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Inhibited Temperament and Parent Emotional Availability Differentially Predict Young Children's Cortisol Responses to Novel Social and Nonsocial Events

ABSTRACT: *Preschool-aged children (n = 274) were examined in the laboratory to assess behavioral and cortisol responses to nonsocial and social threat. Parents also responded to scales on the Children's Behavior Questionnaire reflecting exuberant approach to novel/risky activities (reversed scored) and shyness. Multimethod measures of Nonsocial and Social Inhibition were computed. Parents and children were observed engaging in a series of interactive tasks and the Emotional Availability scales were scored for parental sensitivity, nonintrusiveness, nonhostility, and structuring. These scores were factored to yield one measure of Parenting Quality. Analyses revealed that Nonsocial and Social Inhibition could be distinguished and that associations with cortisol response were stressor specific. Moderation analyses revealed that parenting quality buffered cortisol elevations for extremely socially, but not nonsocially inhibited children. These findings are consistent with evidence that sensitive, supportive parenting is an important buffer of the HPA axis response to threat in infants and toddlers, and extends this finding to the preschool period. © 2009 Wiley Periodicals, Inc.* Dev Psychobiol 51: 521–532, 2009.

Keywords: *fearful temperament; behavioral inhibition; cortisol; parenting*

INTRODUCTION

The relation between temperamental fearfulness and activity of the hypothalamic–pituitary–adrenocortical (HPA) system is of increasing interest both in developmental psychobiology (Buss et al., 2003; Kagan, Reznick, & Snidman, 1988) and biological psychiatry (Bakshi & Kalin, 2000; Smoller et al., 2005). This interest reflects a growing understanding of the role of corticotropin-releasing hormone (CRH), the CRH-l family of receptors, and glucocorticoids (cortisol in humans) in shaping reactivity of the neural systems involved in orchestrating fear and anxiety (Habib et al., 2000; Kalin, Shelton, & Davidson, 2000; Makino, Gold, & Schulkin, 1994; Merali, Anisman, James, Kent, & Schulkin, 2008). It also reflects an expanding knowledge of factors contributing

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to continuity and discontinuity between early fearful/inhibited temperament and later developmental outcomes, including affective pathology (Biederman et al., 1990; Pérez-Edgar & Fox, 2005; Schwartz, Wright, Shin, Kagan, & Rauch, 2003).

One role of the HPA system is to shape future behavioral and physiological responses to threat through affecting the development of fear-orchestrating brain regions such as the amygdala and its connections with regions in the medial prefrontal cortex (Sapolsky, Romero, & Munck, 2000). Accordingly, it is argued that 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). There is evidence that for boys, at least, reactivity of the HPA axis may be a mechanism through which extreme fearfulness in childhood influences risk for maladaptive behavior later in childhood (see Pérez-Edgar, Schmidt, Henderson, Schulkin, & Fox, 2008).

Studies of behaviorally fearful, inhibited children show that while this trait may be one of the most stable reported in childhood, some children who are extreme in this disposition early in life grow up to be socially outgoing, nonanxious older children and adults (Fox, Henderson, Marshall, Nichols, & Ghera, 2005). Qualities of parenting are believed to influence the stability of this temperamental disposition. Degnan, Henderson, Fox, and Rubin (2008) noted that children who were extremely distressed by novelty as infants and extremely socially inhibited as young preschoolers continued to be socially wary in middle childhood if their primary caregiver was lower in sensitivity, higher in intrusiveness, and higher in overprotective care (see also Rubin, Hastings, Stewart, Henderson, & Chen, 1997). Insensitive, intrusive and overcontrolling parental care is associated with larger and more prolonged cortisol responses to threat among fearful infants (see review, Gunnar & Donzella, 2002). Such findings with human infants are consistent with the large literature in animals demonstrating the role of maternal availability in regulation of the HPA axis (Coe, Mendoza, Smotherman, & Levine, 1978; Levine & Wiener, 1988).

In the present article, we examine associations between temperamental fearfulness and cortisol reactivity among preschool-aged children and examine whether qualities of parent–child interaction associated with secure attachment relationships continue to be associated with a buffering of the HPA axis beyond infancy. Because sensitive, responsive parenting and secure attachment relationships help support the development of emotion regulatory capacities in the child (e.g., Smith, Calkins, & Keane, 2006), we expected that preschoolers in relationships with a more supportive, responsive parent would exhibit a greater capacity to regulate activity of the HPA axis, and that this effect might be pronounced for children who were more temperamentally fearful.

This study builds on findings initially reported on a subset of the sample presented by Talge, Donzella, and Gunnar (2008) that described a preliminary analysis of the associations between behavioral inhibition and cortisol activity in a cohort of 162 children recruited through family based care. Results on the initial cohort showed a modest positive correlation between a global measure of fearful, inhibited temperament to social and nonsocial stimuli with a global measure of cortisol response (area under the curve) in response to a series of challenging laboratory events. Recent research with adults, however, suggests some degree of specificity of cortisol responses for individuals prone to fear reactions to particular types of stimuli. Roelofs et al. (2009) report an association of cortisol responses with avoidance behavior following social threat specifically in adult social phobics but not individuals with no disorder or another anxiety disorder (PTSD). In children, fearful, inhibited behaviors during a nonsocial fear provoking event and a social fear-provoking event are not highly correlated (Buss, Davidson, Kalin, & Goldsmith, 2004; Talge et al., 2008). In the present study we test the notion that cortisol responses to social and nonsocial threats will be associated with social and nonsocial inhibition distinctly.

