THE ROLE OF ATTACHMENT IN CHILDREN'S CARDIAC REACTIVITY

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ABSTRACT

THE ROLE OF ATTACHMENT IN CHILDREN'S CARDIAC REACTIVITY

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This thesis is an investigation of how children's cardiac reactivity differed between a baseline and novel stressor episode as a function of their Secure vs. Insecure-Ambivalent attachment status. Children's physiological regulation was measured in response to the novel stressor, an *Interesting-but-Scary* (IbS) mask that spoke to the child and asked him or her to approach. Assessment at 3.5 years ($N = 48$) included heart rate and vagal tone during a baseline episode and the IbS episode. Security of attachment was assessed in the modified Preschool Strange Situation Paradigm. Children only showed meaningful physiological regulation (i.e., vagal withdrawal) to the stressor if they were classified as Secure, not Ambivalent. This finding remained significant even when examined only within children who coped successfully with task demands by approaching the IbS mask. Findings are discussed in relation to children's emerging physiological and emotion regulation skills in the context of their attachment relationship.

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The Role of Attachment in Children's Cardiac Reactivity

Children learn to regulate their behaviour and emotions as they develop, especially in the face of social stress or novelty. Infants derive comfort almost exclusively through their caregivers in early attachment relationships, whereas children over time must increasingly self-regulate and independently adapt to situations they find challenging. One way to measure adaptation to challenge and emotion regulation is to examine physiological measures of stress and coping. Change in cardiac vagal tone, or vagal regulation, provides quantitative information about children's ability to regulate their emotions and adapt to stressful environmental events. The goal of the present study was to examine how quality of the child-caregiver attachment relationship related to children's emotional and physiological regulation in response to a novel social stressor. The literature reviewed herein is an overview of the construct of attachment, and how children's temperamental characteristics might interact with children's quality of attachment to influence physiological correlates of emotion regulation.

Emotion Regulation, Coping Behaviour, and Development

Emotion regulation encompasses those behaviours and strategies that humans enlist to manage emotional arousal, so that successful interactions and personal functioning are possible through modulated emotion experiences and expression (Gross & Thomson, 2007). Examining children's ability to emotionally and physiologically regulate in novel, ambiguous, and socially demanding situations that cause increased arousal provides an opportunity to gauge their developing regulatory capacity. Both internalizing (e.g., depression, anxiety, social withdrawal) and externalizing (e.g., impulsivity, oppositional behaviour and hyperactivity) have been construed as failures in emotion regulation or as emotion dysregulation (Calkins & Hill, 2007).

Developmentalists posit that the period between infancy and the preschool age is a critical time for children's burgeoning emotion regulation capability, that is, the ability to modulate affective states increasingly independently (Kopp, 1989). Previous research has suggested that children's ability to regulate their affect as they age is influenced by extrinsic factors (e.g., responsive and sensitive caregiving; Cassidy, 1994) as well as intrinsic factors (e.g., biologically-based temperament characteristics; Fox, Henderson, Marshall, Nichols, & Ghera, 2005).

Calkins (1997) described temperament as a stable and largely inherited predictor of individual differences in intensity and threshold of affective response to myriad situations. Behavioural inhibition is a temperamental characteristic defined by the propensity to behaviourally show restraint, withdrawal, and reactivity to novelty, concomitant with physiological arousal (Kagan, Reznick, & Snidman, 1987), but findings have been inconsistent in relation to baseline cardiac activity and behavioural inhibition (Calkins & Fox, 1992; Garcia Coll, Kagan & Reznick, 1984). Research has supported the suggestion, however, that relative to less fearful children, children who are reactive, fearful, and considered high in behavioural inhibition, experience elevated autonomic arousal in situations they find novel or ambiguous (Calkins, 1997; Doussard-Roosevelt, Montgomery, & Porges, 2003; Kagan et al. 1987; Marshall & Stevenson-Hinde, 1998; Partridge, 2003). By the same token, children with externalizing behavioural and emotional regulation difficulties show parallel dysregulated responding at a physiological level, with emerging evidence suggesting that uninhibited behaviour relates to physiological underarousal (Beauchaine, 2007; Burgess, Marshall, Rubin, & Fox, 2003). Individual differences in reactivity and temperament are expected to be related to coping both because they relate to the individual's stress threshold activation and because they may promote or dissuade certain coping strategies (Compas, Connor-Smith, Saltzmen, Thomsen & Wadsworth, 2001).

In the stress and coping literature it is put forth that individuals may choose among two broad response orientations in response to a threat: to approach or to avoid (Roth & Cohen, 1986). That is, once a child evaluates the situation, he or she may attempt to cope with the stressor by approaching and engaging with the stressor or by withdrawal and avoidance. As children high in behavioural inhibition show the tendency to respond to challenge with frustration and to novel events or strangers with wariness, it is likely that such children will more often rely on avoidance or passive disengagement as a means to cope; children with uninhibited temperament may, in contrast, be more apt to show approach or active engagement toward stimuli or situations they initially find unnerving.

In the current study, children's use of approach or avoidant behavioural coping strategies toward the stressor was examined in relation to their physiological response.

Physiological Correlates of Emotion Regulation

Emotions affect physiological responses (Beauchaine, 2001; Roisman, 2007). The autonomic nervous system is responsible for the physiological response via its two subdivisions: the parasympathetic and sympathetic nervous systems. The parasympathetic division, responsible for restoration and maintaining homeostasis, and the sympathetic division, responsible for mobilizing resources and upregulating metabolic output (e.g., fight-or-flight response), work antagonistically to promote adaptive physiological and behavioural regulation of emotions and stress responses (Porges, 1995; 2007). Heart rate is affected by both parasympathetic and sympathetic input. It is therefore important to remember that change in heart rate from baseline only reflects reactivity of the autonomic nervous system generally and cannot be used specifically to examine sympathetic nor parasympathetic contributions. The contribution of the sympathetic nervous system to physiological regulation is studied non-invasively by quantifying the stress hormone cortisol (see Gunnar & Quevedo, 2007, for a review) and more recently, salivary α-Amylase (Hill-Soderlund et al., 2008). The influence of the parasympathetic nervous system on heart rate is the measure of interest in the current study.

The parasympathetic branch of the autonomic nervous system is particularly implicated in regulatory processes, including the dynamic change of heart rate via the inhibitory control of the heart by the vagus nerve (Porges, 1995). The vagus nerve, which originates in the brainstem, has complex interconnections with the respiratory center and connections to the midbrain from higher cortical areas (Porges, 2007). In Porges' Polyvagal theory (1995; 2001), the brainstem regulation of heart rate via the vagus nerve (i.e., vagal tone) is an index of homeostasis. For this reason, baseline cardiac vagal tone is considered to be a stable and enduring indicator of autonomic and temperamentally based reactivity in the absence of environmental challenge (Calkins & Keane, 2004; Doussard-Roosevelt, Montgomery, & Porges, 2003).

The vagus nerve regulates heart rate via the vagal "brake" (Porges, 2001). The brake slows heart rate in the absence of challenge, but when the individual is involved in an activity that requires active coping or response to environmental demands, the vagal brake is withdrawn (i.e., vagal tone is inhibited) to support increased heart rate. The brake is re-engaged via activation of vagal tone to promote return to a resting state. Taken together, vagal functioning reflects the ability to shift between internal demands (i.e., maintaining homeostasis) and response to external demands (i.e., vagal withdrawal) and has been related to children's ability to effectively regulate affective and behavioural arousal (Gunnar & Quevedo, 2007; Porges, 2001).

Cardiac vagal tone, which specifically indexes parasympathetic regulation of heart rate, is quantified by measuring respiratory sinus arrhythmia (RSA), that is, beat-to-beat change in heart rate (HR) during respiration (Kennedy, Rubin, Hastings, & Maisel, 2004; Porges, 1995; 2001; 2007). RSA is widely considered to reflect the parasympathetic tonus of the heart, with decreases in RSA indicating release of the vagal brake (i.e., vagal withdrawal or suppression). Brain regions associated with cognition and emotion influence respiratory centers in the brainstem where the vagus nerve originates, especially under conditions of heightened attention, weariness or coping, to suppress vagal control of the heart, reflected in decreased RSA and, in turn, increased HR.

Taken together, as an individual difference measure in developmental science, the measurement of autonomic reactivity through measures of parasympathetic involvement in HR yields divergent information about how children react to stress internally that cannot be gauged through behavioural observation, interview, or questionnaire (Alkon, Goldstein, Smider, Essex, Kupfer, & Boyce, 2003). Indeed, Porges (2001) contends that the vagal branch of the autonomic nervous system mediates affect, which is one reason to believe that baseline and change in RSA correlate with emotion regulation capability.

Across laboratories, researchers are finding that children with behavioural regulation problems show lower baseline RSA and less vagal withdrawal to challenge (Beauchaine, Gatzke-Kopp, & Mead, 2007; Calkins, 1997). Thus, cardiac vagal regulation between baseline and challenge reflects how well children are able to respond flexibly to myriad situations requiring cognitive, emotional, or attentional demands (Bernston, Cacioppo, & Quigley, 1993; Calkins & Keane, 2004; Porges, 2007). In the current study, children's baseline RSA and HR response to a social stressor was examined in the context of their ability to regulate their emotions on a behavioural and physiological level.

Quality of attachment relationship and physiological correlates of emotion regulation

Originally proposed by John Bowlby (1969/1982), attachment theory posits that children internalize a mental representation of themselves and close others in response to early interactions with attachment figures. The young child's attachment system functions to ensure security and survival by maintaining a balance between proximity seeking (being close to, checking in with parent or caregiver) and exploration (seeking out things or others) within their direct environment (Ainsworth, Blehar, Waters, & Wall, 1978). Securely attached children see their caregiver as their secure base, facilitating their exploratory behaviour and regulating reactivity in situations that stress the child (Cassidy, 1994; Shamir-Essakow, Ungerer, & Rapee, 2005). Insecurely attached children appear relatively less confident in their caregiver's availability as a source of comfort: Insecure-Avoidant children tend to ignore or avoid their caregiver in a somewhat premature effort to independently regulate their own negative affectivity, whereas Insecure-Ambivalent children heighten displays of negative emotions in an effort to ensure their caregiver's response.

