only

marginally significant, $t(142) = 1.8, p = .07$. However, for 63% of the children their cortisol response scores were positive, and for some of these children their scores were quite high, indicating a meaningful cortisol response (i.e., $CORT_{AUC}$ over 5 for approximately 10% of the children). Notably, there were no significant associations between cortisol response and either PEP, $r(134) = -.01, ns$, or VT responses, $r(128) = .11, ns$.

Age has sometimes been noted to be associated with these stress-sensitive physiological measures (Finley & Nugent, 1995; Kazuma, Otsuka, Wakamatsu, Shirase, & Matusoka, 2002; Lenard, Studinger, Mersich, Kocsis, & Kollai, 2004; Massin & von Bernuth, 1997; Silveti, Drago, & Ragonese, 2001). As already noted, sex differences were obtained for measures of fearful temperament. We therefore examined relations between age and sex on each of the physiological measures. For age, no significant correlations were obtained with either the baseline measures or response measures (all p 's $> .10$); therefore, age was not included as a covariate in subsequent analyses. T-tests were computed to examine sex differences in each of the physiological measures. None of the tests achieved significance, indicating sex is not a necessary covariate. However, sex could still serve as a moderating variable on the relation between fearful temperament and stress physiology, and thus, formal moderation analyses were conducted. Sex did not moderate any of the relations presented below.

Associations of Fearful Temperament and Stress-Reactive Physiological Systems

Simple correlations were first computed between fearful temperament measures and baseline measures of PEP, VT and cortisol. None of these associations was significant, with all correlations coefficients less than .12. Table 4 shows the simple correlations between measures of fearful temperament and measures of stress response. Not surprisingly, given the absence of a significant PEP response, there were no significant associations between this measure of sympathetic activation and either the summary measure of Fearful Temperament or any of the measures used to construct it. Although changes in vagal tone accounted for most of the increases in heart rate to the fear-eliciting video, there was only a marginal association between VT responses and Fearful Temperament, and this appeared to primarily reflect more inhibited responses to the Risk Room. However, the correlation was negative, not positive, indicating that children who showed *less* parasympathetic withdrawal tended to exhibit more fearful temperament.

As noted in the methods, about 27% of the children had never seen the fear-eliciting video clip and these children exhibited larger decreases in vagal tone to the clip than did those for whom the clip was not novel. We therefore re-analyzed the associations between fearful temperament and vagal response excluding these children. The correlations were nearly identical to those computed for the total group (see footnote to table 4), therefore the association between fearful temperament and vagal response was not due to the larger response of children who had never seen the video before.

The only stress response measure to bear a significant association with Fearful Temperament was $CORT_{AUC}$ (see Table 4), although Fearful Temperament only accounted for a small amount of the variance in cortisol reactions (3%). This effect appears to have reflected CBQ Shyness; although not significant, the correlation with Stranger Approach Fear was in the same direction. Because Stranger Approach Fear and CBQ Shyness were significantly associated, we computed a Social Fear score by standardizing and combining them. Social Fear was correlated highly with the Fearful Temperament measures, $r(161) = .76, p < .001$, thus, not surprisingly, it was associated with $CORT_{AUC}$, $r(143) = .18, p < .05$, at the same magnitude as the total Fearful Temperament index.

Examination of Stress-Reactivity for Children Consistent in Temperamental Fearfulness

To determine whether differences in stress-reactivity would be more apparent for children who were Consistently Fearful versus Consistently Non-Fearful, we recomputed the correlations between measures of fearful temperament and physiology using only these children. Doing so tends to artificially magnify correlation coefficients. However, we still obtained no significant associations with baseline measures of these stress-reactive systems. While the size of the correlation with Fearful Temperament was larger, $r(44) = .36$ versus the .18 noted before, the smaller sample size meant that this level of association was only marginally significant ($p = .08$). As before, it was CBQ Shyness that bore the strongest association with cortisol response, $r(44) = .39$, $p < .05$, with the coefficients for the other measures comprising the Fearful Temperament index ranging from .05 to .19.

No significant differences in mean levels of autonomic or neuroendocrine reactivity were observed between the consistently fearful and non-fearful groups. However, when examining the number of children exhibiting increases or decreases in these measures, some differences were noted. Ninety percent of the children in the Consistently Fearful group showed cortisol increases to the laboratory challenge compared to 50% in the Consistently Non-Fearful group, $\chi^2(1) = 8.1$, $p < .001$. No difference was noted for the percentage of Consistently Fearful and Non-Fearful children exhibiting parasympathetic withdrawal $\chi^2(1) = .003$, *ns*. However, using this analysis, a difference was noted for the percentage of Consistently Fearful and Non-Fearful children exhibiting sympathetic activation to the fear-eliciting video, $\chi^2(1) = 4.7$, $p < .05$. Specifically, 70% of the Consistently Fearful children exhibited an increase in sympathetic activation from the neutral to fear video compared to 38% of the Consistently Non-Fearful children.