The present study combines two cohorts of children, one recruited through family child care settings (Kryzer, Kovan, Phillips, Domagall, & Gunnar, 2007) and a second through a university based preschool (Van Ryzin, Chatham, Kryzer, Kertes, & Gunnar, 2009). To finely tune cortisol-fear relations we examine cortisol change over two periods: one with very little social interaction and more challenge to confront novel situations and objects (nonsocial threat), and another involving a good deal of interaction with a stranger (the experimenter), including body contact while the experimenter placed electrodes for autonomic assessment (social threat). We further examine the possible moderating role of sensitive and supportive parental care on children's cortisol responses to the social and nonsocial threats. Based on preliminary results from the initial cohort, we predicted that measures of social and nonsocial inhibition would be distinct. Based on research on cortisol and avoidance behavior in adult anxiety disorders, we hypothesized that elevations in cortisol for children described as socially or nonsocially inhibited would be specific to the type of stressor. Finally, based on previous literature suggesting a protective effect of sensitive caregiving on cortisol responses to threat in infants, we examined whether

sensitive and supportive parent child interactions would buffer cortisol responses for more fearful, inhibited children, either for those children who could be described as socially or nonsocially inhibited.

The children in this report were not preselected for their extreme scores on behavioral inhibition, although much of the work on this construct has been conducted on children preselected to be extremely inhibited versus uninhibited. However, because the sample was large, it allowed us to also examine the association between cortisol response, behavioral inhibition, and the potential buffering impact of parenting quality for children who were at the extremes on the inhibition measures. Therefore, each of the key analyses examined associations for the total group and for extremely inhibited or noninhibited children. We identified the children in the top and bottom 15% of the sample on inhibition (social and nonsocial) and classified these children as ''inhibited'' and ''exuberant.'' While the 15% criterion was somewhat more liberal than that used by Kagan (2001) in defining extreme inhibition and exuberance, it permitted a sufficient number of subjects for analysis.

METHODS

Participants

The sample was comprised of 274 children who ranged in age from 3.23 to 5.69 years (55.1% female; M age = 3.97, SD = .48). To allow a large sample for analysis, the present sample combined children solicited for studies that were run concurrently (Kryzer et al., 2007; Talge et al., 2008; Van Ryzin et al., 2009), one involving children in a university laboratory preschool ($n = 116$) and the other a study of children in family child care ($n = 158$). Before tax, family income (in 25,000 dollar increments) ranged from <$25,000 to >$200,000, with a mean in the $76–100,000 range. Parent education ranged from high school/GED to professional school/doctorate; median education for both parents was a bachelor's degree. The majority of the children (83%) were White, non-Hispanic.

Procedures

The children were accompanied to the laboratory by at least one parent or primary caregiver, usually (88%) the mother. Session times were blocked as morning ($n = 175$, 10:30 am, time of first sample M = 10:43, SD = 0:26 min) or afternoon ($n = 99$, 3:30 pm, time of first sample, M = 15:11, SD = 0:27 min). When the family arrived at the laboratory, consent and the initial salivary cortisol sample were obtained. Following sampling, the parent and child were escorted into testing rooms that were equipped with unobtrusive videocameras to allow for later behavioral coding of child inhibition and parenting quality. The *Risk Room* assessment was completed first (approximately 7 min). 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.

Once in the electrophysiology room, the child was seated in front of a 13″ monitor and the experimenter (E) placed four sensors for cardiac and impedance monitoring (data reported in Talge et al., 2008). The electrodes were placed behind the ear and on the chest, which required the experimenter to lift the child's shirt, wipe the sensor regions with a mildly abrasive gel, Nuprep, and secure the electrodes. As soon as the sensors were placed, the parent departed and a second cortisol sample was collected ($M = 25$ min from Sample 1). [Ten children (3.6%) could not separate and the parent was allowed to stay during the videos.] The child remained in the room with the E, who was seated at the recording console behind the child and the child watched 3 videoclips (see Talge et al., 2008). Between each video clip, the experimenter spoke briefly to the child and encouraged him/her to continue to sit quietly. After the final video, the E removed the electrodes and a third cortisol sample was collected ($M = 25$ min from Sample 2). After this cortisol sample, the child participated in the *Empty Box* vignette (not discussed further in this report) which ended with the E leaving the room. After the E left, a person whom the child had not yet met entered the room for the *Stranger Approach* vignette.

Immediately after Stranger Approach, the child was reunited with the parent for a 30-min parent–child interaction period. The first 10 min was parent–child free play (with various toys and books supplied in a toy box), concluded by a parent-initiated clean-up. This was followed by approximately 10 min each of two structured tasks: making a sno-cone that required the parent to read directions and direct/assist the child in completing the task, and completing a developmentally appropriate puzzle together. Parents were told to participate in the structured tasks however they deemed appropriate. The E was not present for any of the parent–child interaction except to bring supplies for each of the three segments. Following the interaction period, additional assessments not described in this report were completed.

