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DEVELOPMENT AND VALIDATION OF A DEVELOPMENTALLY-SENSITIVE TASK FOR THE INDUCTION OF STRESS IN PRESCHOOL-AGED CHILDREN

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DEVELOPMENT AND VALIDATION OF A DEVELOPMENTALLY-SENSITIVE TASK FOR THE INDUCTION OF STRESS IN PRESCHOOL-AGED CHILDREN

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Abstract

This study examined the validity of a novel task developed to elicit cortisol reactivity in a group of 215 preschool-aged children. Children participated in a standardized stress task during a home visit. The task was videorecorded for coding of child expressions of positive and negative emotions. Salivary cortisol samples were obtained at baseline and 10, 20, 30, 40, and 50 minutes post-stress. Statistically significant increases in cortisol levels from baseline were found followed by a significant decline defining a quadratic function. Children exhibited a significant increase in negative emotions and a decrease in positive emotions from baseline to the stressful portion of the task. Negative emotions expressed during the task predicted a significantly greater cortisol slope, suggesting a greater increase in cortisol when higher levels of negative emotions were exhibited. No sex differences were found on either child emotionality or on indices of cortisol reactivity to the task. Results confirm that the task successfully elicited the hypothesized cortisol response in three-year-old children.

Keywords: HPA; cortisol; stress; child; developmental; multi-level modeling

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Development and Validation of a Developmentally-Sensitive Task for the Induction of Stress in Preschool-Aged Children

Exposure to stressors and sensitivity to stress have been strongly implicated in the etiology of major depression and anxiety disorders (Heim & Nemeroff, 2001; McFarlane et al., 2005). The hypothalamic-pituitary-adrenal (HPA) axis is activated following exposure to stressors, and this HPA response is excessive in many individuals diagnosed with major depression, as well as in some with anxiety disorders (Barden 2004; Risbrough & Stein, 2006; Shea, Walsh, MacMillan, & Steiner, 2005). In addition, HPA reactivity/recovery from stress may reflect the activity of stress-sensitive physiological systems that correspond to more trait-like individual differences in emotional reactivity (Gunnar & Talge, 2005; Rothbart, Derryberry, & Posner, 1994). Theoretical and empirical work suggest that not all individuals who are stress-sensitive will develop problems and that not all individuals who experience adversity during development will develop problems (Belsky & Pluess, 2009; Burke & Elliott, 1999; Caspi et al., 2003; Ellis & Boyce, 2008; Morris, Ciesla, & Garber, 2010), indicating that context and biological sensitivity interact to predict adverse outcomes. Ellis and Boyce (2008) call this susceptibility to one's environment 'biological sensitivity to context" and have argued that such sensitivity can both augment and reduce risk for negative outcomes. For example, a contextually-sensitive individual reared in an enriched family environment may benefit from such enrichment more so than a less sensitive individual, resulting in highly positive outcomes. However, such sensitivity also means that this same child raised in a context of adversity will experience relatively severe negative outcomes relative to a less sensitive individual. Recent research demonstrates that early intervention is widely beneficial for many child outcomes and that preventative strategies can be employed when markers of pathology risk can be identified (Gwynne, Blick, & Duffy, 2009; Lakes et al., 2009). Being able to index biological sensitivity is an important goal for developmental psychopathologists, as this could aid prevention and early intervention efforts by facilitating the identification of those children most likely to be strongly impacted by adversity. However, the question of how to best index such sensitivity remains open. Interest in the putative role of genetic polymorphisms in reflecting biological vulnerability has generated a large body of research in recent years (Haier, Buchsbaum, DeMet, & Wu, 1988; Johnson, Cuellar, & Miller, 2009; Nantel-Vivier & Pihl, 2008). However, a rich research tradition has also focused on the role of cortisol (Caspi et al., 2003; Hinkelmann et al., 2009). In particular, the work on cortisol in adult psychopathology has led investigators to attempt to identify early emerging disruptions in this system (Dougherty, Klein, Olino, Dyson, & Rose, 2009; Lopez-Duran, Hajal, Olson, Felt, Vazquez, 2009). Cortisol is a glucocorticoid produced by the adrenal glands, one of the body's primary stress hormones. The trajectory of cortisol following a stressor may have important implications for adjustment, as the failure of the cortisol system to successfully downregulate post-stressor leads to increased exposure to the hormone. This increased exposure may have detrimental effects on brain tissue as well as on overall mental health (de Felice et al., 2008; Gunnar & Talge, 2005; Pacak et al., 2002).

Elucidating ways in which psychological stressors activate the cortisol system is important in mental health research for at least two reasons: 1) psychological stressors affect physiology by activating specific cognitive and affective processes and their corresponding central nervous system pathways, and finding specific stressful circumstances that trigger this reaction may inform how the activation proceeds; and 2) HPA activation feeds back into cognitive and affective processing and has been associated with problems such as depression (Dickerson & Kemeny, 2004). In recognition of this importance, many studies have examined cortisol reactivity using laboratory tasks and salivary cortisol as an index of HPA reactivity. However, the methods used to elicit cortisol have varied widely in the literature. This variability may result from early conceptualizations of stress (e.g., Selye, 1956), which held that stress responses were non-specific, such that any stressor, whether psychological or physiological, had the potential to elicit a physiological response. However, more recent research indicates that specific characteristics of tasks increase the likelihood that a pronounced cortisol response will be elicited. In early work, Rose (1980) posited that tasks that were novel to participants were more likely to activate the cortisol system, whereas other researchers suggested that unpredictability (Mason, 1968), lack of control over task outcome (Henry & Grim, 1990; Saplosky, 1993), and threat (Blascovich & Tomaka, 1996; Dienstbier, 1989) were superior means of eliciting a cortisol response. Dickerson and Kemeny's (2004) meta-analysis addressed the issue of which elements of laboratory tasks are most strongly associated with a cortisol response. This meta-analysis of 208 laboratory studies of nonclinical samples of adults synthesized decades of research using stress manipulations to elicit cortisol reactivity and recovery. Results indicated that tasks that incorporated a combination of social-evaluation, perceived uncontrollability, and motivated elements produced the greatest and most prolonged cortisol response, regardless of the type of task (e.g., cognitive; public speaking/verbal interactions; public speaking/cognitive combination; noise exposure; emotion induction) or the length of the stressor (i.e., the duration of stress exposure was not associated with the magnitude of the cortisol response).

To clarify, social-evaluative tasks are those in which participants are asked to perform some type of task, and are told that their performance will be observed and evaluated by others (typically laboratory confederates). Such tasks include the Trier Social Stress Test (TSST) and the Ewart Social Competence Interview, in which participants are evaluated on their performance by an audience or are otherwise exposed to possible negative social comparisons, and/or performance is captured on permanent record (e.g., written or video-recorded) (Dickerson & Kemeny, 2004). Dickerson, Gruenewald, and Kemeny (2004) proposed that it is the self-appraisals generated when under social evaluative pressure that lead to negative emotional experiences (e.g., shame) which, in turn, produce the neuro-cognitive cascade resulting in HPA axis activation. This proposition is supported by research by Mills, Imm, Walling, and Weiler (2008). In fact, tasks employing social-evaluative threat have effect sizes three times that of tasks that do not include this element (Dickerson & Kemeny, 2004). Regarding perceived uncontrollability, tasks often incorporate unavoidable failure or lack of control over task outcome (Cavanagh & Allen, 2008; Pruessner, Hellhammer, & Kirschbaum, 1999) toward the goal of inducing low efficacy or helplessness in participants. For example, manipulation of time constraints such that participants will not have sufficient time to complete a task, regardless of effort expended, can achieve this goal (Dickerson & Kemeny, 2004). Interestingly, perceived uncontrollability alone may not be sufficient to elicit a prominent psychophysiological response; rather, perceived uncontrollability in the context of a desirable goal that is threatened by poor performance appears most likely to elicit a strong cortisol response (Dickerson & Kemeny, 2004). One way of increasing performance motivation, especially in studies of young children, is by having a desirable prize contingent on successful completion of the task.

Tasks that incorporate these elements account for up to 26% of the between-studies variability in effect sizes regarding cortisol reactivity (Dickerson & Kemeny, 2004). Furthermore, Dickerson and Kemeny (2004) showed that stressors containing all three of these elements also influence the recovery of the cortisol system; only social-evaluative and uncontrollable tasks produced a prolonged elevation of participants' cortisol levels. Thus, tasks that incorporate these elements appear most likely to facilitate investigations of cortisol response and recovery in adults.

For researchers interested in the role of cortisol reactivity in developmental psychopathology, the question remains whether these same factors are applicable to eliciting cortisol reactivity in young children. Similar elements have been incorporated in tasks used with older children and adolescents, such as the TSST for Children (Gunnar, Frenn, Wewerka, & Van Ryzin, 2009), and such tasks have yielded intriguing results. However, research on psychophysiological reactivity in younger children, with some exceptions (Lewis & Ramsay, 2002), often uses tasks that elicit fear (e.g., exposure to fear-inducing stimuli such as strangers and/or separation from caregivers) (Blair et al., 2008; Dougherty et al., 2009; Talge, Donzella, & Gunnar, 2008), or frustration (Lopez-Duran et al., 2009), possibly because this type of emotional response is typically easy to elicit in children. Unfortunately, a meta-analytic study comparable to that done by Dickerson and Kemeny has not been conducted on cortisol research on children. Nevertheless, it seems reasonable to develop developmentally appropriate downward extensions of tasks that are known to elicit cortisol responses in older samples, as longitudinal research aimed at examining changes in cortisol reactivity across development will be hampered by the use of tasks that differ widely in terms of the nature of the stress manipulation. Developing tasks that elicit cortisol reactivity in young children that map well onto tasks validated as effective in older age groups will facilitate investigations of continuity and discontinuity of psychophysiological reactivity from early childhood into the age of risk for "stress-reactive" disorders such as depression.

Despite the absence of an extensive literature using such tasks in young children, research on normative child emotional development suggests that children may be capable of experiencing the same emotional responses to stressors as adults in the context of developmentally sensitive laboratory methods. Research examining child conscience in relation to guilt suggests that children are able to appraise their behavior through the eyes of others as early as 22 months of age, and that the degree to which children express guilt is moderated by both child temperament and level of self-development and mothers' socialization style (Kochanska, Gross, Lin, & Nichols, 2002). Indeed, theory of mind researchers have established that children as young as three and four can understand the intentions of others, which is crucial in perceiving that a social evaluation is indeed taking place by an observer (Call & Tomasello, 1998). While guilt and shame are thought to be discrete emotions (Kochanska et al., 2002), the two are sufficiently related to allow investigators to conclude that developmentally appropriate tasks can elicit such emotions in early childhood.