The effect of Insecure attachment on measures of physiological regulation is beginning to be studied. Sprangler and Grossman (1993) found that although Insecure-Avoidant infants masked their behavioural distress during the Strange Situation, they showed greater arousal physiologically than their Secure counterparts, as evidenced by higher heart rate arousal and cortisol levels. More recently, Hill-Soderlund and colleagues (2008) found that children classified as Avoidant had higher vagal withdrawal and higher salivary α-Amylase than infants classified as Secure. They argued that masking negative affect via avoidant coping requires the greatest regulatory efforts and change from homeostasis.

Of interest in the current investigation was the physiological regulation of children classified as Insecure-Ambivalent. Unfortunately, there has been relatively less focus on the physiological regulation of infants and children classified as Insecure-Ambivalent for myriad reasons, although some findings indicate that this small population subset are among the most biologically vulnerable (Cassidy & Berlin, 1994). While always of interest to attachment researchers (Bar-Haim, Dan, Eshel, Sagi-Schwartz, 2007; Cassidy & Berlin, 1994; Moran & Pederson, 1998), young children classified as Ambivalent comprise only a small proportion of many samples studied (e.g., 7-15%) and thus are regularly collapsed with those classified as Insecure-Avoidant (Cassidy & Berlin, 1994). Classically the physiological regulation in Insecure-Ambivalent infants has been difficult to study because, characterized by feisty and resistant behaviour, Ambivalent infants in particular are prone to removing electrodes and cords necessary for cardiac study (Hill-Soderlund et al., 2008). Preschoolers classified in the current study as Ambivalent proved less difficult to study physiologically, and with a disproportionately large number of children given a primary classification of Ambivalent, they were of focus here in comparisons with those classified as Secure.

Attachment theory contends that when children are faced with a stressful or threatening situation, their attachment system is activated and the child seeks protection and reassurance. When a caregiver does not regularly act as a secure base for her child, however, the child learns that she is not a reliable source of comfort and security. Similarly, when a caregiver does not act as a source of support for exploration, a child will feel less confident attempting to navigate in the world away from her (Marvin, Cooper, Hoffman, & Powell, 2002). Indeed, in times of stress, children with Insecure-Ambivalent attachment relationships will seek comfort, but presumably due to a history of inconsistent care, resist the caregiver and as a result cope somewhat unsuccessfully with the stressor (Cassidy, 1994). As the child-caregiver relationship is the primary relational context through which parents can alter children's developing behavioural and physiological regulation and coping (Moore et al., 2009; Porges, 2001; Porter, 2003), studying how attachment status relates to children's regulatory capability provides crucial insight into children's differential adaptability to environmental demands.

Preliminary evidence suggests that the quality of the child-caregiver attachment relationship may moderate physiological stress responses of children and thereby is an important determinant of emotion regulation competencies, and more broadly, psychosocial adaptation in children (Cassidy, 1994). Cassidy has argued that parental behaviour facilitates the development of children's complementary attachment strategies.

Furthermore, sensitive parental behaviour has been associated not only with a secure attachment relationship, but also with high vagal tone. For example, Porter (2003) found that caregivers who played with their infants in a coordinated way (i.e., guiding their own behaviour by the gaze, facial expressions, gestures and vocalizations of the infant) promoted a Secure attachment relationship and facilitated higher resting RSA. Lomax-Bream (2001) found that high maternal sensitivity at 3 months was predictive of infants at 12 months showing RSA suppression in response to a frustration-task. Taken together, these studies provide evidence to support that early sensitive parenting positively affects children's ability to respond flexibly on a physiological level.

In another study, Kidwell and Barnett (2007) found that high baseline RSA was protective in a high risk African American sample only for those children classified as Secure, but placed Insecure children at heightened risk of externalizing emotion regulation difficulties at age 6. These authors raised the possibility that high baseline vagal tone indicates responsiveness to environmental demands generally, either positive or negative, and that this reactivity is detrimental only to emotion regulation development in Insecure children because of the poor caregiver co-regulation in infancy and toddlerhood that characterizes the relationship. It would appear from this and other research (Gilissen, Bakermans-Kranenburg, Van Ijzendoom, & Van der Veer, 2008; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Stevenson-Hinde & Marshall, 1999) that the attachment relationship may moderate biologically based risk such as temperamental characteristics and autonomic nervous system reactivity and in fact, that reactivity might only be a risk factor when coupled with poor dyadic regulation early in life.

Attachment, Temperamental Characteristics, and Physiological Stress Response

With increasing age children's socialized emotion regulation patterns, learned within early attachment relationships, generalize to contexts outside of the child-caregiver system. Securely attached children, who tend to express their emotions openly and directly, are at an advantage relative to Insecure children (both Avoidant and Ambivalent), who tend to express less positive affect and recover from distress less quickly (Cassidy, 1994). Parental responses that fail to support a Secure attachment relationship may be especially problematic for children high in behavioural inhibition. Indeed, mothers of children with Insecure attachment and high behavioural inhibition have been found to interfere with their children's attempt to cope with novelty, interfering with their children's opportunity to practice coping with unfamiliarity and to experience self-efficacy (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996).

It is likely that poor emotion regulation ability in children with high behavioural inhibition and Insecure attachment may be reflected in dysregulated cardiac physiology in times of stress (Beauchaine, 2001). A small number of studies have begun to investigate the potentially interactive roles of inhibition and attachment in predicting physiological indicators of children's emotional reactivity and regulation. Support for the hypothesis that child-caregiver attachment moderates the physiological impact of behavioural inhibition spans different physiological measures and ages including: (1) elevated cortisol in highly fearful and Insecure infants only (Nachmias, Gunnar et al., 1996); (2) high skin conductance reactivity in fearful middle schoolers classified as Insecure only (Gilissen, Bakermans-Kranenburg, Van Ijzendoom, & Van der Veer, 2008); and (3) lack of vagal withdrawal to a frustration-task in infants with insensitive mothering (Lomax-Bream, 2001). Taken together, these studies suggest that quality of caregiving moderates the influence of inhibited or fearful temperament on various physiological stress responses in children of different ages.

Thus far, however, findings are far from conclusive. Stevenson-Hinde and Marshall (1999) used a modified Strange Situation Paradigm (Main & Cassidy, 1988) in their study of behavioural inhibition, cardiac activity and attachment and found no real effects of RSA. Their main finding was that only Secure children low in behavioural inhibition showed decreased HR three minutes after reunion. Oosterman and Schuengel (2006) examined how HR and RSA varied in relation to the separation-reunion procedure in children ranging in age from 3 to 6 years old. All children on average showed vagal withdrawal during the separation, but children's security of attachment and behavioural inhibition did not predict change in RSA between separation and reunion with the parent. The authors explained that the separation may not have been stressful enough to challenge emotion regulation processes of the vagal or attachment system in these children already exposed to preschool and kindergarten. In sum, more research is needed to elucidate how attachment status serves to promote children of differing biological risk factors toward adaptive emotion regulation, particularly across contexts not designed to elicit attachment-based stress (i.e., the Strange Situation Paradigm).

Aims of the Present Study

The current study assessed parasympathetic (via RSA) nervous system contributions to HR, and behavioural approach versus avoidance behaviour, in a paradigm that induces stress within the context of the attachment relationship, the

Interesting-but-Scary (IbS) paradigm (Forbes, 2003; Forbes, Evans, Moran, & Pederson, 2007). Children's cardiac data were used in conjunction with attachment status (as assessed in the modified preschool Strange Situation Paradigm) in order to better understand how preschool aged children respond physiologically to a stressor (i.e., the IbS). Measuring change in children's vagal tone (RSA) from baseline as they explored the novel and potentially frightening *interesting-but-scary* character was considered valuable in understanding how attachment status may protect or predispose children to physiological dysregulation in the face of challenge.

The hypotheses of the present study followed from the available literature. The first hypothesis was that the children sampled, on average, would show a physiological response to the IbS paradigm relative to baseline. It was theorized that overall, children's RSA would decrease and their HR would increase upon exposure to the interesting but moderately frightening stimulus.

The second hypothesis was that children's biologically based individual differences in reactivity to novelty would relate to their resting physiological state (Calkins, 1997). As such, it was expected that children who coped with and met task demands by showing approach behaviour in the IbS paradigm would have lower resting HR and higher RSA at baseline than their more fearful counterparts. It was also expected that resting RSA would be negatively related to children's behavioural inhibition rating in the IbS paradigm, such that children with lower RSA at baseline would have higher behavioural inhibition ratings.

Finally, the third hypothesis was that children's degree of physiological regulation between baseline and IbS episodes would differ depending on their attachment classification. It was theorized that children classified as Ambivalent have had less practice developing emotion regulation skills in the face of challenge, and that this may be evidenced through poorer vagal regulation than those children classified as Secure.

Method

Participants

Mother-child dyads recruited as part of an ongoing study (UWO Review Number 09140S) were invited to participate in a laboratory visit when children were between 3 years, 1 month and 4 years, 1 month. A total sample of 88 dyads participated in the laboratory visit. After electrode failure or technical difficulty (n = 24), abnormal physiology (n = 5), or child refusal (n = 11) there were 23 male children and 25 female children who had useable physiological data (*Mean age* = 3.4, *SD* = .38). Data cleaning procedures and decisions regarding abnormal physiology are described below.

Of the sample with physiological data (N = 48), about 47% percent of mothers reported a household income of less than $30,000, 24% reported a household income between $30,000 and $60,000, while the remaining 29% of participants reported a household income greater than $60,000. Two percent of mothers left this field blank. All dyads were English speaking and children were first born. Children who had useable physiological data did not differ from excluded children in terms of gender [$\chi^2(1, N = 86) = .04$, *ns*], socio-economic status [$t(74) = .368$, *ns*], or age [$t(84) = -.19$, *ns*].