Discussion

The results reveal only modest relations between fearful temperament and reactivity of stress-sensitive autonomic and neuroendocrine systems in preschoolers. Nonetheless, the associations obtained were in the expected direction for measures of cortisol reactivity. Although extreme groups have been the focus of much of the research on biological processes in fearful temperament, the present findings revealed highly comparable results regardless of whether children were classified as consistently fearful or non-fearful. The only novel finding that emerged from these analyses was a suggestion that consistently fearful children might be more sympathetically reactive than consistently non-fearful children. With this exception, there was little evidence that reactivity of either the sympathetic or parasympathetic arm of the autonomic nervous system was associated with variations in fearful temperament. Each of these findings and their implications will be discussed.

The construct of fearful temperament, and the argument that individual differences in stress reactivity may contribute to temperamental fearfulness, are arguments about traits. One of the challenges in assessing trait variations in behavior and physiology is to derive measures that are not highly state- and context-specific. The need to isolate trait from state is one of the reasons, in both temperament and personality research, that researchers have sought to aggregate observations over situations and time (Epstein, 1986). In part, this is why Rothbart (e.g., Rothbart & Bates, 1998) has argued that parent questionnaire measures are important adjuncts to laboratory observations in temperament research. Although use of parents as informants necessarily introduces bias, because parents observe their children over time and contexts, their ratings are aggregates of many individual instances of child behavior.

Laboratory measures of temperament involve assessments obtained in response to specific contexts at specific times. While researchers have reported statistically significant

associations among laboratory measures of temperament both within the same assessment period and over time, generally speaking the magnitude of these associations, when present, are modest, particularly when extreme groups are not the focus of investigation (Kagan et al., 1987; Pfeifer, Goldsmith, Davidson, & Rickman, 2002; Reznick et al., 1986; Rothbart, 1988). Indeed, in the present study we found no correlation between fearful responses to Stranger Approach and to the Risk Room. Buss and colleagues (Buss et al., 2004) noted a similar null association for behavior to these two fear-eliciting LabTAB vignettes in their study with toddlers. Notably, however, parent reports of shyness on the CBQ were modestly correlated with both Risk Room and Stranger Approach measures, perhaps because parents had an opportunity to observe, and thus aggregate, impressions of their children's fearfulness in many different unfamiliar situations.

The problem of isolating trait stability from state variability is also present for reactivity measures of stress-sensitive physiological systems. Furthermore, associations with trait measures of behavior increase when stress-response measures are aggregated (e.g., Pruessner et al., 1997). In the present study, we obtained measures of physiological reactivity to the specific context of our laboratory assessment. The measures of autonomic reactivity were obtained in response to only one eliciting condition, the fear-evocative video clip, while the measure of cortisol response was obtained over much of the session. Cortisol levels in saliva are lagged by about 25 minutes from activation of the HPA axis. Thus, the first sample obtained after baseline assessment should have reflected a combination of response to the Risk Room, sensor placement, and separation from the parent. The second sample after baseline should have reflected Stranger Approach, as well as the vignettes designed to elicit anger/frustration and effortful control (not examined in this report). Finally, the last sample should have reflected the child's ability to contain elevations in cortisol to stressful events after play with their parent and work on cognitive tasks. The fact that $CORT_{AUC}$ was the only physiological measure to reflect the expected association between greater fearfulness and larger stress-reactivity may reflect the greater aggregation over events reflected in the cortisol response measure. This argument might also explain why the strongest associations between fearful temperament and $CORT_{AUC}$ were obtained with CBQ Shyness, another measure that reflected aggregation over time and contexts. According to this argument, we should not expect to obtain high correlations between measures of fearful temperament and physiological measures of stress reactivity unless both the temperament and physiological measures reflect more trait than state activity.

This argument does not explain the direction of the association between parasympathetic withdrawal to the fear video and measures of fearfulness in the Risk Room. From the perspective of stress-reactivity, more fearful children would be expected to show greater parasympathetic withdrawal (to allow heart rate to increase); however, the results of the present study suggested the opposite. Children who were more tentative in the Risk Room showed smaller decreases in vagal tone to the fear video. There are several possible explanations for this finding. First, given the mild challenge these children faced, lifting what Porges has called the vagal brake might reflect an adaptive response. Certainly the majority of children in this study did show parasympathetic withdrawal, suggesting this is a normative response. The lack of parasympathetic withdrawal among the most fearful children might reflect poor autonomic regulation in the face of challenge. Alternatively, Porges and colleagues (Bazhenova & Porges, 1997) have argued that one function of lifting the vagal brake is to allow the child to engage mildly arousing stimuli. In response to a mild stressor like watching a fear-evocative segment of an age-appropriate movie, larger decreases in vagal tone may reflect the child's willingness to engage or attend to the video clip. More fearful children might be less willing to engage the stimulation provided by the video segment, and these same children might be less willing to engage a stimulating environment like the Risk Room. Additionally, it is important to note that children explored

the Risk Room in the presence of their parent. This likely buffered the children's fear, which may have resulted in an attenuation of tentative behaviors in this particular context.

