THE ORIGINS AND HETEROGENEITY OF SHYNESS

THE ORIGINS AND HETEROGENEITY OF SHYNESS: A DEVELOPMENTAL, BIOLOGICAL PERSPECTIVE

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Lay Abstract

Shyness is a trait that is characterized by fear and nervousness during new social situations or in situations of perceived social evaluation. Although there has been an abundance of research examining the psychosocial correlates of childhood shyness, we know considerably less about the developmental and biological origins of shyness. In this dissertation, I examined individual differences in the biology, developmental onset, and developmental trajectory of shyness. This work illustrated that shyness is a heterogenous phenomenon, with individual differences in the developmental onset and developmental course. As well, this work provided evidence that shy children tend to be more sensitive to perceiving threat in social situations, and their brain and body may be “primed” to overreact when they are faced with this perceived social threat. Shy children may have a biological profile associated with avoidance and threat processing, which may be one factor underling their fear in new social contexts.

Abstract

Temperamental shyness is a trait characterized by fear and avoidance in response to situations of social novelty and/or perceived social-evaluation. Although there has been an abundance of research examining the psychosocial correlates of childhood shyness, we know considerably less about the developmental and biological origins of shyness and its subtypes. Chapters 2 to 5 of this dissertation include empirical studies that examine the developmental and biological foundations of temperamental shyness in general, and Chapter 6 examines subtypes of shyness in particular. In Chapter 2, I found that individuals who were born extremely premature and also exposed to exogenous corticosteroids prenatally displayed a stable trajectory of high shyness from childhood to adulthood, possibly due to the programming of threat sensitivity. In Chapter 3, I found that children who had greater relative right frontal brain activity at rest (a neural correlate of fear and avoidance) demonstrated increases in shyness across the early school age years. In Chapters 4 and 5, I examined patterns of autonomic physiology among shy children during two types of social threat processing. I demonstrated that shy children show stability in autonomic arousal while viewing socio-affective threat from age 6 to 7.5 years (Chapter 4), and that shy children show arousal and excessive regulation on autonomic and affective levels during the anticipation of socio-evaluative threat (Chapter 5). Finally, Chapter 6 reports that the developmental onset of shyness is associated with distinct behavioral and biological correlates in shy children. Children with early-developing shyness showed greater relative right frontal brain activity at rest, while children with later-developing shyness showed greater salivary cortisol production to a socio-evaluative task. Collectively, the studies and findings from this dissertation highlight that shyness is related to distinct developmental and biological processes associated with avoidance and threat processing, which may underlie fear in novel social contexts.

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Dedication

In loving memory of my grandmothers Millie Dubeau (1924–2002) and Joyce Poole (1934–2017), cousin Jesse Wright (1982–2018), and Aunt Judy Ritchie (1946–2020).

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List of Abbreviations and Symbols

-2LL: -2 log likelihood
°C: degrees Celsius
α: Cronbach's alpha
ANOVA: analyses of variance
β: beta
BI: behavioural inhibition
χ^2: Chi-square
CCTI: Colorado Child Temperament Inventory
DFT: discrete Fourier transform
d*:* Cohen's d effect size
ECG: electrocardiogram
EEG: electroencephalogram
ELBW: extremely low birth weight
ELBW+NS: extremely low birth weight and not exposed to exogenous corticosteroids
ELBW+S: extremely low birth weight and exposed to exogenous corticosteroids
F: F-test statistic
HPA: hypothalamic–pituitary–adrenal
HP: heart period
HR: heart rate
HRV: heart rate variability
Hz: hertz
ICC: intraclass correlation
IQ: intellectual quotient
κ: Kappa
LFA: left frontal asymmetry
M: mean
MCAR: missing completely at random
ms: millisecond
mg: milligram
ml: milliliter
NBW: normal birth weight
N: total sample size
n: sample size
p: *p*-value
r: Pearson correlation coefficient
RFA: right frontal asymmetry
RSA: respiratory sinus arrhythmia
SCARED: Screen for Child-Anxiety Related Disorders
SD*:* standard deviation
SE: standard error
SES: socioeconomic status
t: *t*-test statistic
z: *z*-score

Declaration of Academic Achievement

This thesis consists of four studies published in scientific journals (Study 1 in Chapter 2, Study 2 in Chapter 3, Study 3 in Chapter 4, Study 5 in Chapter 6), and one study that is submitted for publication (Study 4 in Chapter 5). The author of this thesis is the primary author, and her supervisor, Louis A. Schmidt (McMaster University), is the final author on all manuscripts. The contributions of each author in each study are outlined below.

Study 1 (Chapter 2) is a reprint of the following published journal article with permission from Cambridge University Press, Copyright © 2020:

Poole, K.L., Saigal, S., Van Lieshout, R.J., & Schmidt, L.A. (2020). Developmental programming of shyness: A longitudinal, prospective study across four decades. *Development and Psychopathology, 32,* 455–464.

This study examined the influence of early pre- and postnatal stressors on the development of shyness from childhood to adulthood using a prospective, longitudinal study design across four decades. Kristie L. Poole, the primary author, conceptualized the research and experimental design, performed data analyses, and wrote the manuscript. Saroj Saigal (McMaster University), the second author, began the study when participants were infants and was responsible for research design and gave feedback on the manuscript. Ryan Van Lieshout (McMaster University), the third author, was responsible for research design and gave feedback on drafts of the manuscript. Louis A. Schmidt, the last author, was responsible for conceptualization of the research and experimental design, and gave feedback on drafts of the manuscript.

Study 2 (Chapter 3) is a reprint of the following published journal article with permission from Springer Nature, Copyright © 2019:

Poole, K.L., Santesso, D.L., Van Lieshout, R.J., & Schmidt, L.A. (2019). Frontal brain asymmetry and the trajectory of shyness across the early school years. *Journal of Abnormal Child Psychology, 47,* 1253–1263.

This short-term longitudinal study examined how patterns of brain activity influenced the development of shyness in early school-aged children. Kristie L. Poole, the primary author, conceptualized the research and experimental design, performed data analyses, and wrote the manuscript. Ryan Van Lieshout (McMaster University), the second author gave feedback on the manuscript. Diane Santesso (University of Winnipeg), the second author was responsible for research design, data collection, and gave feedback on the manuscript. Louis A. Schmidt, the last author, was responsible for conceptualization of the research and experimental design, and provided feedback on drafts of the manuscript.

Study 3 (Chapter 4) is a reprint of the following published journal article with permission from John Wiley and Sons, Copyright © 2018:

Poole, K.L., & Schmidt, L.A. (2018). Trajectory of heart period to socio-affective threat in shy children. *Developmental Psychobiology, 60,* 999–1008.

Study 3 (Chapter 4) examined patterns of autonomic activity in response to socio-affective threat in shy children across two years. Kristie L. Poole, the primary author, conceptualized the research and experimental design, performed data analyses, and wrote the manuscript. Louis A. Schmidt, the last author, was responsible for conceptualization of the research and experimental design, and provided feedback on drafts of the manuscript.

Study 4 (Chapter 5) examined patterns of autonomic and affective responses to an impending social stressor in shy children. Kristie L. Poole, the primary author, conceptualized the research and experimental design, collected data, performed data analyses, and wrote the manuscript. Louis A. Schmidt, the last author, was responsible for conceptualization of the research and experimental design, and provided feedback on drafts of the manuscript.

Poole, K. L. & Schmidt, L.A. (Under Review). While a shy child waits: Autonomic and affective responses during the anticipation and delivery of a speech. *Manuscript submitted for publication.*

Study 5 (Chapter 6) is a reprint of the following published journal article with permission from John Wiley and Sons, Copyright © 2019:

Poole, K.L. & Schmidt, L.A. (2020). Early- and later-developing shyness in children: An investigation of biological and behavioral correlates. *Developmental Psychobiology, 62,* 644–656.

Study 5 (Chapter 6) examined how the developmental onset of shyness was related to neuroendocrine, neural, and behavioral correlates in children. Kristie L. Poole, the primary author, conceptualized the research and experimental design, processed psychophysiological data, performed data analyses, and wrote the manuscript. Louis A. Schmidt, the last author, was responsible for conceptualization of the research and experimental design, and provided feedback on drafts of the manuscript.

CHAPTER 1

General Introduction

Shyness is characterized by fear and discomfort in response to situations of social novelty and/or perceived social-evaluation (Kagan, Reznick, & Snidman, 1988; Rubin, Coplan, & Bowker, 2009). Although shyness is a ubiquitous phenomenon with up to 90% of the population feeling shy at some point in their lives (Zimbardo, 1977), a smaller proportion of approximately 15-20% of people are characterized by temperamental shyness which is conceptualized as a dispositional trait (Kagan, 1994). Behaviorally, shyness can manifest as social reticence (i.e., onlooking, unoccupied behavior), avoidance (e.g., gaze aversion), and/or inhibition (e.g., freezing behaviors) during interactions with unfamiliar social partners, exposure to novel social contexts, or situations in which an individual is the object of social attention (Coplan et al., 2004, 2009; Kagan, Reznick, & Snidman, 1987, 1988; Schmidt et al., 1999).

A body of work has extensively studied the psychosocial correlates and developmental outcomes associated with shyness. For example, researchers have found that childhood shyness is correlated with academic difficulties (Crozier & Hostettler, 2003; Hughes & Coplan, 2010), lower self-esteem (Crozier, 1995), internalizing difficulties including anxiety (Coplan, Arbeau, & Armer, 2008), and poorer peer relationships (Eggum-Wilkens, Valiente, Swanson, & Lemery-Chalfant, 2014; Rubin, Coplan, & Bowker, 2009). Longitudinal work also has investigated the life course outcomes of shy children and this work found that childhood shyness was predictive of distinct developmental milestones in adulthood such as later age for marriage, parenthood, and stable careers, and lower levels of education (Caspi, Elder, & Bem, 1988; Kerr, Lambert, & Bem, 1996).

There has also been interest over the last several decades in trying to better understand the origins of childhood shyness. Some researchers have focused largely on the role of socialization factors on the development of shyness (see e.g., Stevenson-Hinde, 2002), while another line of research has been largely interested in the biological bases of temperamental shyness (Fox et al., 1995, 2001; Kagan et al., 1987, 1988; Schmidt, et al., 1997, 1999). This has been motivated in part by the fact that shyness is rooted in early biologically-based temperamental biases, as well as appears to be a heritable trait (Cherny, Fulker, Corley, Plomin, & DeFries, 1994; Eggum-Wilkens, Lemery-Chalfant, Aksan, & Goldsmith, 2015; Plomin & Daniels, 1986). The focus of my program of research has been largely on trying to better understand the development of temperamental shyness from a biological perspective.

Developmental Antecedents of Shyness

The developmental antecedents of shyness are thought to emerge during infancy (Poole, Tang, & Schmidt, 2018). As early as four months of age, infants display distinct individual differences in their motor and affective reactivity in response to novel visual and auditory stimuli (García-Coll, Kagan, & Reznick, 1984; Kagan, Reznick, & Snidman, 1987). This reactive responding style to novel stimuli in infancy has been shown to predict behavioral inhibition in toddlerhood, which is a temperament characterized by wariness in response to novel social (i.e., strangers) and non-social (e.g., objects, places) stimuli (Calkins, Fox, & Marshall, 1996; Kagan & Snidman, 1991; Kagan, 1994). During early childhood, toddlers undergo further cognitive development responsible for the social self, self-conscious emotions, and the ability to take on the perspective of others (Crozier & Burnham, 1990; Lagattuta & Thompson, 2007). Thus, as behaviorally inhibited toddlers undergo this further socio-cognitive development across early childhood, their inhibition can become particularly salient in novel *social* contexts, reflected in temperamental shyness (Poole, Tang, & Schmidt, 2018). Indeed, longitudinal, prospective studies have shown that temperamental behavioral inhibition in toddlerhood prospectively predicts shyness in later childhood according to both maternal-report using questionnaires as well as observed behavior in the laboratory (e.g., Degnan et al., 2014; Fox et al., 2001; Schmidt et al., 1997; Volbrecht & Goldsmith, 2010).

It is thought that temperamentally shy children are more likely to perceive and detect threat in social contexts, which has been supported by empirical work examining behavioral indices of threat processing. For example, eye-tracking studies have demonstrated that shy infants (Matsuda, Okanoya, & Myowa-Yamakoshi, 2013) and children (Brunet et al., 2009) tend to focus their attention on the eyes of unfamiliar faces, demonstrating their heightened vigilance to social threat. Additional work has found that temperamentally shy children are more sensitive to detecting threat than non-shy children, and that this is specific to *social* threat-related stimuli (i.e., angry faces) and not to non-social threat-related stimuli (i.e., snakes; LoBue & Pérez-Edgar, 2014). Finally, individual differences in attentional biases to social threat play a developmental mechanism linking early temperamental behavioral inhibition to later shyness. Specifically, among toddlers who were behaviorally inhibited, only those who showed attentional biases towards social threat in childhood demonstrated higher levels of shyness in childhood (Pérez-Edgar et al., 2011) and adolescence (Pérez-Edgar et al., 2010).

The early appearing behavioral responses to novelty that underlie the development of shyness are thought to be influenced in part by a low threshold for arousal in central and peripheral physiological systems implicated in threat processing and the regulation of the emotion fear (Kagan, 1994). There is some empirical support for this hypothesis, which is briefly reviewed below.

Biological Correlates of Shyness

There are a number of experimental approaches and biological measures across both central and peripheral physiological systems, that have previously been shown to be implicated in avoidance and threat processing. These biological systems may underlie the expression, experience, and regulation of fear, and be particularly relevant to the study of shyness.

Most commonly, physiological correlates are assessed during resting, baseline conditions, which is referred to as a biological stress *vulnerability*, and is conceptualized as an individual's dispositional physiological profile. The early antecedents of shyness (i.e., behavioral inhibition) have been correlated with the following neural, autonomic, and endocrine correlates of fear and arousal: (1) greater relative right frontal brain electrical activity at rest (Calkins, Fox & Marshall, 1996; Fox & Davidson, 1987; Fox et al., 2001; McManis et al., 2002); (2) a higher resting heart rate (Garcia-Coll, Kagan, & Reznick, 1984; Kagan et al., 1984), and (3) higher baseline salivary cortisol (Kagan et al., 1987, 1988).

Relatively little work, however, has been devoted specifically to shyness, but there is some evidence to suggest that shy children and adults also show those similar biological correlates of fear at rest as inhibited infants and toddlers, including (1) greater relative right frontal brain electrical activity at rest (Schmidt, 1999); (2) a higher resting heart rate (Schmidt, Santesso, Schulkin, & Segalowitz, 2007; Schmidt & Fox, 1994), and (3) higher baseline salivary cortisol (Schmidt et al., 1997; Schmidt et al., 2007).

An additional approach is to examine an individual's physiology during different experimental manipulations, for example, during threat processing (e.g., viewing of threatening stimuli), or during threat induction (e.g., anticipating or completing a speech or social interaction). This is commonly referred to as biological stress *reactivity*, and may reflect an individual's ability to regulate during social challenge, and provide insight into the level of perceived social threat experienced by an individual.

Considerably less work has examined how shyness is related to biological indices of fear and threat in response to social threat/challenge. However, some existing work has found that shyness is correlated with: (1) increases in relative right frontal brain electrical activity (Fox & Davidson, 1987; Schmidt et al., 1999); (2) increases in heart rate (Schmidt et al., 1999), and (3) increases in salivary cortisol (Granger, Weisz, & Kauneckis, 1994) in response to a social challenge in the laboratory.

In addition to the relative scarcity of empirical work investigating the relation between shyness and biology at rest and in response to social stressors in the laboratory, an additional limitation of existing studies is that they are cross-sectional in nature. As a result, we know considerably less about: (1) the influence of biology on the *trajectory* of prospective shyness, and (2) shy children's pattern of physiology across development.

Further, we know comparably less about the biological correlates of shyness *subtypes* that have previously been theorized in the literature. As is highlighted in the next section, there exists individual variability in the developmental onset and trajectory of shyness, which may be differentially related to underlying biological processes.

Heterogeneity in the Developmental Onset and Course of Shyness

There is substantial heterogeneity in the phenomenon of shyness including individual differences in both the onset and the trajectory of shyness across development (Poole, Tang, & Schmidt, 2018). Early theoretical work by Arnold Buss (1986a,b) proposed that there is an early-developing shyness which typically has its onset in toddlerhood and is maintained due to heightened sensitivity to fear, as well as a later-developing shyness which has its onset after toddlerhood (i.e., early to middle childhood or adolescence) and which is closely tied to the experience of self-conscious emotions such as embarrassment. Buss proposed that early-developing shyness manifests in response to social novelty and intrusiveness (e.g., close proximity of a stranger, or interaction with an unfamiliar peer), and may be maintained by biological systems underlying the regulation of fear and avoidance. Individuals with later-developing shyness are thought to be particularly concerned when he/she is socially exposed and/or the object of social attention, and thus should show physiological arousal when faced with situations of social evaluation and exposure. Despite the assertions proposed by Buss (1986a,b) that developmental onset of shyness may be related to distinct behavioral and biological correlates, to date, there exists no empirical research on this topic in childhood.