Procedural Overview

Mothers who had previously participated in the longitudinal study were contacted and given a full description of the visit. Mothers who had not participated in a previous study were also recruited via telephone to participate. If mothers were interested in participating in a visit they were mailed a questionnaire package and demographic information booklets. Dyads were booked to come into the University of Western Ontario Child Development Research Laboratory for a 1 hour and 30 minute visit. Upon arrival at the lab mothers were required to read a letter of information (Appendix A), and upon signing consent for participation, observation, and training (Appendix B), the study began.

Upon arrival, dyads were fitted with electrodes and physiological recording equipment. A stuffed cow with sticker electrocardiogram (ECG) electrodes was used to show the children the procedure for monitoring heartbeats during the study. Electrode stickers were placed by trained experimenters in an inverted triangle configuration on opposite sides of each participant's chest just below the collarbone, with a ground lead positioned on the lower left rib. Participants sat alongside their mothers and watched a short video (Teletubbies clip) for a minimum of 2 minutes to provide baseline physiological data. Other laboratories have used a similar procedure to collect resting cardiac from young children (Calkins, Graziano, Berdan, Keane, & Degnan, 2008), acknowledging that although children's attention was held by an external stimulus, this procedure also helps young children to sit quietly, thereby limiting artifacts in the IBI series. Mother-child dyads also participated in the Preschool Strange Situation Procedure (see measures; Cassidy & Marvin, 1992). All interactions in the laboratory room were video and audio recorded.

In the final reuniting sequence of the Strange Situation Procedure the dyads played for 3 minutes. Then, the mother was cued to open a cupboard containing a mask of an unusual looking creature (Appendix C). This marked the onset of the *Interesting-but-Scary* (IbS) paradigm modified for preschoolers (Forbes, 2003; Forbes, Evans, Moran, & Pederson, 2007). Although this paradigm was originally developed to assess attachment security (i.e., Forbes, 2003) the current modified pre-school IbS was utilized to assess children's physiological stress response and behavioural inhibition. The IbS paradigm represents a novel and ambiguous social situation for the child similar to that of a well validated paradigm (Behavioural Inhibition Paradigm) that assesses children's inhibition using a variety of probes, based on their ability to interact with a novel, oddly dressed experimenter (Garcia Coll, Kagan, & Reznick, 1984).

During this task, dyads were presented with an ambiguous and potentially frightening situation for the child. The mask of the *interesting-but-scary* creature was mounted into the cupboard and had a speaker inserted behind it. The mother was shown the mask prior to the onset of the study and was instructed to act as she normally would if her child became frightened. The mother was instructed that she could close the cupboard if her child became highly distressed by the task, but was reassured that the task was brief. The experimenter, from another room, engaged the child in conversation (via the mask) in a friendly voice. During the first minute the examiner engaged in interactive conversation with the child about what toys they had played with. At the onset of the second minute the experimenter invited the child to touch her nose (the mask). The full script is an adapted version of Stevenson-Hinde's (1991) behavioural inhibition script (Appendix D). When the two minutes expired the mother was signaled by a knock to close the cupboard door.

Following the laboratory visit dyads were invited to ask any questions about the study. A debriefing script was prepared for explaining the purposes and use of the collected data (Appendix E). Both the mother and child had their physiological equipment removed prior to leaving the laboratory and children were provided with a souvenir of their visit to take home. At this time, mothers were reimbursed $30 for their time and incidental expenses.

Materials

Physiological Recording. Interbeat interval (IBI), the time between two consecutive heart beats, must be measured in order to calculate HR and RSA. There are several techniques to extract RSA from IBI data: these techniques are classified as frequency domain measures and time domain measures. In the current study RSA was quantified using a time domain measure, RMSSD, the root mean of the squared successive differences in IBI. RMSSD is sensitive to fluctuations in IBI in the respiratory range and because of this it functions as a high-pass filter that captures predominately respiratory sinus arrhythmia. There are unresolved controversies (Allen, Chambers, & Towers, 2007; Goedhart et al., 2007; Porges, 2007) in the field regarding measurement of vagal tone (i.e., effect of respiration rate, lung volume). In some literature, RSA and cardiac vagal tone are equated, considered by others as inappropriate because, although RSA reflects vagal tone, there are other factors that influence heart rate variability independent of vagal tone. The current study, acknowledging the imperfect measures of vagal functioning that all currently used indices provide, used the RMSSD statistic to quantify RSA (Bernston, Lozano & Chen, 2005; Richards, 1995; Ritz & Dahme, 2006). For our purposes, the term RSA is equivalent with cardiac vagal tone, while a decrease in RSA is equivalent with vagal suppression, decrease, or withdrawal to challenge, reflecting release of the vagal brake from baseline (Hill-Soderlund et al., 2008; Porges, 2007).

Children's IBI cardiac data was derived from an electrocardiogram (ECG) through electrodes with a BIOPAC^{TM} MP100 unit and an ECG 100B amplifier acquiring data at 500 samples per second. The unit wirelessly transmitted data to a Pentium computer running Acknowledge 3.8 software (BIOPAC, California) and was synchronized with video feed via Noldus Observer 7.0. After a threshold had been set in the AcqKnowledge software for detection of the R-waves, the program created a list of the successive IBI lengths. The raw ECG data was passed through a high pass filter to reduce low frequency variability and noise.

Following this first filter, each waveform was examined for artifacts by the author. An artifactual peak of similar magnitude to a cardiac peak (i.e., R-wave) would interfere with accurate extraction of each IBI length, resulting in two short false intervals being extracted instead of one genuine IBI. Therefore, each ECG was visually scanned by the author before and after extraction of the IBIs. Artifacts were edited from the data by replacement with non-voltage peak values, so that the integrity of the IBI series was maintained.

Two measures were derived from each IBI series in 15 second epochs (i.e., segments): heart rate (HR as measured in beats per minute) and respiratory sinus arrhythmia (RSA), across the baseline and IbS episodes. Acknowledge was then used to convert the IBIs into heart rate with units of beats per minute (BPM).

$$\textbf{HR} = \frac{1}{IBI} x \ 60{,}000 \ ms$$

The estimated HR based on each IBI was also scanned to ensure accurate extraction and that no artifacts were missed. An Excel template designed by the author was used for each child to average their 15 second epoch estimates (i.e., an average of the averages), to yield the mean heart for that episode, which was referred to in the analyses simply as HR (in units of BPM).

RSA was estimated using a time domain measure of the variability contained in the IBI series, the root mean squared successive differences (RMSSD) of the IBI data.

$$\textbf{RMSSD} = \sqrt{\frac{1}{n}\sum(IBI_i - IBI_{i-1})^2}$$

RMSSD was calculated using an Excel template designed by the author, converted to units of $\ln(\text{msec})^2$ as is conventional in the field, and was referred to as RSA. RMSSD has recently been shown to be a sensitive measure to primarily vagal influences on HR (Berntson, Lozano, & Chen, 2005), to be a reliable estimate of longer duration RSA measures (Hamilton, Mckechnie & Macfarlane, 2004; Richards, 1995), and to be robust to the influence of respiration rate and movement (Goedhart et al., 2007). As described previously, one alternative method of estimating RSA is a frequency domain technique, spectral analysis. For a randomly selected participant, the natural logarithm of the RSA measure (RMSSD) for the IbS episode was compared to values estimating RSA with spectral analyses approach, as calculated by Dr. Thomas Hollenstein at Queen's University. There was a moderate-strong correlation (r = .65) between the two measures providing additional pragmatic assurance that the estimate of RSA in this study is indexing respiratory-associated variance in the IBI series as accurately as other commonly used methods of quantifying RSA.

Attachment Classification. Following baseline physiological periods, children and their mothers were asked to participate in a modified Strange Situation Procedure (SSP; Cassidy & Marvin, 1992; Main & Cassidy, 1988). The SSP is the standard behavioural assessment of the quality of attachment relationship, involving a series of separations from and reunions with the caregiver (SSP; Ainsworth, Blehar, Waters, & Wall, 1978). In order for the SSP to be developmentally appropriate for preschoolers, the modified SSP has longer separations (approximately 4 minutes in duration) during which the child remained alone rather than with a stranger. It is widely held that the separations and reunions serve to activate the attachment system by eliciting a moderate level of attachment-related stress, particularly during reunions with the mother following this activation.

The SSP consisted of a 3-minute baseline period where the mother remained with her child in a room with chairs and a variety of age-appropriate toys. The mother was asked to handle the separation periods as she normally would. Following the baseline period, in response to an auditory cue, the mother exited the room, leaving the child alone for 4 minutes (separation 1). After this period, the mother re-entered the room (reunion 1) where she remained for 3 minutes thereafter. Again, the mother heard the knock at the window at the conclusion of the reunion period, when she left the room again for 4 minutes more (separation 2). Finally, the mother was instructed to go back to the room (reunion 2), at which time the dyad played for a minimum of 3 minutes.