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 Laboratory Temperament Assessment Battery (LabTAB; Goldsmith, Reilly, Lemery, Longley, & Prescott, 1999). Following procedures in the LabTAB manual, children were given 5 min 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). The parent was present in the room at all times, although s/he was asked to interact minimally with the child.

Coding was completed using the LabTAB instructions with data from Phase I used to assess nonsocial fear. Two variables, *Tentativeness of Play* and *Total Play* were used to compute a measure of *Risk Room inhibition*. 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 s. Total Play reflected the duration of play (in seconds) with the objects in the Risk Room. Based on 10% of the sessions, the intraclass

correlations for coder agreement were .95 and .98 for tentativeness of play and total play respectively. To compute *Risk Room Inhibition*, total play was reverse scored, both measures were standardized and averaged, Cronbach's $\alpha = .84$.

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 absent E. The Stranger adopted a neutral expression, queried the child on mundane facts, for example, ''Have you been here before?,'' and engaged in 10–20 s periods of silence following each question. Using the LabTAB instructions, two variables were examined in this report. ''Hesitancy'' or conversational nonresponsiveness to the stranger (on a 0–2 scale), and ''activity decrease'' or the extent to which the child displayed decreased gross motor activity (on a 0–3 scale) were scored during the interval between each prompt, and averaged across the intervals. 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. These measures were standardized and combined to yield a measure of *Stranger Approach Inhibition*, Cronbach's $\alpha = .66$.

Children's Behavior Questionnaire (CBQ). Parents completed the CBQ short form (Putnam & Rothbart, 2006). The CBQ scales factor onto three higher-order factors (Ahadi, Rothbart, & Ye, 1993); one of them, Surgency, served to guide selection of scales for analysis in this report. From the scales which load on Surgency we selected three that exemplified exuberant approach to novel and strange objects: High Pleasure, which assesses the child's enjoyment of highly stimulating and somewhat risky activities, Approach to novel events, and Activity Level, which is highly correlated with the other two scales (see Ahadi et al., 1993). We standardized and combined these into a measure of exuberant approach and reversed the scale to reflect cautious approach to novel objects or events which we labeled CBQ Nonsocial Inhibition (Cronbach's $\alpha = .81$).

Nonsocial and Social Inhibition. To compute multi-method measures of inhibition, we subjected the following measures to a principal component analysis: Risk Room Inhibition, CBQ Nonsocial Inhibition, Stranger Approach Inhibition, and CBQ Shyness. Two factors were obtained accounting for 70% of the variance. Following varimax rotation, factor 1 with an eigenvalue of 1.7, explained 43% of the variance and consisted of Risk Room Inhibition and CBQ Nonsocial Inhibition. Factor 2 with an eigenvalue of 1.1 explained 27% of the variance and consisted of Stranger Approach Inhibition and CBQ Shyness. The two factor scores computed from the analysis were labeled, *Nonsocial Inhibition* and *Social Inhibition*, respectively.

To identify extremely inhibited and uninhibited children for analysis, we identified the top and bottom 15% of the distributions of each of these factor variables for children who provided cortisol samples. Children in this range were labeled ''inhibited'' or ''exuberant.'' This yielded 37 children in each of the four extreme groups: Socially Inhibited, Socially Exuberant, Nonsocially Inhibited, Nonsocially Exuberant. The remaining children were grouped into Average Socially Inhibited and Average Nonsocially Inhibited groups.

Quality of Parental Interaction was evaluated from the 30-min videotaped parent–child interaction using the Emotional Availability scales (EAS; Biringen, Robinson, & Emde, 1998). Data from five dyads were missing because of equipment problems ($n = 2$) or because the dyad spoke a language other than English (two Spanish, one Chinese). Thus, we coded 269 parent–child interaction sessions. We scored the four parent scales on the EAS: sensitivity, structuring, nonintrusiveness, and nonhostility. [Note we also scored the two child scales; however, including them in the analysis with the parent scales did not change the results. Thus to maintain a focus on qualities of the parent's interaction with the child, the child scales were not included in the present article]. Parental sensitivity (9-point scale) is a global measure of the parent's affect and ability to share pleasure in activities with the child, along with appropriate responsiveness to the child's communications, awareness of timing during interactions and transitions, flexibility, acceptance, clarity of perceptions, and appropriate handling of conflict situations. Parental structuring (5 point scale) measures the degree to which the parent appropriately organizes the child's play by providing a supportive framework for interaction without diminishing the child's autonomy. Parental nonintrusiveness (5 point scale) assesses the degree to which the parent is available and supportive to the child without being overprotective, over-stimulating, over-directive, or interfering. Finally, parental nonhostility (5 point scale) measures both overt and covert hostility directed towards the child. Specifically, this scale assesses the degree to which a parent is able to engage with his or her child in a way that is positive in nature and not antagonistic, abrasive, impatient or rejecting. Coders initially scored each of the three 10-min segments then provided the overall score used for analyses that took into account the entire 30 min session. Following training, all coders scored reliability tapes provided by Biringen and were certified as reliable. Based on 18% of the sessions, intraclass correlations were $>.80$ for all coders.