There are also individual differences in the developmental course of shyness. Although some individuals may show relative stability in shyness across developmental periods (particularly among extreme groups), it also has been noted that some individuals may show increases or decreases in shyness across different developmental periods. For example, in toddlerhood, behavioral inhibition is only modestly stable (Fox et al., 2001). Similarly, shyness has been reported to be moderately stable from early to middle childhood (Asendorpf, 1989; Fordham & Stevenson-Hinde, 1999; Kagan, Reznick & Snidman, 1988; Pedlow, Sanson, Prior, & Oberklaid, 1993; Rubin et al., 1997; Sanson, Pedlow, Cann, Prior, & Oberklaid, 1996) and during late childhood to adolescence (Schneider, Younger, Smith, & Freeman, 1998). Recent work has also illustrated that shyness may increase during young adulthood (Brook & Schmidt, 2019; Kwiatkowska & Rogoza, 2017). Together, these studies suggest that there is moderate stability in shyness across developing, there is plasticity in the growth or decline in shyness across time. Accordingly, is important to examine developmental and biological factors that may influence both the onset of shyness as well as change in shyness levels across repeated assessments.

Overview of Dissertation

This dissertation broadly examines the developmental origins, biological correlates, and heterogeneity of temperamental shyness. In a series of five studies, I used a multi-method, multi-measure approach that includes both cross-sectional and longitudinal study designs, as well as integrate observational and psychophysiological methodology to investigate developmental influences and biological substrates of shyness across central, autonomic, and neuroendocrine levels of analysis. This integrative approach allows for a better understanding of the origins and heterogeneity of shyness from a developmental and biological perspective.

In Chapter 2 (Study 1), I tested the hypothesis that temperamental shyness may have its earliest developmental origins in utero for some individuals. As a developmental model of early adversity, I used a sample of individuals who were born at extremely low birth weight (ELBW; < 1000 grams). This longitudinal, prospective study spanned four decades, in which shyness was assessed in childhood (age 8), adolescence (age 12), young adulthood (age 22), and adulthood (age 32). I found that individuals who were exposed to the greatest relative levels of early stress (i.e., born at ELBW and exposed to synthetic steroids prenatally) exhibited the highest levels of childhood shyness which remained stable into their early thirties compared individuals born ELBW and not exposed to synthetic steroids and normal birth weight controls. I interpret these findings to suggest that for some individuals, there may be adaptations that take place in utero in response to early stressors that prepare the fetus for a threatening postnatal environment through physiological modifications. These individuals may be more prone to manifest threat sensitivity, fear, and hypervigilance, which may lay the developmental blueprint for temperamental shyness.

In the remaining empirical chapters (Chapters 3, 4, 5, 6), I examined the biological bases of shyness in children – across both central and peripheral physiological systems – that have previously been shown to be implicated in avoidance, fear sensitivity, and threat processing, and may underlie the regulation of fear using longitudinal and cross-sectional designs. Using longitudinal and cross-sectional designs, these chapters investigate biological substrates of shyness including both resting, baseline measures, and well as measurement during social threat processing and induction in the laboratory.

In Study 2 (Chapter 3), I examined if patterns of resting state frontal brain electrical activity were related to the development of shyness in early school-aged children. In this short-term longitudinal, prospective study, children's shyness was reported by mothers across five visits spanning approximately two years in early childhood. I found that children who had greater relative right frontal brain activity (a neural correlate of avoidance) at rest experienced increases in maternally-reported temperamental shyness across visits. This study illustrates that brain-based measures of avoidance may serve to influence the growth in children's shyness during the early school age years.

Studies 3 and 4 extended and complemented Study 2 in two ways. First, these two studies focused on how autonomic nervous system activity was related to children's shyness. Further, while Study 2 examined a resting state, baseline measure of physiological stress vulnerability, Studies 3 and 4 examined children's physiological stress reactivity in response to laboratory tasks designed to assess socio-affective threat processing (Study 3) and induce socio-evaluative threat (Study 4). Given that both Studies 3 and 4 examined autonomic nervous system activity in relation to shyness, there is some overlap in the background literature in the introduction in each of these chapters.

Study 3 (Chapter 4) used a short-term longitudinal, prospective study design in order to examine if shy and non-shy children differed of their patterns of autonomic physiology (i.e., heart rate) in response to socio-affective threat processing. Across four visits separated by approximately six months, children had their heart rate measured while they viewed age-appropriate video clips designed to induce threat-related processing. I found that shy children exhibited a trajectory of stable, relatively higher heart rate from age 6 to 7.5 years while they viewed video clips designed to induce threat processing. In contrast, non-shy children showed relative decreases in their autonomic arousal across each repeated assessment. These findings suggest that longitudinal patterns of heart rate to socio-affective threat among shy children may reflect a stable, characteristic way of responding to threat and possibly a physiological mechanism underlying shyness in some children.

In Study 4 (Chapter 5), I used a cross-sectional study design to examine if shy children exhibited distinct patterns of autonomic and affective reactions in response to an experimental paradigm designed to induce socio-evaluative threat: a videotaped self-presentation task. This experiment included three task phases including a baseline condition, speech anticipation period, and speech delivery period. I found that temperamentally shy children showed arousal and overcontrol on an autonomic level, as well as increased nervous affect specifically during the *anticipation* of socio-evaluative threat relative to non-shy children. These findings suggest that temperamentally shy children may experience autonomic and emotion dysregulation particularly during the anticipation of novel social encounters, which may be reflective of their tendency to over react to perceived impending social threat on both physiological and affective levels.

Finally, in Study 5 (Chapter 6), I extended the previous studies by examining if subtypes of children's shyness were distinguishable on biological and behavioral measures. This study further extended the previous studies by including a neuroendocrine measure, specifically salivary cortisol. I found that children who had early-emerging shyness (i.e., onset in toddlerhood) demonstrated greater relative resting right frontal brain activity, while children with later-emerging shyness (i.e., onset in early childhood) exhibited greater relative cortisol production in response to a self-presentation task, higher levels of embarrassment, and lower social skills. These findings provide support for distinction of early-developing and later-developing shyness in childhood, and demonstrate that differences in the developmental onset of shyness may be differentially related to behaviour and biological correlates.

Collectively, these series of studies and findings from this dissertation highlight that shyness is related to distinct developmental and biological processes in childhood. Specifically, I demonstrated that early developmental programming in response to prenatal stress may occur to shape the trajectory of shyness from childhood to adulthood, illustrating heterogeneity in the developmental course of shyness. Further, it appears that patterns of resting frontal brain activity may be associated with growth in shyness during the early school age years, as well as related to an early-emerging subtype of shyness. Finally, shy children appear to demonstrate greater autonomic and affective over arousal during the processing of socio-affective threat during a passive viewing task, as well during the anticipation phase of impending socio-evaluative threat in middle childhood.

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CHAPTER 2

Study 1: Prenatal Exposure to Stress and the Development of Shyness from Childhood to Adulthood

Poole, K.L., Saigal, S., Van Lieshout, R.J., & Schmidt, L.A. (2020). Developmental programming of shyness: A longitudinal, prospective study across four decades. *Development and Psychopathology, 32,* 455–464.

Abstract

Although shyness is a ubiquitous phenomenon with early developmental origins, little research has examined the influence of prenatal exposures on the developmental trajectory of shyness. Here, we examined trajectories of shyness from childhood to adulthood in three groups (*N* = 254), with varying degrees of prenatal adversity as indicated by the number of stressful exposures: extremely low birth weight (ELBW; <1000 grams) survivors prenatally exposed to exogenous corticosteroids (ELBW+S); ELBW survivors not prenatally exposed to exogenous corticosteroids (ELBW+NS); and normal birth weight (NBW) controls. Multilevel modeling revealed that the ELBW+S individuals exhibited the highest levels of childhood shyness, which remained stable into adulthood. The ELBW+NS and NBW controls had comparably low levels of childhood shyness; however, the ELBW+NS individuals experienced patterns of increasing shyness, while NBW controls displayed decreases in shyness into adulthood. We speculate that individuals exposed to multiple prenatal stressors (i.e., ELBW+S) may be developmentally programmed to be more sensitive to detecting social threat, with one manifestation being early-developing, stable shyness, while increasing shyness among ELBW+NS individuals may reflect a later-developing shyness influenced by postnatal context. We discuss the implications of these findings for understanding the developmental origins and developmental course of human shyness from childhood through adulthood.

Introduction

Development is a dynamic process in which very early perturbations can alter the psychobiological systems responsible for temperament and personality (Gartstein & Skinner, 2017; Krzeczkowski & Van Lieshout, 2018). Developmental programming hypotheses posit that individuals who are exposed to prenatal and early postnatal stressors may have altered maturation of central and peripheral physiological systems responsible for the regulation of stress, such as the prefrontal cortex, amygdala, and hypothalamic–pituitary–adrenal (HPA) axis (Gluckman, Hanson, & Buklijas, 2010; Harris & Seckl, 2011). These biological changes are thought to be adaptive in that they increase the fetus' *immediate* survival within a stressful prenatal environment (Bateson, Gluckman, & Hanson, 2014; Van Den Bergh, 2011). However, when the postnatal context is not comparably harsh and threatening as the prenatal environment, the individual manifests *long-term* programmed changes in the ability to handle stress and a predisposition to stress-reactivity and threat sensitivity (e.g., Bolten et al., 2013; Davis et al., 2007; DiPietro, Hodgson, Costigan, & Johnson, 1996; Pesonen, et al., 2006; Werner et al., 2007; see also Gartstein & Skinner, 2017, for a review).

While emerging work has highlighted general behavioral domains impacted by early exposure to stressors, we know relatively little about *specific* temperaments or personalities that may be developmentally programmed. Some work has found that prenatal stress and administration of glucocorticoids in utero have resulted in fearful behaviors in response to novelty in rodent models (Dickerson et al., 2005, Van den Hove et al., 2005, Weinstock, 2005) and among non-human primates (Schneider, 1992, Schneider et al., 1992). These behavioral manifestations are comparable to behavioral inhibition in humans (Garcia-Coll, Kagan, & Reznick, 1984). Indeed, limited work in humans has likewise found that early exposure to glucocorticoids can predict cortisol dysregulation, behavioral inhibition, fearfulness, and anxiety in offspring (Davis et al. 2004; Davis et al., 2005, de Weerth et al., 2003; Trautman et al., 1995). Collectively, these data suggest that prenatal exposure to glucocorticoids may result in programmed changes in the infant with one of the main behavioral outcomes being inhibition and fear in response to novelty – early developmental precursors of shyness. Therefore, we hypothesized that one ubiquitous temperamental characteristic that may be particularly relevant to study in the context of developmental programming is shyness.

Shyness is a trait characterized by fear and inhibition in response to social novelty and/or situations of perceived social-evaluation (Melchior & Cheek, 1990). Shyness is characterized by physiological and behavioral stress reactivity (e.g., Kagan et al., 1987, 1988; Poole & Schmidt, 2018; Schmidt, 1999; Schmidt & Fox, 1994; Schmidt et al., 1997, 1999) as well as increased detection of threat-related stimuli (LoBue & Pérez-Edgar, 2014; Pérez-Edgar et al., 2010, 2011). Likewise, exposure to prenatal adversity including glucocorticoids can result in altered development of neural systems, including limbic regions and prefrontal cortex involved in fear regulation as well as increase the sensitivity of the HPA axis (see Bock et al., 2014; McEwen et al., 2015, for a review). Thus, it is possible that the experience of early adversity may result in alterations in stress response systems and expression of negative affect, behavioral inhibition, and hypervigilance which lay the developmental foundation for prospective shyness (Gartstein & Skinner, 2017).

Some cross-sectional work has looked at the influence of prenatal stressors on the development of shyness or closely related constructs. These studies found evidence that lower birth weight (Pesonen, et al., 2009) and prenatal corticosteroid exposure (dexamethasone; Trautman et al., 1995) were correlated with more shyness and avoidance at 2 years of age. Additional work has found that prematurity (Tessier, Nadeau, Boivin, & Tremblay, 1997) and prenatal corticosteroid exposure (betamethasone; Erni et al., 2012) are correlated with more social withdrawal and social-evaluative stress, respectively, in childhood (age 10-12 years). Still other studies have found that lower birth weight was associated with higher levels of introversion (Allin et al., 2006; Hertz et al., 2013; Pesonen et al., 2008), behavioral inhibition (Pyhälä et al., 2009), social withdrawal (Eryigit-Madzwamuse et al., 2015; Hack et al., 2004) and shyness in young adulthood (early twenties; Schmidt et al., 2008) and adulthood (early thirties; Waxman et al., 2013; Xu et al., 2018), as well as harm avoidance in later adulthood (age 60 years; Lahti et al., 2008).

Although individuals exposed to early stressors may display behavioral profiles characterized by shyness in cross-sectional studies, considerably less is known about prospective *longitudinal* development of shyness from childhood to adulthood. The reason for this is that existing studies generally measure or analyze shyness or related behavior during single developmental periods and employ cross-sectional analyses. While such approaches are important, they do not reveal whether these phenotypes exhibit *stability* or *change* across development. It is important to have information on development starting in childhood and measured across repeated assessments, as this allows a valuable opportunity to examine *trajectories* of personality and information on the emergence of specific traits and the generation of hypotheses around factors involved in altering these trajectories. This is particularly relevant in the study of shyness, as previous work has illustrated heterogeneity in the developmental onset of shyness, including both early- and later-developing subtypes (Booth-LaForce & Oxford, 2008; Oh et al., 2008; Tang et al., 2017), which may have different underlying biological origins, different contextual influences, and different developmental outcomes (see Poole, Tang, & Schmidt, 2018, for a review). Thus, studying early developmental influences on prospectively and longitudinally measured shyness has implications for understanding heterogeneity in its developmental onset and course.

An additional gap in the context of developmental programming is that it remains unclear if the relative number of prenatal exposures may influence personality development. There is some evidence of possible cumulative effects of early adversity on levels of social-evaluative stress in a cross-sectional study of children (Erni et al., 2012). However, to our knowledge, no work has tested this within a longitudinal framework. Doing so may reveal whether multiple prenatal exposures can result in cumulative effects of early adversity that contribute to differing long-term effects on the development, stability, or change in shyness across time (Gartstein & Skinner, 2017).

It appears that independent exposure to prematurity or prenatal corticosteroids may result in shyness, thus it is plausible that individuals who experience *both* of these exposures may be particularly susceptible to experience developmental programming effects. Infants born at extremely low birth weight (ELBW; <1000 grams) are the tiniest and most at-risk babies and are susceptible to a number of stress-related problems across development (see Mathewson et al., 2017, for a review), and also exhibit physiological correlates of stress-vulnerability (Krzeczkowski et al., 2018; Schmidt, Miskovic, Boyle, & Saigal, 2010). In addition, some individuals born at ELBW are *also* exposed prenatally to exogenous corticosteroids, which are given to women at risk for preterm labor (Waffrin & Davis, 2012). Although these synthetic corticosteroids are an effective and important therapy for reducing infant mortality, exposure to these steroids prenatally can alter the developing brain and stress regulation systems (Davis et al., 2011; Savoy et al., 2016; see Welberg & Seckl, 2001, for an exhaustive review). For example, exposure to glucocorticoids can result in altered development of neural systems, including limbic regions and prefrontal cortex involved in fear regulation as well as increase the sensitivity of the HPA axis (see Bock et al., 2014; McEwen et al., 2015, for a review). The reason is that while approximately 80% of maternal cortisol is metabolized prior to entering fetal circulation, synthetic corticosteroids such as those administered for preterm labor *are not* metabolized (Waffrin & Davis, 2012). Consequently, the developing fetus brain is exposed to abnormally high levels of synthetic cortisol, which can lead to HPA axis dysregulation (Moisiadis & Matthews, 2014). There exists some work suggesting that ELBW infants who are also exposed prenatally to corticosteroids may exhibit greater stress-vulnerability relative to those who were not exposed, as indicated by greater relative right frontal brain activity (Krzeczkowski et al., 2018) and increased risk for anxiety disorders (Savoy et al., 2016; Van Lieshout et al., 2015); interestingly, these are also physiological and psychological correlates of shyness.

The Present Study

We conducted a longitudinal, prospective study spanning four decades to test whether increasing levels of perinatal adversity, specifically prematurity and prenatal exposure to exogenous corticosteroids, were associated with the developmental onset and developmental course of shyness from childhood to adulthood. To this end, we used multilevel modeling to delineate the trajectory of shyness from age 8 to age 32 in three groups with varying degrees of exposure to prenatal adversity. From the highest to lowest levels of prenatal adversity, these groups included: ELBW survivors who were also prenatally exposed to exogenous corticosteroids (ELBW+S); ELBW survivors who were not prenatally exposed to exogenous corticosteroids (ELBW+NS), and normal birth weight (NBW; > 2500 grams) controls.

We tested three hypotheses. First, since ELBW+S individuals had ostensibly more stressful early exposures relative to the other groups, we hypothesized they would manifest high levels of shyness in childhood that remain stable into adulthood, resulting from a predisposition to experience heightened postnatal stress-reactivity, threat sensitivity and emotion dysregulation (Gartstein & Skinner, 2017). Second, we hypothesized that ELBW+NS individuals would display moderate, stable levels of shyness given their intermediate level of early stress exposure. Finally, we hypothesized that NBW controls would display low, stable levels of shyness, or decreases in shyness across development in line with previous work in typically developing individuals (Dennissen, Asendorpf, & Van Aken, 2008).