Children's responses to the separations and reunions during the SSP were classified by coders trained and certified as reliable using the Preschool Attachment Classification System (Cassidy & Marvin, 1992). Each dyad was assigned one of four primary attachment classifications (Secure, Insecure-Avoidant, Insecure-Ambivalent, and Disorganized/Controlling) in accordance with their behavioural and emotional response to the procedure (Solomon & George, 2008). Children are classified as Secure if they demonstrate comfort and ease in interaction with the mother, show minimal distress in response to separation and are easily comforted and quickly re-engage with play upon the mother's return at reunion, and negotiate freely with the mother around their needs and wants. Children are classified as Insecure-Avoidant if they appear to avoid using the mother as a secure base, despite the stressful nature of the SSP. The children outwardly appear neutral and rarely express significant displays of positive or negative emotion. Children are classified as Insecure-Ambivalent if they demonstrate fussy, whiny or resistant behaviour toward the mother. Ambivalent children may protest separation strongly, showing heightened intimacy and dependency, but the reunion is mutually unfulfilling, characterized by the child's ambivalence, subtle hostility, or babyish or coy behaviour. Children who demonstrate role-reversal, whereby they appear to take on an either caregiving or punitive parental role toward their mother are classified as Insecure-Controlling, whereas children who appear to lack a coherent strategy for approaching their attachment relationship are classified as Insecure-Disorganized. Disorganized attachment reflects a breakdown in strategy (i.e., children attempt to employ a strategy to relieve their distress, however the attempt is interrupted by oddly disoriented behaviours, thus distress is not minimized), whereas the other three organized classifications represent a learned strategy (i.e. a strategy that children have adopted to maximize caregiver availability based on past interactions with that caregiver). Finally, children who demonstrate myriad strategies or who fail to fit one of the aforementioned categories, are labeled Insecure-Other.

It is sometimes appropriate to add a secondary classification if the child demonstrated some additional behaviours characteristic of another attachment classification, although not enough to consider this classification as primary. If no secondary classifications are added, it can be inferred that the child's attachment behaviour during the Strange Situation procedure more consistently followed a single attachment strategy (that of the primary classification) and relational elements characteristic of other attachment classifications were not observed (or only very minimally observed).

In the current study, primary attachment classifications were the focus, and those children who had primary and secondary classifications were excluded to promote comparisons of homogenous groups (i.e., children with the same primary classification only). At the time this thesis was written, 22 children had assigned attachment classifications. The children classified as Secure or "B" only (N = 10) and Ambivalent or "C" only (N = 5) were selected for analyses, such that the homogeneity of the samples would yield more powerful comparisons (Kramer & Rosenthal, 1999).

Behaviour during the Interesting-but-Scary (IbS) *Paradigm.* Children's approach and avoidance behaviour were assessed by determining whether the child met the IbS task demands, to approach the potentially fear-inducing stimulus. Children's approach was recorded dichotomously. If the child approached the mask to touch its nose at any point, then the child was considered to show approach behaviour and to have met task demands. On the other hand, if the child refused or avoided touching the mask's nose, even when requested, the child was considered to have showed avoidance behaviour and to not have met the task demand.

To better understand physiological differences, the author assigned ratings of the child's motor movement during the episode, rated on a 7-point Likert-type scale ranging from 1 *(little to no movement)* to 7 (*vigorous motor activity).* It was also recorded if the child was sitting or standing (or did both).

Behavioral Inhibition Scales (Stevenson-Hinde, 1991). The behavioural inhibition scales were coded by two independent and reliable coders during the IbS paradigm to provide a clearer picture of children's inhibition based on a measure used by Stevenson-Hinde and Marshall (1998; 1999). Two scales were used to rate child behaviour: (1) low verbal responsiveness and (2) nonverbal anxiety. Ratings were made on scales from 1 (*relaxed/responsive*) to 9 (*high tension/no verbal response*). A global rating of behavioural inhibition was also made on a scale from 1 (*no signs of inhibition*) to 9 (*extreme inhibition*) with 4 denoting a child who has mild or brief signs of inhibition. Coding criteria outlined by Stevenson-Hinde (1991) for the Behavioural Inhibition Scales can be seen in Appendix F. To ensure laboratory consistency in coding, eight videos were used for training and reviewed collaboratively by the two primary coders to distinguish between Stevenson-Hinde's basic descriptors. As such, decision rules were employed at this time to discern how the codes were different (e.g., Nonverbal Anxiety Scale: (2) *Relaxed* = full body relaxed, some darting eyes, no other tensions; (3) *Generally at ease* = full body mostly relaxed, with some mild tenseness in hands or legs). Twenty-five percent of the remaining videos were coded by two trained coders and interrater reliability was excellent for the global ratings of inhibition ($r = .90$, $p < .001$); the nonverbal anxiety scales, ($r = .90$, $p < .001$); and the low verbal responsiveness scale ($r = .81$, $p < .001$).

Statistical Analyses

As RSA was skewed in both episodes of the procedure, its natural logarithm was used in the analyses (i.e., RSA therefore represents lnRSA). Reporting the natural logarithm of RSA is a widely held standard among physiological researchers in the literature. Repeated measures analysis of variance (repeated measures ANOVA) techniques were used to examine changes in the physiological measures across episodes. All probability values given in the text for main effects and interactions involving episode have been adjusted using the Greenhouse-Geisser estimate, which accounts for the correlation between observations on the same individual and violations of sphericity (Geisser & Greenhouse, 1958, in Howell, 2002). To result in more conservative estimates of the degrees of freedom (*df*) provided with the Greenhouse-Geisser correction, the *df* were rounded down.

Data Reduction

HR and RSA were calculated every 15 seconds for the baseline period and the IbS paradigm. Fifteen second epochs are commonly used for episodes shorter than 2 minutes (Moore et al., 2009), and have been shown to be reliable estimates of measures over longer periods (Hamilton, Mckechnie & Macfarlane, 2004; Richards, 1995). Consistent with other researchers (Calkins & Keane, 2004) epochs requiring more than 10% editing were not included in the analyses and if the grand average of the epochs had a standard deviation greater than 1.00, calling into question the validity of the mean, the data file was excluded from analyses.

Results

Descriptive statistics for the study are presented in Table 1. Preliminary analyses examined whether there were any relations between sex and household income and the physiological measures RSA and HR between the baseline and IbS episodes. These correlations were not significant. Children's motor movement rating, and posture (standing vs. sitting), also were not related to the physiological results. There were no significant sex, age or SES differences across the physiological measures.

Main Effects of the Interesting-but-Scary Paradigm On HR and lnRSA

To determine the effects of the *Interesting-but-Scary* paradigm on HR and autonomic nervous system functioning across the entire sample (N = 48), repeated-measures analyses of variance (ANOVAs) were performed for each physiological variable (HR and lnRSA) to identify changes from baseline.

As expected, HR significantly increased in all children, on average, between the baseline (M = 103.47, SD = 10.21) and the IbS paradigm (M = 111.55, SD = 11.00), $F(1, 47) = -55.46$, $p < .001$, partial $\eta^2 = .54$ (large effect size). Similarly, RSA decreased between the baseline (M = 7.37, SD = 1.08) and the IbS paradigm (M = 6.68, SD = 1.11), $F(1, 47) = 28.41$, $p < .001$, partial $\eta^2 = .38$ (large effect size), indicating withdrawal or suppression of the parasympathetic nervous system in response to challenge.

A regression analysis was used to examine the amount of variance in HR accounted for by adaptation of the parasympathetic nervous system (i.e., vagal withdrawal). Change in RSA accounted for 53.2% of the mean increase in HR between the episodes, $t(47) = 7.24$, $p < .001$.

Individual Differences in HR and lnRSA Across the Sample

Children who showed vagal withdrawal (any positive change score from baseline to IbS paradigm indicating a decrease in lnRSA) had significantly higher baseline RSA (M = 7.51, SD = 1.09) than children who did not show vagal withdrawal (M = 6.77, SD = 0.84), $t(15) = 2.25$, $p < .05$, $d = .72$ (large effect size). In contrast, suppression versus non-suppression groups did not differ on baseline HR. Thus, children who showed greater physiological adaptation to their environment, in the form of vagal withdrawal in relation to novelty or stress, also had higher resting vagal tone than children who adapted less readily.

Behavioural Inhibition Ratings & Approach vs. Avoidance Behaviour Across the Sample

Children's baseline RSA and HR were not related to observer's ratings of low verbal responsiveness, nonverbal anxiety, or the global rating of inhibition based on both verbal and nonverbal indicators in the IbS (all correlations $p > .05$). Recall, children who approached the mask and touched the nose were classified in the "Approach" behaviour group, whereas children who did not meet task demands were classified as showing "Avoidant" behaviour toward the mask. Indeed, children who showed avoidance and did not meet task demands were rated as significantly more behaviorally inhibited on the 9-point Likert-type scale (M = 7.31, SD = 1.35) than children who approached and met task demands during the IbS (M = 4.50, SD = 1.95), $t(46) = 2.81$, $p < .001$.

Children who met task demands (n = 32) by approaching the mask during the IbS paradigm had marginally lower resting HR during baseline (M= 101.81, SD = 11.08) than children who avoided the mask (n = 16; M = 106.82, SD = 7.42), $t(42) = 1.86$, $p = .07$, d

= .51 (medium effect size). There were no significant group differences in RSA. Taken together, children who showed approach behaviour in the IbS paradigm had lower resting HR on average than their more fearful counterparts who avoided the mask.

Chi-square analyses were conducted to determine whether the distribution of IbS paradigm behaviour (approach vs. avoidance) differed based on the child's sex. There was a significant difference in the distribution of IbS paradigm behaviour groups based on sex, χ^2 (1, N = 48) = 8.18, $p < .01$, Cramer's V = .41 (medium effect size). Examination of the observed versus expected frequencies indicated that boys approached the mask more often and avoided it less often than would be expected by chance, whereas girls were slightly more likely to avoid the mask than to approach it. As stated earlier, however, gender was not associated with physiological measures and there were no 3-way interactions between gender, IbS paradigm behaviour, and vagal suppression.

Relationship between Attachment Status, HR and lnRSA, In a Sample Subset

A repeated-measures ANOVA was used to examine whether children classified as Secure (n = 10) showed significantly different profiles of vagal regulation (i.e., RSA change) across episodes than children classified as Insecure-Ambivalent (n = 5).

There was not a significant main effect of either attachment classification or episode in this subsample; however, there was a significant two-way interaction between episode (baseline, IbS paradigm) and attachment (Secure, Insecure-Ambivalent), $F(1, 13) = 18.89$, $p < .001$, partial $\eta^2 = .59$ (large effect size; see Figure 1).