We first examined the four parent scales to determine whether it was psychometrically feasible to enter them separately into regression analyses (i.e., colinearity). They were highly correlated, with nonhostility revealing the lowest correlations with the other measures (r's $= .50–.67$), but this likely reflected the fact that all but 5% of the sample scored between 4 and 5 on this scale. Based on the high inter-correlations, we subjected the four measures to a principal component analysis. Following varimax rotation, one factor with an eigenvalue of 2.77 accounted for 69.3% of the variance. Variable loadings ranged from .78 for nonintrusiveness to .92 for sensitivity. The factor score, labeled *Parenting Quality*, was retained for analysis.

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 needleless syringe and the saliva was expressed into a 1.5 ml Eppendorf Safe-Lock microtube, sealed and frozen at −20°C until assaying. Samples were assayed in duplicate for cortisol using a time-resolved fluorescence immunoassay (DELFIA). Intra- and interassay coefficients of variation were at or less than 6.7% and 9.0%, respectively, and duplicates correlated highly, $r = .997$, $p < .001$. The distribution of values was not positively skewed, as is sometimes the case, thus no log-transformations were applied.

Three samples obtained during testing were the focus of the present report. Sample 1 was obtained soon after arrival. Sample 2 was obtained approximately 25 min later, 20 min after entry into the Risk Room and immediately after placement of electrodes for autonomic assessment. Sample 3 was obtained approximately 20 min later immediately after electrode removal. Peak elevations of cortisol are obtained approximately 20–25 min after the onset of a stressor (Gunnar & Talge, 2007). Every attempt was made to obtain the first sample within 5 min of arrival to the laboratory, thus this level should have reflected activity of the HPA axis during the trip to the laboratory. In contrast, the second sample should have predominantly reflected activity during first 10 min or so in the laboratory. This would include the child's entry into the laboratory, obtaining consent, initial saliva sampling, and entry into the Risk Room. During this period, although the experimenter (E) was present for some of the time, the E's attention was largely focused on the parent, gaining consent, explaining the Risk Room procedures, and showing the parent how to collect saliva samples. The third sample, in contrast, should have reflected events in which the child was heavily involved in interaction with the E, still a relative stranger, while the E attempted to get the child to cooperate with novel and likely strange activities. This included the period when the E had the child sit with her while she read the Curious George ™ story, and the period when the E made contact with the child's body while she placed the electrodes for autonomic recording. This period also included the E directing the mother to leave the room, at which point the child was alone with the E during the video portion of the session.

After determining that none of the key variables were correlated with cortisol levels at laboratory arrival (r's ranged from −.01 to .02), we computed difference scores for Sample 2 minus Sample 1 and Sample 3 minus Sample 2. These were labeled: *Response 1* and *Response 2*, respectively. Distribution of these variables did not violate normality and thus were not log 10 transformed. Twenty-two samples were missing (Response 1 = 11.3%; Response 2 = 9.9%). We chose not to use imputation procedures because cortisol was the key dependent variable. Children with and without missing cortisol data did not differ on any of the other measures. We found no evidence that either Response 1 or 2 differed by whether the child was tested during the morning or afternoon testing block, t's < 1.0, ns. Thus data were not blocked by time of day in subsequent analyses. To determine the number of children exhibiting a biologically significant cortisol stress response, we used the criteria of an increase of at least .10 μg/dl and 10% above baseline levels derived from work on previously used in adult studies (Kirschbaum, Pirke, & Hellhammer, 1993; Kirschbaum et al., 1995).

In addition to the samples obtained in the laboratory, for 104 children drawn from the child care study we had home cortisol measures obtained within several weeks of the laboratory assessment at times that approximated (i.e., 10 AM or 4 PM) the time of laboratory arrival. We compared these values to the initial cortisol levels in the laboratory to determine whether the initial sample might reflect a stress response to laboratory arrival. If so, this would alter our interpretations of the cortisol response variables.

Preliminary Analyses

Using t-tests and Pearson correlation coefficients, we first examined the three laboratory cortisol measures to determine whether they exhibited any gender, parent education, family income, or child age effects. In these analyses, we also examined whether cortisol levels differed by whether the child came from the child care or preschool study cohort. None of these factors were significant, p's > .10. Next we examined Nonsocial and Social Inhibition. Because behavioral or inhibitory control typically increases during early childhood, we tested child age as a potential confound for our measures of inhibition. Child age was not associated with either of the inhibition measures, (p's > .18). Girls scored higher on both of the inhibition measures than did boys (Nonsocial Inhibition, $t = 2.67$, df = 272, $p < .01$; Social Inhibition, $t = 2.55$, df = 272, $p < .05$). In addition, Nonsocial Inhibition was significantly correlated with parent education $r = .21$, df = 266, $p < .001$ and household income, $r = .16$, df = 261, $p < .05$. Finally, we examined the Parenting Quality measure which was found to be associated with parent education, $r = .32$, df = 261, $p < .001$. When entered into the regression models described below, none of these variables was significant ($t > -1.8$ or < 1.8, ns). Thus we do not report results for these potential covariates in this manuscript.