The results of research examining children's self-conscious emotions (i.e., embarrassment and shame) and how these relate to differences in cortisol responses to stress, suggest that children do indeed engage in self-appraisal, and that the degree and type of self-appraisal affects cortisol reactivity (Lewis & Ramsay, 2002). In particular, even very young children (e.g., four-year-olds) have been shown to exhibit negative self-evaluations, embarrassment, and shame during failure tasks. In fact, the extent to which children express such behaviors is related to cortisol response (Lewis & Ramsay, 2002). Timely, research by Lewis and Ramsay (2002) contrasted embarrassment in failure situations, when task outcomes are uncontrollable and cannot be completed on time, with what the authors referred to as exposure embarrassment, where children are the object of attention but are not receiving negative feedback. Exposure embarrassment was not associated with increases in cortisol, suggesting that, like the literature on adults, the social-evaluative nature of the task and negative feedback was critical to producing increases in cortisol (Lewis & Ramsay, 2002).

This research indicates that socially evaluative, uncontrollable tasks akin to those shown to elicit a cortisol response in adults (Dickerson & Kemeny, 2004) may be successfully adapted for use with young children. However, additional methodological issues remain. These are largely centered on issues related to obtaining cortisol samples and to timing of sampling procedures.

Cortisol Sampling

In the large literature examining change in levels of cortisol in response to a laboratory stressor, the vast majority of studies have assessed cortisol at two time points (Earle, Linden, & Weinberg, 1999; Matthews, Gump, & Owens, 2001; Roy, Kirschbaum, & Steptoe, 2001; Smeekens, Riksen-Walraven, & van Bakel, 2007) (for several recent exceptions, see Mills, Imm, Walling, & Weiler, 2008; Zoccola; Dickerson & Zaldivar, 2008). While such procedures minimize expense and facilitate participant compliance, especially with respect to child participants who may find repeated sampling aversive, this practice has almost certainly hampered the ability to accurately capture most participants' peaks in cortisol response. In other words, individual differences in when peak responses occur may be "missed" when only a single sample is collected post-stress. Additionally, obtaining a minimal number of samples hinders the ability to characterize post-stress downregulation. Research has suggested that peak cortisol response is a good predictor of recovery time (i.e., greater peak levels are associated with a slower return to baseline) (Gunnar, 1986); however, it is also possible that some stress tasks best capture an impairment in the ability to downregulate the HPA system, resulting in prolonged cortisol elevations that are not dependent on peak response (Linden, Earle, Gerin, & Christenfeld, 1997). Without sufficient sampling of cortisol over time, it is difficult to disentangle these two competing hypotheses.

Additionally, it is likely that individuals vary in terms of how rapidly a maximum cortisol response is expressed post-stressor (Gunnar & Talge, 2005). In fact, Lewis and Ramsey (2003) reported equal proportions of infants peaking at 15, 20, and 25 minutes post-stressor, with some exhibiting a peak as late as 30 minutes post-stress. Studies with older toddlers have found peaks as late as 40 minutes post-stressor (Goldberg et al., 2003) with durations to a peak response being influenced by the type of stressor administered (e.g., fear versus frustration, with the former tending to elicit a relatively early or rapid peak when compared to the latter) (Lopez-Duran et al., 2009). Capturing this variability by obtaining multiple samples post-stress is critical, not only to accurately index participants' peak responses, but to permit an investigation of whether such variation is meaningful with respect to adverse outcomes and to capture the trajectory of an individual's cortisol recovery over time. In addition, there are implications of slow versus quick response in terms of cortisol reactivity (Gunnar & Talge, 2005), which can be more accurately assessed with multiple samples post-stressor.

In addition to the nature of the stress task and sampling over time, there are other considerations to take into account when planning to measure psychophysiological reactivity via cortisol response. Cortisol varies naturally throughout the day on a diurnal rhythm; peak levels are reached at the time of awakening, and the lowest levels are seen after the onset of sleep (de Weerth, Zijl, & Buitelaar, 2003; Gunnar & Talge, 2005). This decrease in cortisol throughout the course of the day can be seen in children as young as 6 weeks of age (Gunnar & Talge, 2005) although it is not present at birth (de Weerth et al., 2003). Basal (morning) levels of cortisol have been linked to the heritability or stability of the HPA system, while samples taken later in the day when levels have declined are best used for analyzing reactivity of the system to stressors (Gunnar & Talge, 2005). This information outlines how crucial it is to control for time of day when assessing cortisol reactivity, preferably by conducting sampling procedures at the same time of day with study participants. Additionally, since cortisol secretion is relatively stable across the afternoon, this time of day is optimal for sampling as the effects of normative variation are minimized (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007).

Other methodological considerations include where assessment of cortisol reactivity takes place, and the method of assessment. Many previous studies have assessed cortisol reactivity in children in laboratory settings (Earle et al., 1999; Matthews et al., 2001; Roy et al., 2001; Smeekens et al., 2007). While laboratory settings have advantages, this approach raises the concern that the novelty of the setting itself might influence children's cortisol levels. Studies by Tottenham, Parker, and Lui (2001) and others support this idea by showing an increase in baseline cortisol when comparing samples collected in the home to those collected in a laboratory setting (Gunnar & Talge, 2005). The opposite effect has been seen in younger children (Legendre & Trudel, 1996; Goldberg et al., 2003). Indeed, these studies suggest that baseline samples in many studies may reflect a psychophysiological reaction to coming to a laboratory, and further indicate that testing done in the home may better identify true increases and recoveries in cortisol levels in response to stressors of interest.

Most studies of children rely on cortisol samples obtained from saliva, a valid and reliable indicator of cortisol response, as cortisol is secreted into the saliva via the largest of the salivary glands, the parotid (Walker, Riad- Fahmy, & Read, 1978). Salivary cortisol reflects the unbound or biologically active fraction of the hormone (Kirschbaum & Hellhammer, 1989). Salivary cortisol assays are easily available and demonstrate an efficacious and efficient means of obtaining information on cortisol reactivity (Gunnar & Talge, 2005). Studies have consistently reported high correlations between serum and saliva cortisol, indicating that salivary cortisol levels reliably estimate serum cortisol levels (Francis et al., 1987; Hiramatsu, 1981; Vining, McGinley, Maksvytis, & Ho, 1983). While saliva sampling is undoubtedly more acceptable to children than attempting to obtain samples from blood, obtaining multiple saliva samples from children is still challenging (Gunnar & Talge, 2005) which can lead to missing data in many studies. For example, most studies of child cortisol report rates of missing data attributable to sampling non-compliance at around 8-10% of the samples (Lewis & Ramsay, 2002; Mills et al., 2008; Blair et al., 2008). Compliance might be enhanced by having sampling occur in familiar environments (e.g., the home) as well as by making the sampling process enjoyable, such as by making it into a game or by offering salient and/or meaningful incentives for the child's compliance (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005).

Summary

As exposure to stressors and stress sensitivity have been strongly implicated in the etiology of major depression and anxiety disorders, research into the role of HPA reactivity to stress in the development of psychopathology is important. However, little consistency exists in the literature regarding how this reactivity is measured. Research on adults has provided strong evidence for task characteristics that produce the greatest increase in cortisol reactivity and recovery time (Dickerson & Kemeny, 2004), as well as what the appropriate contextual and sampling procedures are for these tasks (Gunnar & Talge, 2008). Extending these methods to younger age groups will facilitate the examination of continuity in psychophysiological stress responses across development. With these issues in mind, the current project reports the development and validation of a developmentally sensitive task that incorporates these key characteristics. The capacity of this task to elicit a cortisol response was evaluated in two ways: 1) the task needed to evoke a statistically significant increase in cortisol levels from baseline; and 2) this increase needed to meet or exceed increases reported elsewhere in the literature. In addition, the task was considered successful in eliciting negative emotions in the children sampled if children displayed more negative emotions during the task then they did in a baseline period.

Method

Participants

Participants were 215 three-year-old children from southwestern Ontario participating in a larger study of biological and contextual influences on child temperament and psychopathology risk. Children were recruited by contacting families through a university's developmental research participant pool and by advertisements placed in local daycares, preschools, recreational facilities, and on websites. All child participants were administered the Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997) in order to screen for the presence of cognitive impairment and English proficiency. Children with significant medical, physical, or other problems were excluded from participation via a screening procedure administered by trained study personnel at the recruitment stage.

Procedure

As part of a larger study protocol, each child participated in a stress task, adapted from previous work by Lewis and Ramsey (2002) in which 4-year-old children matched colored stickers to four different animals on a worksheet using a key. Observations of pilot participants indicated that the task used by Lewis and Ramsey was too challenging for three-year-olds in its original form (2002). Therefore, the task was simplified to make it developmentally appropriate for three-year-olds, and was further modified to include a greater number of features thought to increase cortisol reactivity (Dickerson & Kemeny, 2004; see section describing task stimuli) and to increase task comprehension for three-year-olds. This task was conducted by trained study personnel during a visit to the child's home. Families' homes were chosen as the setting for this assessment in part to reduce extraneous influences (e.g., travel to a novel laboratory setting) on children's cortisol levels. To further reduce irrelevant influences on cortisol, all children were already familiar with the female experimenter conducting the cortisol task, having met her previously during a visit to a research laboratory for other study procedures. All home visits began between 12:00pm and 3:30pm in the afternoon to address diurnal variation in children's cortisol levels. Parents were instructed to not allow their child to eat or drink anything but water for one half hour prior to the visit, as certain substances, such as bovine cortisol in milk products, can cross-react with anti-cortisol antibodies and cause false results in cortisol assays derived from human saliva (Magnano, Diamond, & Gardner, 1989), and because acidic or high sugar foods can alter saliva pH and compromise assay performance (Salimetrics, 2008).

At the beginning of the home visit, the child and experimenter played together quietly with a set of standardized toys (e.g., books, coloring, children's videos, blocks, sticker, and puzzles) for 30 minutes. This quiet play period was to allow any increases in salivary cortisol due to the arrival of study personnel to return to baseline levels before baseline samples were taken. During this time, the child was encouraged to stay seated and engage in minimal activity, as cortisol levels are also influenced by physical activity (Wellhoener, Born, Fehm, & Dodt, 2004). After 30 minutes had passed, a baseline salivary cortisol sample was collected, followed by the stress task described below. Following the stress task, the child and experimenter again resumed quiet play while the remaining cortisol samples were collected at 10, 20, 30, 40, and 50 minutes post-stressor. The stress task was videotaped by a female research assistant for subsequent coding.

Stress Task

To assess children's psychophysiological reactivity to stress, each participant participated in a color-matching game that was designed to be impossible to successfully complete. For the task, each child and the experimenter were seated beside each other at a table (usually a dining room or kitchen table) in front of a large felt board on which numerous bear and frog icons had been affixed. A large toy replica of a traffic light with a green, yellow, and red light was placed adjacent to the board, and the experimenter had an unobtrusive remote control used to manipulate the traffic light. A research assistant operating a video camera was seated opposite the experimenter and child to videorecord the task and to contribute to the social-evaluative nature of the task.