Simple main effects analyses were conducted to explore this interaction. With respect to the simple main effect of attachment classification within episode, resting RSA did not differ between children classified as Secure and Insecure-Ambivalent, $F(1,15) = 0.18$, *ns*. During the IbS paradigm, however, children's level of physiological adaptation differed significantly depending on their attachment classification, $F(1,15) = 4.59$, $p < .05$, partial $\eta^2 = .82$ (large effect size). More specifically, children classified as Secure showed significantly lower RSA during the IbS episode than those classified as Ambivalent.

There were also significant simple main effects of episode within the attachment classifications: the Secure group showed meaningful decrease in RSA across episodes reflecting vagal suppression, $F(1, 9) = 47.80$, $p < .001$, partial $\eta^2 = .84$ (large effect size), whereas the Insecure-Ambivalent group did not, $F(1, 4) = 1.48$, *ns*.

A repeated-measures ANOVA also indicated a significant episode x attachment interaction effect on HR change between the baseline and IbS paradigm, $F(1, 13) = 13.31$, $p < .01$, partial $\eta^2 = .41$ (large effect size). This interaction became non-significant when the change in RSA was statistically controlled for (Figure 2), $F(1, 12) = 1.45$, *ns*.

The Impact of Attachment on RSA Withdrawal in Children who Approached

Of the 15 participants with Secure or Insecure-Ambivalent attachment classifications, approximately two-thirds ($n = 9$, 6 Secure) met task demands by showing approach behaviour. Within this group, a repeated-measures ANOVA was conducted to examine the effect of attachment classification. There was a significant two-way interaction between episode (baseline, IbS paradigm) and attachment (Secure, Ambivalent), $F(1, 7) = 11.64$, $p = .01$, partial $\eta^2 = .62$ (Figure 3). This was a large effect size.

Simple main effects analysis of attachment classification within episode revealed no significant effect of attachment classification on RSA during the baseline, $F(1,9) = 0.26$, *ns,* or during the IbS paradigm, $F(1,9) = 1.09$, *ns*. That is, among children who met task demands during the IbS paradigm, baseline and IbS paradigm RSA did not differ by attachment classification.

Conversely, a significant main effect of episode within attachment revealed that the Secure group showed a meaningful decrease in RSA from the baseline to IbS paradigm, $F(1,5) = 19.45$, $p < .01$, partial $\eta^2 = .80$ (large effect size) whereas those classified as Ambivalent did not, $F(1, 2) = 1.12$, *ns.* This means that despite children in the Insecure-Ambivalent group showing approach behaviour in the IbS paradigm challenge, physiologically they did not show vagal withdrawal in response to the stressor (unlike their Securely attached counterparts). There was not a significant main effect of either attachment classification or episode in this subsample.

Discussion

The current study focused on cardiac indicators of emotion regulation. Cardiac functioning is one key non-invasive psychophysiological marker of emotion regulation capabilities and the reactivity of children's autonomic nervous system to stress (Beauchaine, 2001; Porges, 1995). The present study investigated, within the context of the child-caregiver attachment relationship, the effect of a novel social stressor on children's physiological stress response. Previous research has shown that vagal withdrawal in response to challenge, as evidenced by decreased RSA from baseline, is indicative of adaptive functioning in response to emotional or cognitive challenge (Calkins, Graziano, Berdan, Keane & Degnan, 2008; Hill-Soderlund et al., 2008).

Consistent with the first hypothesis, children, on average, had a physiological response to the IbS paradigm as indicated by increased HR and decreased RSA relative to baseline. This finding parallels that of Calkins (1997) who found that across three affect tasks designed to elicit temperamental reactivity (whether negative, positive, or neutral) vagal tone decreased sample wide. This finding is also in line with evidence from the Strange Situation Paradigm that children's behavioural distress and HR increase during separations (Sroufe & Waters, 1977; Stevenson-Hinde & Marshall, 1999) suggesting that the separations stress children's physiological and coping resources. Paralleling these findings, infants have shown decreased RSA from baseline to the still-face episode of the Still Face Paradigm, a stressful experience in infancy (Moore et al., 2009). Thus the current findings attest to the validity of the IbS Paradigm as a novel stressor and an appropriate paradigm for studying children's physiological stress response alongside their caregivers.

The second hypothesis predicted that differences in children's baseline physiological state would relate to their adaptability to the stressor. Children who showed vagal suppression in the IbS paradigm had higher resting levels of RSA. This finding accords with findings in other samples (Calkins, 1997; Santucci et al., 2007) in particular Calkins (1997) who found that young children (ages 2 and 3) who showed vagal withdrawal across tasks designed to elicit affect had higher resting baseline vagal tone. The findings of the present study also support Porges' Polyvagal Theory (1995; 2001) that baseline vagal tone is an indicator of propensity to regulate physiologically in response to environmental demands. However, children's baseline RSA and HR were not related to observers' ratings of low verbal responsiveness, nonverbal anxiety, or a global rating based on both verbal and nonverbal indicators. This finding contrasts those supporting that behaviourally inhibited children display higher baseline heart rates (Doussard-Roosevelt, Montgomery and Porges, 2003; Partridge 2003) but concurs with findings of Stevenson-Hinde and Marshall (1999) who found no relationship between behavioural inhibition (using the same measure as the present study) and cardiac activity, until attachment status was taken into consideration. The way in which behavioural inhibition is measured (e.g., parental rating of shyness, frustration-task, meeting a stranger) seems to differentially predict baseline physiological findings. The interested reader is referred to Fox, Henderson, Marshall, Nichols and Ghera (2005) for an integrative review of studies examining the relationship between physiological measures and behavioural inhibition across development.

Children who showed approach behaviour in the IbS paradigm also had lower baseline HR, on average, compared to their avoidant counterparts. During periods of rest and low environmental demand, it is indeed adaptive to have low heart rate, which is a correlate of engagement of the vagal brake (i.e., higher vagal tone). This finding lends further credence to Porges' (2007) contention that children who employ active coping strategies to deal with challenge are better able to balance between homeostatic demands when at rest, and inhibition of vagal tone when coping physiologically with task demands.

Finally, as predicted in the third hypothesis, children's physiological response between baseline and the IbS Paradigm differed depending on whether the child was classified as Secure or Insecure-Ambivalent. More specifically, children classified as Secure showed vagal withdrawal in response to the stressor, whereas children classified as Ambivalent did not. This is a crucial finding, as most research combines Ambivalent and Avoidant Insecurely attached children to increase power, but finds mixed results in the Secure versus Insecure comparisons of physiological regulation (Stevenson-Hinde & Marshall, 1999). Whereas the physiological stress responses of Insecure-Avoidant children has been recently examined relative to Secure children (Hill-Soderlund et al., 2008), that of Ambivalent children has been the focus of relatively less research. Thus, for the first time, evidence of parasympathetic physiological dysregulation in Ambivalent children specifically exists to potentially explain their characteristic high negative affect and difficulty being comforted (Calkins & Fox, 1992; Cassidy & Berlin, 1994; Moran & Pederson, 1998).

Although not interpreted as such, Stevenson-Hinde and Marshall (1999) may have found parallel results in their study of the stress responses of 4.5 year old children in the Strange Situation Paradigm. The authors mention as an aside that children classified as Ambivalent had the highest mean RSA across the four assessment periods interspersed within the Strange Situation episodes. Stevenson-Hinde and Marshall explained that the Ambivalent pattern presented a contradiction in its association with high RSA (and relatedly, low HR). In light of the current findings, however, their findings may not have been contradictory: rather, children in an Ambivalent relationship appeared not to show appropriate vagal withdrawal to challenge and their non-changing RSA from baseline in fact may have represented physiological dysregulation rather than a protective factor. Taken together, there is mounting evidence that children classified as Ambivalent do not show adaptive physiological regulation in response to both stress of the attachment system at separation (i.e., the SSP) and social stressors in the context of their caregiver (i.e., the IbS paradigm).

Calkins and colleagues (2008) also found a main effect of maternal-child relationship quality (as assessed by degree of maternal hostility, positive affect, responsiveness and stress level) on children's physiological regulation. More specifically, in that study, indicators of dyadic dysfunction were related to children's reduced vagal regulation and HR change to six challenging tasks, attempted both independently and with their caregivers. The authors argued that their findings provided evidence that the caregiver relationship affects not only general arousal (i.e., HR change) but also the adaptability of the complex regulatory mechanisms of the parasympathetic nervous system via inhibitory vagal control (i.e., RSA). The current study replicates and extends the contribution of Calkins and colleagues (2008) by using attachment classifications, as opposed to maternal behaviours across brief emotion eliciting tasks, to gauge child-caregiver relationship quality. Both studies support the impact of insensitive parenting on children's enduring pattern of physiological dysregulation across situations.

The present study also investigated whether children classified as Secure or Ambivalent differed physiologically, even when behaviourally they all coped with the stressor by showing approach behaviour. Indeed, children who were in the group that approached had significantly lower ratings of behavioural inhibition, on average, than children who avoided the mask or failed to meet task demands. Even so, this study found that among all of the children who met task demands during the IbS by approaching the strange character, only those classified as Secure showed the adaptive physiological response of vagal suppression. This means that although the Ambivalent group showed approach toward the novel stimulus, a possible indicator of behavioural regulation and coping, physiologically they did not show vagal suppression. This is in contrast to the adaptive physiological regulation shown in the children in Secure relationships, whether or not they showed behavioural regulation. Taken together, children classified as Secure might show greater behavioural and physiological concordance than children classified as Ambivalent (present study) or Insecure-Avoidant (Hill-Soderlund et al., 2008; Sprangler & Grossmann, 1993).