As noted, many studies of fearful or inhibited temperament have involved selection of extreme groups. Kagan among others has argued the value of studying extreme groups, particularly when trying to understand the role of biological processes in temperament (Kagan, Snidman, & Arcus, 1998). In most cases, extreme groups are followed and subsequent measures of fearful behavior and physiological reactivity are examined to determine if the groups still differ in fearful temperament (e.g., Kagan et al., 1987; Pfeifer et al., 2002). In the present study, we grouped children according to the consistency of their fearful behavior across contexts and informants, and then re-examined the associations we had already examined for the group as a whole. This should have increased the size of the correlation coefficients and it did slightly. It also allowed new associations to emerge. Specifically, children who were consistently fearful across all of the indices of fearful temperament might be more sympathetically-reactive to the fear video than the children who were consistently non-fearful or uninhibited.

Consistent with other studies using measures of pre-ejection period with young children, when all the children were included in the analysis, we did not observe a significant PEP response (Alkon et al., 2003; Buss et al., 2004; Talge et al., 2003). Furthermore, changes in PEP were not associated with either changes in vagal tone or heart rate in response to the fear video. For the sample as a whole, PEP responses were not correlated with any of the measures of fearful temperament, and this conclusion held when correlations were computed using only the children in the consistently fearful and non-fearful groups. This would seem to oppose Kagan's argument that the high and unvarying heart rates associated with extremely fearful or inhibited temperament reflect heightened sympathetic reactivity. However, when we merely counted the number of children in the subgroups who did and did not show a decrease in PEP (i.e. sympathetic increase) to the fear video, we did find a suggestion of greater sympathetic reactivity among the consistently fearful (70%) versus consistently non-fearful (38%) children. It seems possible that the video clip paradigm was not intense enough to activate the sympathetic arm of the mammalian stress system, and that this attenuated any associations between sympathetic reactivity and fearful temperament. If so, the suggestion of a slight bias towards greater sympathetic reactivity for more consistently fearful children might be revealing. However, because we had to perform a number of analyses to uncover this bias, this finding needs to be replicated, preferably with a stimulus that elicits a sympathetic response in more of the children.

Returning to the positive association between fearful temperament and cortisol reactivity, this association did correspond to predictions from the literature (e.g., Kagan et al., 1987). However, what is not clear in the present study is whether greater reactivity of the HPA axis was merely a correlate of temperamental fearfulness, or whether it may have played a role in sustaining and enhancing fearfulness over the child's development. One of the roles of cortisol is to shape and stabilize neural systems involved in regulating fearful behavior, and there is evidence that chronic elevations in corticosterone (the rodent version of cortisol) are associated with heightened reactivity of the amygdala in rats (Rosen & Schulkin, 1998). However, as noted, while this study has shown a significant and positive association between cortisol reactivity and fearful temperament, many other studies have failed to reveal this association, or even more strikingly, have found more fearful children to be less cortisol reactive (see review, Gunnar, 2001). Thus although this finding is in line with predictions, it should be viewed cautiously, particularly given its modest size. Furthermore, longitudinal analyses are needed to determine whether the association with cortisol is merely a correlate of fearful temperament or plays a role in stabilizing and enhancing fearful temperament over development.

Although not a focus of this research, this study also revealed a sex difference in fearful temperament, with girls showing higher levels of fear than the boys. This sex difference was significant for CBQ Fear and Stranger Approach Fear. Finding that girls score higher on fearful temperament than boys is consistent with a recent meta-analysis of temperament studies which concluded that fearfulness may show a modest gender bias (Else-Quest, Hyde, Goldsmith, & Van Hulle, 2006). Importantly, however, we observed no sex difference in any of our physiological measures. Sex differences have been noted for measures of cortisol and sympathetic reactivity obtained in studies of adults (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kirschbaum, Wust, & Hellhammer, 1992) and adolescent-aged children (e.g. Frankenhaeuser et al., 1978). In these cases, males tend to produce larger cortisol and catecholamine responses than females. This may be because performance stressors have been used to elicit elevations in cortisol and catecholamines. When a social rejection stressor was used, women were shown to respond more strongly than men (Stroud, Salovey, & Epel, 2002). In studies of young children, reliable sex differences in stress reactivity have not been found (see for review, Gunnar & Vazquez, 2006). Thus, this small but reliable bias towards greater temperamental fearfulness in girls (Else-Quest et al., 2006) does not appear to be paralleled by a similar bias towards greater stress reactivity among young girls than boys, at least when stress responses are elicited with the type of mild provocation that can be imposed in the laboratory.