Method

Sample Overview

The ELBW sample was recruited at birth and comprised 397 predominantly Caucasian infants born at less than 1000 g between 1977 and 1982 to residents of central-west and has been prospectively followed since birth. Of these, 179/397 (45%) survived

to hospital discharge. In the present study, all participants with neurosensory impairment (NSI; n = 51), defined as the presence of at least one of cerebral palsy, blindness, deafness, intellectual disability, or microcephaly diagnosed in childhood by a neonatologist or developmental pediatrician, were excluded because they have unique challenges that are not generalizable to the majority of those born preterm.
Follow-up assessments on this group have been conducted during childhood (8 years), adolescence (12 to 16 years), young adulthood (22 to 26 years), and adulthood (30 to 35 years). We will use the developmental period to describe each assessment for the remainder of the paper. At the childhood assessment, 108 (84.4%) ELBW survivors had complete shyness data; at the adolescent assessment, 102 (80.0%) ELBW survivors had complete shyness data; at the young adulthood assessment, 109 (85.2%) ELBW survivors had complete shyness data; and at the adulthood assessment, 71 (55.4%) ELBW survivors had complete shyness data. Participants who had shyness data for at least one visit (n = 112) were included in the analyses. Of these ELBW participants, a total of 56 (50%) mothers received prenatal administration of betamethasone (ELBW+S), whereas 56 (50%) mothers did not receive prenatal administration of betamethasone (ELBW+NS).

Participants in the NBW control group were recruited when they and the ELBW survivors were 8 years old. These 145 children were selected from a random sample of students in the Hamilton Public School System (Ontario) who were born at full term and matched with the ELBW participants on age, sex, and family socioeconomic status (SES) at the childhood assessment. Subsequent assessments have occurred at the same ages as the ELBW cohort. At the childhood assessment, 139 (95.9%) NBW children had complete shyness data; at the adolescent assessment, 120 (82.8%) NBW adolescents had complete shyness data; at the young adulthood assessment, 129 (90.0%) NBW participants had complete shyness data; and at the adulthood assessment, 85 (58.6%) NBW controls had complete shyness data. Participants who had shyness data for at least one visit (n = 142) were included in the analyses.

Procedures

Study assessments were conducted at McMaster Children's Hospital for the childhood and adolescent assessment, and at the Child Emotion Laboratory at McMaster University for both adulthood assessments. After a complete description of the study was provided, written informed consent was obtained from the parents of all participants during the childhood and adolescent assessments, and by the participants themselves during the adult assessments. The Hamilton Health Sciences Research Ethics Board approved all study procedures.

Shyness Measure

Childhood Assessment. Shyness was measured in childhood using 7 items from the parent-report Child Behavior Checklist (CBCL; Achenbach & Edelbrock, 1983). Sample items included: "Child is shy or timid" and "Child is self-conscious or easily embarrassed", and parents' rate how characteristic each item is on a 3-point Likert scale (0 = *not true,* 1 = *sometimes or somewhat true,* 2 = *very true or often true*). This scale demonstrated acceptable internal reliability in our sample (α = .63).

Adolescent Assessment. Shyness was measured in adolescence using 5 items from the parent-reported Ontario Child Health Study-Revised (OCHS-R; Boyle et al., 1987) Questionnaire. Sample items from this included: "Adolescent is self-conscious/easily embarrassed", and "Adolescent is withdrawn", and parents' rate how characteristic each item is on a 3-point Likert scale (0 = *not true,* 1 = *sometimes or somewhat true,* 2 = *very true or often true*). This scale demonstrated acceptable internal reliability in our sample ($\alpha = .66$).

Young Adulthood and Adulthood Assessments. At both adult visits, participants completed the Young Adult Self-Report (YASR; Achenbach, 1997), and 7 items comprised the shyness scale. Sample items included: "I am too shy or timid", and "I am self-conscious or easily embarrassed", and participants rate how characteristic each item is on a 3-point Likert scale (0 = *not true,* 1 = *sometimes or somewhat true,* 2 = *very true or often true*). The scale demonstrated good internal reliability at both visits (Age 22-26: $\alpha = .77$; Age 30-35: $\alpha = .78$). This shyness scale demonstrated concurrent validity with the Cheek and Buss Shyness Scale (CBSS; Cheek, 1983; Cheek & Buss, 1981) that was administered at each adult visit (Age 22-26: $r = .60$, $p < .001$; Age 30-35: $r = .68$, $p < .001$).[1]

Prenatal Corticosteroid Exposure

ELBW survivors were identified as being exposed to steroids prenatally if they were born to mothers who received one or two doses (12 mg/dose) of betamethasone administered intramuscularly in a single 24-hour period. Mothers were administered betamethasone at the discretion of their attending physician if they were at risk for

[1] Given that the CBSS was only administered at the adult visits, it was not included in our composite shyness score, and was reported to provide convergent validity with our shyness measure using items from the YASR. However, when computing a shyness composite including both the CBSS and YASR shyness items for the adult visits and using this as the dependent measure, the statistical significance and direction of the reported results remain unchanged.

preterm delivery. Information regarding the dose and exposure was obtained from medical records.

Covariates

We considered a number of covariates in our analyses that may alter socio-emotional development, including participant sex, intellectual quotient (IQ), and socioeconomic status.

Sex. Data on the biological sex of the participant were drawn from the ELBW participants' medical charts and were reported by parents of NBW participants at age 8.

Intellectual Quotient. IQ was assessed by using the Wechsler Intelligence Scale for Children-Revised (WISC-R) that was administered at age 8. The WISC-R consists of 10 subtests and combining these subtests creates a performance IQ scale (i.e., assessment of fluid intelligence) and a verbal IQ scale (i.e., assessment of reading, verbal, and language abilities). Together, these two subscales are combined to reflect an overall IQ, estimating overall intelligence, which was used in the present study (Wechsler, 1974).

Socioeconomic Status. SES was modeled as a time-varying covariate, meaning that we accounted for changes in SES across each of the four visits. At the childhood and adolescent assessment, parental SES was assessed via parental reports using the Hollingshead 2-factor index that uses educational attainment and occupational prestige as indicators of social position (Hollingshead, 1969). This index has 5 levels, and for comparison with our adult measures of SES, we recoded SES such that 1 indicated the lowest SES level and 5 indicated the highest SES level. In order to attempt to remain as consistent as possible, for both adulthood assessments we used educational attainment and household income as our primary indicators of SES. Educational attainment was self-reported and calculated by summing the years of education completed at the time of testing, and mean household income was self-reported. These two indices of SES were averaged and combined into a composite measure at each adult visit. To ensure consistency across measures, indicators of SES at each assessment were *z*-scored and included as a time-varying covariate.

Statistical Analyses

Sample characteristics between groups (i.e., ELBW+S, ELBW+NS, NBW) were examined using analyses of variance (ANOVA) for continuous measures, and chi-squared tests for categorical variables. Pearson's correlations assessed the relation of shyness scores across visits.

Shyness trajectories from childhood to adulthood were delineated using multilevel modeling in which repeated measures (i.e., shyness) are regressed on the timing of these assessments (i.e., participant age) to estimate rates of change at an individual level. Growth curve analysis provides estimates pertaining to variability in baseline shyness (i.e., intercept variance) as well as the possibility that individuals' shyness changes at different rates (i.e., slope variance) (Delucia & Pitts, 2006). Because the items comprising the measures of shyness varied slightly across some visits, *z*-scores were computed and used as the dependent measure.

We examined whether prenatal exposure status (i.e., ELBW+S, ELBW+NS, NBW) affected the initial status (i.e., shyness at childhood) and the rate of change of shyness across assessments (i.e., trajectory of shyness from childhood to adulthood). The reference category for this analysis was the ELBW+NS group. Maximum likelihood was used to account for missing data in the growth models to present unbiased estimates. We examined two models including an unadjusted model, and a fully adjusted model controlling for covariates (i.e., sex, IQ, SES).

Results

Descriptive Statistics

Sample characteristics are presented in Table 1.1. Pearson's correlations and mean levels of shyness scores across each of the four visits are presented in Table 1.2. Shyness at each visit was normally distributed.

Trajectories of Shyness

The parameter estimates for the unadjusted and adjusted growth curve models are shown in Table 1.3. Results indicated that ELBW+S participants had significantly higher levels of shyness in childhood relative to the ELBW+NS participants ($\beta = 0.42$; $p < .05$), with a stable trajectory into adulthood (as indicated by a non-significant slope: $\beta = -0.01$; $p = ns$). The NBW and ELBW+NS participants did not significantly differ on childhood shyness ($\beta = 0.34$; $p = ns$), but there was divergence in shyness levels from childhood to adulthood in the ELBW+NS and NBW participants. As illustrated in Figure 1.1, the NBW participants demonstrated decreases in shyness into adulthood relative to the ELBW+NS participants ($\beta = -0.02$; $p < .05$) who displayed relative increases, with levels comparable to the ELBW+S participants by adulthood.[2]

[2]Because previous research has suggested that males and females may be differentially vulnerable to the physiological and behavioral effects of prenatal environmental exposures (Gartstein & Skinner, 2017), we also considered sex as a *moderator* on the influence of prenatal stress and trajectories of shyness, and no significant effects were observed.

Table 1.1

Sample characteristics

	ELBW+S (n = 56)	ELBW+NS (n = 56)	NBW (n = 142)	
Birth Weight in grams, Mean (*S.D*)	836.33 (112.26)[a]	836.50 (128.73)[b]	3376.18 (492.11)[a,b]	$p < .001$
Gestational Age in weeks, Mean (*S.D.*)	26.98 (1.64)[a]	27.60 (2.89)[b]	40[a,b]	$p < .001$
Small for Gestational Age, *n* (%)	18 (32.14%)	15 (26.78%)	N/A	$p > .05$
Males, *n* (%)	25 (41.66%)	29 (42.65%)	66 (45.52%)	$p > .05$
Childhood IQ, Mean (*S.D.*)	94.00 (11.81)[a]	94.88 (13.38)[b]	104.16 (12.01)[a,b]	$p < .001$
Age, Mean (*S.D.)*				
Childhood Assessment	7.64 (0.34)[a]	7.74 (0.39)[b]	8.10 (0.50)[a,b]	$p < .001$
Adolescent Assessment	13.52 (1.51)[a]	14.00 (1.62)[b]	14.50 (1.30)[a,b]	$p < .001$
Young Adulthood Assessment	23.05 (1.09)[a]	23.25 (1.22)[b]	23.63 (1.02)[a,b]	$p < .01$
Adulthood Assessment	31.80 (1.63)	32.11 (1.69)	32.47 (1.36)	$p > .05$
Household SES (*z*-scored), Mean (*S.D.*)				
Childhood Assessment	-0.05 (0.90)	-0.11 (1.03)	0.09 (1.02)	$p > .05$
Adolescent Assessment	-0.07 (0.88)	-0.12 (1.01)	0.07 (1.00)	$p > .05$
Young Adulthood Assessment	-0.04 (0.63)[a]	-0.13 (0.66)[b]	0.19 (0.62)[a,b]	$p < .01$
Adulthood Assessment	-0.09 (0.63)[a]	-0.31 (0.68)[b]	0.21 (0.83)[a,b]	$p < .01$

Note: identical superscripts denote significant group differences. ELBW+S = extremely low birth weight and prenatal steroid exposure; ELBW+NS = extremely low birth weight and no prenatal steroid exposure; IQ = intellectual quotient; NBW = normal birth weight, SES = socioeconomic status

Table 1.2

Descriptive statistics and correlations for shyness across assessments from childhood to adulthood

	1	2	3	Mean (S.D.)
1. Childhood	--			0.03 (0.99)
2. Adolescence	.27**	--		-0.04 (0.97)
3. Young Adulthood	.23**	.22**	--	-0.01 (0.98)
4. Adulthood	.17*	.34**	.69**	-0.06 (0.94)

***p* < .001; **p* < .05; *Note*: shyness scores are *z*-scored

Table 1.3

Growth curve models of shyness predicted by birth weight status and prenatal corticosteroid exposure

	Unadjusted Model β (S.E.)	Adjusted Model β (S.E.)
Initial status		
Intercept	-0.20 (0.14)	0.14 (0.61)
ELBW+S	0.44 (0.22)*	0.42 (0.22)*
NBW	0.30 (0.19)	0.34 (0.20)
Sex	--	-0.05 (0.15)
IQ	--	-0.00 (0.01)
Slope		
Age	0.01 (.01)	-0.03 (0.03)
ELBW+S × Age	-0.01 (.01)	-0.01 (0.01)
NBW × Age	-0.02 (.01)*	-0.02 (0.01)*
Sex × Age	--	-0.00 (0.01)
IQ × Age	--	0.00 (0.00)
SES	--	-0.15 (0.04)*

ELBW+S = extremely low birth weight and prenatal steroid exposure; IQ = intellectual quotient; NBW = normal birth weight, SES = socioeconomic status; *Note*: ELBW+NS = reference category; $N = 254$. $*p < .05$

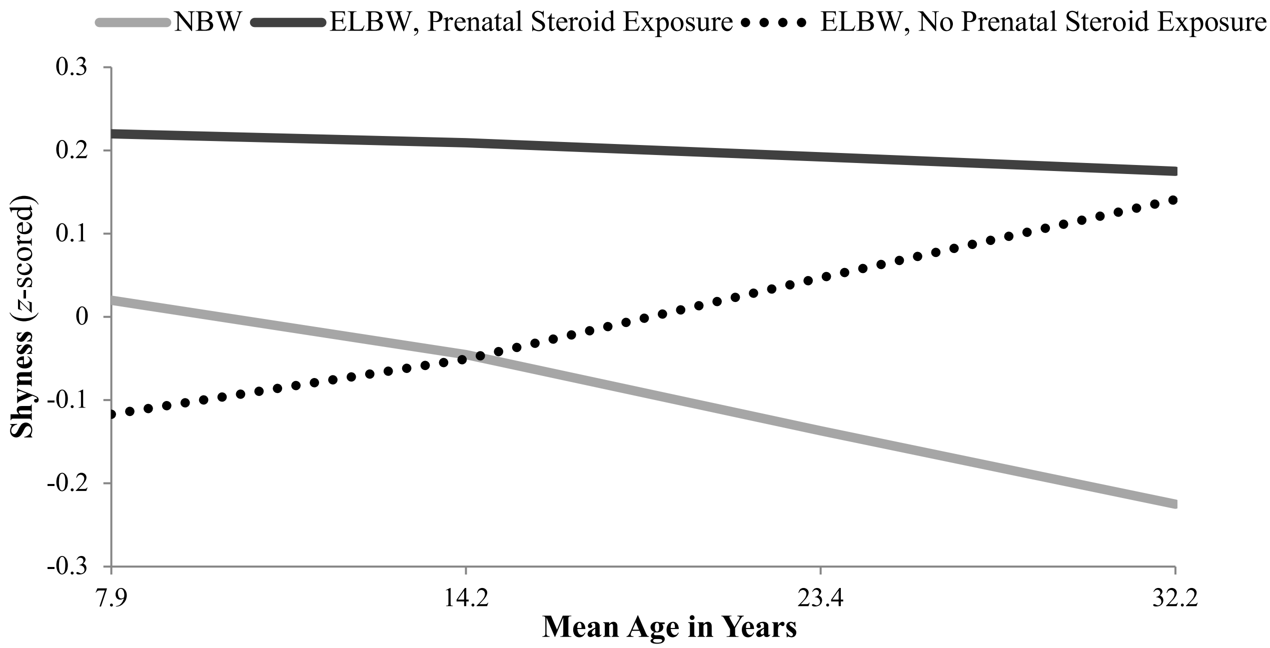

Figure 1.1. The mean developmental trajectory of shyness from childhood to adulthood based on birth weight status and prenatal corticosteroid exposure. ELBW – extremely low birth weight; NBW – normal birth weight. *Note*: estimates from the growth curve analysis are plotted. $N = 254$.

Discussion

Previous theoretical frameworks have posited that in-utero exposure to adversity and stressful environments can exert long-term influences on behavior and socio-emotional development (Gartstein & Skinner, 2017; Gluckman, Hanson, & Buklijas, 2010). Our findings converge with this notion and suggest that the relative severity of early prenatal stressors may influence the emergence and trajectory of shyness across different developmental periods. Using a prospective, longitudinal study, we found that individuals who were born at ELBW *and* exposed prenatally to corticosteroids exhibited the highest levels of shyness in childhood that remained stable into their early thirties. We further report that ELBW survivors who *were not* exposed prenatally to corticosteroids had comparably low levels of childhood shyness relative to the NBW controls; however, whereas the NBW controls displayed decreases in shyness from childhood to adulthood, the ELBW+NS individuals experienced patterns of increasing shyness into their thirties.