We also examined whether gestational age, illness (parent report that the child had a cold), or medication (12.8% were on Tylenol or medication for ear infection) was associated with any of the measures and found no robust effects. Ten children (3.6%) insisted that their parent remain with them during the videos. These children did not differ from the other children on either measure of cortisol change and or in terms of social inhibition (t's < 1.5 or > −1.5, ns); however, these children were more nonsocially inhibited [$t(272) = -2.69$, $p < .01$]. Recomputing analyses without these 10 children did not alter the findings reported below.

Finally, we examined the subset of the children with a reference home baseline measure at the same time of day as their laboratory cortisol data. Using paired t-tests, we found that all three of the laboratory cortisol measures were lower, not higher, than home reference baseline levels. The laboratory minus home reference mean difference were −.03, −.11, and −.12 μg/dl for the first, second, and third laboratory samples, respectively. The t's with df's of 103 were −1.9, $p = .06$, −4.2, $p < .01$, and −3.8, $p < .01$, respectively. Thus there was no evidence that cortisol was already elevated at the time of laboratory arrival.

Table 1. Descriptive Data

Variables	*N*	*M*	*SD*
Time 1: Cortisol in μg/dl	252	.108	.117
Time 2: Cortisol in μg/dl	243	.097	.116
Time 3: Cortisol in μg/dl	249	.099	.129
Nonsocial Inhibition	274	.003	.783
Social Inhibition	274	.013	.843
Parenting Quality	269	.000	1.000
Sensitivity (1–9)	269	6.676	.951
Structuring (1–5)	269	4.392	.468
Nonhostility (1–5)	269	4.522	.457
Nonintrusive (1–5)	269	4.511	.475

Note: Cortisol variables are reported in a linear scale to three decimal places. Valid *N* (listwise) = 230.

RESULTS

Descriptive Analyses of Cortisol Change

A repeated measures analysis of variance, RM-ANOVA, was computed entering the first, second, and third sample (see Tab. 1 for descriptive data). A significant quadratic term was obtained [$F(1, 229) = 7.37$, $p < .01$]. Overall, cortisol levels decreased significantly from Sample 1 to Sample 2 [M change $= -.014$, $t(242) = -2.10$, $p < .05$], while the change from Sample 2 to Sample 3 was not significant [M change $= .004$, $t(246) = .87$, ns]. We then examined the distributions of Response 1 and Response 2 values. For Response 1, only 26% of the sample showed any increase in cortisol and only 4% met criteria for a stress response. For Response 2, only 33% showed any increase in cortisol, and only 6% met criteria for a stress response. Of the 26 children showing stress magnitude elevations on either Response 1 or Response 2, only 2 did so consistently on both measures.

Descriptive Analysis of Social and Nonsocial Inhibition

Table 1 also shows the means and standard deviations for the two inhibition measures. The correlation of these two measures was significant but modest, $r = .28$, $n = 274$, $p < .001$. We examined whether children who might be described as Inhibited (top 15%) or Exuberant (bottom 15%) were consistently classified across the two types of inhibition measures. Twenty-six percent of the children with cortisol data were classified as Inhibited on one or the other inhibition measures, but only 4.1% were inhibited on both measures. Twenty-five percent of children were classified as Exuberant on either of the two measures, but only 4.9% were exuberant on both measures.

Association of Inhibition With Cortisol Change

Pearson correlations were computed between the two inhibition measures and the two cortisol change measures. As expected, Response 1 was significantly, but modestly, associated with Nonsocial Inhibition ($r = .17$, $n = 243$, $p < .01$) but not with Social Inhibition ($r = .09$, $n = 243$, ns). In contrast, Response 2 was significantly, but modestly, associated with Social Inhibition ($r = .16$, $n = 247$, $p < .05$) but not Nonsocial Inhibition ($r = -.02$, $n = 247$, ns). Next, we examined whether these associations were being carried by children on either extreme of the inhibition measures. When we removed the Nonsocially Inhibited children, the correlation between Nonsocial Inhibition and Response 1 was reduced to $r = .10$, $n = 206$, ns. Removing the Socially Inhibited children reduced the association between Social Inhibition and Response 2 to $r = .07$, $n = 210$, ns. When removing the Exuberant children, in contrast, correlations did not change for Nonsocial Inhibition with Response 1 (r's $= .18$ $p < .05$) and for Social Inhibition with Response 2 $r = .14$, $p < .05$). Thus, it was the more extremely fearful/inhibited children who carried these associations.

Parenting Quality and Associations With Inhibition and Cortisol Response

Descriptives for the Parenting Quality measure along with descriptive data on the four EA scales on which this measure was based are also shown in Table 1. The distribution of EA scores was similar to other reports of typically developing children (e.g., Aviezer, Sagi, Joels, & Ziv, 1999; Biringen, Robinson, & Emde, 1994; Ziv, Aviezer, Gini, Sagi, & Koren-Karie, 2000). The mean for sensitivity was close to 7 on the 9-point scale, while the means for the other measures were above 4 on 5-point scales. Parenting Quality was not significantly correlated with Nonsocial Inhibition, $r = .10$, $n = 269$, ns, but there was a small positive correlation with Social Inhibition, $r = .12$, $n = 269$, $p < .05$. We also examined whether parenting quality differed when the extremely inhibited or exuberant children were contrasted with the remainder of the children. There were no significant differences in parenting quality between the Exuberant, Average, and Inhibited children for either the Social ($F(2, 239) = .52$, ns) or Nonsocial ($F(2, 235) = .18$, ns) Inhibition measures. Parenting quality was then examined with the cortisol response measures. Neither correlation was significant, r's were .04 and .00, respectively.