Accumulating evidence supports the premise that quality of parenting is particularly influential for children with greater behavioural inhibition and temperamental reactivity: the least adaptive outcomes have been found for highly fearful children with insensitive parenting or Insecure attachment relationships (Gilissen, Bakermans-Kranenburg, Van Ijzendoom, & Van der Veer, 2008; Kidwell & Barnett, 2007; Lomax-Bream, 2001). In particular, research has supported a moderating role of attachment status on the relationship between behavioural inhibition and sympathetic nervous response. Of infants identified as inhibited, only those in Insecure attachment relationships have shown elevated cortisol responses to an inoculation (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996) and to the Strange Situation (Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996). Taken together, more research is needed to understand how quality of the caregiving relationship influences children across development (i.e., infancy to middle school) across measures of emotion and behaviour regulation, as well as physiological regulation via indicators of both parasympathetic and sympathetic nervous systems. Fascinatingly, emerging evidence suggests that maternal sensitivity moderates even genetic risk, wherein children with the dopamine receptor risk allele (DRD2) show vagal regulation to challenge commensurate with those infants without the risk allele (DRD4) only if they have sensitive mothering (Propper et al., 2008).

Understanding the protective impact of quality caregiving on physiological regulation in children remains an important research avenue, especially in light of preliminary research suggesting that attachment and regulatory processes of the limbic system are intimately related (Calkins & Hill, 2007; Gunnar & Quevedo, 2007; Schore, 2001). The limbic system is the area of the brain primarily responsible for emotion responding and adaptation, and is implicated in autonomic nervous system responsivity throughout the lifespan. As the limbic system is in a critical period of development during the first few years of life, adaptive maturation of a child's limbic system is contingent on the emotion based communication that occurs primarily within attachment relationships. In sum, then, studying how the children's attachment relationship predicts or protects children from physiological dysregulation will illuminate one key way to understand children's development using a biopsychosocial perspective.

Strengths and Limitations

Strengths of the current study include measurement of attachment status using a Strange Situation Paradigm, a stable indicator of relationship quality that has been found to generalize outside the laboratory context (Pederson & Moran, 1996). Moreover, measuring children's physiological reactivity in a different paradigm than the SSP means that children's physiological responses are not immediately dependent on the same behaviours used to classify attachment. Secondly, measuring both change in HR as well as vagal regulation was utile in examining how the parasympathetic nervous system specifically was implicated in physiological regulation in response to the stressful stimulus. Measuring indicators of children's physiological and emotion regulation in a paradigm other than the Strange Situation provides an important complement to what is known about children's reactivity in that attachment-based stressor. Finally, identifying a distinct group of children with Ambivalent classifications only allowed their physiological stress responses to be examined in a way that has previously been difficult to capture.

There were various limitations of the current research. First, children's physiological responses were sampled only at one time point, rendering developmental conclusions difficult to draw. Secondly, not having cardiac data after the IbS Paradigm, to index children's period of vagal recovery (i.e., return to baseline), limits conclusions regarding children's ability to return to a resting state, after coping with environmental challenge, in relation to the quality of their child-caregiver attachment relationship. Third, limited sample size of children with both attachment classification and physiological measures, for myriad reasons, precluded more extensive analysis of how attachment status may moderate the relationship between behavioural inhibition and physiological regulation. Indeed, the relative lack of Avoidant dyads in this sample was unexpected based on the distributions of attachment classifications in other research (Hill-Soderlund et al., 2008) and warrants further investigation. Although speculative, possible explanations for this difference include cultural or socioeconomic influences or demographic trends.

Future Directions

The findings of the current study represent a crucial step in understanding how physiological stress responding relates to children's attachment status, particularly the discovery that children classified as Ambivalent children do not show vagal withdrawal in response to a novel stressor, the IbS paradigm. Indeed, this finding may begin to explain the mechanism underlying the finding that infants classified as Ambivalent are at increased risk for onset of anxiety disorders relative to those classified as Secure or Avoidant (Warren, Huston, Egeland & Sroufe, 1997).

Continuing to pursue understanding of individual differences in physiological regulation, and how the quality of the child-caregiver relationship may serve to be moderate this relationship, is theoretically and clinically significant. As the development of behavioural and emotion regulation capability burgeons during the preschool period, measuring physiological adaptability via vagal withdrawal during this time period lends researchers a window through which to gauge children's psychosocial developmental trajectory. Additional measures of physiological risk (i.e. both sympathetic and parasympathetic nervous system correlates) as well as genetic risk factors (e.g., dopamine receptor alleles) will enrich our understanding of predisposing and protective factors in the relationship between caregiving quality and regulatory adaptability, in particular contributing greatly to our understanding of temperamentally fearful youngsters.

Summary and Conclusions

The present study investigated, within the context of the child-caregiver attachment relationship, the effect of a novel social stressor on children's physiological stress response. Independent of attachment status, children who showed vagal suppression had higher resting RSA, and children who showed approach behaviour had lower resting HR. Both high levels of baseline RSA and relatively low heart rate were indicative of children's adaptive coping in response to task demands.

Children's change in physiological response from baseline to the *Interesting-but-Scary* Paradigm also differed according to attachment classification. More specifically, children classified as Secure showed vagal withdrawal in response to the stressor, whereas children classified as Ambivalent did not. Moreover, even among all of the children who met task demands and showed approach behaviour toward the strange character, only those classified as Secure showed vagal suppression.

In conclusion, past research and the current findings underscore the importance of facilitating sensitive parenting and a Secure attachment relationship in infancy and early childhood, the time when physiological and emotional regulation abilities are developing that underlie children's well-regulated behaviour and independent functioning. Helping parents in both a prevention and intervention context (e.g., Marvin, Cooper, Hoffman, & Powell, 2002) to be responsive and to provide a secure base in relation to their Ambivalently attached children would be justified in light of the current study, as a step toward promoting more adaptive physiological and emotion regulation in their children across future contexts.

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Table 1

Descriptive Statistics Across the Sample and as a Function of Gender, Attachment Status, and IbS Behaviour

	lnRSA		HR	
	Baseline	IbS	Baseline	IbS
Sex				
Male (*n* = 23)	7.37 ± 1.18	6.76 ± 1.15	101.48 ± 10.85	110.03 ± 11.90
Female (*n* = 25)	7.36 ± 1.01	6.61 ± 1.09	105.31 ± 9.44	112.96 ± 10.15
Attachment Status				
Secure (*n* =10)	7.59 ± 1.15	6.54 ± 1.21	100.06 ± 10.31	110.21 ± 13.13
Ambivalent (*n* = 5)	7.33 ± 0.72	7.84 ± 1.10	101.63 ± 6.08	103.25 ± 9.11
IbS Behaviour				
Approach (*n* = 32)	7.46 ± 1.08	6.71 ± 1.08	101.81 ± 11.08	110.56 ± 12.02
Avoidance (*n* = 16)	7.20 ± 1.09	6.62 ± 1.20	106.81 ± 7.42	113.53 ± 8.62

Note. The values represent mean (± *SD*). RSA = respiratory sinus arrhythmia, HR = beats per minute.

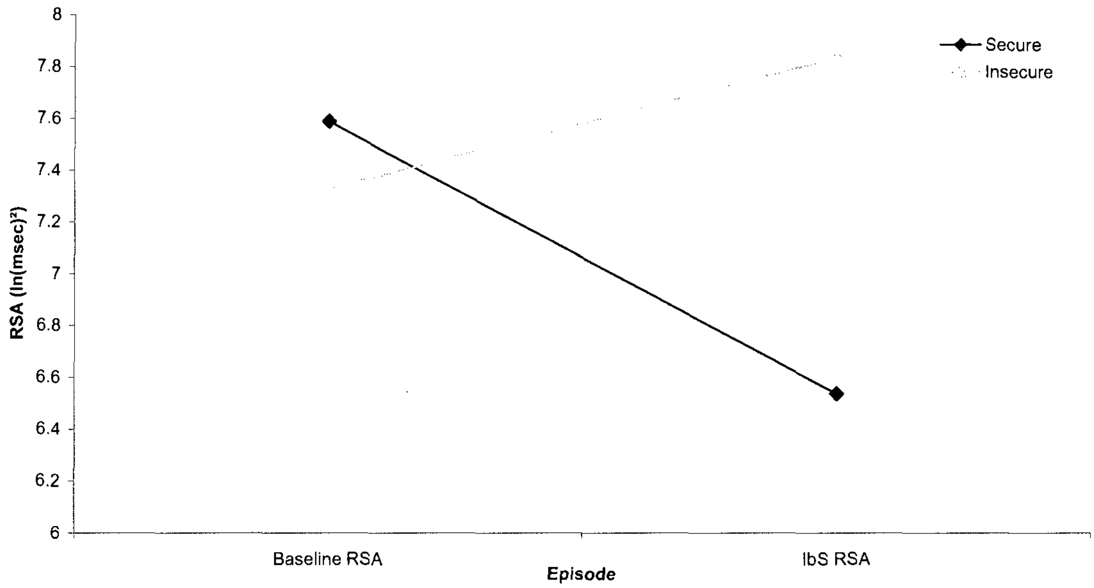


Figure 1. Significant relationship between attachment classification and episode, supporting RSA withdrawal across episodes for Securely attached children only.