The origins of shyness are multifaceted, with both biological and contextual influences affecting its developmental course (e.g., Poole, Tang & Schmidt, 2018; Schmidt, Polak, & Spooner, 2005; Stevenson-Hinde, 2002). One line of evidence argues that some infants enter the world with a biological predisposition to become physiologically and behaviorally aroused in response to novelty (i.e., behaviorally inhibited; Garcia-Coll, Kagan, & Reznick, 1984). As these individuals undergo further socio-cognitive development across early childhood, this inhibition can become particularly salient in novel *social* contexts for some children (i.e., shyness), resulting in the perception of social situations as threatening in nature. The typical biological modifications (e.g., increased HPA axis and amygdala activity) accompanying stressful prenatal environments are similar to those biological systems implicated in shyness and social threat processing (Beaton et al., 2012; Kagan et al., 1987, 1988; Schmidt, et al., 1997; Tang et al., 2016), and we speculate that these developmental antecedents of shyness may be programmed in-utero in response to prenatal stressors.

The pattern of shyness among the ELBW+S individuals likely reflects an early-emerging shyness that is stable across development. It has been proposed that prenatal alterations that occur in order to increase postnatal survival can occur at the expense of physiological and behavioral flexibility (i.e., ability to change across development), and consequently may lead to behavioral phenotypes that are less likely to change across time (Duckworth, 2015). The selection of a stable system during a stressful prenatal period is viewed as adaptive as this allows the individual to optimize survival in an equally threatening post-natal environment through the programming of traits such as hypervigilance, threat-sensitivity, and stress-reactivity– key features of shyness. Being born at ELBW is a significant early stressor resulting in programmed changes in the central and physiological systems responsible for the stress response (Gluckman, Hanson, & Buklijas, 2010; Harris & Seckl, 2011). Likewise, administration of prenatal synthetic steroids stimulates the fetal HPA axis and mimics the effects of a natural occurring stressor (Benediktsson et al., 1993). Thus, exposure to synthetic glucocorticoids *in addition* to the stresses of being born at ELBW may result in a cumulative effect by which set points in the brain and body are programmed during sensitive periods of early development, and lead to stability in shyness across development. Given that these hypothesized mechanisms were not directly measured in the present study, it will be informative to systematically test the speculated mechanisms in future work.

Our findings also illustrate the concept of equifinality, which is the notion that individuals may have different initial starting points but have similar later outcomes (Cicchetti & Rogosch, 1996). Indeed, the ELBW+S and ELBW+NS groups had different initial starting points of steroid exposure versus no exposure and levels of childhood shyness, but the ELBW+NS group had patterns of increasing shyness with levels similar to the ELBW+S group by adulthood. We further find that the ELBW+NS and NBW groups had similarly low levels of childhood shyness, but there was divergence in shyness between ELBW+NS and NBW groups around adolescence, such that the ELBW+NS individuals demonstrated patterns of increasing shyness into adulthood, while the NBW controls showed decreases in shyness. It is possible that this divergence in shyness occurs due to differences in developmental circumstances and processes between the groups (Cicchetti & Rogosch, 1996).

Beyond biological influences on the development of shyness, additional developmental models of shyness highlight the important role of social influences and context (Coplan, Arbeau, & Armer, 2008; Gazelle, & Ladd, 2003; Hastings et al., 2010; Rubin, Bowker, & Gazelle, 2010; Schmidt, Polak, & Spooner, 2005; Stevenson-Hinde, 2002). Previous work has shown that those born prematurely are more prone to be the recipient of overprotective parenting (e.g., Indredavik et al. 2005; Jaekel et al. 2012; Wightman et al. 2007), victims of bullying, peer victimization, social exclusion (see Day, Van Lieshout, Vaillancourt, & Schmidt, 2015, for a recent review), and have lower social competence and social skills (Dahl et al., 2006; Hoy, Sykes, Bill, Halliday, McClure, & Reid, 1992; Ross, Lipper, & Auld, 1990) relative to their typically-developing peers. Interestingly, these are key social influences that play a role in later-developing or increasing patterns of shyness (Booth-LaForce & Oxford, 2008; Hastings, et al., 2010; Karevold et al., 2012; Oh et al., 2008; Poole, Tang & Schmidt, 2018; Rubin, Bowker, & Gazelle, 2010, Tang et al., 2017). These social factors may be particularly influential during adolescence as this coincides with the onset of puberty, increases in socio-cognitive development, and an increased reliance on peers and need for social acceptance (Cheek, Carpentieri, Smith, Rierdran & Koff, 1986; Damon, 1983). These hormonal, neural, and social changes can affect the development, expression, and regulation of emotions (Del Piero, Saxbe, & Margolin, 2016), and some work has found that shyness and social fears may increase during adolescence due to these factors (Cheek et al., 1986; Cheek & Krasnoperova, 1999; Tang et al., 2017; Westenberg, Drewes, Goedhart, Siebelink & Treffers, 2004). Taken together, we predict that social influences may place ELBW+NS individuals on a path toward increasing shyness beginning in adolescence that reflects a later-developing shyness that is influenced by postnatal context. Although we suspect that these social experiences are also present for ELBW+S individuals, these factors may play a role in the maintenance of shyness as opposed to increasing patterns given the relatively high initial childhood shyness of the ELBW+S group, likely resulting in ceiling effects.

Our findings also shed light on the relative trajectory of shyness from childhood to adulthood in typically developing samples (i.e., those born at NBW). Although the findings should be interpreted with appropriate caution as the trajectories are *relative* to the ELBW survivors, they nonetheless provide information on how shyness develops in typically developing individuals. This is important given that there exists relatively little work examining the mean-level developmental course of shyness from childhood to adulthood. One longitudinal study found decreasing patterns of shyness from age 4 to 23 years among “resilient” children (Dennissen, Asendorpf, & Van Aken, 2008), which is similar to what we found for the NBW group from age 8 to 32 years. As individuals enter developmental periods of stability and achieve important milestones (e.g., establish romantic relationships, obtain a career, start a family), they may become more socially dominant and emotionally stable (Neyer & Asendorpf, 2001; Roberts et al., 2006; Robins, Fraley, Roberts, & Trzesniewski, 2001). Indeed, relative to individuals who were born at ELBW, NBW individuals are more likely to obtain these milestones (Saigal et al., 2016), and this may be one mechanism underlying their relative decreases in shyness into adulthood.

Although it appears that survivors of ELBW exposed to synthetic glucocorticoids may be on a path towards the development of shyness and some work has shown that shyness is predictive of psychopathology and maladaptive outcomes (e.g., Clauss & Blackford, 2012), it is important to point out that shyness is not always inherently maladaptive. Indeed, other research has reported adaptive aspects of some shyness subtypes (e.g., Colonnesi et al., 2013, Poole & Schmidt, 2018, Schmidt & Poole, 2018). Likewise, although we hypothesize that the development of shyness may result from programmed changes in the physiological systems underlying hypervigilant and fearful reactions due to early stressors, we further reiterate that these changes are not necessarily pathological, but are thought to be functional adaptations that may promote resilience in the faces of future environmental challenges and stressors (Del Giudice, Ellis, & Shirtcliff, 2010; Gluckman et al., 2005).

Limitations

There were several limitations of the present study that should be acknowledged. First, shyness was assessed using different informants during the different developmental periods (i.e., parent- versus self-report). Although this is an inherent methodological challenge to long-term developmental research (Biesanz, West, & Kwok, 2003; Caspi, Roberts, & Shiner, 2005), and while we standardized measures for consistency, it nonetheless is prone to possible informant discrepancies across assessments and issues of measurement variance. Second, although this work provides valuable information on the development of shyness from childhood to adulthood, we do not have data pertaining to earlier developmental periods such as toddlerhood and so we cannot directly assess the early developmental precursors of shyness (e.g., behavioral inhibition) that may have influenced the trajectories. Third, we relied on questionnaire-based indices of shyness, and thus future work could aim to integrate observational measures of shyness, notwithstanding the challenges associated with accurately assessing the same phenotype across development given differences in the expression, experience, and overt behaviors of shyness depending on age. Fourth, although Cronbach's alphas were acceptable, they were somewhat low during the childhood and adolescent assessments; however, this is not uncommon for scales with less than ten items, as internal consistency tends to be an underestimate in scales with fewer items (Taber, 2017). Fifth, we acknowledge that there were likely additional unmeasured influences on the development of shyness beyond those investigated in the present study including parental traits such as social anxiety which likely exerts both biological (i.e., genetic) and environmental (i.e., social modelling) influences on the expression of shyness in offspring (Bögels, Stevens, & Majdandžić, 2011; Lieb et al., 2000; Poole et al., 2017; Smith et al., 2012), as well as additional early stressors aside from prematurity and prenatal steroid exposure. Sixth, given the cohort study design, women were not randomized to receive corticosteroids (i.e., NBW babies were not prenatally exposed to corticosteroids), and thus it is possible that there were unmeasured confounding factors influencing our findings. Finally, it is important to replicate our findings in contemporary samples to ensure generalizability to more recent birth cohorts given advancements in neonatal care and increased survival rates among individuals who are born extremely prematurely.

Implications and Conclusions

The present study highlights the importance of employing a longitudinal framework when testing developmental programming hypotheses as this allows for identification of how prenatal stressors may result in the prospective emergence of personality traits during different developmental periods. Our findings inform developmental models of shyness, and illustrate that while biological susceptibility may influence the development of shyness, postnatal contextual influences may also impact the development of shyness for some individuals. This study provides evidence for heterogeneity in the developmental origins, onset, and course of shyness. We recommend that future studies continue to investigate how early life experiences may influence the manifestation of shyness in particular, and personality styles in general across development, and how this may be related to the development of psychopathology.

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CHAPTER 3

Study 2: Frontal Brain Activity and the Development of Shyness in Childhood

Poole, K.L., Santesso, D.L., Van Lieshout, R.J., & Schmidt, L.A. (2019). Frontal brain asymmetry and the trajectory of shyness across the early school years. *Journal of Abnormal Child Psychology, 47,* 1253–1263.

Abstract

Although resting right frontal electroencephalogram (EEG) asymmetry has been linked to avoidance and withdrawal-related behaviors such as shyness in previous cross-sectional studies, relatively little research has examined the influence of frontal brain electrical activity on the development of shyness in children using a prospective, longitudinal study design. Here, we tested whether resting frontal EEG asymmetry predicted the trajectory of children's shyness across five assessments. Children were enrolled in the study during the summer prior to grade 1 (N = 37; M_{age} = 6.39 years, *S.D.* = 0.15 years), at which time resting frontal EEG activity and maternal report of children's shyness were collected. Mothers then reported on their child's shyness over another four follow-up assessments, spanning two years (winter of grade 1, summer prior to grade 2 entry, winter of grade 2, and summer prior to grade 3). Growth curve analysis revealed that children displaying greater relative right frontal EEG activity had lower levels of shyness relative to children exhibiting greater relative left frontal EEG activity at study enrollment (i.e., age 6), but displayed statistically significant linear *increases* in shyness across time, with the highest levels of shyness by the summer prior to grade 3 (i.e., age 8). There was, however, no relation between left frontal EEG asymmetry and change in shyness across time. These preliminary findings suggest that right frontal EEG asymmetry may reflect a biological diathesis for the growth of shyness during the early school years.

Introduction

Shyness is a trait characterized by fear and inhibition in novel social situations and under conditions of perceived social-evaluation (Kagan et al., 1988; Rubin et al., 2009). Shy children tend to face greater socio-emotional difficulties across several domains, including poorer language skills, lower social functioning, and more internalizing problems such as anxiety relative to their less shy peers (Coplan, Prakash, O'Neil & Armer, 2004; Coplan et al., 2013; Crozier, 1995; Miers, Blote, & Westenberg, 2010; Spere, Schmidt, Theall-Honey & Martin-Chang, 2004).

Shyness shares conceptual overlap with a number of related constructs used in developmental research. For example, *behavioral inhibition* is an early-emerging temperamental antecedent of shyness marked by fear and wariness in response to social and non-social novelty (Garcia-Coll, Kagan & Reznick, 1984). Additional constructs used in children and adolescents include *anxious-solitude* which is operationalized as solitary behavior that is prompted by social anxiety in the presence of familiar peers (e.g., Avant, Gazelle, & Faldowski, 2011; Gazelle & Ladd, 2003; Gazelle & Rudolph, 2004; Markovic & Bowker, 2017; Shell, Gazelle & Faldowski, 2014; Shell, Gazelle & Faldowski, 2014); *social withdrawal* which encompasses both fearful and non-fearful preferences for solitude when encountering familiar and/or unfamiliar peers (Oh et al., 2008); and *social reticence* which is characterized by onlooking, unoccupied behavior in response to familiar or unfamiliar peers, and is common in shy children (Rubin et al., 2009). These constructs share conceptual similarities with shyness in that they describe phenomena characterized by discomfort and/or withdrawn behaviors in social situations.

Developmental Course of Shyness and Related Constructs

While shyness is moderately stable across development for some children (particularly among extreme groups) (Asendorpf, 1989; Fordham & Stevenson-Hinde, 1999; Kagan, Reznick & Snidman, 1988; Pedlow, Sanson, Prior, & Oberklaid, 1993; Rubin et al., 1997; Sanson, Pedlow, Cann, Prior, & Oberklaid, 1996), there exist individual differences in the developmental trajectory (i.e., growth, decline, or stability) of shyness during childhood (Engfer, 1993; Karevold et al., 2012). Several studies have identified subgroups of children who follow common developmental trajectories of shyness, or closely related constructs, beginning in infancy through to adulthood. For example, during infancy and early toddlerhood (6 to 36 months), one study examining stranger fear (i.e., wariness in response to unfamiliar adults) found four trajectories of parent-reported social fear, characterized by: stable-high trajectory; decreasing trajectory; slow increasing trajectory; and steep increasing trajectory (Brooker et al., 2013). Other research examining the stability of observed behavioral inhibition has found that it is only modestly stable across the first few years of life (Fox et al., 2001; Rubin, Burgess & Hastings, 2002).

Additional work in young children aged 2 to 5 years found three developmental pathways of observed social reticence during an interaction with an unfamiliar peer. These trajectories in early childhood included: a low, increasing trajectory; a high, decreasing trajectory; and a high, increasing trajectory (Degnan et al., 2014). In older school-aged children, two studies examining trajectories of social withdrawal found evidence for three developmental pathways in children from grades 1 to 6 according to teacher-report (Booth-LaForce & Oxford, 2008), and grades 5 to 8 according to peer nominations (Oh et al., 2008): a low, stable trajectory; a high, decreasing trajectory; and an increasing trajectory. In a long-term prospective study of individuals followed from ages 8 to 35, Tang and colleagues (2017) recently found three trajectories of shyness from childhood to adulthood (using parent-report and self-report depending on age): a low, stable trajectory; a low, increasing trajectory; and a high, decreasing trajectory. Collectively, this body of work converges on the notion that although shyness (or related constructs) may be stable for some individuals, there is individual variability in the developmental course of shyness with some individuals displaying non-stable patterns.

Right Frontal Brain Activity and Shyness

Although the origins of shyness are multifaceted, interest in the biological foundations of shyness has received considerable attention over the past several decades (Fox et al., 2001, 2005; Kagan, Reznick, & Snidman, 1987, 1988; Schmidt & Miskovic, 2014; Schmidt & Schulkin, 1999). One line of neurobiological evidence for shyness comes from studies of ongoing brain electrical activity (EEG) indexed from the anterior portions of the scalp during resting states.

Motivational models of frontal brain activity (see Coan & Allen, 2004; Davidson, 1993, 2000; Fox, 1991, 1994; Reznik & Allen, 2018, for reviews) posit that approach-avoidance related tendencies are lateralized in different hemispheres of the frontal region of the brain. These models have described frontal brain asymmetry as a trait-like measure (i.e., a biological diathesis) that is stable across time and context (e.g., Davidson, 2000), and there is a wealth of empirical evidence corroborating these assumptions (see, e.g., Coan & Allen, 2004; Reznik & Allen, 2018, for reviews). Greater relative activity in the left frontal brain region is presumed to facilitate approach-related behaviors such as sociability, whereas greater relative activity in the right frontal region has been implicated in the development and maintenance of avoidance-related behaviors such as shyness (Schmidt, 1999).

Electroencephalography (EEG) allows for relatively non-invasive collection of information pertaining to brain activity in the left and right frontal hemispheres and is a helpful tool for measuring biological predispositions underlying approach-avoidance tendencies. Indeed, researchers have used EEG-based data to derive asymmetries of frontal brain activity and the frontal activation motivational model as a theoretical platform to test hypotheses related to individual differences in temperament (including shyness and related constructs) and affective style across development (see Schmidt & Miskovic, 2014, for a review). Typically, these studies examined frontal asymmetry as the difference in EEG alpha power in the right frontal hemisphere *minus* EEG alpha power in the left frontal hemisphere. Because EEG alpha power is inversely related to cortical activity, negative scores reflect greater relative right frontal brain activity (Tomarken et al., 1992).

During different developmental periods, researchers have provided support for the relation between right frontal asymmetry (RFA) and avoidance-related tendencies. For example, in infants, resting RFA has been associated with behavioral inhibition and reactivity (Calkins, Fox & Marshall, 1996; Davidson, & Fox, 1989; Fox & Davidson, 1987; Fox et al., 2001; Hane, Fox, Henderson, & Marshall, 2008; Howarth, Fettig, Curby, & Bell, 2016; McManis, Kagan, Snidman, & Woodward, 2002). In preschool children, those described as socially inhibited and withdrawn during interactions with peers show RFA at rest (Fox et al., 1996), as do temperamentally shy preschoolers (Theall-Honey & Schmidt, 2006). In adults, higher levels of behavioral inhibition, shyness, and social anxiety also have been linked to RFA at rest (Moscovitch et al., 2011; Schmidt, 1999; Sutton & Davidson, 1997).