At the beginning of the task, the child was allowed to choose a prize from an assortment of small toys. The desired toy was then placed where the child could see it. The child was told that the experimenter would like them to play a matching game (see Appendix A for script and an image of the task). Children were told that each bear on the felt board needed a blue colored ball and that each frog needed a red colored ball (the "balls" being blue and red game pieces with adhesive Velcro backing to allow them to adhere to each animal on the game board). The child was shown how to place each game piece of the appropriate color on a bear or frog on the board based on a key at the top of the board. To ensure comprehension of the task, children were given several opportunities to practice matching the animals with the correct color game piece prior to starting the task. The child was then told that he or she did not have much time to complete the task, and that the traffic light would show how much time they had to finish. More specifically, the children were told that they had plenty of time to work when the light was green, but that when the light turned yellow they were running out of time, and that when the light turned red, they were out of time. The red light was accompanied by a loud buzzer sound. The experimenter demonstrated each light color for the child while explaining. Children were told that they must match all the animals on the board with the right "ball" to get their preferred prize (the previously selected toy). If they did not finish in time, children were told that they would receive a sticker instead (actually a white hole punch reinforcement). To enhance the stress-inducing nature of the task, children were also told that matching all the pieces was easy to do, and that even "little kids" could do so.

The matching portion of the task began when the experimenter cued the child to start matching by saying "ready, set, go." For most children[1], the green light on the stop light was allowed to shine for 2 minutes and 20 seconds. Next, the light was changed to yellow and the experimenter exclaimed that the child was running out of time. After another 40 seconds, the experimenter switched the light to red, which triggered the loud buzzer. At this time, the experimenter told the child that they did not finish in time and that they would not get the preferred toy; instead, children were given the white "sticker" (i.e., the hole punch reinforcer). Throughout the task the child was corrected verbally and the adhesive piece removed whenever a game piece was matched incorrectly, and the experimenter recorded how many pieces the child placed correctly and incorrectly for each consecutive trial (see Appendix B for recording sheet). After the first attempt to complete the matching task, two subsequent and identical trials occurred in which children were again unsuccessful at finishing the task. Upon the third failure, the

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[1] As some children were very good at the task, time was adjusted at the discretion of the experimenter to ensure that all children were unsuccessful at completing the task. Such adjustments were not usually needed as the times used for the task were established using pilot data, which indicated that most children would not be able to complete the task in three minutes.

experimenter looked at the stop light in a puzzled fashion, and explained to the child that the light was broken and that the child hadn't been given enough time to finish the task. The child's matching skills were then praised and the child received his or her preferred toy. The duration of the task, including the instruction period, ranged from 5.9 to 18.5 minutes (*SD* = 1.99), depending on how quickly the child mastered the matching rules and how quickly they transitioned between trials. This length is consistent with most tasks used in the literature (Dickerson & Kemeny, 2004). Due to extreme negative reactions by some children (N = 26, 12.5%), all three trials of the task could not be completed, thus reducing the length of the task for these individuals. As task length varied somewhat between children, it was used as an independent variable in analyses. However, task duration has no known influence on cortisol reactivity and longer tasks do not elicit stronger responses, even when comparing tasks ranging from 3 to 60 minutes in duration (Dickerson & Kemeny, 2004).

Cortisol Sampling Procedure

Cortisol samples were obtained at baseline immediately before the introduction of the stress task and at ten minute intervals following completion of the task, for a total of five samples post-stressor. The samples were taken 10, 20, 30, 40, and 50 minutes following the end of the matching task. To facilitate appropriate timing of the samples, as well as to keep record of the time the samples were obtained, the start and stop times of the matching task and each cortisol sample were recorded (see Appendix C for recording sheet).

To collect saliva, children were asked to chew on an absorbent cotton dental roll until it was wet. To facilitate ease of sampling with three-year-olds, the sampling was presented as a game in which the child raced the main experimenter to get a few grains of Kool-Aid out of a colorful Dixie cup, receiving stickers upon completion of each sample. The child received a new cup and dental roll for each "game" to avoid cross-contamination of samples. This approach not only made sampling pleasant for the child, thus promoting compliance, but also promoted the flow of saliva since the Kool-Aid stimulated the salivary glands. Kool-Aid was used sparingly, and previous work shows that its use does not compromise the quality of the assays as it does not significantly alter the pH of the saliva (Talge et al., 2005). Red colored Kool-Aid was used as the color red has an optical density of upwards of 600nm and was the least likely to interfere with assay protocol. After each sample was obtained, a research assistant expunged the saliva into a labeled micro tube, and all samples were frozen immediately upon return to the laboratory following the visit. Samples were later taken to a laboratory at the University of Western Ontario where they were assayed in duplicate using an expanded range, high sensitivity, salivary cortisol enzyme immunoassay kit (Salimetrics, PA). Optical density was read on a standard plate reader at 450 nm (Bio-Rad Labs). All samples from the same child were assayed in the same batch and duplicates varying more than 5% were re-assayed. This test employs a principle of competitive binding and provides precise results while using a minimal test volume (25 µl per determination), with a lower limit of sensitivity at .003 µg/dL, a standard curve range of 0.007- 3.40µg/dL, and average intra- and interassay coefficients of 3.5 and 5.1% respectively. Values from matched serum and saliva samples show the expected strong linear relationship, $r(47) = 0.91$, $p < .0001$ (Salimetrics, 2008). Levels that exceed 3.5 to 4.0 µg/dl should be considered suspect (Gunnar & Talge, 2005) but no levels met that range in this sample.

As mentioned previously, non-compliance rates for cortisol sampling with young children generally range anywhere for 8-10% for samples (Lewis & Ramsay, 2002; Mills et al., 2008; Blair et al., 2008). Non-compliance rates can be reported in two ways: by the number of children failing to provide one or more samples; and by the number of failed samples out of those attempted, the prior method being a conservative estimate. In order to be included in analyses, children had to have provided at least a baseline and a 30 minute post-stress sample, which was the case for all participants. Two hundred and six children provided all six cortisol samples (95.8%) while the remaining nine children (4.2%) did not provide a sample at one to three time points (one child refused to provide a 50 minute sample; two children did not provide 40 and 50 minute samples; 5 children failed to provide 10 and 20 minute samples; and one child refused to provide 10, 20, and 50 minute samples). Non-compliance rates calculated based on the number of samples collected was 18 samples out of 1290 attempted, which is a non-compliance rate of 1.4%.

Manipulation check/video coding

In order to confirm that children experienced the stress task as stressful, facial, verbal, and physical displays of the children's positive and negative emotions (PE and NE, respectively) were coded to examine whether NE increased and/or PE decreased as a result of the task. As a baseline measure of child PE and NE, relevant child behaviors during the experimenter's introduction of the task, until the point where children were shown the red light and told that they could run out of time, were coded[2]. The rate of instances of PE and NE expressed during the baseline relative to the stressful portion of

[2] Pilot data suggested that this juncture of the task was when most children began to perceive the task as stressful.

the task were calculated as a manipulation check. Results from these analyses are reported later.

Ratings of facial, verbal, and bodily, PE and NE were derived from judgments about the frequency, intensity, and duration of expressions of affect during the episode. A NE score comprised of the sum of all instances of NE during the baseline was created, and a similar score was derived for the stressful portion of the task. As the baseline period was much shorter than the task itself, these summed NE and PE scores were divided by the number of minutes to the half minute comprising each interval in order to control for the length of the task. These scores were created to be used as an index of the amount of NE and PE expressed by the child during the stressful portion of the task. Next, a difference score was obtained by subtracting the NE scores for baseline from those for the stressful portion of the task. A PE difference score was created in a similar manner (see Appendix D for scoring sheet and exemplars). Difference scores were used as an index of change in negative and positive emotion from baseline to the stressful portion of the task in order to examine potential sex differences in emotional reactivity to the task. In addition, children's activity level was coded as activity level can produce changes in cortisol (Wellhoener et al., 2004). Activity level was assessed via a global rating ranging from 0-3; scores of 1 and 2 were written to reflect typical child behavior during the task, whereas a score of 0 would reflect especially low activity, and a 3 would reflect especially high activity (see Appendix E for activity level coding description).

Videos were coded by trained graduate and undergraduate students. Training was initiated by having the trainees code videos together with a trained and reliable "master" coder. Next, trainees coded sets of 5-10 videos on their own until they were able to code at least 5 videos with interclass correlation (ICCs) of .83-.85 derived from comparisons with the master coder's coding. At this time, a trainee was considered competent to code independently. Interrater reliability was assessed periodically throughout the coding process, with coders expected to maintain an average ICC of .85. When an ICC for a particular video fell below .85, the recording was reviewed with the master coder and consensus ratings between the coder and master coder regarding the child's affect and activity level during the task were made. For reliability purposes, an additional 15% of the coded videos were coded by the master coder. The average ICC for 15% of the videos was high (.92).

Results

Sample Characteristics

Participating children in this sample were largely Caucasian (90%). The mean age of children in the sample was 42 months (range= 36 to 47 months) and 53.5% of the children were female. It is often the case that cortisol distributions are positively skewed (Gunnar & Talge, 2005) and this was true for the data obtained in this study. Therefore, as is standard in this literature, a log10 transformation of the raw cortisol values yielded unskewed cortisol values and these transformed variables were used in all analyses.

Data Analysis Techniques

Correlations were used to examine the interrelationships among demographic and all major study variables. Paired t-tests were used to examine changes in cortisol levels between specific time points in more detail than is allowable in multi-level modeling. In addition, paired t-tests were conducted to analyze changes in NE and PE from the introduction of the stressor to the stressful portion of the task. Independent samples t-tests were used to examine whether sex differences were present on cortisol, NE and PE difference scores, and NE and PE expressed during task intervals.

As participants had multiple cortisol samples collected over time, these data were analyzed using multi-level modeling (MLM) conducted in HLM 6 (Scientific Software International Inc, IL). MLM has a number of advantages, such as allowing data to be modeled at two levels (Level 1, describing within-individual change over time; and Level 2, relating predictors to any interindividual differences in change; e.g., activity level, sex, and change in affect), and accounting for missing values at level 1 (Singer & Willett, 2003). As the study's goal was to test the task's ability to produce an increase in salivary cortisol and to examine the recovery of cortisol following a peak response, a quadratic equation was built to examine the effects of level two variables on the intercept, instantaneous rate of change (hereby referred to as slope), and curvature (Equation 1). Confirming the selection of a quadratic model, a chi square test of the deviance statistics between unconditional linear and quadratic models indicated that adding a quadratic term to the model resulted in a significant improvement in model fit ($p < .05$). For the model, Level 1 consisted of cortisol time points (baseline, 10, 20, 30, 40, and 50 minutes) while level two consisted of individual measures of activity level, time of day, length of task, child sex, child age, and child NE and PE expressed during the task after controlling for baseline levels. In this way, the level one variable, cortisol samples, was nested in the level two variable, participant. For all analyses, log 10 transformed cortisol values were treated as the dependent variable. This model can be understood as a within-subjects regression of an individual's cortisol values onto the time of each assessment. To evaluate the model, the following function was specified to describe the data from each individual:

$$\text{Level 1: } Y_{ij}=\beta_{0j} + \beta_{1j}(\text{Time}) + \beta_{2j}(\text{Time}^2) + r_{ij}$$

Level 2

$$\text{Intercept: } \beta_{0j} = \gamma_{00} + u_{0j}$$

$$\text{Instantaneous Rate of Change: } \beta_{1j} = \gamma_{10} + u_{1j}$$

$$\text{Curvature: } \beta_{2j} = \gamma_{20} + u_{2j}$$

(Equation 1)

where Y_{ij} is cortisol values of individual j at time i; β_{0j} is the cortisol value of individual j at Time = 0 (i.e., the baseline cortisol value of individual j); β_{1j} is the instantaneous rate of the linear change in cortisol for individual j at Time = 0^2; β_{2j} is the rate of the curvature in cortisol; and r_{ij} is the residual variance in repeated measurements for individual j.