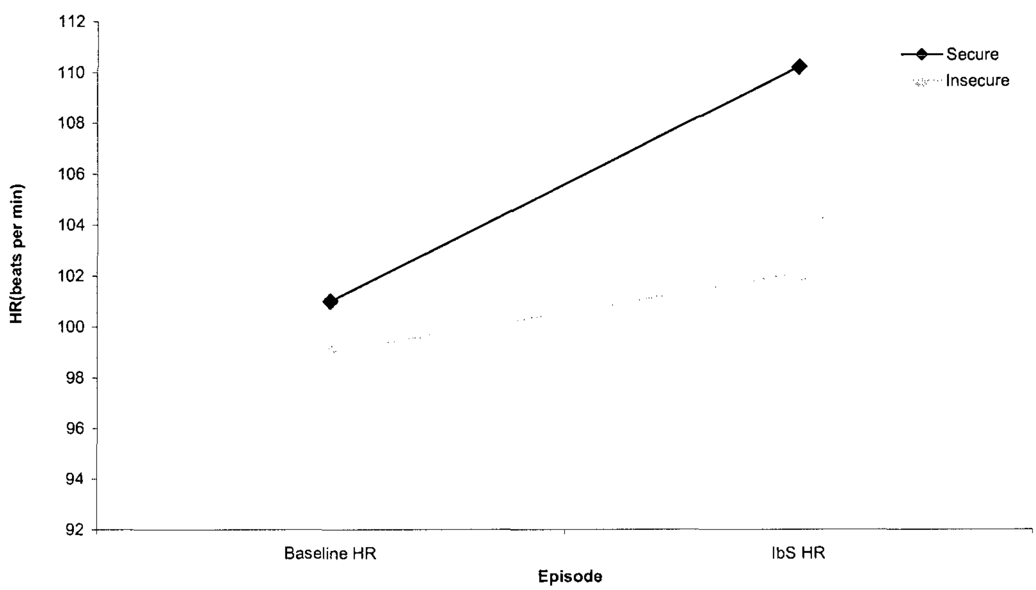


Figure 2. Non-significant relationship between attachment classification and episode on HR when the change in RSA is statistically controlled.

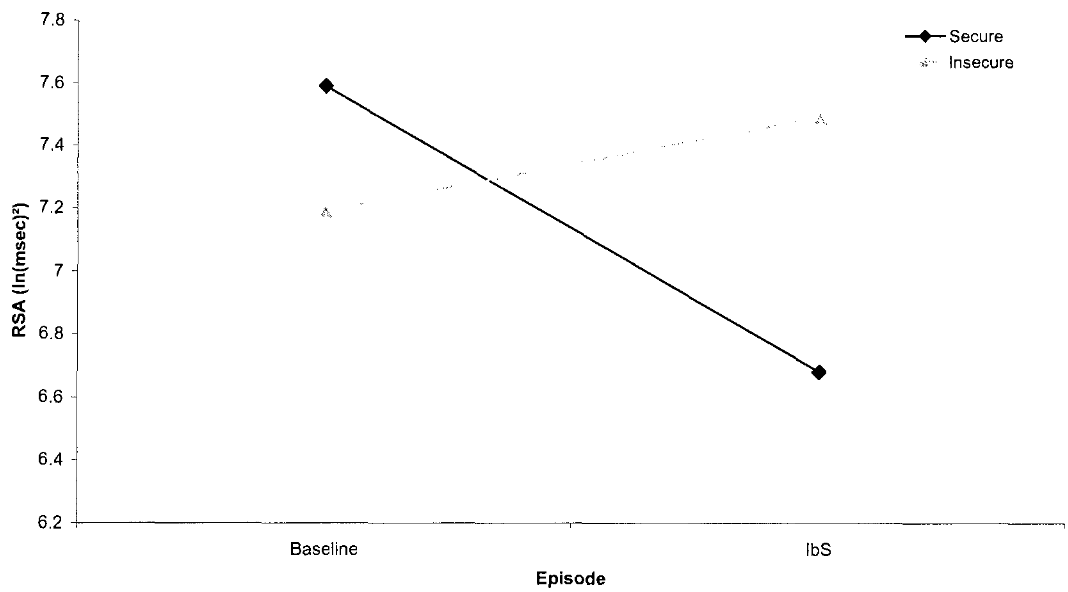


Figure 3. In a subsample of children that showed approach behaviour in the IbS paradigm, a meaningful relationship between attachment and episode stayed significant such that RSA withdrawal across episodes occurred for Securely attached children only.

Appendix A

Letter of Information

Exploring Parent-Child Relationships, Emotion Regulation,

Children's Thinking and Heart and Respiration Responses

at the Preschool Age

Dear Parent,

We are conducting a study with 3 ½ year olds and their parents to learn more about preschoolers' development in the areas of relationships, emotions and thinking. We want to understand how children's relationships with their parents develop at this age, how they talk about emotions and how they learn to think about different things. We will be asking you and your child to do a variety of activities both together and apart. The visit will last approximately an hour and a half and will be videotaped. If you do not wish to be videotaped you should not participate in the study. You are always free to choose not to participate in an activity or answer a question.

In this visit, we will have both you and your child wear some special equipment. You will have a small sensor clipped to your waist and your child will wear a light-weight vest with the sensor inside. These will monitor and record your heart rate and your breathing as you go through the activities. All of the equipment is safe to wear and the electrodes feel a lot like putting on and then removing a sticker. A new area of research has been looking at how mothers and their children regulate at a physiological level, that is, how their heart rates are often in "sync", so we are interested to see how this relates to other areas of preschoolers' development.

- After you are both set up with the special equipment, we will ask your child to do two quick activities that tell us about his/her thinking. In the first activity, your child will be instructed to provide one of two words depending on the picture card presented. In the second task, your child will be asked to find the correct targets on a page of different pictures. We are interested to see if preschoolers can remember and correctly employ the rules of the game and if they can successfully focus their attention to find the correct targets. While your child is doing those activities, we will give you a package of questionnaires to begin filling out. These will help us to further understand your child's development.

- Then, because we are interested in how your child plays in new surroundings both when you are present and when you are not, we will then ask you to leave your child for two brief periods (approximately 4 minutes each) during this part of the visit. If your child becomes upset, we will send you back in immediately.

- Following that, when you and your child are in the room together, we will observe how your child reacts to an interesting, but unusual mask. The mask will speak to your child and your child may find this a bit scary. If you feel that your child has become too upset, we will stop the task. We would like to observe what your child does in response to the unusual mask as a way of better understanding preschoolers' emotion regulation skills.

- You and your child will then separate again, and your child will be asked to make up some stories involving dolls. Your child will not be alone: someone from our research team will be with him/her during this time. In the meantime, you will be completing some questionnaires and brief activities in an adjacent room. The questionnaires and activities that you complete will parallel the things that your child does, and will help us to understand where children learn different skills and abilities.

- Next, you and your child will get together for a snack and you will be asked to talk about some past experiences involving various emotions. This will help us to understand preschoolers' behaviour in relationships with parents and will provide more information about their emotion regulation skills.

- Finally, we will ask you some questions about demographic information while your child is asked to tell us the names of some pictures and objects. If, at this point, you haven't had a chance to complete all of the questionnaires in the package that you received at the start of the visit, we will provide you with a self-addressed, stamped envelope and ask you to complete them at home and mail them back to us.

All information collected from you for the study will be kept confidential. All written and videotaped records and questionnaires will be assigned numbers to maintain confidentiality. Any identifying information such as names and place of birth will be changed to maintain confidentiality. Only those directly involved in the study will see the transcripts and videotapes unless you agree that fragments can be used for professional training. The family names will only be available to direct members of the research group. Absolute confidentiality cannot be guaranteed as we may have to disclose certain information as required by law according to provisions under the Child and Family Services Act. This includes any suspicion that a child under the age of 16 years is or has been abused or if you are in imminent danger of hurting yourself or another person. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published.

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time. Even if specific questionnaires request that you answer every question you do not have to do so. There are no known risks associated with any of the procedures. As mentioned above, the electrodes, feel a lot like putting on and then removing a sticker. If you feel that this is too upsetting for your child, we will discontinue this part of the visit. This study will not result in any direct benefit to you or your child, but will help us to further understand the development of preschoolers. In appreciation for your assistance with the study you will receive $30.00 and a DVD with selected excerpts from your visit.

Throughout the study we will ask you if you have any questions about any of the procedures. We would also appreciate any ideas or advice about your experience as a participant. We hope that participating in this study will be an interesting time for you and your child. If at any time you have questions or concerns, please do not hesitate to let the researcher know or you can contact the principal investigators or research coordinator listed below:

Dr. Greg Moran
Department of Psychology
University of Western Ontario
(519) 661-2111 extension 83109

Dr. Heidi Bailey
Department of Psychology
University of Guelph
(519) 824-4120 extension 56399

Dr. Jean-Francois Bureau
Department of Psychology
University of Ottawa
(613) 562-5800 extension 4484

Dr. David Pederson
Department of Psychology
University of Western Ontario
(519) 661-2111 extension 84672

Sandi Bento
Research Coordinator
Child Development Centre
(519) 661-2111 extension 84660

If you have questions about the conduct of this study or your rights as a research subject you may contact:

Office of Research Ethics
The University of Western Ontario
519-661-3036 or email at: ethics@uwo.ca

Appendix B

Consent for Participation, Observation and Training purposes

Exploring Parent-Child Relationships, Emotion Regulation, Children's Thinking and Heart and Respiration Responses At the Preschool Age

Consent for Observations of my Participation in Research

We would like your permission for visiting graduate students or professionals such as psychiatrists, psychologists and social workers not directly involved in the research study to observe your participation in our research. Any observation will be under the control and discretion of the principal investigators. You may still participate in the research study even if you do not wish to consent to this.

I consent to visiting graduate students or professionals such as psychiatrists, psychologists and social workers to observe my participation in the study. Any observation will be under the control and discretion of the principal investigators.

____

Parent's Name (Please Print)

____________________________________ ____________________

Parent's Signature Date

____

Name of Person Obtaining Informed Consent

____________________________________ ____________________

Signature of Person Obtaining Informed Consent Date

Exploring Parent-Child Relationships, Emotion Regulation,

Children's Thinking and Heart and Respiration Responses

At the Preschool Age

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

____

Parent's Name (Please Print)

_____________________________________ __________________

Parent's Signature Date

____

Name of Person Obtaining Informed Consent

_____________________________________ __________________

Signature of Person Obtaining Informed Consent Date

☐ Please check this box if you would like to receive a summary of study findings after the study has been completed.