Although the relation between resting RFA and avoidance-related tendencies is well established (e.g., see Reznik & Allen, 2018, for a review), the existing body of research is limited in at least three important ways. First, the majority of the existing work utilizes samples that were selected for extreme temperamental profiles in childhood (e.g., Calkins et al., 1996; Fox et al., 2001; Theall-Honey & Schmidt, 2006) and adulthood (Schmidt, 1999) or adults with clinical levels of social fear (e.g., Davidson et al., 2000; Moscovitch et al., 2011), allowing generalization to these selected temperamental profiles and clinical samples. However, whether the frontal activation-motivational model generalizes to studies examining continuous variation in avoidance-related phenotypes and/or typically developing samples not selected for shyness has not been fully investigated. Second, the majority of the existing work has focused on the developmental periods of infancy, toddlerhood, early childhood, and adulthood, so whether frontal asymmetry plays a role in the development of shyness in school age children, a key time period for socio-emotional development, remains an empirical question. Third, a large proportion of existing work has utilized cross-sectional designs. Although this provides important correlational information, it does not reveal how resting RFA may act as a biological diathesis to the *prospective* stability or change in shyness, limiting potential causal developmental interpretations and inferences.

There is, however, some limited longitudinal work on the topic of frontal EEG asymmetry and shyness. For example, Henderson and colleagues found that frontal EEG asymmetry moderated the association between negative reactivity (i.e., signs of distress including crying, fussing, and motoric agitation) in infancy and social reticence at age 4, such that this relation was stronger for infants with resting RFA (Henderson, Fox, & Rubin, 2001). In another study, resting and on-task RFA in four-year-old children was associated with poorer emotion regulation during a speech task when children were 9 years of age (Hannesdóttir et al., 2010). These studies highlight the possible influence of resting RFA on prospective socio-emotional development, but only measured a shyness-related construct at one prospective time point. By employing a longitudinal study design in which shyness is measured across several repeated assessments and resting RFA is measured at an initial starting point, we may be better able to uncover the predictive utility of resting RFA on the growth or decline in the trajectory of shyness across time in childhood and in so doing provide empirical support for the theoretical notion that resting RFA may reflect a biological diathesis or vulnerability factor that manifests in withdrawal-related behaviors over time.

The Present Study

We examined whether resting RFA predicted shyness across the early school years in a sample of typically developing children. To address this question, maternal-report of children's shyness was collected on a total of five separate occasions across two years. We first measured children's shyness during the summer prior to grade 1, with the remaining four assessments over the early school years: winter of grade 1, summer prior to grade 2 entry, winter of grade 2, and the summer prior to grade 3. We tested whether resting frontal EEG asymmetry measured at the first assessment predicted children's trajectory of shyness across the early school years (summer prior to grade 1 through summer prior to grade 3). If resting RFA reflects a biological vulnerability (i.e., diathesis) to stress, then it may manifest as shyness over time as children encounter normative developmental stresses of middle childhood. Accordingly, we predicted that shyness would increase over time in children with right, but not left, frontal EEG asymmetry.

Method

Sample and Recruitment

Thirty-seven typically developing children (16 males, 21 females) and their mothers were recruited into a study examining the biological predictors of socio-emotional development from the summer before grade 1 to the summer before grade 3. Children were recruited from a database containing the birth records of healthy children whose parents were recruited upon their child's birth for future developmental research studies conducted at McMaster University. The children were born either at McMaster University Medical Centre or St. Joseph's Healthcare Hamilton, both located in Hamilton, Ontario, Canada. All children were typically developing with no significant pre-, peri-, or post-natal health problems and were primarily Caucasian and from middle class backgrounds.

In Canada, children typically enter primary school in kindergarten, and we recruited children into the study the following year in the summer prior to grade 1 [Time 1 (T1); M_{age} = 6.39 years, *S.D.* = 0.15 years]. There was a total of four subsequent follow-up visits spanning approximately two years which occurred in the winter of grade 1 [Time 2 (T2); M_{age} = 6.79 years, *S.D.* = 0.25 years], the summer prior to grade 2 [Time 3 (T3); M_{age} = 7.39 years, *S.D.* = 0.16 years], the winter of grade 2 [Time 4 (T4); M_{age} = 7.77 years, *S.D.* = 0.20 years], and finally the summer prior to Grade 3 [Time 5 (T5); M_{age} = 8.43 years, *S.D.* = 0.19 years].

Procedures

At all five visits, the mother and child were briefed about study procedures, written consent was obtained, and mothers completed measures pertaining to the child's temperament. At T1-T4, children completed a self-report of their emotional experience in response to age-appropriate socio-affective video clips and psychophysiological data were collected from the child as part of the larger study that have been published elsewhere, including baseline EEG (Poole et al., 2018) and electrocardiogram (ECG; Poole & Schmidt, 2018) recordings and are not presented here. The mother waited in an adjacent room during the psychophysiological recordings and could watch her child on a closed-circuit TV. At T4, children participated in a videotaped self-presentation task. At all five visits, children received a "Junior Scientist Certificate" and a toy prize as a token of appreciation for their participation. All procedures were approved by the McMaster University Research Ethics Board.

In the present study, we were particularly interested in the predictive utility of resting RFA at enrollment on the prospective development of shyness. Accordingly, the analyses below focus specifically on EEG collected at T1, and maternal-reports of shyness collected at T1-T5.

Maternal Report of Shyness

Mothers assessed their child's shyness at each of the five assessments using the shyness subscale of the parent-report Colorado Child Temperament Inventory (CCTI; Rowe & Plomin, 1977). Five items comprise the shyness subscale including: "My child tends to be shy" and "My child takes a long time to warm up to strangers", "My child makes friends easily (reversed)", "My child is very friendly with strangers (reversed)" and "My child is very sociable (reversed). Each item is rated on a 5-point Likert scale ranging from 1 ("strongly disagree") to 5 ("strongly agree") and an average shyness score is computed that can range from 1 to 5, with higher scores reflecting more shyness. This subscale demonstrated good to very good internal reliability in our sample (T1: $\alpha = .88$; T2: $\alpha = .83$; T3: $\alpha = .83$; T4: $\alpha = .72$; T5: $\alpha = .80$). This measure also has been shown to correlate with behavioral indices of child shyness and social anxiety in previous studies (e.g., Smith et al., 2012; Theall-Honey & Schmidt, 2006), and also correlated with behavioral indices of social anxiousness in the present study.[3]

EEG Data Collection and Reduction

EEG Recording. Baseline electroencephalogram (EEG) recordings were obtained for two minutes (1 minute with the child's eyes open, and 1 minute with the child's eyes closed) while the child was seated. Resting EEG data of 2 minutes duration have been shown to be sufficient for deriving reliable estimates of frontal asymmetry (e.g., Allen et al., 2004; Miskovic et al., 2009; Schmidt, 2008; Theall-Honey & Schmidt, 2006). The child was instructed to simply relax during the baseline testing and try not to move around.

EEG was recorded using a lycra stretch cap (Electro-Cap, Inc.) with electrodes positioned according to the international 10/20 Electrode Placement System (Jasper, 1958). Electrode impedances below 10 K ohms per site were considered acceptable. EEG was recorded from the left and right mid-frontal (F3, F4), central (C3, C4), parietal (P3,

[3]At Time 4, children participated in a videotaped self-presentation task in which we coded children's behavioral display of observed social anxiousness. The concurrent relation between maternal-rated shyness on the CCTI and observed social anxiousness at Time 4 was statistically significant, $r = .41$, $p = .02$. Because this task was only administered at T4, we were unable to examine how frontal asymmetry at T1 affected the trajectory of observed behavior in the present study. An independent samples t-test revealed that the two frontal asymmetry groups from T1 did not significantly differ on observed behavior at T4.

P4), occipital (O1, O2) sites. These sites represent the left and right hemispheres and anterior and posterior regions of the brain. All electrodes were referenced to the central vertex (Cz). For the analyses below, we only examined the data collected from the mid-frontal sites as this region has been reliably linked to individual differences in affective style and socio-emotional development (Davidson, 2000). The channels were amplified by individual SA Instrument Bioamplifiers. The filter settings for the channels were set at .1 Hz (high pass) and 100 Hz (low pass). The data from all channels were digitized on-line at a sampling rate of 512 Hz.

EEG Data Reduction and Quantification. The EEG data were visually scored for artifacts due to eyeblinks, eye movements, and other motor movements using software developed by James Long Company (EEG Analysis Program, Caroga Lake, NY). This program removes data from all channels if artifact is present on any one channel. The amount of artifact in edited EEG data was examined among all participants in order to ensure that it did not systematically differ between participants. The number of 1-second artifact-free [i.e., discrete Fourier transform (DFT)] EEG windows was not correlated with any study measures. Eyes-open and eyes-closed conditions were combined as they were highly related and provide a more reliable and stable estimate of asymmetry (see Tomarken et al., 1992).

For the purposes of this study, we examined EEG power in the 6 to 8 Hz alpha frequency band. We chose this band because the majority of power tends to reside at this band at this age, and previous work has shown relations between frontal EEG asymmetry scores in this band and personality in preschool and early school-aged children (e.g., Fox et al., 1995; Schmidt, Fox, Schulkin, & Gold, 1999; Theall-Honey & Schmidt, 2006).

An EEG asymmetry score was computed using the mid-frontal data (i.e., *ln* right EEG power *minus ln* left EEG power). Because EEG power is inversely related to activity, negative scores on this metric reflect greater relative right activity, and positive scores reflect greater relative left activity (Tomarken et al., 1992). Using these scores, we classified children based on their frontal EEG asymmetry (RFA: n = 17; left frontal asymmetry (LFA): n = 18) at T1. Our rationale for using a dichotomous outcome is that RFA and LFA are thought to reflect qualitatively different traits (e.g., Fox, 1991), thus a group-based approach aids in the interpretation and theoretical significance of the findings.

Descriptive statistics for the two groups were as follows: RFA (M = -.04, *S.D.* = .02, Range: -.08 to -.01) and LFA (M = .03, *S.D.* = .02; Range: .01 to .08). Children's frontal asymmetry group at T1 did not significantly differ compared to frontal asymmetry groups at T2 [X^2 (2, N = 29) = 0.54, p = .81], T3 [X^2 (2, N = 29) = 0.78, p = .38], or T4 [X^2 (2, N = 30) = 1.20, p = .27], suggesting that frontal asymmetry group membership at T1 remained preserved across the other assessments in which regional baseline EEG was recorded.

Statistical Analyses

Child shyness trajectories were estimated using growth curve analysis in which repeated measures (i.e., shyness) were regressed on the timing of these assessments (i.e., participant age) to estimate rates of change at an individual level. Growth curve analysis provides information on two estimates: (1) estimates pertaining to variability in shyness at T1 (i.e., intercept variance), and (2) estimates regarding *rate of change* in shyness across visits (i.e., slope variance) (Delucia & Pitts, 2006). Five waves of longitudinal data contributed to the models described below. If the unconditional growth curve model reveals significant individual variation in the intercept and slope of shyness across time, then it is possible to examine the influence of predictors (including moderators) in this model. In our conditional model, our primary predictor was child's resting frontal EEG asymmetry group at T1 which was dummy coded (RFA = 0; LFA = 1) and the outcome was maternal report of shyness (i.e., CCTI shyness subscale) across the five assessments.

All statistical analyses were performed using SPSS Version 24.0, with significance levels set at $\alpha = 0.05$.

Missing Data

Growth curve analysis allows partial data on trajectory variables (i.e., shyness), but does not allow missing data on the predictor variable (i.e., frontal EEG asymmetry at T1). There were missing EEG data on 2 participants due to technical issues, resulting in a sample size of 35. Of these 35 children, 33 (94%) returned at T2, 33 (94%) returned at T3, 31 (89%) at T4, and 21 (60%) at T5. Reasons for missing data on the shyness measure were due to participants being unable to be contacted and scheduled to return to follow-up visits. Missing data across visits were not systematically associated with child shyness, EEG asymmetry, or child sex (*p*s >.05), thus maximum likelihood (ML) was used to account for missing data on our trajectory variable (i.e., shyness) to present unbiased estimates.

Results

Descriptive Analyses

Table 2.1 provides study variable means, standard deviations, and intercorrelations. All continuous variables were normally distributed as indicated by skewness statistics, and there were no significant outliers (defined as 3 standard deviations above or below the mean). Males and females did not significantly differ in resting frontal EEG asymmetry, or shyness scores at any of the five visits (*p*s >.05).

Table 2.1

Descriptive statistics and correlations among study variables

	1	2	3	4	5	6	Skew	Mean (*S.D.)*
1) T1 Resting Frontal EEG Asymmetry	--						-0.07	-0.001 (0.04)
2) T1 CCTI Shyness	.29	--					0.26	12.34 (4.63)
3) T2 CCTI Shyness	.19	.80**	--				0.10	11.91 (4.15)
4) T3 CCTI Shyness	.14	.76**	.76**	--			-0.40	12.41 (3.77)
5) T4 CCTI Shyness	-.02	.67**	.71**	.74**	--		-0.26	12.09 (3.55)
6) T5 CCTI Shyness	-.45*	.30	.22	.32	.48*	--	0.28	13.13 (4.19)
7) Child Sex	.21	.17	.20	-.16	-.28	-.13	N/A	N/A

** $p < .001$; * $p \leq .05$

Note: Negative values of frontal EEG asymmetry correspond to greater relative right frontal brain activity, and positive values correspond to greater relative left frontal brain activity. CCTI Shyness scores were according to maternal-report. Child sex is dummy coded such that males = 0 and females = 1. EEG = Electroencephalogram, CCTI = Colorado Child Temperament Inventory, N/A = not applicable; T1 = Time 1 (Summer prior to Grade 1), T2 = Time 2 (Winter of Grade 1), T3 = Time 3 (Summer prior to Grade 2), T4 = Time 4 (Winter of Grade 2), T5 = Time 5 (Summer prior to Grade 3), *S.D.* = standard deviation

Trajectories of Child Shyness Predicted by Frontal Asymmetry at Time 1

To evaluate which model best fit the growth pattern for the whole sample, the following models were evaluated: intercept-only (-2 log likelihood (LL) = 783.38), intercept + linear (-2LL = 778.63), and intercept + linear + nonlinear (quadratic, -2LL = 788.30; cubic, -2LL = 796.89). The intercept + linear growth model was selected as the baseline model given that it appeared to provide the most parsimonious fit to the data, as reflected by the smallest -2LL value, an index of model fit. This unconditional model revealed significant individual variation in the intercept and linear slope of shyness across the five visits. Therefore, a conditional model was computed to test child frontal EEG asymmetry group at T1 (i.e., RFA versus LFA) as a predictor of individual variation in shyness trajectories.

Our conditional model revealed that resting RFA group was a significant predictor of both intercept and slope (See Table 2.2). Specifically, children with resting RFA had *lower* levels of shyness at T1 (i.e., summer prior to grade 1; $\beta = -2.27$, $p = .02$), but experienced significant linear *increases* in shyness from the summer prior to grade 1 to the summer prior to grade 3 ($\beta = 0.03$, $p = .02$; See Figure 2.1) relative to children with resting LFA. Children with resting LFA displayed no significant changes in levels of shyness from the summer prior to grade 1 to the summer prior to grade 3 ($\beta = -0.02$, $p = .48$). The model fit indices of the model were as follows: -2LL = 737.32, Bayesian Information Criterion = 772.01, Akaike's Information Criterion = 749.77.