There are several limitations to this study that need to be considered. These analyses of temperament and stress reactivity were abstracted from a larger study focused on understanding the impact of child care quality assessed in family child care homes on children's physiology and behavior. Consequently, all of the children in the study attended family child care for at least 30 hours per week, an experience that has been associated with discontinuity in inhibition during childhood (Fox et al., 2001). Likewise, most of the children were Caucasian and had parents with at least a high school education. The results of the present study may not generalize beyond children with similar backgrounds. Another limitation is the use of only two LabTAB vignettes to assess fearful temperament. As we have discussed, if aggregation across contexts is needed to obtain more reliable indices of temperamental fearfulness, then in subsequent studies more fear-evoking vignettes should be used. A final limitation is that in this analysis we have only examined associations between fearful temperament and stress reactivity. We have not attempted to examine whether aspects of temperament (e.g. effortful control) or the child's history of relationships (e.g. secure attachment) that might moderate associations between fearful temperament and reactivity measures of stress-sensitive physiological systems. These measures are available in this data set, pending further behavioral coding. Thus it remains possible that, as in the analysis of extreme groups, associations between fearful temperament and stress reactivity may be larger for children whose relationships and self-regulatory competences do not help buffer them from stress evoked by exposure to strange, novel events.

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Table 1a

Means and Standard Deviations for the Four Measures of Fearful Temperament

	<i>M</i>	<i>SD</i>	<i>n</i>
Stranger Fear	−.01	.88	156
Risk Room Fear	.02	.94	162
CBQ Shyness	4.07	1.21	161
CBQ Fear	4.12	1.06	161

Table 1b

Inter-correlations among measures of fear behaviors

	1	2	3	4
1. Stranger Fear	—			
2. Risk Room Fear	.01	—		
3. CBQ Shyness	.39***	.17*	—	
4. CBQ Fear	-.02	.07	.07	—

Note.

*
 $p < .05$.

 $p < .001$.

Table 2

Descriptive Data for Measures of Stress-Sensitive Autonomic and Neuroendocrine Activity

	<i>M</i>	<i>SD</i>	<i>n</i>
Baseline HR	99.15	9.30	147
Response HR	2.72	2.93	147
Baseline VT	6.54	1.15	141
Response VT	0.44	0.04	141
Baseline PEP	87.94	8.73	147
Response PEP	−0.01	3.98	147
Cortisol @ Arrival +24 min	0.078	0.25	144
Cortisol @ Arrival +47 min	0.071	0.28	145
Cortisol @ Arrival +87 min	0.078	0.26	147
Cortisol @ Arrival +109 min	0.081	0.24	150
Cortisol AUC	0.82	5.40	143

Note. VT and PEP Response measures are computed as Baseline – Fear. Cortisol values are recovered from log10 for purpose of presentation. HR = Heart Rate in beats per minute, VT = Vagal Tone in $\ln(\text{msec}^2)$, PEP = Pre-Ejection Period in msec, AUC = Area under the curve, corrected for baseline cortisol, or Cortisol @ Arrival +24 min.

Table 3

Inter-correlations among autonomic and neuroendocrine measures

	1	2	3	4	5	6	7
1. Baseline HR	—						
2. Response HR	-.15 [†]	—					
3. Baseline VT	-.76 ^{***}	.13	—				
4. Response VT	-.15 [†]	-.60 ^{***}	.08	—			
5. Baseline PEP	-.07	-.08	.01	-.01	—		
6. Response PEP	.09	.10	-.14 [†]	-.10	-.10	—	
7. Cortisol AUC	.12	.00	-.04	-.11	.06	.01	—

Note.[†] $p < .10$.^{***} $p < .001$.

Table 4

Pearson Correlation Coefficients between Measures of Fearful Temperament and Stress Reactivity

Cort_{AUC}	PEP response	VT response	
Fearful Temperament	-.01	-.15 [†]	.18*
Stranger Fear	.01	-.12	.12
Risk Room Fear	-.10	-.17*	.07
CBQ Shyness	.04	-.05	.18*
CBQ Fear	.08	.10	.03

Note. Fearful Temperament was the standardized and averaged sum of the observational and parent reported measures. Because children who had never seen the fear video before exhibited larger VT responses, associations with VT response were recomputed without these children. The correlation with Fearful Temperament was $r(101) = -.18$, $p < .07$, while the correlation with Risk Room Fear was $r(101) = -.20$, $p < .05$.

[†]
 $p < .10$.

*
 $p < .05$.