Exploring Parent-Child Relationships, Emotion Regulation,

Children's Thinking and Heart and Respiration Responses

At the Preschool Age

Consent to use Videotapes for Training and Education

In addition to using the videotapes for the research study, we would like your permission to use the videotapes for training and education of professionals such as psychiatrists, psychologists and social workers. The use of the videotapes will be under the control and discretion of the principal investigators. You may still participate in the research study even if you do not consent to the additional use of the videotapes.

I consent to the videotapes being used for the training and education of professionals such as psychiatrists, psychologists and social workers under the control and discretion of the principal investigators.

____

Parent's Name (Please Print)

____________________________________ __________________

Parent's Signature Date

____

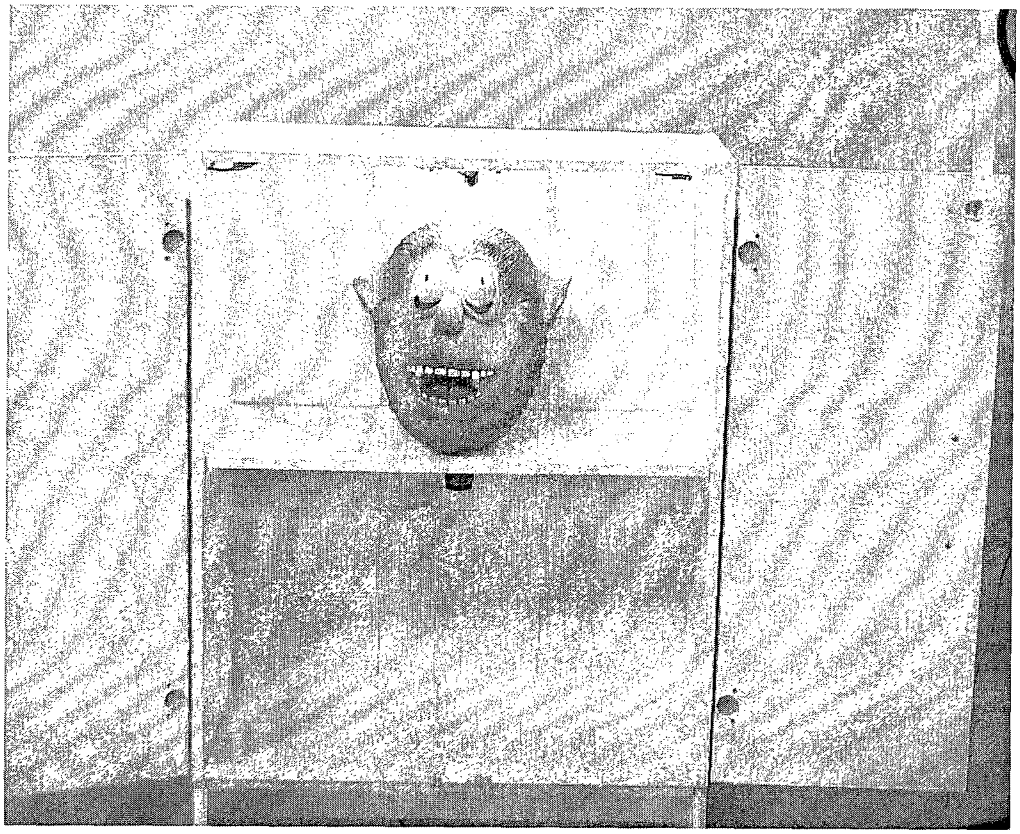
Name of Person Obtaining Informed Consent

____________________________________ __________________

Signature of Person Obtaining Informed Consent Date

Appendix C

Interesting-but-Scary Stimulus



Appendix D

Interesting-but-Scary Mask Script

1. "Hi <u>Child's Name</u> . It's nice to meet you. How are you today? (Pause for child to respond, either way continue) What are you doing?"

10" pause before any probes

<u>If no response</u> to Question 1 (followed by 10" pause) then probe . . . . . .

"Have you been doing anything fun?"

If no response to above, then . . . . . .

"What have you have played with?"

If no response to above, then . . . . . .

"Did you do some activities with your mom?" If "NO" then <u>Move to 2.</u>

<u>Once there is a response,</u> then probe If "NO" then <u>Move to 2.</u>

"Anything more to tell me?" If "NO" then <u>Move to 2.</u>

"Anything else?" <u>Move to 2.</u>

2. "What toys have you played with today?"

10" pause before any probes

If <u>no response</u> to Question 2 (followed by 10" pause) then probe . . . . . .

"Is there a toy you really like?"

If no response to above, then . . . . . .

"Something that you play with your mom?" If "NO" then <u>Move to 3.</u>

<u>Once there is a response,</u> then probe . . . . . .

"Anything more to tell me about that toy?" If "NO" then <u>Move to 3.</u>

"What do you like about that toy?" If "NO" then Move to 3.

"Anything else?" Move to 3.

NO MATTER WHAT THIS QUESTION SHOULD CONCLUDE AT 1:20 and next session should begin

3. "Child's Name", Why don't you come over here and touch my nose!'

If a child moves forward and touches nose, Say

"Good job you touched my nose, Child's Name, would you like to touch my nose again? That would be alright with me! "

(wait for child to respond)

If child responds with another nose touch or if child does not touch nose you can say

"It has been really fun getting to know you Child's Name. Good bye!"

If child does not immediately touch nose after initial prompt,

Probe . . . . . .

"Come over here and just give my nose a little touch- it will be fun!"

If still no response, then say

It's okay that you don't want to touch my nose Child's Name.

"Can you wave to me Child's Name ?"

END.

CONCLUDE AT APPROXIMATELY 2:00 min

Notes: Mask can repeat what child says, just do not expand after that. When the child answers before re-prompting them with the appropriate prompt you can repeat the child's answer (this often happens in conversation with 3 ½ year olds). "Anything more to tell me" would also be an appropriate lead in.

Appendix E

Debriefing Script

If Mom asks about the purpose of the maternal measures, say:

"The reason that we had you complete those tasks is because we gave some similar tasks to [C's name] so that we can better understand children's word- and school-related abilities. It is really helpful to know how parents do on similar tasks designed for adults because if we find that parent-child relationships are related to children's developing school-related skills, we can check to see if it's actually just because moms and kids have similar abilities."

If Mom asks about the Strange Situation Procedure, say:

"The reason that we asked you to leave your child alone in the room briefly is because we're interested in children's reactions to brief separations from their parents. This information helps us to understand how children cope with such situations at this age. For example, how they might deal with going to daycare or being left with a babysitter. It also helps us to better understand the parent-child relationship."

If Mom asks about the executive functioning tasks, say:

"We've asked your child to complete these activities because they help us to understand how things like attention and inhibition develop at this age. These abilities are what allow children to do things such as stay on task, or wait their turn and so become very important as children enter school. We're curious to see whether a child's

relationships with the important adults in his/her life can impact the development of these abilities."

If Mom asks about the IbS Procedure, say:

"This task allows us to see how children this age respond to novel, but potentially frightening things. Mostly, we're looking at your child's temperament, or his/her tendency to be shy or. outgoing in these types of situations."

If Mom asks about the physiological equipment, say

"Having a read on both your and your child's heart and breathing rates allows us to see how different activities can influence physiological responses. We know that things like stress and relaxation can show up in our heart and breathing rates, but we still have a lot to learn about how other emotional states influence these things. In addition, as we mentioned in the letter you read, some new research suggests that Moms and children's heart rates are often in 'sync' with each other, so we're interested to see if this is in fact the case in our study."

If Mom asks about the Doll-Play Procedure, say:

"That task helps us to gather more information about how children perceive relationships. The dolls help to make the task more like real-life and can also help children to be able to express themselves without having to use words."

If Mom asks about the Emotion Discussion task, say:

"We asked you and your child to speak about past experiences with different emotions because we are interested in understanding how children learn about emotions and how they talk about them with their parents. We would like to see if the way children talk about emotions with their parents is related to some of the other things we are looking at, such as children's school-related abilities"

If Mom asks about the EVT, say:

"We had your child complete the EVT so that we can check to ensure that their ability to express themselves using language is not accounting for their performance on some of the other tasks. For example, a child who has difficulty finding the words to express themselves might also have difficulty engaging in a discussion about emotions. We would need to know that it is language that explains this and not something related to emotions"

Appendix F

Behaviour Inhibition Rating Scales

NONVERBAL ANXIETY

1. Very relaxed posture and movement. Ease of orientation to S. Open facial expression. Eye contact. Clear, unrestricted voice with good range
2. Relaxed
3. Generally at ease
4. Mild and brief indication of anxiety
5.
6. Fairly tense throughout
7. Strong tension
8.
9. Extreme physical/visual avoidance and tension. Turns away. Minimal eye contact. Tense/frozen posture and movements. Nervous gestures or coyness. Apprehensive/anxious expression (e.g. underbrow look, appeasement/ambivalent smile). Whispering, restrained voice. Restricted range. (anxiety rather than relaxed non-compliance).

LOW VERBAL RESPONSIVENESS

1. Extremely responsive with full extensions &/or intitiations or spontaneous comments
2. Ready responsiveness. Chats with ease. Responds with full extensions
3. Responsive
4. Fairly forthcoming. Needs the odd prompt. Full/adequate responses to probes.
5. Slightly reticent. Needs prompts.
6. Short answers. Lots of pauses. Hesitant
7. Does respond verbally but few extensions. Very short answers. Some long pauses.
8. Minimal verbal responses. Adequate non-verbal responses.
9. Extremely low responsiveness. Few, if any, responses(verbal or non-verbal)

BEHAVIOURAL INHIBITION

(balancing all relevant behaviour to reflect the whole)

1. No signs of inhibition. Very relaxed and confident. Extremely responsive. May introduce self or offer spontaneous comments.
2. Relaxed. Readily responsive
3. Generally at ease. Responsive
4. Mild and brief indications of inhibition
5.
6. Fairly inhibited throughout.
7. Strong inhibition. Low responsiveness.
8. Very strong Inhibition
9. Extreme inhibition and very low responsiveness.