Table 2.2

Conditional growth curve model of maternal-report child shyness from age 6 to 8 years predicted by resting frontal EEG asymmetry groups in the summer before grade 1

	Estimate	Standard Error
Fixed effects		
Initial status		
Intercept	3.02**	0.71
Right Frontal EEG Asymmetry Group	-2.27*	0.98
Linear Slope		
Intercept	-0.01	0.01
Right Frontal EEG Asymmetry Group	0.03*	0.01
Random effects		
Within-age variance	6.77**	0.93
Between subject variance	10.06**	2.89

EEG = Electroencephalogram;
**p < .01; *p < .05

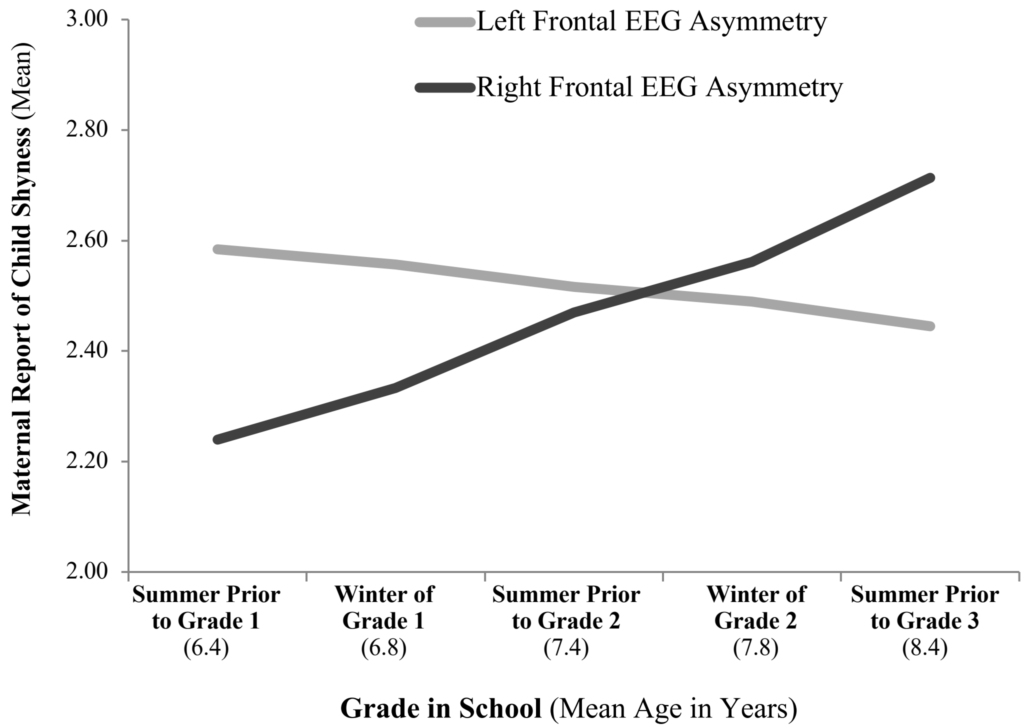

Figure 2.1. Right frontal EEG asymmetry group in the summer before grade 1 predicts an increasing mean trajectory of maternal-reported shyness from the summer prior to grade 1 to the summer prior to grade 3. *Note*: estimates from the growth curve analysis are plotted.

Post Hoc Analyses: Frontal Asymmetry as a Time-Varying Covariate

Although in the present study we were particularly interested in the predictive utility of resting RFA at enrollment on the prospective development of shyness, we also ran exploratory post-hoc analyses with RFA group across the first 4 visits as a time-varying covariate (EEG was not collected at T5). As shown in Table 2.3, these results largely mirrored the results above and revealed that RFA group as a time-varying covariate was predictive of lower shyness at T1 (i.e., summer prior to grade 1; β = -2.09,

p = .05), but associated with marginally significant linear *increases* in shyness from the summer prior to grade 1 to the summer prior to grade 3 (β = 0.02, p = .06) relative to children with resting LFA.

Table 2.3

Conditional growth curve model of maternal-report child shyness from age 6 to 8 years predicted by time-varying resting frontal EEG asymmetry groups from the summer before grade 1 to the winter of grade 2

	Estimate	Standard Error
Fixed effects		
Initial status		
Intercept	3.66**	0.72
Right Frontal EEG Asymmetry Group	-2.09*	1.03
Linear Slope		
Intercept	-0.01	0.01
Right Frontal EEG Asymmetry Group	$0.02^{\dagger}$	0.01
Random effects		
Within-age variance	4.51**	0.67
Between subject variance	11.56**	3.07

EEG = Electroencephalogram;
**p < .01, * p = .05, † p < .06

Discussion

Using a prospective, longitudinal study design, we found that resting RFA differentially associated with levels of shyness in children over time. We found that, although resting RFA was associated with the lowest levels of shyness in the summer prior to grade 1 entry (i.e., age 6), RFA was associated with linear increases in shyness across the early school years with the highest levels of shyness by the summer prior to grade 3 (i.e., age 8). Left frontal asymmetry, however, was not correlated with shyness across the early school years. The present findings are consistent with the notion that resting RFA may reflect a biological diathesis to the growth of shyness in cross-sectional studies linking shyness and resting RFA (e.g., Schmidt, 1999; Theall-Honey & Schmidt, 2006). Two provocative questions arise from the current results that we discuss below.

Why is right frontal asymmetry associated with lower shyness at age 6?

Our finding that resting RFA was associated with *lower* shyness at enrollment conflicts with motivational models of frontal brain activity, and a previous body of work examining frontal EEG asymmetry and avoidance-related tendencies in cross-sectional studies (e.g., Calkins, Fox & Marshall, 1996; Davidson, & Fox, 1989; Fox & Davidson, 1987; Fox et al., 1995, 2001; Howarth, Fettig, Curby & Bell, 2016; McManis, Kagan, Snidman, & Woodward, 2002; Schmidt, 1999; Sutton & Davidson, 1997). Although it is unclear exactly why this relation may exist, we speculate on some possible explanations. First, the present relation may have been due to methodological and measurement differences between our study and previous research. For example, the majority of previous research has employed designs in which participants have been selected for extreme temperamental characteristics from larger samples, such as observed behavioral inhibition (Calkins et al., 1996), extreme shyness (Schmidt, 1999; Theall-Honey & Schmidt, 2006), or elevated social anxiety (Davidson et al., 2000; Moscovitch et al., 2011). In contrast, we utilized a typically developing sample of children who were not selected for extreme shyness. These extreme group and clinical samples may be more sensitive for detecting RFA than a typically developing sample not selected for individual differences in shyness scores.

Second, it is possible that the role of resting RFA may differ depending on developmental period and the personality construct under investigation. That is, resting RFA may subserve and maintain temperamental inhibition in infancy and toddlerhood where temperament (i.e., *reactive* aspects of personality) largely characterizes the phenotype, but resting RFA may not play the same role in shyness in childhood, where shyness is characterized not only by temperament but higher order cognitive processes such self-consciousness (i.e., *reflective* aspects of personality; see Poole, Tang, & Schmidt, 2018; Schmidt & Poole, 2018). Although temperamental inhibition and shyness overlap conceptually and empirically, they are not the same phenomenon, and thus RFA may serve different roles in the present study versus previous studies.

Third, it is important to point out that RFA does *not* always predict avoidance-related tendencies. Some studies have failed to find relations between resting RFA and shyness and anxious tendencies in previous work (e.g., Beaton et al., 2008; Blackhart, Minnix, & Kline, 2006; LoBue, Coan, Thrasher, & DeLoache, 2011; Schmidt & Fox, 1994). Interestingly, other work has found that resting RFA was linked to *approach*-related behaviors such as empathy in young adults (Tullett, Harmon-Jones & Inzlicht, 2012). In addition, the seemingly paradoxical pattern found in our study is similar to what was observed in a cross-sectional study of anxious children by Baving and colleagues (2002) who found that *non-anxious* (ostensibly low shy) school-aged males (age 8 years and age 11 years) exhibited significantly greater resting RFA compared to anxious males (Baving, Laucht, & Schmidt, 2002). However, these authors did not examine whether this pattern of brain activity was predictive of the *longitudinal* course of symptoms of anxiety. In our study, we found that although resting RFA was associated with lower maternal-reported shyness at enrollment, it was associated with prospective increases in shyness.

Other longitudinal research designs have also reported the predictive utility of resting RFA on anxious behaviors. For example, Blackhart and colleagues demonstrated in a sample of young adults that although RFA was not associated with concurrent symptoms of anxiety, resting RFA nevertheless predicted greater symptoms of anxiety one year later (Blackhart, Minnix, & Kline, 2006). Collectively, these inconsistent findings highlight (1) the importance of examining how RFA may contribute to the *development* of anxious behaviors and profiles across time using longitudinal designs, and (2) although resting RFA previously has been viewed as a vulnerability factor, it may not always confer contemporaneous risk.

Why might right frontal asymmetry predict growth in maternal-reported shyness across the early school years?

There are at least two possible explanations for this finding. First, our findings lend support to the idea that resting RFA may be best conceptualized as a diathesis for the development of shyness. Although speculative, it is possible that resting RFA may result in shyness as the child encounters normative developmental stresses across the early school years. For example, during this time there may be development in the neural circuits involved with the stress response, changes in social environment, peer comparisons, and social-cognitive development (Crozier & Burnham, 1990; Lagattuta & Thompson, 2007; Piaget, 1970). Thus, RFA may reflect a biological diathesis towards withdrawal and avoidance of novel or stressful stimuli, that in concert with normative development across the early school years may result in continual priming of the brain circuits regulating normal fear responses (Rosen & Schulkin, 1998), lower the threshold for stimulation in response to social stressors, and result in the child to find the expectations for social maturity and independence challenging, placing him/her on a pathway towards shyness (Crozier & Burnham, 1990; Schmidt, Polak, & Spooner, 2005). A second explanation is that maternal-perception of children's shyness may change during the early school years. For example, there are new social demands in the academic domain during grade school such as increased expectation of social participation and interactive group work, larger classroom sizes, and increased child-to-staff ratios relative to preschool and kindergarten (Coplan & Arbeau, 2008; Early, Pianta, & Cox, 1999; Evans & Bienert, 1992; Ladd & Price, 1987; Rimm-Kaufman & Kagan, 2005). With continued contact with the child's teacher, mothers may have a more accurate perception of their child's shyness relative to other children than they did before entry into grade school. Similarly, mothers may become more aware that the solitary behaviors often characteristic of shy children become less accepted and less benign across early childhood as the importance of establishing peer relationships increases dramatically (Coplan, Ooi, & Baldwin, 2018; Werner & Crick, 2004).

Limitations and Future Directions

Although the present study provides preliminary evidence that resting RFA may influence maternal-reported levels of shyness in children utilizing a longitudinal framework, there are several limitations that should be discussed and addressed in future work in order to ensure the reliability of our findings. First, although comparable sample sizes (i.e., 20-40 participants) have been used in previous longitudinal research using similar analytic approaches (e.g., Huttenlocher et al., 1991; MacNeill, Ram, Bell, Fox, & Pérez-Edgar, 2018; Raz et al., 2012; Tang et al., 2018), and although our retention rate was high, our sample size was relatively small. Thus, it remains important to regard our findings as preliminary in nature, and replication is required using larger samples of children following longitudinally. Our small sample size was underpowered to reliably test more complex models, including interactions between frontal asymmetry and other factors internal and external to the child that may alter shyness trajectories (Leon & Heo, 2009).

Second, maternal-report of shyness was used. Although parent-report is considered a reliable and valid indicator of temperament (Garstein, Bridget, & Low, 2012), is correlated with observational measures (Smith et al., 2012; Theall-Honey & Schmidt, 2006) and was concurrently related to behavioral measures of anxiousness coded from direct observation in the present study, it would be, nonetheless, informative to examine trajectories of shyness using additional informants, such as teachers and behavioral nominations from peers. Third, we focused on one biological measure and potential mechanism underlying the development of shyness in children. Future studies should consider multiple contextual factors that influence the growth or decline of shyness during the early school years, particularly the influence of parents, peers, and teachers (Coplan et al. 2004, 2008; Gazelle & Ladd, 2003; Kagan et al., 1987; Nelson et al., 2005; Rubin & Coplan, 2010; Spangler & Gazelle, 2009) and multiple biological measures (see, e.g., Schmidt & Buss, 2010; Schmidt et al., 1999; Schmidt & Miskovic, 2013). The investigation of contextual factors would help further our understanding of the developmental course of subtypes of shyness that do not necessarily have a biological foundation. Fourth, our objective was how frontal asymmetry at one time point predicted change in shyness across time. Future work should examine bidirectional relations between frontal asymmetry and shyness using cross-lagged analytic approaches in order to see if changes in frontal asymmetry also influence concurrent and prospective levels of shyness, or vice versa (see, for example, Howarth, Fettig, Curby & Bell, 2016).

Conclusion and Implications

This preliminary study illustrates that biological predispositions towards avoidance-related tendencies might exert longitudinal influences on social behavior such as shyness during the early school years. These findings highlight that developmental experience and developmental change may be important factors to consider when examining individual differences in resting frontal brain asymmetry on children's social and emotional development. Our findings also show that although shyness may be relatively stable in some children, there are individual differences in growth of children's shyness during the early school years.

It will be important for future work to first replicate our preliminary findings utilizing larger sample sizes, as well as to examine multiple contextual and biological factors implicated in the stability and developmental course of shyness across other development periods including earlier childhood, later childhood, and adolescence. This is important given that there are unique normative stresses accompanying each of these developmental transitions.

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CHAPTER 4

Study 3: Longitudinal Patterns of Autonomic Nervous System Activity to Socio-Affective Threat in Shy Children

Poole, K.L., & Schmidt, L.A. (2018). Trajectory of heart period to socio-affective threat in shy children. *Developmental Psychobiology, 60,* 999–1008.

Abstract

Although shyness is characterized by distinct psychophysiological correlates, we know very little about the development of these correlates. In this longitudinal study, we examined how children's shyness was associated with trajectories of heart period (HP) to socio-affective threat across four assessments spanning approximately two years. Children (M_{age} = 6.39 years) viewed age-appropriate, socio-affective videos at each visit while having their HP measured concurrently. A growth curve analysis revealed that low shy children had a relatively lower HP at enrollment, but experienced *increases* in HP across visits, while high shy children exhibited relatively *stable* low HP across visits while viewing threat-related socio-affective video stimuli. These patterns did not exist for HP during resting baseline or HP to non-threatening video stimuli. These findings suggest that longitudinal patterns of HP among shy children may reflect a stable, characteristic way of responding to socio-affective threat and possibly a physiological mechanism underlying shyness in some children.

Introduction

Shyness is a relatively stable trait characterized by fear and anxiety in response to social novelty or situations of perceived social-evaluation (Kagan et al., 1988; Rubin et al., 2009). Although the origins of shyness are multi-faceted, shyness appears to be rooted in an early temperamental style of behavioral inhibition, which is marked by wariness in response to novel stimuli (Garcia-Coll, Kagan, & Reznick, 1984). Shy children perceive social situations as threatening in nature and consequently tend to avoid novel social encounters. Some shy children are at risk for poorer psychosocial adjustment (e.g., Coplan et al., 2004, 2013; Crozier, 1995) and the development of psychopathology -- particularly social anxiety disorder -- relative to non-shy children (Clauss & Blackford, 2012). Across development, shyness has distinct psychophysiological correlates associated with stress-reactivity on central, neuroendocrine, and autonomic levels (e.g., Fox et al., 1996, 2001, 2005; Kagan et al., 1987, 1988; Poole, Santesso, Van Lieshout, & Schmidt, 2018; Schmidt, 1999; Schmidt & Fox, 1994; Schmidt et al., 1997; Schmidt, Fox, Schulkin, & Gold, 1999; Tang et al., 2016; Theall-Honey & Schmidt, 2006).

The autonomic nervous system in particular is regarded as one of the primary regulators of behavior (Porges, 2001). One measure of autonomic functioning is heart period, which is defined as the timing between sequential heart beats (Porges & Byrne, 1992). A lower heart period is conceptualized as an index of physiological arousal and has been a focus in research examining stress responses in general and also has been examined in relation to individual differences in temperament in particular. For example, some existing work has examined a series of cross-sectional relations between behavioral inhibition and heart period at multiple time points in infants and toddlers and has found a lower HP among those who are behaviorally inhibited (Garcia-Coll, Kagan, & Reznick, 1984; Kagan et al., 1984; Reznick, et al., 1986). Other work has found relations between lower heart period and higher shyness in children (Schmidt et al., 1999) and adults (Bruch, Gorsky, Collins, & Berger, 1989; Schmidt & Fox, 1994). It has been posited that increased sympathetic reactivity in inhibited/shy children stems from a lower threshold of excitability in limbic sites, particularly the amygdala and hypothalamus (Kagan, 1987, 1988), and may result in these children becoming physiologically aroused in response to perceived social threat. It is, however, important to note that although some work has found lower heart period in relation to higher inhibition and shyness, these findings are inconsistent, with other work failing to find significant relations (Asendorpf, & Meier, 1993; Calkins & Fox, 1992; Kagan et al., 1988; Theall-Honey & Schmidt, 2006).

These inconsistencies in relations between shyness and heart period may be due to a number of issues, and thus there remain a number of important questions that still need to be addressed. First, relatively little research has examined heart period specifically in relation to shyness in *school-aged children*, with the majority of existing research conducted in infants and toddlers in relation to behavioral inhibition. Thus, it remains less clear whether autonomic patterns manifesting in behaviorally inhibited young children are sustained into later childhood in the phenotype of shyness. Second, relatively little research has examined shy (or inhibited) children's autonomic activity during the processing of *threat*-related socio-affective stimuli. This is important given current theoretical hypotheses that the origins and maintenance of shyness may be related to an inability to regulate negative emotions, particularly those associated with threat such as fear and anger (LeDoux, 1996; Miskovic & Schmidt, 2012a; Schmidt, Polak, & Spooner, 2005; Tang et al., 2016). A further limitation of existing work on threat-related processing of shy and socially anxious profiles is that they often use static images (e.g., Beaton et al., 2008; Miskovic & Schmidt, 2012b), usually faces, to induce threat that are presented for brief time points, limiting the capturing of the dynamic affective experience. Finally, we know of no research that has examined how individual differences in shyness are associated with *trajectories* of heart period to perceived socio-affective threat using growth curve analyses to model change and/or continuity in autonomic functioning within a longitudinal framework.