Between subjects predictors of individual change were also modeled to allow examination of cortisol levels at each sampling time across participants, or for each individual, while taking into account between persons predictors (e.g., NE and PE expressed during the task controlling for baseline levels, sex and age of the child, activity level during the task, length of task, and time of day). We also examined whether any of the between-subject variables interacted to predict children's HPA axis reactivity. Prior to analyses, time was anchored at baseline (at baseline, time = 0) so that the cortisol intercepts (β_{00}) would reflect the average of individual's cortisol levels at baseline. All level-2 between-person variables were centered at their grand mean. MLM is equipped to handle missing data at level-1 by estimating the trajectory based on the existing data for that participant. Therefore, missing data were accounted for in this way during the creation of the multivariate data matrix file. Individuals with data missing at level 2 were excluded during analysis.

Correlational Analyses

Table 1 presents correlations between mean cortisol levels at each sampling time and all major between subject variables. As expected based on the association between activity level and cortisol (Wellhoener et al., 2004), higher mean cortisol levels at ten, twenty, thirty, forty, and fifty minutes post-stress were significantly correlated with higher ratings of child activity during the stress task. Lower ten minute post-stress cortisol levels were positively and significantly associated with child age and higher twenty, thirty, forty, and fifty minute cortisol levels were significantly associated with more NE expressed during the task. Lower NE difference scores were significantly associated with a longer length of task and with lower PE difference scores and with higher NE levels during the task. Child activity level was positively associated with time of day and child age, indicating that older children and children who were tested later in the afternoon were more active during the task. In addition, activity level was positively associated with both NE and PE expressed during the task, indicating that children who were more active during the task also expressed more emotion. Females generally had a shorter length of task than males. Lastly, NE during the task was negatively associated with length of task indicating that children who performed the task for shorter amounts of time were more negative.

Table 1

Correlations among variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Baseline Cort. *M* = -1.28(SD = .27)	--														
2. Ten Min. Cort. *M* = -1.06(SD =.32)	.62**	--													
3. Twenty Min. Cort. *M* = -1.03(SD = .35)	.56**	.83**	--												
4. Thirty Min. Cort. *M* = -1.04(SD = .33)	.56**	.64**	.77**	--											
5. Forty Min. Cort. *M* = -1.08(SD = .33)	.57**	.53**	.60**	.80**	--										
6. Fifty Min. Cort. *M* = -1.15(SD = .36)	.50**	.43**	.50**	.60**	.79**	--									
7. PE Difference *M* = -.49(SD = 1.69)	-.04	-.12	-.13	-.05	-.03	.07	--								
8. NE Difference[1] *M* = 1.7(SD = 1.8)	-.02	-.03	.02	.10	.11	.09	.14*	--							
9. Activity Level *M* = 1.5(SD = .9)	.10	.20**	.24**	.26**	.23**	.17*	-.03	.09	--						
10. Length of Task *M* = 12.3(SD = 2.0)	.01	-.05	-.06	-.11	-.09	-.00	-.06	-.22**	-.08	--					
11. Time of Day *M* = 2.00pm(SD = 1.25h)	-.06	.08	.09	.13	.12	.06	-.09	-.09	.17*	.03	--				

[1] Difference scores were calculated by subtracting the summed emotion score (either NE or PE) during the baseline portion from the summed emotion score during the stressful portion of the task after first controlling for length of baseline and task by dividing the sums by the lengths of the intervals.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
12. Child Age *M* = 3.5(SD = .31)	-.03	-.14*	-.09	-.08	-.10	-.12	-.04	-.04	.14*	-.07	-.01	--			
13. Child Sex	-.06	-.08	-.08	-.02	-.02	-.08	.01	.02	-.05	-.17*	-.05	.04	--		
14. NE during task *M* = 2.97(SD = 2.00)	.05	.08	.14*	.20*	.15*	.14*	.01	.60**	.32**	-.21**	.03	-.00	-.00	--	
15. PE during task *M* = 1.91(SD = 1.78)	.09	.07	.02	.05	.03	.08	.14*	.07	.22*	-.05	.04	.10	-.04	.01	--

Note. Cortisol levels are measured in microgram per deciliter (μg/dl)

Child Sex: Male = 1 and Female = 2; Min. = Minutes; Cort. = Cortisol; h = hours

*$p < .05$, **$p < .01$

Results of Manipulation Check

A manipulation check was performed by conducting paired t-tests between PE and NE expressed during baseline and during the stressful portion of the task after first controlling for length of the baseline and stressful portion of the task by dividing the sum of NE and PE during each segment by the total time of the segment. Results revealed a significant decrease in PE from the baseline to the stressful portion of the task and a significant increase in NE from baseline to the stressful portion of the task, $t(215) = 3.68$ and $t(215) = -13.95, p < .001$ respectively (see Table 1 for NE and PE difference means). No significant sex differences were found for NE or PE difference scores or for NE and PE expressed during baseline or during the stressful portion of the task ($p > .42$) although there was a significant variation in levels NE and PE expressed during the task amongst all participants (see Figures 1 and 2).

Cortisol Level Comparison across Sample

Paired samples t-tests were conducted to examine the differences between mean cortisol levels across time points. Mean cortisol levels increased significantly from baseline to ten minutes, $t(214) = -11.36$, and from ten to twenty minutes, $t(214) = -3.61, p < .001$, post-stress. There was no significant change from twenty to thirty minutes post-stress ($p > .05$) (see table 2). Mean cortisol levels then began to decrease and significant reductions were found in mean levels from thirty to forty minutes, $t(214) = 3.08, p < .001$, and forty to fifty minutes, $t(214) = 4.28, p < .001$, post-stress (see Table 1 for mean cortisol values). In summary, a significant change in mean cortisol levels across participants was seen across time points with the exception of the twenty to thirty minutes samples post-stress. This lack of a significant change from twenty to thirty minutes post-

Table 2

Changes in cortisol over time using paired t-tests.

Effect(minutes)	*M*	*SD*	*SE*	*t*-value
Baseline-Ten	-.20	.26	I.02	-11.36**
Baseline-Twenty	-.25	.30	.02	-12.27**
Baseline-Thirty	-.24	.29	.02	-12.21**
Baseline-Forty	-.20	.29	.02	-9.97**
Baseline-Fifty	-.13	.32	.02	-5.78**
Ten-Twenty	-.05	.19	.01	-3.61**
Ten-Thirty	-.04	.27	.02	-2.06*
Ten-Forty	.01	.32	.02	.24
Ten-Fifty	.07	.36	.03	2.91*
Twenty-Thirty	.01	.23	.02	.62
Twenty-Forty	.05	.31	.02	2.55*
Twenty-Fifty	.12	.35	.02	4.99**
Thirty-Forty	.04	.21	.01	3.08*
Thirty-Fifty	.11	.31	.02	5.27**
Forty-Fifty	.07	.23	.02	4.28**

* = $p < .001$, ** = $p < .05$

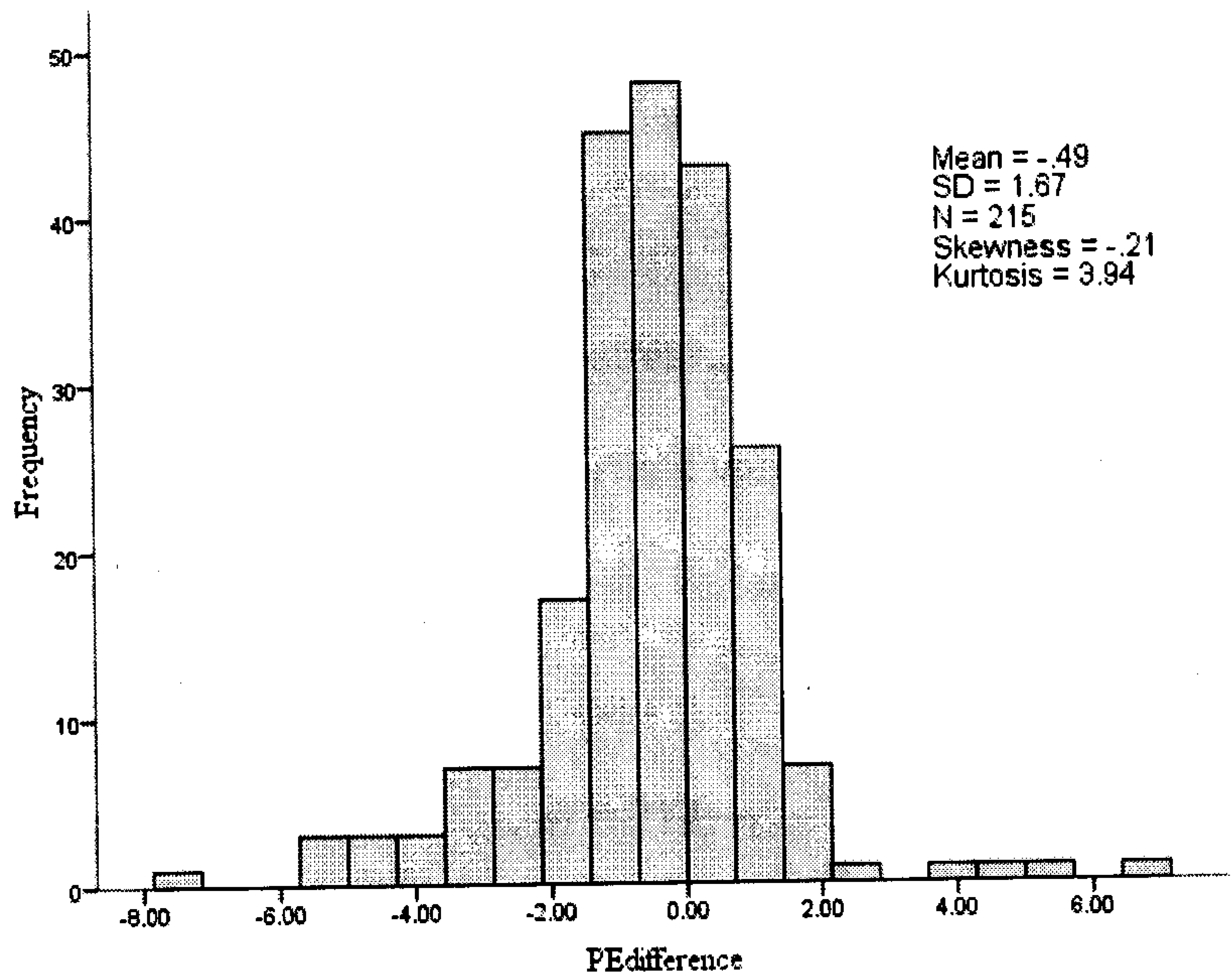

Figure 1. Frequency distribution of NE difference scores.