Moderation Analyses

We then turned to the question of whether Parenting Quality might moderate the associations between

inhibition and cortisol change. We approached this first for the total sample and then for the children who were extreme on each of the dimensions of inhibition. For the total sample, we computed two regression models, one each for Response 1 and Response 2. In each, we entered the inhibition measures (i.e., Nonsocial Inhibition and Social Inhibition, respectively), Parenting Quality, and the interaction of Parenting Quality by the relevant inhibition measure. For Response 1, the overall model was significant [$F(3, 234) = 8.01$, $p < .001$, $R^2 = .09$] and the interaction term, Nonsocial Inhibition x Parenting Quality, was significant [$t = 4.03$, $p < .001$]. However, regression diagnostics indicated that the interaction effect was due to one child. Removing this child from the analysis yielded a nonsignificant interaction term. For Response 2, the overall model was only marginally significant [$F(3, 238) = 2.47$, $p = .062$, $R^2 = .03$], and the interaction term, Social Inhibition × Parenting Quality, was not significant [$t = -.81$, ns].

Next we turned to the extreme group analysis. We computed ANOVAs contrasting the Inhibited (top 15%) and Exuberant (bottom 15%) of children with the remaining (Average) children by quality of parenting (median split). Of concern were interaction effects. For Nonsocial Inhibition, the interaction of Extreme Nonsocial Inhibition and Parenting Quality was not associated with Response 1, $F(2, 232) = 1.65$, ns. For Social Inhibition, we obtained a main effect of Parenting Quality (median split), $F(1, 236) = 4.5$, $p < .05$ and Extreme Social Inhibition, $F(2, 236) = 7.05$, $p = .001$, that was qualified by a Parenting Quality by Extreme Social Inhibition interaction, $F(2, 236) = 5.00$, $p < .01$, see Figure 1. Post-hoc tests with Bonferroni correction indicated that the Socially Inhibited children significantly differed from both the Socially Exuberant ($p = .01$) and Average ($p = .01$) children, whereas there was no significant difference between the Socially Exuberant and Average children. Tests for simple effects within lower versus higher Parenting Quality, indicated no effect of extreme Social Inhibition on cortisol rise for children whose parents scored higher on Parenting Quality, $F(2, 120) = 1.29$, ns whereas there was significant effect of Extreme Social Inhibition among the children with lower Parenting Quality, $F(2, 116) = 6.73$, $p < .01$. Post-hoc tests showed significant difference between the Socially Inhibited children and both the Socially Exuberant ($p < .01$) and Average ($p < .05$) groups. Tests for simple effects within the Socially Inhibited group also indicated larger cortisol increases for the Socially Inhibited children with lower Parenting Quality, $F(1, 34) = 3.89$, $p = .06$.

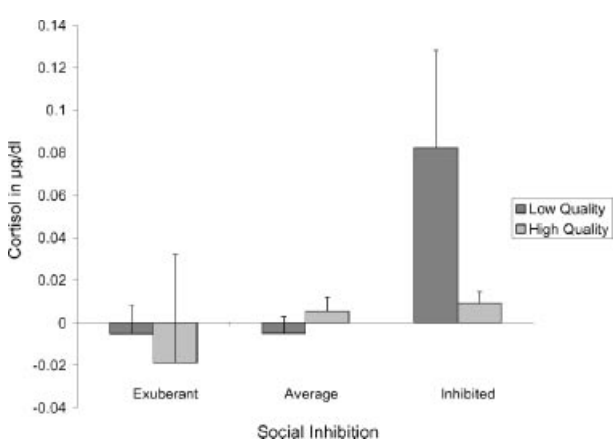

FIGURE 1 Cortisol response in μg/dl to social challenge comparing Socially Exuberant, Average and Socially Inhibited children as a function of median split on Parenting Quality as assessed during parent–child interaction tasks. N's = 23, 14, 82, 87, 14, and 22.

Because most of the children did not exhibit a biologically significant stress response of the HPA axis, we extended our analysis to examine whether Parenting Quality buffered Extreme Social Inhibition contrasting children who did and did not produce a stress response to the social stressor period (Response 2). First we examined the 6% of the sample who exhibited a biologically significant Response 2. Of these children, 80% had a parent who scored below the median on parenting quality, Fisher exact $p = .004$. Then we limited this analysis to the children who were labeled Socially Inhibited on the Social Inhibition measure ($n = 37$), of whom 14% versus 4% of Average and 0% of the Exuberant children exhibited a biologically significant Response 2. Among the children classified as both Socially Inhibited and exhibiting a biologically significant response ($n = 5$), all had a parent who scored below the median on parenting quality. Thus, focusing on extreme groups in either social inhibition or social stressor cortisol response, or both, indicated an effect of parenting quality, while this effect was not observed when extremes were not the focus of analysis.