Some recent research has examined developmental patterns of autonomic activity in a longitudinal framework, illustrating the utility and importance of such analytic approaches. However, these existing studies have not specifically examined shyness, but rather have been restricted to atypical samples, including sleep studies (El-Sheikh, Hinnant, & Philbrook, 2017), children at-risk for autism (Perdue, Edwards, Tager-Flusberg, & Nelson, 2017), children at-risk for depression (Gentzler et al., 2012), or young children from low-income countries (Alkon, Boyce, Davis, & Eskenazi, 2011; Jewell, Suk, & Luecken, 2017). Some work in typically developing samples has used longitudinal samples to examine a series of cross-sectional comparative analyses in early childhood (e.g., Bar-Haim, Marshall, & Fox, 2000) and found that heart period shows an *increase* from 4 months to 4 years of age, suggesting that a longer heart period may reflect the development of a more mature physiological profile across early childhood. Other work in typically developing children has charted general developmental patterns of autonomic activity using longitudinal analytic strategies in middle childhood and found relative stability from age 8 to 10 years (Hinnant, Elmore-Staton, & El-Sheikh, 2011). However, none of these studies have focused on how individual differences in shyness may be associated with psychophysiological trajectories across repeated study visits.

From a developmental perspective, investigating longitudinal patterns of autonomic activity is important to understand temporal continuity or change in shy children's physiological responses. This would not only advance our understanding and confidence in regarding these physiological measures as "trait-like" individual differences as is routinely done in developmental research (see Schmidt & Segalowitz, 2008, for a review), but may also help to identify possible psychophysiological correlates underlying threat biases in shy children. Importantly, this will help to elucidate putative biological mechanisms implicated in threat-related processing that may underlie the development and maintenance of shyness. Given the plasticity in physiological systems in childhood, this may provide an important opportunity to identify targets for modification in shy children before the onset of poor developmental sequelae.

In the present study, we examined how shyness was associated with longitudinal patterns of heart period to the presentation of age appropriate, threat-related video clips of social scenes in a sample of typically developing six-year old children. Children's heart period was collected in response to these stimuli across four separate, repeated visits spanning approximately two years. Given that shy children tend to have a characteristic way of responding to social threat (Brunet et al., 2009), which appears to be associated with autonomic physiology (Schmidt et al., 1999), then there should be a characteristic, trait-like pattern of autonomic activity to social threat processing in shy children. To that end, we predicted that children classified as relatively high in shyness would show a stable pattern of relatively lower heart period during the processing of threat-related video clips of social scenes across the four visits relative to children classified as relatively low in shyness.

Method

Participants

Thirty-seven typically developing children (16 boys, 21 girls) and their mothers participated in this longitudinal study examining socio-emotional development in children. Children entered into the study in the summer prior to grade 1 [Time 1 (T1); M_{age} = 6.39 years, *S.D.* = 0.15 years]. There was a total of three subsequent follow-up visits that occurred in: the second term of grade 1 [Time 2 (T2); M_{age} = 6.79 years, *S.D.* = 0.25 years], the summer prior to grade 2 [Time 3 (T3); M_{age} = 7.39 years, *S.D.* = 0.16 years], and the second term of grade 2 [Time 4 (T4); M_{age} = 7.77 years, *S.D.* = 0.20 years].

Participants were recruited from a database containing the birth records of children whose mothers were recruited upon their child's birth for future developmental research studies conducted at the Department of Psychology, Neuroscience & Behaviour at McMaster University. The children were born either at McMaster University Medical Centre or St. Joseph's Healthcare Hamilton, both located in Hamilton, Ontario, Canada. All children were typically developing with no significant pre-, peri-, or post-natal health problems and were primarily Caucasian and from middle-class backgrounds.

Procedures

Upon arrival to the Child Emotion Laboratory at McMaster University, the mother and child were briefed about study procedures and consent was obtained. At all visits (T1-T4), the child was given time to acclimatize to the laboratory, and then a baseline, resting electrocardiogram (ECG) recording was collected. The child then watched identical age-appropriate video clips of social scenes, designed to induce different emotions. The order of the video clips remained fixed across participants (sad, anger, fear; described in detail below). While the child was watching these video clips, an ECG recording was obtained from the child. The mother completed measures of child temperament in the adjacent room during psychophysiological data collection. The child received a "Junior Scientist Certificate" and a toy prize as a token of appreciation for their participation. All procedures were approved by the McMaster University Research Ethics Board.

Psychophysiology Data Collection

Electrocardiogram (ECG) Recording. Heart period was continuously recorded during a 2 minute baseline condition and concurrently during the 1 min socio-affective video clips using two disposable pediatric ECG electrodes applied to the left and right sides of the child's chest in line with the heart. A ground electrode was attached to the scalp surface (FCz) as part of a larger EEG study (see Poole, et al., 2018). There was a 60 second "washout period" before the viewing of subsequent video clips. A separate SA Instrument Bioamplifier was used to amplify the heart period signal, with the bandpass filters set at .1 Hz (high pass) and 100 Hz (low pass). The heart period data were digitized on-line at a sampling rate of 512 Hz.

Electrocardiogram (ECG) Data Reduction. The heart period data were scanned visually for artifacts (e.g., missing beats) and reduced using software by James Long Company (ECG Analysis Program, Caroga Lake, NY). Cardiac R-waves from each resting condition were detected offline with a four-pass algorithm, which produced a visual display of the cardiac signal with the R-waves marked. Missing or spurious R-waves were edited manually according to recommendations by Berntson and Stowell (1998). The ECG program calculates the mean inter-beat interval (i.e., heart period) in milliseconds.

Socio-Affective Video Clips

The video clips were selected from children's videos of social scenes and have been previously rated as reliable elicitors of each emotion (Theall-Honey & Schmidt, 2006). Each film was approximately 60 seconds in duration, was in color, and did not have audio. We chose to focus on emotion-eliciting *visual* stimuli, as this has been regarded as more ecologically valid in childhood research whereas threat-related *vocal* stimuli are more prone to subjective interpretability (Mogg & Bradley, 1999).

The video chosen to elicit sadness was from a segment of *The Lion King,* depicting a distraught Simba trying desperately to awaken his dead father. The video designed to elicit anger was a segment from *101 Dalmatians*, portraying a heated argument between the villainous Cruella deVille and the kind owners of the Dalmatian puppies. The video designed to elicit fear was a segment from *Snow White and the Seven Dwarfs*, portraying Snow White running through a dark haunted forest with eyes appearing everywhere and trees coming to life to attack her.

For our primary objective, we were particularly interested in children's heart period during videos designed to elicit threat-related emotions, including fear and anger, in social scenes. Because heart period during these two conditions were highly correlated across each visit (rs = .87 to .96; ps < .001), an average heart period composite measure during these two videos was computed to reflect autonomic activity during the processing of threat-related stimuli.

Although it has been argued that fear and anger are avoidance- and approach-related emotions, respectively, they are both conceptualized as threat-related emotions and previous research has shown links between shyness and threat-related biases in children (e.g., LoBue & Pérez-Edgar, 2014; Muris, Merckelbach, & Damsma, 2000; Pérez-Edgar et al., 2010, 2011). Neuroimaging studies provide further support of both anger and fear being conceptualized as threat-related emotions, with each being associated with heightened amygdala activity, a key brain region implicated in social and threat-processing and perception (e.g., Etkin, & Wager, 2007; Klumpp, Angstadt, Nathan & Phan, 2010; Phan, Fitzgerald, Nathan & Tancer, 2006).

Maternal Report of Child Shyness

Mothers completed the Colorado Child Temperament Inventory shyness subscale (Rowe & Plomin, 1977). Five items comprise the shyness subscale, each of which is rated on a 5-point Likert scale ranging from 1 (“strongly disagree”) to 5 (“strongly agree”). A mean shyness score was computed with higher scores reflecting more shyness. Example items from the shyness subscale include: “Child tends to be shy” and “Child takes a long time to warm up to strangers”. The shyness subscale demonstrated good to very good internal reliability in our sample across the four assessments (T1: α =.88; T2: α = .83; T3: α = .83; T4: α = .72). This measure also has been shown to correlate with behavioral indices of child shyness and social anxiety in previous studies (e.g., Smith et al., 2012; Theall-Honey & Schmidt, 2006).

Person-Oriented Approach

We adopted a person-oriented approach in the present study. Person-oriented approaches are regarded as useful in classifying individuals who share common characteristics and effectively categorize individuals who have conceptually and empirically different behavioral profiles (see Bergman & Trost, 2006, for a review). Indeed, previous research studying shyness (LoBue & Pérez-Edgar, 2014; Poole & Schmidt, 2018; Schmidt, 1999; Wolfe & Bell, 2014) and related constructs such as social withdrawal (Coplan et al., 2016; Nelson, 2013) and behavioral inhibition (Coplan, Wilson, Frohlick, & Zelenski, 2006; Kagan et al., 1987, 1988) have illustrated the utility of using a person-oriented approach in studying social behavior. Likewise, children in the present study were classified as high shy and low shy using a median split (high shy: n = 19, M = 3.18; low shy: n = 18, M = 1.64; t (35) = -9.45, $p < .001$, d = 3.15). These groups did not significantly differ in proportion of boys versus girls [X^2 (1, N = 37) = 2.67, p = .10].

To ensure the reliability and stability of children's shyness in the two groups across follow-up visits, we examined group (i.e., high shy, low shy) differences in shyness at each of the three subsequent assessments using independent samples t-tests, and also examined Pearson's correlations of shyness scores at each assessment. As expected, children in the high shy group remained significantly more shy than the low shy group at T2 [t (30) = -4.50, $p < .001$, d = 2.14], T3 [t (31) = -3.92, $p < .001$, d = 1.85], and T4 [t (30) = -4.29, $p < .001$, d = 1.74]. Further, shyness scores were significantly correlated across each visit (T1 to T2: r = .73, $p < .001$; T1 to T3: r = .76, $p < .001$; T1 to T4: r = .68, $p < .001$; T2 to T3: r = .76, $p < .001$; T2 to T4: r = .71, $p < .001$; T3 to T4: r

= .74, $p < .001$). Collectively, these findings are supportive of the internal reliability and stability in children's level of shyness across the follow-up visits for each group.

Data Analysis

Trajectories of children's heart period were estimated using growth curve analysis in which repeated measures (i.e., heart period) were regressed on the timing of these assessments (i.e., participant age) to estimate rates of change at an individual level. Growth curve analysis provides information on: (1) estimates pertaining to variability in heart period at T1 (i.e., intercept variance), and (2) estimates regarding *rate of change* in heart period across visits (i.e., slope variance) (Delucia & Pitts, 2006). Four waves of longitudinal data contributed to the models described below. In our conditional model, our primary predictor was child's shyness group classified at T1 (high shy = reference category). Our primary dependent measure was children's heart period during the viewing of the socio-affective threatening stimuli across visits.

There were complete data on shyness group for all 37 children. There were heart period data on 36 (i.e., 97%) children at T1, 32 (i.e., 86%) children at T2, 33 (i.e., 89%) children at T3, and 31 (i.e., 84%) children at T4. Missing ECG data across visits was due to equipment failure and being unable to contact families to return to the laboratory for follow-up visits. Growth curve analysis allows partial data on trajectory variables (i.e., heart period), but does not allow missing data on the predictor variable (i.e., child shyness). Maximum likelihood was used to account for missing data on our trajectory variable (i.e., heart period) to present unbiased estimates for all 37 children. All statistical analyses were performed using SPSS Version 24.0, with significance levels set at $\alpha = 0.05$.

Results

Descriptive Statistics

All continuous heart period variables were normally distributed as indicated by skewness statistics, and there were no significant outliers (defined as 3 standard deviations above or below the mean). Means and standard deviations for resting baseline heart period and heart period during the socio-affective video clips are presented in Table 3.1. The minimum amount of ECG data was 58.40 s for T1, 60.50 s for T2, 58.80 s for T3, and 55.50 s for T4. There were no significant differences for the amount of useable ECG data across visits between the two shyness groups.

Trajectory of Heart Period to Threat-Related Socio-Affective Video Clips

Our unconditional model revealed significant individual variation in the intercept and linear slope of heart period across the four visits. Therefore, a conditional model was computed to test if children's shyness predicted individual variation in heart period trajectories. Our conditional model revealed that shyness was a significant predictor of both intercept and slope (See Table 3.2). Low shy children had relatively lower initial heart period at T1, but experienced significant linear increases in heart period relative to high shy children across visits during the viewing of the threat-related socio-affective video stimuli. Conversely, high shy children displayed stability in heart period (p = .48), exhibiting a relatively lower heart period across the four assessments during the viewing of the threat-related socio-affective video stimuli.[4] Figure 1 illustrates these findings.

[4] We also computed a heart period reactivity difference score (i.e., threat-related condition *minus* baseline condition) and examined whether shyness group affected the trajectory of heart period reactivity score. However, this analysis did not reach statistical significance.

Table 3.1

Means and standard deviation of heart period (milliseconds) during the resting baseline condition and socio-affective video clips across visits

	Full Sample	Low Shy Group	High Shy Group
	Mean (SD)	Mean (SD)	Mean (SD)
Resting Baseline HP			
Time 1	644.00 (81.26)	627.98 (77.85)	668.05 (83.37)
Time 2	669.85 (70.79)	668.82 (77.34)	671.47 (62.24)
Time 3	662.31 (73.60)	670.70 (75.29)	652.25 (72.80)
Time 4	685.43 (64.85)	683.57 (74.06)	688.01(52.27)
Threat-Related Stimuli HP			
Time 1	644.17 (81.70)	636.83 (80.29)	654.43 (85.35)
Time 2	662.89 (66.71)	654.96 (68.76)	673.87 (64.29)
Time 3	673.71 (69.72)	690.07 (72.17)	654.09 (63.49)
Time 4	683.44 (61.00)	690.86 (72.74)	673.17 (40.11)
Non-Threatening Stimuli HP			
Time 1	654.64 (83.99)	658.48 (88.64)	650.99 (81.58)
Time 2	679.86 (73.44)	692.50 (75.71)	666.36 (70.97)
Time 3	691.06 (75.80)	703.27 (76.58)	681.56 (75.99)
Time 4	698.45 (65.14)	702.57 (75.20)	692.75 (50.46)

HP = heart period; SD = standard deviation

Note. Heart period is inversely related to heart rate.

Table 3.2

Summary of growth curve models for trajectories of heart period as predicted by shyness group for (A) threat-related stimuli, (B) non-threatening stimuli, and (C) resting baseline.

	Dependent Measure (Heart Period)								
	(A) Threat-Related Stimuli			(B) Non-threatening Stimuli			(C) Resting Baseline		
	Beta	S.E.	*p*-value	Beta	S.E.	*p*-value	Beta	S.E.	*p*-value
Initial status									
Intercept	**622.40**	**82.62**	**<.001**	**565.76**	**89.02**	**<.001**	**591.52**	**85.86**	**<.001**
Low Shy Group	**-231.47**	**112.37**	**.04**	-161.58	122.31	.19	-154.20	116.12	.19
Slope									
Age	0.43	0.96	.66	1.21	1.03	.25	0.73	0.99	.47
Low Shy Group × Age	**2.86**	**1.31**	**.03**	2.12	1.43	.15	1.90	1.35	.16

Note: S.E. – standard error; $N = 37$; bolded estimates are significant

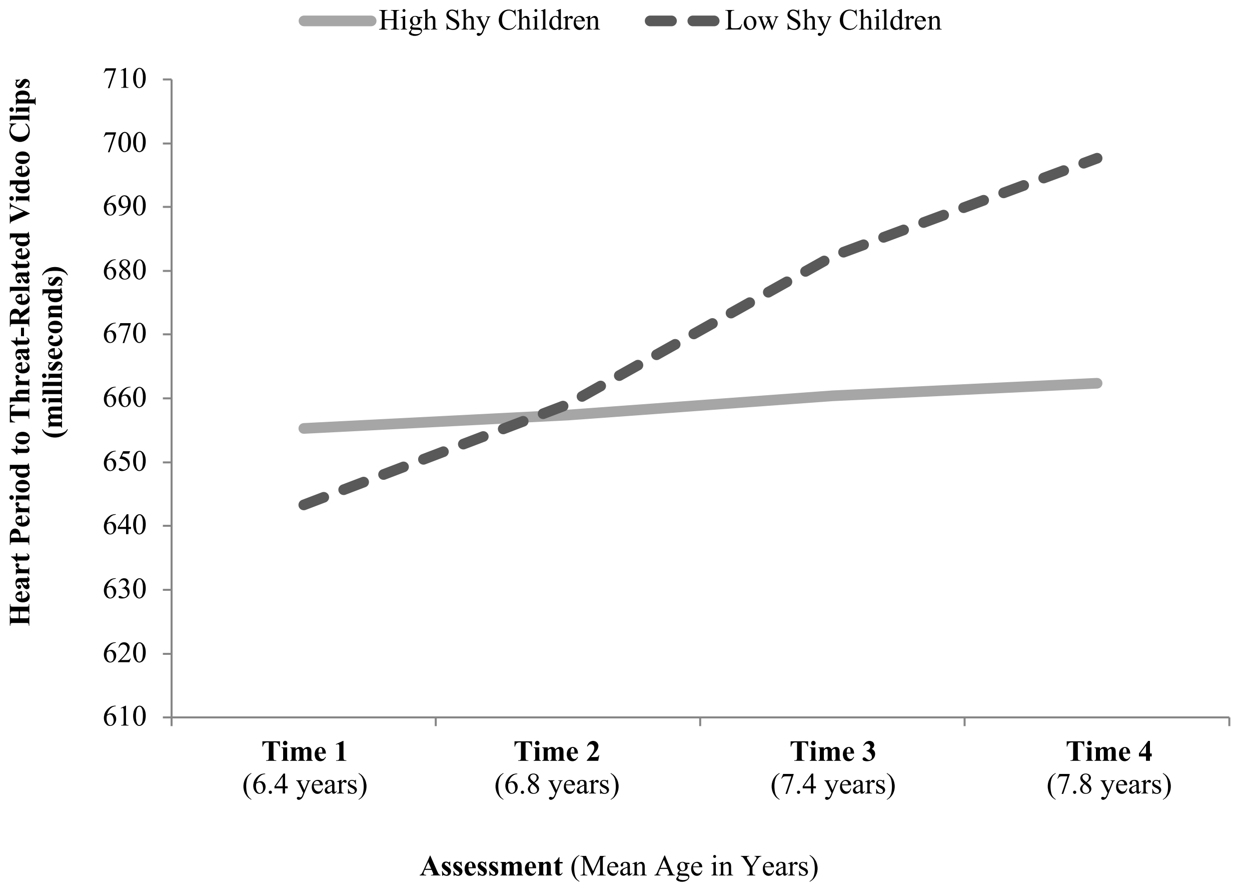

Figure 3.1. Trajectories of mean heart period while viewing threat-related socio-affective video clips in high shy and low shy children across repeated assessments. *Note*: estimates from the growth curve models were plotted. Heart period is inversely related to heart rate, so lower heart period is reflective of a higher heart rate.