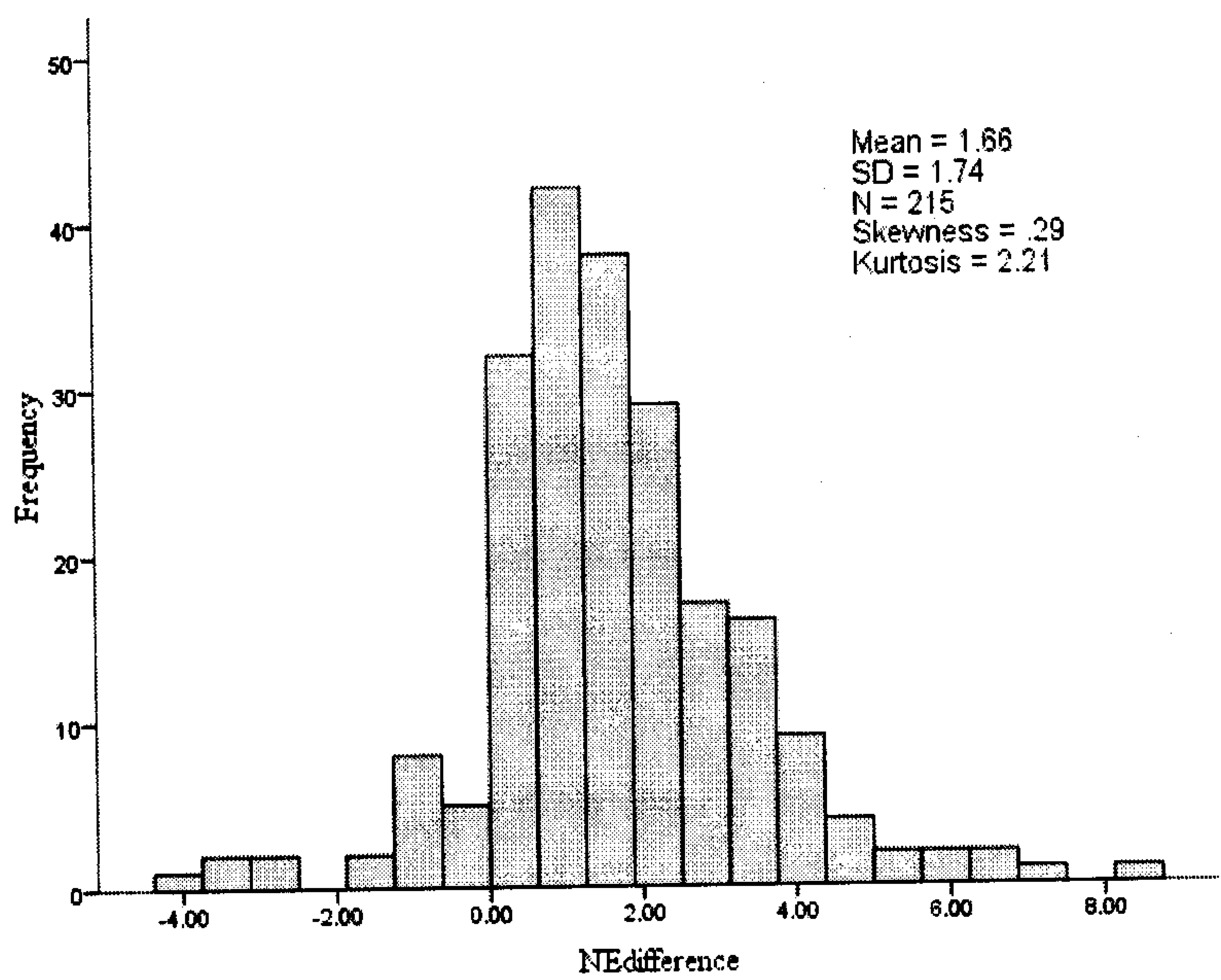

Figure 2. Frequency distribution of PE difference scores.

stress may be due to differing times to peak cortisol level across participants. The average and individual cortisol trajectories can be seen in Figures 3 and 4. Untransformed data was used to calculate the average increase in cortisol levels across participants from baseline to twenty minutes post-stress, revealing a value of .125 μg/dl.

Regressing individual cortisol values onto time showed that the random error terms associated with the intercept (variance component = .07, $df = 214$, $\chi^2 = 412.60$, $p < .001$), slope (variance component = .05, $df = 214$, $\chi^2 = 461.03$, $p < .001$), and curvature (variance component = .001, $df = 214$, $\chi^2 = 514.09$, $p < .001$) components were significant, indicating that the baseline (intercept) cortisol value and slope differed significantly from zero and that there was significant variation rate of quadratic curvature within the sample. There were no significant sex differences in cortisol levels at any time point ($p > .23$).

Task Variables and Cortisol Reactivity

We examined the main effects of NE and PE during the task after controlling for baseline levels, activity level, age, child sex, length of task, and time of day on the intercept, slope, and curvature for individuals' cortisol trajectories. First, the univariate effects of these variables were examined in a quadratic growth model (see Table 3). PE expressed during the stressful portion of the task was significantly associated with slope and curvature after controlling for baseline PE levels by entering them simultaneously into the level 2 equation. The cortisol slope coefficient suggests that for every unit increase in the PE expressed during the task there is a .002 decrease in slope and a .00004 increase in curvature ($p < .05$). This suggests that children who displayed more PE during the task had a less linear and more quadratic trajectory than children who displayed less

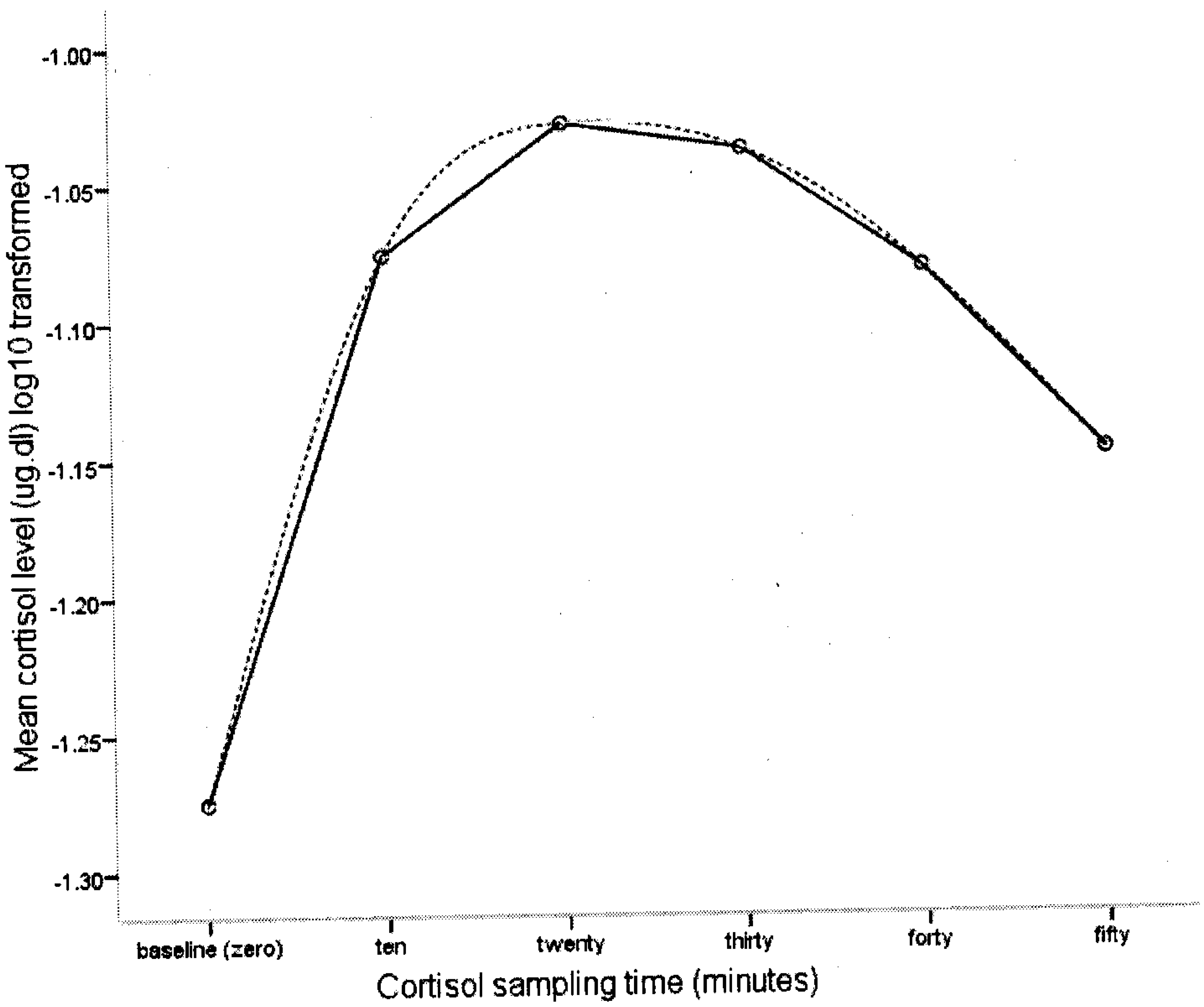

Figure 3. Log10 transformed mean cortisol level (μg.dl) as a function of cortisol sampling time.