DISCUSSION

The results of the present study provide evidence of stressor-specific associations between dimensions (nonsocial and social) of temperamental fearfulness/inhibition and reactivity of the HPA axis. It also provides evidence that for children extremely high on social inhibition, sensitive, responsive and supportive parenting continues to play a role in regulating reactivity of the HPA axis during the preschool years, similar to what has been documented in studies of infants and toddlers. However, the effects of parental buffering of the HPA axis were only observed when extreme groups were the focus of the

analyses. Each of these aspects of the results will be discussed.

Previous studies have shown that behavioral inhibition to the unfamiliar is not a unitary trait, but differs if the unfamiliar, novel or strange experiences are primarily nonsocial versus social (Buss et al., 2004). The results of the present study were consistent with these findings. Our multi-method measure of nonsocial inhibition was composed of how much and how tentatively children played in the Risk Room that confronted the child with opportunities to engage in physically risky activities (climb stairs, jump onto a mattress, approach a gorilla mask on a stick, stick one's hand into the ''mouth'' of a box with big teeth) combined with parent report measures from the CBQ (reversed) reflecting low approach and pleasure in novel, risky or high stimulating activities. This measure can be viewed as indexing the child's inhibition in face of objects or events that might produce physical harm to the self. In contrast, our measure of social inhibition was comprised of inhibited responses during the approach of a strange adult and parent reports on the shyness scale of the CBQ. This measure can be interpreted as indexing the child's caution in the face of social threats to the self. The fact that these two measures were only very modestly associated and the fact that very few children were extreme on both measures suggests that physical and social threats to the self may be differentially organized (see discussion by Dickerson & Kemeny, 2004).

The need to differentiate social and nonsocial inhibition was further supported by their distinct associations with the cortisol response measures. Thus nonsocial inhibition was associated with the cortisol response to the risk room (Response 1), while social inhibition was associated with the cortisol response to the period when the experimenter interacted and also prepared the child for the electrophysiological assessment (Response 2). These modest associations suggest that neither social nor nonsocial inhibition is associated with a generally more reactive HPA axis but that the behavioral inhibition-cortisol relation may be specific to the nature of the stressor.

The modest size of the correlations likely reflected the fact that these situations did not provoke a stress response of the HPA axis in most of the children tested. Indeed, for most of the children, cortisol decreased over the risk room period and showed little change over the period of interaction with the experimenter. Declining values over the first 30 to 40 min in the laboratory are also typical in studies of older children and adults; however, in these studies initial laboratory levels are elevated over home baselines, potentially reflecting a negative feedback mediated acclimation of the HPA system following a mild stress evocation to laboratory arrival (e.g., Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). Young children, in contrast, tend to exhibit lower cortisol levels during laboratory assessments than under home baseline conditions (see review, Gunnar, 2003). Based on data available for a subset of our sample, our observation of lower cortisol levels in the laboratory relative to home baseline levels is consistent with prior reports of young children. Thus it is unlikely that the decrease over the risk room period reflected negative feedback regulation of the HPA axis in response to an elevation evoked by coming to the novel laboratory setting. Why cortisol levels decreased in response to the novel and strange risk room setting is a puzzle, but is consistent with findings obtained in many studies of young children (e.g., Gunnar, Talge, & Herrera, 2009). It may be that because children had control over approach to these strange objects and the parent was present, the majority of children had the resources to cope with its threatening elements. But while this might explain the lack of elevation in cortisol, it does not provide a satisfying explanation for the declining values. We readily admit that this pattern, seen in so many studies of infants and young children, is in need of explication.

Despite the fact that most of the children did not exhibit a stress response to the risk room or to interacting with the unfamiliar adult during electrode preparation, some of the children did exhibit stress magnitude elevations in cortisol. When we removed children characterized as extremely high on the Nonsocial Inhibition measure, the association of Nonsocial Inhibition with Cortisol Response 1 approached zero, despite the fact that 85% of the children were still in the analysis. The same pattern was observed for the association between Social Inhibition and Cortisol Response 2 when the extremely Socially Inhibited children were removed. Notably, the pattern of association did not change for either Social or Nonsocial Inhibition when Exuberant children were removed. Thus while the two stressor periods were not potent enough to activate the HPA axis for most children, they did evoke responses that were associated with individual differences in temperamental fearfulness/inhibition, and these associations reflected the responses of the most extremely nonsocially and socially inhibited children, respectively. We can only speculate that had more evocative stressors been used which were capable of provoking a stress response in more of the children, then the correlation between cortisol reactivity and inhibition might have been stronger across the whole range of variations in inhibition. Nonetheless, the present results suggest that even such mild stressor conditions are capable of provoking elevations in cortisol for children who are more fearful/inhibited, but that social and nonsocial stressors specifically provoke cortisol responses among socially and nonsocially inhibited children, respectively.