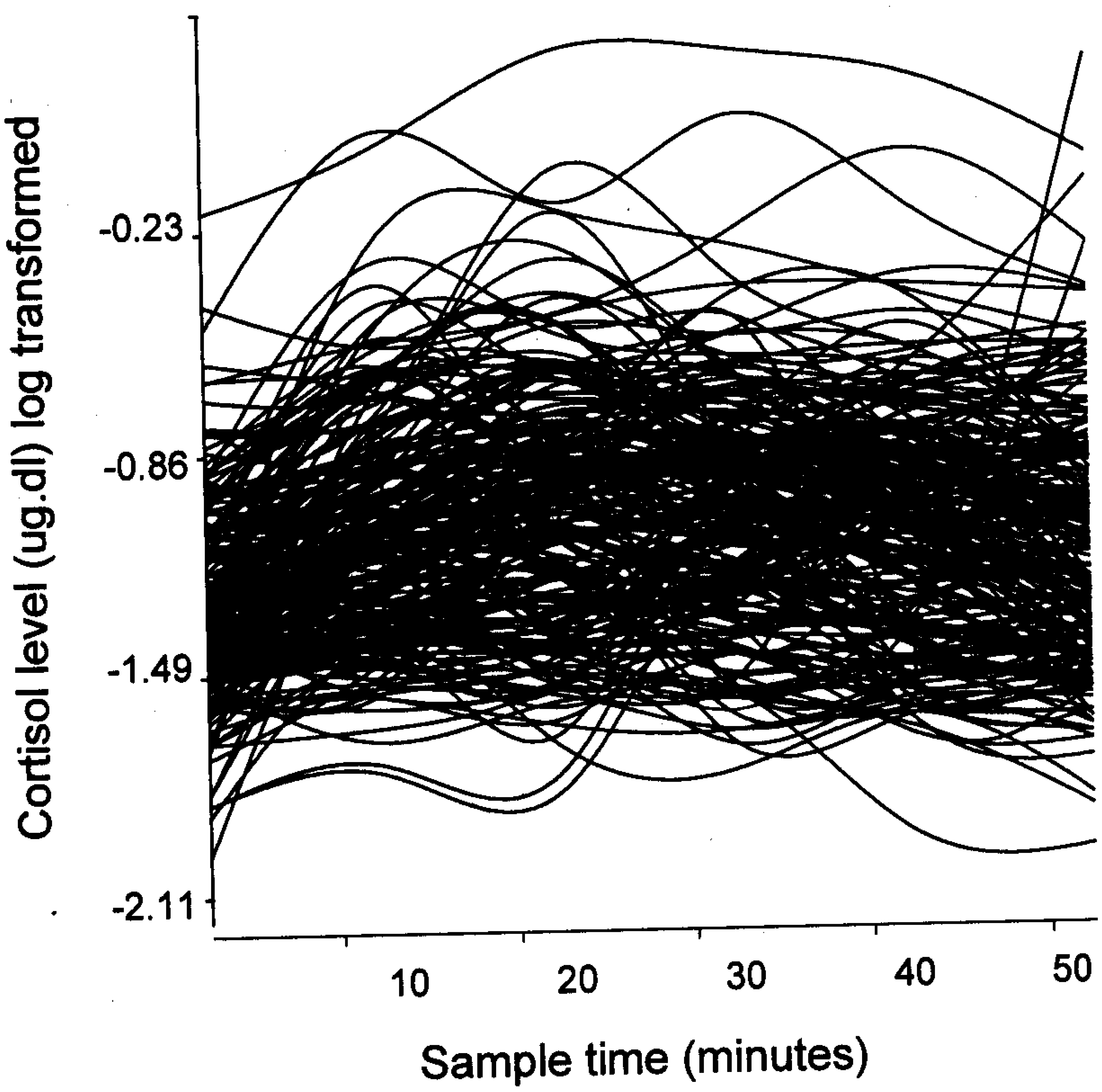

Figure 4. Log 10 transformed individual cortisol level (μg.dl) as a function of cortisol sampling time.

Table 3

HLM exploratory univariate analyses: Children's cortisol reactivity predicted from child sex, child age, NE and PE difference score, global NE and PE during task, activity level, length of task, and time of day in a quadratic model.

Variable	Coefficient Intercept Coefficient (*SE*)	*t*-value	Cortisol Instantaneous Rate of Change Coefficient (SD)	*t*-value	Cortisol Slope Coefficient (SD)	*t*-value
NE during task	.003(.011)	.250	.001(.001)	2.267*	-.000(.000)	-1.628
PE during task	.016(.012)	1.331	-.001(.001)	-1.169	.000(.000)	1.147
NE control baseline	-.004(.013)	-.336	.001(.001)	1.720	-.000(.000)	-.863
PE control baseline	.008(.015)	.533	-.002(.001)	-2.014*	.000(.000)	2.489*
Activity level	.032(.023)	1.399	.004(.001)	2.778**	-.000(.000)	-2.312*
Length of task	.005(.011)	.448	-.001(.001)	-2.184*	.000(.000)	2.077
Time of day	-.012(.017)	-.682	.003(.001)	2.906**	-.000(.000)	-2.437*
Child sex	-.049(.044)	-1.126	.001(.002)	.233	-.000(.000)	-.246
Child age	-.062(.070)	-.884	-.002(.004)	-.420	.000(.000)	.118

child sex: male = 1, female = 2; *df* = 208

* = < .05; ** = < .01

of an increase or a decrease in positive emotions from baseline to the stressful portion of the task. NE expressed during that stressful portion of the task was significantly related to cortisol slope such that a one unit increase in global NE during the task was associated with a .001 increase in linear slope at Time = 0^2 ($p < .05$). This suggests a more rapid increase in cortisol following the stressor for children who were increasingly negative during the task. However, this relationship was no longer significant when baseline levels of NE were controlled for at level 2. The implications of this will be discussed later. Activity level during the task was significantly associated with both slope and curvature such that a one unit increase in activity level was associated with an .004 slope ($p < .01$) increase and a .00006 decrease in quadratic curvature ($p < .05$). This suggests that children who were more active during the task had a slower return to baseline values following stress.

Length of task was significantly associated with slope such that a unit increase in length of task was associated with a .001 decrease in slope ($p < .05$) suggesting that children who performed the task longer had smaller linear increase in cortisol following baseline. Lastly, time of day was significantly associated with slope ($p < .01$) and curvature ($p < .05$) such that a unit increase in time of day (task was performed later in the day) was associated with a .003 increase in cortisol slope and a .00005 decrease in quadratic curvature.

Interactions between Level 2 Predictors

To further explore possible sex differences in the cortisol trajectory, we examined whether child sex interacted with NE and PE expressed during the stressful part of the task both alone and while controlling for baseline levels, child age, length of task, time of day, or activity level to predict intercept, slope, and/or curvature. In addition, we examined interactions between all correlated variables (Table 1) in predicting a quadratic model and between NE and PE expressed during the task alone and controlling for baseline NE and PE. Interaction terms were made by first centering continuous variables, then multiplying two terms to create a term reflecting the product of both level two predictors (Aiken & West, 1991). The grand mean centered level 2 predictor variables were entered in the model followed by the interaction term. Only one significant interaction emerged. Activity level and child age interacted to predict both intercept, instantaneous rate of change, and curvature (see table 4). To probe the interaction with child age, child age was recentered at values 1 *SD* above the mean (3.45) and 1 *SD* below the mean and a new interaction term was created. The model was rerun allowing activity level to predict intercept, slope, and curvature. Activity level significantly predicted slope, and curvature for children whose ages were 1 *SD* above the mean but not for those whose ages were 1 *SD* below the mean ($p > .45$), suggesting that, for older children, higher activity level during the task predicted a higher slope and slower rate of curvature (slope: unstandardized coefficient = .005, $SE = .002$, $t = 2.990$, $df = 160$, $p = .003$; and curvature: unstandardized coefficient = -.000, $SE = .000$, $t = -2.716$, $df = 160$, $p = .007$).

Discussion

The results of this study support the validity of the task used to elicit psychophysiological reactivity indexed by salivary cortisol. Methodological issues appear to have hampered research on cortisol reactivity in children, such that few studies have reported the expected pattern of quadratic reactivity. As such, it is unusual to find a quadratic function in such data, despite the fact that this is what would be expected given

Table 4

Interaction between activity level and child age in predicting children's cortisol reactivity in a quadratic model.

Fixed effect	Coefficient (*SE*)	*t*-value	Variance component (SD)	Chi-squared test of variance
Predicting cortisol intercept				
Intercept	-1.443(.026)	-54.617***	.070(.265)	403.420***
Activity level	-.018(.029)	-.637		
Child age	-.027(.085)	-.311		
Activity X child age	-.195(.093)	-2.111*		
Predicting cortisol instantaneous rate of change				
Intercept	.224(.018)	12.494***	.035(.187)	434.050***
Activity level	.059(.019)	3.065**		
Child age	-.053(.058)	-.914		
Activity X child age	.131(.063)	2.087*		
Predicting cortisol curvature				
Intercept	-.029(.003)	-11.064***	.001(.029)	488.427***
Activity level	-.007(.003)	-2.619*		
Child age	.005(.009)	.628		
Activity X child age	-.021(.009)	-2.333*		

SE = standard error; SD = standard deviation; † = < .10 (trend level); *df* = 211; * = < .05; ** = < .01, *** = < .001

the nature of the physiological response (Earle et al., 1999; Gump, & Owens, 2001; Roy et al., 2001; Smeekens et al., 2007), even in the few studies in which adequate sampling post-stress would permit the successful detection of such a response (Mills et al., 2008; Zoccola et al., 2008). In contrast, the data from this study best fit a quadratic function (see Figure 3). Also, a significant increase in children's cortisol levels was found from baseline to ten and ten to twenty minutes post-stressor accompanied by a significant decrease from thirty to forty and forty to fifty minutes post-stress.

The average salivary cortisol increase from baseline to twenty minutes post-stress was .125 μg/dl, which is high relative to values reported in the literature. For example, in a sample of girls ages 9-14, Gotlib, Joorman, Minor, and Hallmayer (2009) report increases in cortisol of approximately .075 μg/dl from baseline to 30 minutes post-stress, assessed over the course of a backwards counting task and the Ewart Social Competence Interview. This smaller increase existed even though initial baseline levels and peak responses reported in the study were higher (about .15 and .23 μg/dl, respectively) than those reported in the current study (.07 and .14 μg/dl, respectively). The research on which this task was based (Lewis & Ramsey, 2002) found no significant increase in the cortisol levels of 60 four-year-old children from baseline to twenty minutes post-stressor. In fact, a slight decrease in cortisol was noted (.42 to .41 μg/dl from baseline to twenty minutes post). Both the higher baseline cortisol levels and smaller cortisol responses following the stressor reported by these two studies may be due to the fact that both studies were conducted in the laboratory. Gunnar and Talge (2005) have shown that samples obtained at laboratory arrival do not match samples obtained at home at the same time of day as the laboratory visit, suggesting that samples obtained in the laboratory may reflect the psychophysiological response to coming to the laboratory. By conducting our assessments in the home, a more accurate reflection of children's baseline cortisol levels may have been obtained, thus improving measures of baseline cortisol levels and increasing estimates of reactivity. In addition, while Gotlib and colleagues report an average initial sampling time of 12:15pm and no significant difference in time of visit among participants, Lewis and Ramsey reported more variability in terms of the times children were tested; hence, the normal decline of cortisol throughout the day may have prevented them from detecting a response. Lewis and Ramsay also obtained only one post-stress sample, following a lengthy combination of tasks, which may also have contributed to their ambiguous results. Indeed, it is possible that both elevated baseline levels from coming to the laboratory and their sampling procedure prevented Lewis & Ramsey from finding significant increases in cortisol. The present study assessed participants within a relatively consistent time frame and addressed the issue of laboratory reactivity by conducting assessments following a 30-minute acclimatization period, and in the child's home.

No sex differences in cortisol levels at any sampling point were found. This is typical when examining salivary cortisol levels in young children and is frequently reported in the literature (Dettling, Gunnar, & Donzella, 1999; Lewis & Ramsay, 2002; Lundberg, 1983) despite the fact that child sex has been shown to influence the development of negative emotions such as shame and embarrassment (Lewis & Ramsay, 2002). Sex differences in cortisol levels tend to emerge later in childhood and adolescence as children move through puberty (Stroud, Papandonatos, Williamson, & Dahl, 2004). Hence, the present findings are consistent with the larger literature.

The components of social evaluation and lack of control over task success incorporated into the design of this task would all be expected to elicit negative emotions in children and, potentially, to decrease positive emotion expression. Analyses aimed at testing whether there was an increase in negative emotionality and a decrease in positive emotionality were consistent with this expectation; children showed significantly more negative emotionality during the task than at baseline, and also exhibited a significant decrease in positive emotionality. There was variation in children's emotional responses to the task, such that varying degrees of NE and PE were expressed (see Figures 1 and 2). Further exploration of factors contributing to children's emotional responses to the task (e.g., temperament, coping styles) is warranted. Children who expressed more negative emotions during the task (had higher NE scores during the stressful portion of the task) also had significantly higher cortisol slopes. Additionally, NE expressed during the stressful portion of the task was significantly associated with cortisol levels at twenty, thirty, forty, and fifty minutes post-stress, suggesting that children who displayed more negative emotions during that task had higher cortisol levels at these time points. This indicates that children who showed more negative emotions during the task exhibited more prolonged elevations in cortisol following the stressor than children who displayed fewer negative emotions during the task. However, this relationship was no longer significant when controlling for baseline levels of NE. This suggests that children who were more negative during the task and in turn had greater slopes could have also had higher levels of NE during the baseline period, indicating that children with higher levels of NE in general have a more linear cortisol trajectory following stress. This supports the future examination of temperamental differences in emotionality as a potential predictor of both basal cortisol and cortisol reactivity to stress.

Interestingly, length of task was significantly correlated with child sex such that females generally had shorter task durations than males. As females and males did not differ significantly on the level of negative emotion expressed during the task this finding could suggest two things. First, it is possible that females expressed more negativity in a shorter amount of time and were therefore less likely to complete the full duration of the task. This is consistent with results showing that children who performed the task longer had a lower slope and greater quadratic curvature than children performing the task for a shorter time as children performing the task longer did not exhibit "extreme" negative reactions. Second, it is also possible that females performed the task better and needed fewer corrections thereby completing the task before their allotted time (three minutes) had elapsed and forcing the experimenter to end each trial early.

PE expressed during the stressful portion of the task was significantly associated with slope and curvature of the quadratic model when controlling for baseline levels in that higher PE scores during the task predicted a lower slope and a significantly higher rate of quadratic growth than lower levels of PE during the task. This indicates that children who displayed more positive emotions during the stressful portion of the task than during baseline displayed less linear growth and a more rapid return to baseline levels than children who displayed a negative change in PE from baseline to task. While speculative, this may suggest that greater PE helps children adapt more effectively to a mild social evaluative threat and results in less of an increase in cortisol following stress, accompanied by a more rapid return to baseline. This is consistent with literature suggesting that positive emotions facilitate broadening of attention and thought-action repertoires during stress thereby promoting more positive outcomes (Folkman & Moskowitz, 2000; Fredrickson & Branigan, 2005). This also warrants future research into what other variables related to positive emotionality (e.g., child temperament) predict growth trajectory in linear or quadratic forms.

Exploring the effect of activity level during the task is important as activity itself can influence cortisol levels (Wellhoener et al., 2004). Activity level during the task was significantly correlated with all cortisol levels at all post-stress time points and significantly predicted cortisol slope and curvature. This suggests that children who are more active during the task have a greater slope and slower rate of quadratic curvature. This could be a genuine effect of child activity level during the task, or could be a manifestation of children who were more active during that task also being more active following the task preventing their cortisol levels from returning to baseline at the same rate as less active children.

In addition, activity level interacted with age in that older children with higher activity level had a higher slope, and a slower rate of curvature relative to younger children who had the same level of activity during the task. This suggests that older children who were more active throughout the task demonstrated a more linear cortisol increase, suggesting a delayed return to baseline levels. Two possible hypotheses can be suggested for why older children who are more active would have more reactive cortisol. First, it is possible that children who are older put more effort into the task thereby grasping and reaching for matching task pieces at a higher rate and receiving higher activity level scores. Secondly, it is possible that children who are older and display more activity during a task in which they are asked to remain seated in their chair have poor inhibitory control and may be at greater risk for psychopathology.

Other main effects of time of day and length of task were found. Children tested later in the day had higher slopes, and slower rates of curvature. This finding suggests that as the afternoon progresses children exhibit a more rapid increase in cortisol following stress coupled with a slower return to baseline. Finally, increasing length of task was found to predict a lower slope and a faster rate cortisol curvature. This indicates that children who performed the task longer had a less pronounced increase in cortisol following the stressor and better fit a quadratic trajectory than children who became too negative to complete the full duration of the task. This again suggests that children so high on NE that they refused to complete the three trials of the task better fit a linear growth model suggesting a delay in return to baseline. This could have psychological and physiological implications as discussed previously, and may be examined further with the inclusion of measures relating to child temperament and psychopathological symptoms.

Summary

The purpose of this study was to develop and validate an age appropriate task for the induction of psychophysiological stress in three-year-old children. Using salivary cortisol as an index of stress reactivity, this study indicates that the task used significantly influenced children's cortisol levels. Further supporting the validity of the task, children also showed both a significant increase in negative emotionality and a decrease in positive emotionality as a result of the task.

The need for the development of a validated and standardized task and procedure to measure reactivity is evident in the literature on child psychophysiology. Too frequently, methods and tasks are used to elicit a stress response from participants that are not grounded in theory and have not been validated. This paper describes a task that was developed to incorporate task aspects shown to maximize cortisol responses in adults (Dickerson & Kemeny, 2004). Results of this study confirm that the current task can effectively and reliably elicit a cortisol response in three-year old children.

It should be noted that while components of social evaluation, lack of control of task outcome, and motivation were all employed in the task, it is unclear which task characteristics played the strongest role in eliciting the cortisol response. As children between three years and four years of age undergo significant cognitive development, it is likely that some characteristics were more effective at eliciting a response for particular children than they were for others. A dismantling study design, in which task characteristics are added and removed to examine their effect on participants' cortisol levels, would be best able to provide insights into the individual and joint effects of task characteristics on children's cortisol levels.

Study Strengths

This study attempted to minimize multiple potentially confounding variables that have likely influenced previous research on cortisol reactivity in children, such as laboratory effects and effects of diurnal cortisol variation. In doing so, a clear pattern of psychophysiological reactivity was measured that is rarely found in the child cortisol literature to date. The task and sampling procedures used in this study provide a way of eliciting psychophysiological reactivity that is theory-driven and relatively easy and brief to administer.

Future Research

Future research that further breaks down which task characteristics are active in eliciting a cortisol response may help identify those that are most salient to a cortisol response, although Dickerson and Kemeny's work (2004) indicates that it is likely that all task features contributed to the induction of psychophysiological reactivity in preschool aged children. In addition, further breakdown of the negative emotions elicited by the task (i.e., anger, fear, and sadness) could further inform which emotions are relevant in producing different patterns of cortisol reactivity. In addition, it is possible that children used different styles of coping in order to deal with the stress of the task and that these varying styles affected their cortisol reactivity to the stressor. Future research to examine differences in coping styles expressed by children during the task is warranted. Lastly, child temperament and parenting are also likely to affect the emotional response and subsequent cortisol response displayed by children during this task (Dougherty et al., 2009; Kochanska et al., 2002; Locke, 2006) and exploration into how these variables influence task response is needed.

The results of this study suggest that research on child temperament and other individual difference variables as potential influences on cortisol reactivity is warranted, as there was significant variation in cortisol intercepts, slopes, and curvatures across children in this sample that was not accounted for by the variables tested in this research.

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Appendix A: Script for Matching Task

Here I have some stickers and some cool bouncing balls. Which do you like best? [allow the child to pick and set the preferred toy and a sticker by the matching task stimuli, putting the other toys 7 stickers aside]

I want to see how good you are at games, so let's play a matching game. This is how you play: up here at the top of my board, we have Bear and Frog. Bear and Frog both have a ball that is a special color—Bear has a blue ball and Frog has a red ball [point to each animal and "ball" as you name them].

Here [point to a bear] is this guy. Who is he?

> ***[If child says 'Bear']*** *That's right! It's Bear! But he doesn't have a ball. [point to where the ball should be].*
>
> ***[If child gives incorrect response or doesn't know]*** *This guy is Bear! But look, he doesn't have a ball [point to where the ball should be].*

What color ball does Bear get [point to the top]?

> ***[If child says 'blue']*** *That's right! Bear gets a blue ball. Here's where the balls are. [indicate basket] Can you give Bear a blue ball? [Help if necessary]*
>
> ***[If child gives incorrect response or doesn't know]*** *Look up here at the top of the board. Bear gets a blue ball. Here's where the balls are. Can you give Bear a blue ball?*

Right here I have Frog, but he doesn't have his ball. Can you find the right ball and put it by Frog? [Help if necessary. <u>Do additional examples if necessary</u> until the child understands. Remove all "balls" when you are done explaining the game].

To play the game, you have to give Bear and Frog the right color ball. You only have a little bit of time, just a few minutes, to give all these animals the right ball, so you've got to hurry!

*So you know how much time you have, we're going to use this thing [**indicate Yacker Tracker**.] When it's green, you have plenty of time to work. When it turns yellow [turn the light yellow], you are running out of time and better hurry if you are going to finish. And when it turns red and makes an ugly noise, that means you are all out of time. [demonstrate by making the red light buzz].*

If you finish, you get this cool toy [indicate preferred toy]. But if you don't finish, all you get is this [indicate sticker]. This is an easy game, for little kids, so you should be able to finish it and get the toy.

*[**Dramatically**] Are you ready? Set? Go!*

With a neutral demeanor, observe the child as (s)he works on the matching task, marking correct and incorrect matches on the appropriate sheet. If (s)he makes a mistake in his/her matching, remove the incorrect "ball" immediately and quietly and neutrally say:

Remember, [insert animal name] gets a [insert color name] ball.

After **2 minutes, 20 seconds** (or sooner if the child actually comes close to completing the task, have the timer switch to yellow and say (in a neutral tone):

Uh oh, you're running out of time!

After **3 minutes** (or sooner if the child actually comes close to completing the task, have the timer go off and say:

Uh oh, you ran out of time. So you just get this sticker. [hand child the sticker]

Let's try again. Remember, if you finish, you can still get this cool toy [indicate preferred toy]. But if you don't finish, all you get is this, another sticker [indicate sticker].

Ready, set, go!

With a neutral demeanor, observe the child as (s)he works on the matching task, pretending to "take notes" on your clipboard. To make the task more stressful, you should be seated such that the child can see you "taking notes." If (s)he makes a mistake in his/her matching, remove the incorrect "ball" immediately and quietly say:

Remember, [insert animal name] gets a [insert color name] ball.

After **2 minutes, 20 seconds** (or sooner if the child actually comes close to completing the task, have the timer switch to yellow and say (in a neutral tone):

Uh oh, you're running out of time!