Turning now to the parenting data, as shown in Table 1, we expected based on the previous literature (e.g., Degnan et al., 2008) that more inhibited children would have parents who scored lower on parenting quality. In fact, we found a modest linear association between parenting quality and social inhibition, which is consistent with arguments that highly protective parenting may foster greater fearfulness in children (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Rubin, Nelson, Hasting, & Asendorpf, 1999). Because the measures of inhibition and parenting were obtained in the same laboratory session, we cannot determine whether more sensitive, responsive and supportive parenting encouraged more socially inhibited behavior in the children, or whether the parents may have been responding to their children's social anxiety during the laboratory testing by being more sensitive and responsive to their signals during the parent–child observation period. The analysis of the cortisol data nonetheless suggests that the parent's responsiveness to their extremely socially inhibited children was associated with a buffering of these children from exhibiting a stress response of the HPA axis.

We obtained no evidence of social buffering by higher quality parenting when we examined the whole range of variation in either nonsocial or social inhibition. But when we turned to the extreme group analyses, we found clear evidence that higher quality parenting was buffering cortisol elevations for the socially inhibited children. Specifically, cortisol increases over the social stressor period were larger for extremely socially inhibited children whose parents scored below the median on parenting quality compared to those whose parents scored above the median. For children whose parents scored above the median on parenting quality, we observed no difference in cortisol response as a function of extreme social inhibition. Finally, all of the children rated extremely high in social inhibition who also exhibited a significant stress response to the social stressor period had parents who scored below the median on parenting quality. Thus, even within the generally high range of parenting quality observed in this study, lower parenting quality was associated with a failure of social buffering of the HPA system among extremely fearful or inhibited children.

The evidence for social buffering of the HPA axis by parenting quality is consistent with research with infants and toddlers (for review, see Gunnar and Donzella, 2002). However, the psychological processes reflected in this finding are still unclear. In the infant and toddler work, it has been assumed that the parent's presence along with active and supportive engagement with the child is necessary to produce the buffering effect. Indeed, a buffering effect is not observed among toddlers adapting to a new child care setting once the parent is no longer present (Ahnert, Gunnar, Lamb, & Barthel, 2004). By early childhood, however, a history of supportive and sensitive parenting helps children develop better self-regulatory capacities (e.g., Smith et al., 2006). This suggests that for preschoolers, sensitive, supportive care may buffer stress responses even in the parent's absence. In the present study, although the parent was present during all of the risk room period and during the time that the experimenter prepared the child for electrophysiological assessment, the parent was relegated by the research protocol to a passive role in which she (or he) was asked to respond minimally to the child's signals. And, following electrode placement, the parent left the room. Thus, in the present study it seems unlikely that the buffering function associated with higher parenting quality reflected the parent's active regulation of the child's internal state. And, while it may have been mediated by the child's emerging self-regulatory capacities, these regulatory capacities were not being reflected in fear behavior. Thus, some other mechanism is likely involved that, in a sense, is preventing more extreme fear reactions from activating the HPA axis. Possible mechanisms include a history of responsive care in development of stress counter-regulatory activity of the parasympathetic system (Calkins, Graziano, Berdan, Keane, & Degnan, 2008; Kennedy, Rubin, Hastings, & Maisel, 2004; Porges, 1995).

There are a number of limitations to this study that need acknowledgement. First, this was a relatively low-risk, middle-class, and Caucasian sample. These sample characteristics are relatively consistent with past research on behavioral inhibition (see Fox et al., 2001; Kagan, Snidman, Kahn, & Towsley, 2007). Nonetheless, generalizability of the findings must be constrained by these factors. Second, the experimental tasks used in this study did not provoke significant elevations in cortisol for most of the children. The majority of the children exhibited stable or decreasing levels of cortisol over the session. The laboratory tasks may not have been optimal to detect associations with behavioral inhibition or the role of parenting quality in moderating HPA axis reactions to threat. Third, we had a limited range of parenting quality scores. Consistent with the low risk nature of the sample, scores on the Emotional Availability Scales were high and variability was generally low. Another reason why we were not able to evoke HPA stress responses in most of the children may have been the relatively high parenting quality observed in our sample. It may be that all of the children experienced a high degree of parental stress buffering. Moreover, although our distribution of scores was similar to other reports of typically developing children, the nature of parenting effects in clinically ascertained (e.g., abused/neglected) samples may differ. In addition, children were not preselected for extreme

scores on behavioral inhibition as in some studies. Preselecting individuals for extreme social or nonsocial inhibition and exuberance may have increased the magnitude of effect. However, effects even of small magnitude on biological response to threat in an unscreened sample are noteworthy. Finally, from the standpoint of assessing HPA axis reactions to threat, it would have been preferable to have only physically healthy, nonmedicated children who had not been exposed to antenatal steroids due to prematurity. While we examined the data with and without these children and noted no significant change in results, excluding all these children a priori might have helped reduce unexplained sources of variance. Doing so, however, would have further reduced the representativeness of the sample and decreased power to detect statistical significance.

With these limitations in mind, the results clearly showed that the HPA axis is sensitive to variations in behavioral inhibition and provide two novel findings. Associations between cortisol and behavioral inhibition appear specific to the source of threat or challenge and should not be generalized across nonsocial and social threats. The results further showed that parenting quality buffers HPA axis response to social threat among preschool-aged children, similar to results previously reported for infants and toddlers. This finding raises questions about whether the sequelae of extreme social inhibition, particularly affective pathology, will differ for children who receive higher versus lower quality parenting. Longitudinal studies are needed to address this question.

NOTES

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