After **3 minutes** (or sooner if the child actually comes close to completing the task, have the timer go off and say (in a neutral tone):

Uh oh, you ran out of time. So you just get this sticker. [hand child the sticker]

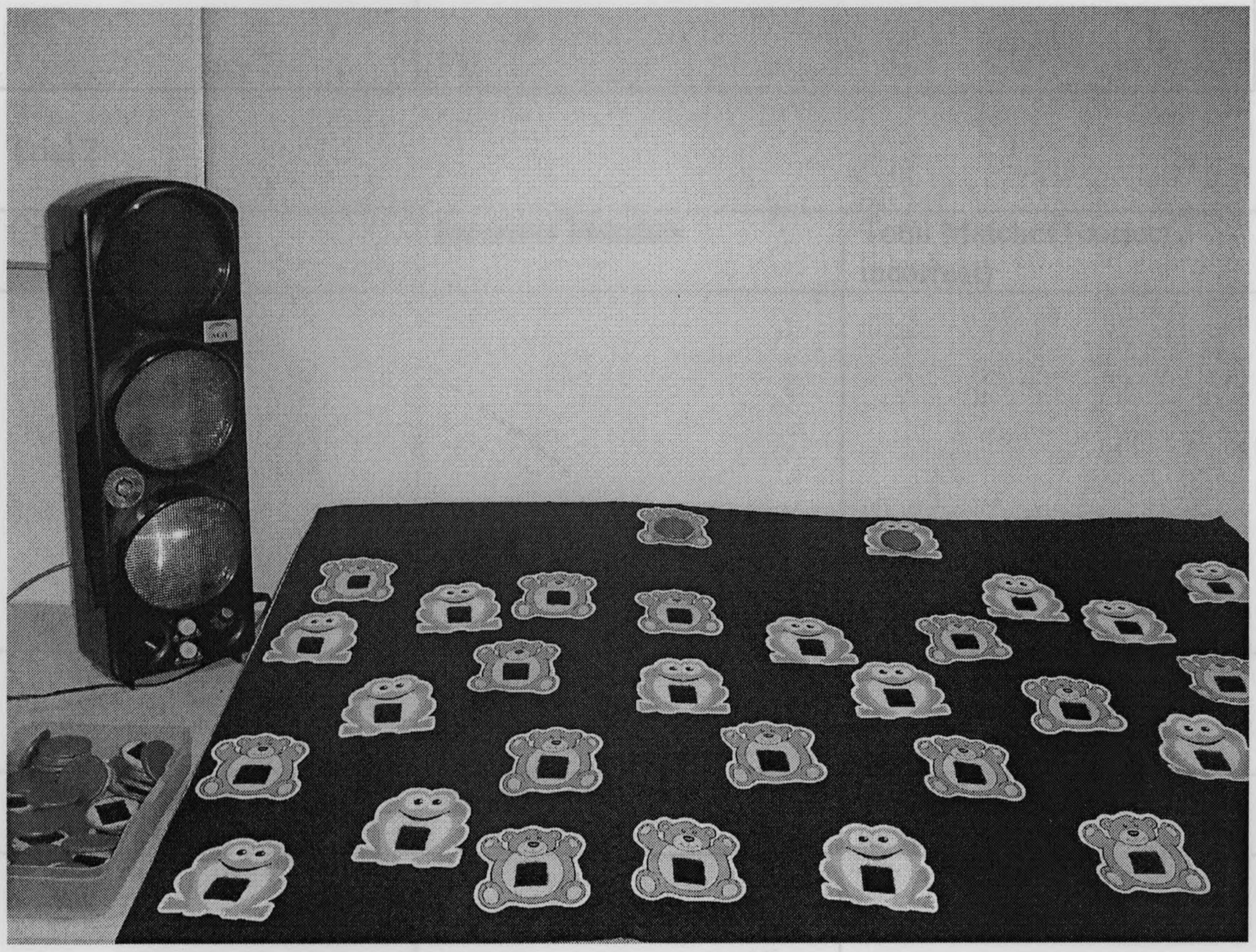
Let's try again. Remember, if you finish, you can still get this cool toy [indicate preferred toy]. But if you don't finish, all you get is this, another sticker [indicate sticker].

Ready, set, go!

Repeat this procedure one last time (for a total of three trials), then say:

Uh oh, time is up. You didn't finish in time. [Pause for a moment and look at the timer as though you are confused]

Wait a minute! My timer isn't working right! It's been going off after only 2 minutes, not 3 minutes, so you didn't have enough time to finish. You know what? Let's work on this together a little while, just for fun. I also think you should get this cool toy for trying so hard.



Appendix B: Matching task recording sheet.

Trial 1: Date: Subject Number:

Correct Matches	Incorrect Matches	Total Matches (correct + incorrect)

Trial 2:

Correct Matches	Incorrect Matches	Total Matches (correct + incorrect)

Trial 3:

Correct Matches	Incorrect Matches	Total Matches (correct + incorrect)

Notes on child's performance:

Appendix C: Recording sheet for matching task and cortisol sampling times.

Subject ID:_________________ Date:______________________

To be completed by home visitor during home cortisol assessment

Time matching task begins:
Time matching task concluded:

		Post stressor				
	Baseline***	10 minutes	20 minutes	30 minutes***	40 minutes**	50 minutes*
Exact start time/ finish time of sampling (e.g., HH:MM)						

***-very important samples, try your very best to obtain; **-important, try hard to obtain; *-useful to have, try to obtain.

Notes (e.g., reasons for missing samples, other problems):

Appendix D: Coding sheet and exemplars.

Episode Start Time: Episode Stop Time: Total time (secs):

Matching Task Part 1 Part 2 (Circle either part 1 or 2)

Subj No	
Coder	
Date	

Positive affect	***Low***	***Mod***	***High***	**Overall score**
Facial PA				
Vocal PA				
Bodily PA				
Negative affect	***Low***	***Mod***	***High***	
Facial fear				
Facial sadness				
Facial anger				
Vocal fear				
Vocal sadness				
Vocal anger				
Bodily fear				
Bodily sadness				
Bodily anger				
Behavioral ratings (complete once for part 1 and 2).	***Low***	***Mod***	***High***	
Activity level/vigor				

EXEMPLARS OF LOW, MODERATE, AND HIGH INTENSITY AFFECT CODES

Positive affect

Facial:

LOW = slight raising of corners of mouth – no teeth visible – no contraction of outer eye corner; or, smile is fleeting

MODERATE = corners of mouth definitely raised – teeth visible – no contraction of outer eye corner

HIGH = full smile – corners of mouth definitely raised – teeth visible – contraction of outer eye corner

Vocal:

LOW = somewhat lilting tone of voice; brief giggle or hiss

MODERATE = giggle or extended laugh; clearly exuberant tone of voice; statement with overtly positive content (e.g., "I like this!", "neat", "cool")

HIGH = full, extended laugh; screech, shriek, or whoop; statement with *both* overtly positive content and positive tone

Bodily:

LOW = perky/snappy movement; floating motion of arms or hands

MODERATE = brief hop or skip with clearly positive tone; slight wiggle or contortion

HIGH = clearly jubilant motions, "dance of joy", clapping, arm shaking/quivering, knee slapping

Sadness

Facial:

LOW = droopy cheeks; slightly downturned mouth; slight raising of inner corners of eyebrows; or, expression is fleeting

MODERATE = definitely downturned mouth or definite raising of inner corners of eyebrows

HIGH = both definitely downturned mouth *and* definite raising of inner corners of eyebrows

Vocal:

LOW = slightly whiny or dejected tone; slight sigh

MODERATE = definite sigh; definite whiny or dejected tone; statement with possible/probable sad content

HIGH = deep sigh; crying sound; statement with obvious sad content

Bodily:

LOW = somewhat slumped posture; lifeless motion with arms, dejected gait/walk

MODERATE = definitely slumped posture; shoulders slumped; dejected kick of feet or dropping of arm

HIGH = head in hands; head slump; clearly dragging feet

Anger

Facial:

LOW = eyebrows drawn slightly down & together, mouth slightly tense or squarish; or, expression is fleeting

MODERATE = eyebrows definitely drawn down & together; mouth definitely tense or squarish

HIGH = *both* eyebrows definitely drawn down & together *and* mouth definitely tense or squarish

Vocal:

LOW = irritable or cranky tone; slight grunt

MODERATE = definite grunt, groan, or sharp exclamation; statement with possible/probable angry content

HIGH = statement with definite angry content; definite angry/irritable tone; yelling

Bodily:

LOW = slight tension in neck or shoulders; irritable foot tapping or shaking

MODERATE = definite tension in neck or shoulders; forceful movements; arm shaking

HIGH = kicking, punching or other aggressive motion; fists balled; stomping

Fear

Facial:

LOW = eyebrows slightly raised & tightened; mouth corners drawn slightly down & back

MODERATE = eyebrows definitely raised & tightened; mouth corners definitely drawn down & back

HIGH = *both* eyebrows definitely raised & tightened *and* mouth corners definitely drawn down & back

Vocal:

LOW = whispering or cautious tone

MODERATE = quavering tone of voice; statement with possible fearful/wary content; frightened "ooh", "yikes"

HIGH = "eek", yelp; statement with definite fearful/wary content

Bodily:

LOW = cautious or wary gait; slight tension; nervous twitching, hand tapping, foot swinging, etc.; diminished activity level

MODERATE = slight defensive body posture; fearful tension

HIGH = definite defensive body posture, jumping back in fear

Appendix G: Activity level global code

CODES FOR MATCHING TASK ACTIVITY LEVEL

Activity level/energy/vigor

Normative Considerations: Children are asked to sit at a table to complete the matching task. This task is not designed to elicit very much movement and scores of 1 and 2 will be typical (e.g., most children will remain seated and will not move around the table; most movement should be limited to reaching to place matching task pieces).

0 = extremely low vigor/activity level - child remains seated for the entire episode, and appears sluggish in his/her handling of the matching task pieces.

1 = mild vigor/activity level - child remains seated sits most of the episode, but exhibits occasional instances of vigor in his/her handling of the matching task pieces.

2 = moderate vigor/activity level –child's manipulation of matching task pieces is typically firm or vigorous; they may periodically bounce around in the chair.

3 = extremely high vigor/activity level - child's handling of matching task pieces is typically vigorous; child moves about in chair consistently, bounces around; child may leave chair.

Western

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. E.P. Hayden
Review Number: 15121S **Review Level:** Full Board
Review Date: May 2, 2008

Protocol Title: Gene-Environment Interplay and the Development of Child Temperament
Department and Institution: Psychology, University of Western Ontario
Sponsor: CANADIAN INSTITUTE OF HEALTH RESEARCH
Ethics Approval Date: June 11, 2008 **Expiry Date:** July 31, 2013
Documents Reviewed and Approved: UWO Protocol, Letter of Information and Consent (Parent Consent for Self), Letter of Information and Consent (Parent Consent for Child), Advertisement.

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Non-Medical Research Involving Human Subjects (NMREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the applicable laws and regulations of Ontario has granted approval to the above named research study on the approval date noted above.

This approval shall remain valid until the expiry date noted above assuming timely and acceptable responses to the NMREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the study or consent form may be initiated without prior written approval from the NMREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the NMREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the NMREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the NMREB.

Chair of NMREB: Dr. Jerry Paquette

Ethics Officer to Contact for Further Information			
☑ Grace Kelly	☐ Janice Sutherland	☐ Elizabeth Wambolt	☐ Denise Grafton

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