Specificity Analysis

We performed two identical growth curve specificity analyses as conducted above. The first specificity analysis used a measure of children's heart period during the viewing of a video clip designed to induce sadness. We were interested in examining this as a dependent measure as sadness is a negatively-valenced emotion but is considered to be non-threatening in nature and this ensures the findings with the threat-related socio-affective stimuli were not reflective of attentional processes involved in viewing video stimuli in general. The second specificity analysis used a resting baseline measure of children's heart period. Although the relative development of heart period during the age range studied (i.e., age 6 to 8 years) has not been well established, work in younger children has found increases in heart period across development (e.g., Bar-Haim, Marshall, & Fox, 2000). Accordingly, in order to ensure the findings with the threat-related socio-affective stimuli were not related to developmental differences in autonomic functioning across the four visits and/or anxious arousal of shy children coming to the laboratory for testing, we also examined the trajectory of resting baseline heart period.

Results from both of these specificity analyses are presented in Table 2 and revealed that shyness group was not a significant predictor of intercept or slope for heart period during the sad video or resting baseline heart period, suggesting specificity to the processing of threat-related socio-affective video stimuli. As well, the lack of between group differences between the high versus low shy children on the baseline HP measure suggest that the stable patterns of low heart period among the high shy children to the socio-affective threat stimuli did not merely reflect anxious arousal due to their coming to the laboratory.

Discussion

We examined whether individual differences in children's shyness was associated with trajectories of heart period to threat-related video clips across four separate assessments spanning two years. We found that children classified as relatively low in shyness displayed a significantly lower heart period during the initial presentation of the threat-related stimuli (i.e., visit 1), but experienced increases in heart period across the repeated study visits relative to children classified as relatively high in shyness. As predicted, high shy children displayed stable levels of relatively lower heart period to the threat-related video clips during the repeated follow-up visits across the two years. We did not find any significant relations between shyness and trajectories of resting baseline heart period, suggesting that there was no developmental change in heart period across the age range studied (i.e., age 6 to 8 years). Further, there was no relation between children's shyness and their trajectory of heart period in response to a video designed to induce sadness. Importantly, these findings suggest that shyness had a specific influence on the trajectory of heart period to socio-affective threat-related conditions. We discuss at least two possible explanations underlying these findings.

One explanation is that the stable pattern of heart period to the processing of threat in shy children may reflect a trait-like characteristic that is involved in the maintenance of shyness, and/or reduced flexibility in autonomic functioning. Previous work has shown that individual differences in flexibility of the autonomic system may emerge as early as infancy and childhood, with lower flexibility being linked to increased stress-vulnerability, deficits in modulating emotion, and behavioral inhibition (Friedman & Thayer, 1998; Porges, 1991, 1992; Porges et al., 1994). More recent work has shown evidence of restricted autonomic flexibility in children with social anxiety disorder (Schmitz et al., 2011), a clinical phenotype that shares conceptual and empirical overlap with shyness on cognitive, behavioral, and physiological levels (Heiser, Turner & Beidel, 2003; Poole, Van Lieshout, & Schmidt, 2017). For shy children, the ability to attend and physiological adjust to threatening stimuli may be a more rigid, inflexible response. This style may be detrimental in that it limits the shy individual's ability to distinguish threat from non-threat. Indeed, the relatively stable physiological state manifested in the shy children may reflect a characteristic way of responding and a processing bias towards threat-related information, possibly a basic mechanism subserving their shyness.

Conversely, the increase of heart period over time to the processing of the threat-related video clips in the low shy children may reflect the fact that they have more flexible autonomic responses than do high shy individuals. Previous research has found evidence of autonomic flexibility in non-anxious individuals relative to anxious individuals (Friedman & Thayer, 1998; Friedman, 2007; Hoehn-Saric, & McLeod, 1988; Thayer & Friedman, 1993, 1997; Thayer et al., 2000). According to this conceptualization, heightened autonomic activity to a stressor (e.g., threat-related stimulus) is not necessarily considered to be a marker of risk if the system is able to demonstrate flexibility to such stimuli (Beauchaine, 2001; Porges, 2007; Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996; Thayer & Brosschot, 2005; Thayer & Lane, 2000). Indeed, although the low shy children displayed a lower initial heart period to the threat-related stimuli, they nevertheless demonstrated an increase in heart period across visits. This variability in the responding of the autonomic system serves an adaptive function in attending and acting upon threat-related stimuli.

Relatedly, a second speculative explanation of our findings is that low shy and high shy children differ in their habituation responses to threat. Habituation has a long and rich history and has been well studied in terms of nervous system plasticity and its role in predicting behavior (Graham & Clifton, 1966; Graham & Slaby, 1973; Sokolov, 1963). Typically, the initial presentation of a novel stimulus involves a heightened response, but after repeated exposure to the stimulus without detrimental consequences, a learning process occurs (i.e., habituation) and the individual reduces his/her response to the stimulus (Rankin et al., 2009). Although habituation is regarded as a fundamental learning mechanism, there exists variation in habituation responses, and these individual differences in habituation may emerge as early as infancy (e.g., Bornstein & Suess, 2000; Moehler et al., 2006).

Previous theoretical work has emphasized the role of both *affective valence* and *expectancy* of the stimulus on the prediction of one's autonomic responses in the context of habituation (Cook & Turpin, 1997). Here, we focused on negatively-valenced, threat-related socio-affective stimuli. We suspect that the initial presentation of these stimuli is particularly novel and salient to *low* shy children. Our rationale for this is that we suspect that these children do not have underlying biases and expectancies to experience and process threat-related stimuli compared to high shy children who are primed to process and experience threat-relevant social cues on a daily basis. Indeed, we found that low shy children showed a heightened initial response to the novel threat-related stimuli, but after repeated exposure to the stimuli across the follow-up visits without detrimental consequences, these children showed habituation and experienced a reduction in their physiological response to the stimuli. The effective habituation response in low shy children is considered to be an adaptive process because it allows for the optimal allocation of both cognitive and physiological resources required to detect and process novelty in their environment, and importantly, a learning mechanism that the stimuli are no longer threatening (Rankin et al., 2009).

In contrast to the low shy children, the high shy children did not display a heightened initial response to the threat-related stimuli, nor did they manifest a typical habituation response across repeated assessments. The failure to habituate to the stimuli across repeated exposure is considered to be a maladaptive strategy because it results in greater allocation of information processing resources and may result in these shy children being unable to accurately detect relevant stimuli in their environment. That is, shy children may be deficient in the basic learning of threat-stimuli and the corresponding physiological regulation during these situations.

Failure to habituate has been viewed as one mechanism implicated in the development and maintenance of fear- and anxiety-related behavioral profiles in clinical samples (e.g., Thayer et al., 2000), but considerably less attention has been devoted to the role of habituation in temperamental styles characterized by fearful and/or anxious tendencies within typically developing samples (see Moehler et al., 2006, for an exception in relation to behavioral inhibition). Recently, a series of studies by Blackford and her colleagues has examined how individual differences in social fearfulness affect neural habituation in cross-sectional studies using samples of adults. In both dichotomous group designs (Blackford et al., 2009, 2012) and when examining social fearfulness across a continuous spectrum (Avery & Blackford, 2016), this work has shown that socially fearful (i.e., shy, socially anxious) adults display impairments in neural habituation during the presentation of faces in the hippocampus and amygdala, key brain regions implicated in threat processing. Our findings extend this work by examining the relation between temperamental shyness and long-term habituation to threat on an autonomic level in a sample of typically developing children within a developmental framework.

It is important to note that longitudinal patterns of autonomic functioning in the present study were assessed in response to the *viewing* of social signs of threat, which can be considered a *passive* task. Although the longitudinal patterns of heart period between groups differed when modeled in a growth curve, we did not find large differences in heart period cross-sectionally between baseline and socio-affective threat, which may suggest that, on average, children are not reacting strongly to the affective stimuli. Although these preliminary findings are informative in understanding shy children's physiological responses during threat processing, it would be particularly interesting to examine longitudinal physiological responses in shy children during an *active* task, such as while they are *participating* in social situations typically perceived as threatening, such as interactions with unfamiliar peers or the delivery of a speech. This would reveal information about how shy children habituate (or fail to habituate) in socially threatening contexts. Importantly, this would also allow investigation of whether similar inflexible responses revealed here on a physiological level may also generalize to behavioral and/or cognitive components that are hallmarks of shyness (e.g., avoidance, apprehension, self-consciousness).

Limitations

Our findings should be interpreted in the context of the following limitations. First, although our retention rate was high and although comparable sample sizes have reliably been used in previous longitudinal research (e.g., Huttenlocher, Haight, Bryk, Seltzer, & Lyons, 1991; Lahat et al., 2017; MacNeill, Ram, Bell, Fox, & Pérez-Edgar, 2018; Raz et al., 2012; Tang et al., 2018), our sample was relatively small and thus our findings should be interpreted with appropriate caution and replicated using larger sample sizes of children. Second, maternal-report of shyness was used. Although parent-report is considered a reliable and valid indicator of temperament and is correlated with observational measures (Smith et al., 2012; Theall-Honey & Schmidt, 2006), it would be informative to have measures of shyness using additional informants and/or behavioral observation. Third, it is possible that some children may have been more familiar with the socio-affective stimuli video clips selected, which may have influenced exposure, habituation, and possibly physiological arousal in response to viewing these clips. Fourth, we did not randomize the order in which the video clips were presented which could have led to practice effects. Fifth, we examined children's physiological response to age-appropriate threat-related stimuli but did not collect data to precisely measure attentional biases, such as reaction times or eye-tracking data. These data would be important to more precisely understand whether shy children are showing threat-related attentional biases *toward* or *away from* threatening stimuli, as inconsistent findings in this domain have been mixed (e.g., Monk et al., 2006; Roy et al., 2008; White, Helfinstein, & Fox, 2010). Finally, we have examined one autonomic measure (i.e., heart period) and acknowledge this does not provide a complete picture of the multifaceted nature of autonomic functioning. Future work should aim to integrate additional indices of autonomic activity such as respiratory sinus arrhythmia.

Implications and Conclusion

The preliminary findings of this study have both methodological and theoretical implications. First, our study highlights the importance and utility of collecting and examining psychophysiological measures within a developmental framework. Such longitudinal designs allow us to employ growth curve modeling and an important opportunity to track how individual differences in temperament may be associated with physiological processes in children across time, allowing for possible developmental inferences.

Second, these findings have theoretical implications for furthering our understanding of developmental mechanisms underlying shyness in children. Particularly, we find that shy children may display deficits in habituating to threat-related stimuli, possibly reflecting early biological biases manifesting as reduced autonomic flexibility relative to low shy children. These findings have implications for understanding early social threat sensitivity in shy children and fundamental learning mechanisms that may be a target for modification in shy children at risk for later socio-emotional maladjustment.

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CHAPTER 5

Study 4: Autonomic Nervous System Activity to Socio-Evaluative Threat in Shy Children

Poole, K. L. & Schmidt, L.A. (Under Review). While a shy child waits: Autonomic and affective responses during the anticipation and delivery of a speech. *Manuscript submitted for publication.*

Abstract

Shyness is a temperament characterized by wariness to social novelty and perceived social evaluation. However, we know relatively little about temperamentally shy children's psychophysiological and affective responses to impending novel social events. We examined whether children's temperamental shyness was related to distinct patterns of autonomic and affective responses across three conditions: baseline, speech anticipation, and speech delivery. Participants included 152 children (M_{age} = 7.82 years, SD = 0.44) who had their autonomic nervous system activity [i.e., respiratory sinus arrhythmia (RSA) and heart rate (HR)] and subjective nervousness assessed across each experimental condition. Children's temperamental shyness was assessed using parent- and child-report. Results revealed that shy children exhibited significant decreases in RSA from baseline to speech anticipation followed by increases in RSA from speech anticipation to delivery. Non-shy children showed no significant changes in RSA across task phases. Further, shy children showed linear increases in HR across all task phases, while non-shy children only showed increases in HR from speech anticipation to delivery. Finally, shy children showed increases in subjective nervousness from baseline to the anticipation phase with no further increase in response to the speech delivery, while non-shy children showed linear increases in subjective nervousness across all task phases. These findings suggest that temperamentally shy children may experience autonomic and emotion dysregulation particularly during the anticipation of novel social encounters, which may be reflective of their tendency to over react to perceived impending social threat on both physiological and affective levels.

Introduction

Shyness is characterized by wariness in response to social novelty and/or perceived social-evaluation (Kagan, Reznick, & Snidman, 1988; Rubin, Coplan, & Bowker, 2009). Although shyness is a ubiquitous phenomenon with up to 90% of the population feeling shy at some point in their lives (Zimbardo, 1977), a smaller proportion of approximately 15-20% of the population are described as being dispositionally or temperamentally shy (Kagan, 1994). Temperamental shyness is relatively stable across context and development and is presumed to be rooted in early biologically-based biases, specifically a behaviorally inhibited (BI) temperament which is characterized by the tendency to react to social and non-social stimuli with fear and wariness (García-Coll, Kagan, & Reznick, 1984; Kagan, 1994). It has been hypothesized that shy children may have a low threshold for arousal in physiological systems that play a key role in regulating the emotion of fear (Kagan, 1994).

The autonomic nervous system has been regarded as important for regulating responses to perceived threat and may be particularly important in understanding the biological processes underlying shyness. According to the polyvagal theory (Porges, 1995, 2007), the vagus nerve is responsible for maintaining a state of homeostasis. Under baseline conditions or in the absence of perceived threat, it is thought that relatively higher parasympathetic nervous system activity, as reflected by higher vagal tone or respiratory sinus arrhythmia (RSA), allows for a relatively lower heart rate (Porges, 1995, 2007). However, when an individual is faced with a situation of perceived threat, there is decreased activity in the parasympathetic nervous system (i.e., lowering of RSA) and activation of the sympathetic nervous system which results in increased arousal (i.e., increased heart rate; HR) in order to allow an individual to employ a fight or flight response to the anticipated stressor (Bush, Alkon, Obradović, Stamperdahl, & Boyce, 2011; Porges, 1995, 2007). Collectively, these changes in autonomic activity are thought to serve an important function in the social engagement system (Porges, 2007).

There exists some work that has examined indices the autonomic nervous system activity in relation to temperamental BI and shyness during baseline conditions and in response to social challenges. Behaviorally inhibited toddlers and preschoolers have been shown to exhibit a higher baseline HR and lower vagal tone than their non-inhibited peers (García-Coll, Kagan, & Reznick, 1984; Kagan, Reznick, Clarke, Snidman, & García-Coll, 1984; Kagan, Reznick, & Snidman, 1987; Partridge, 2003; Rubin, Hastings, Stewart, Henderson, & Chen, 1997). As well, it has been found that having a higher baseline HR during preschool predicts stability in an inhibited temperament into early childhood (Marshall & Stevenson-Hinde, 1998). Temperamental shyness also has been positively correlated with a higher baseline HR and lower RSA in early childhood (Doussard-Roosevelt, Montgomery, & Porges, 2003), late childhood (Schmidt, Santesso, Schulkin, & Segalowitz, 2007), and emerging adulthood (Schmidt & Fox, 1994).

Although studies suggest an elevated baseline HR and lower vagal tone in relation to shyness and related constructs, it is important to note that other studies have found no relation between temperamental shyness and baseline HR or RSA in preschoolers (Sulik, Eisenberg, Silva, Spinrad, & Kupfer, 2013; Theall-Honey & Schmidt, 2006; Zhang, Spinrad, Eisenberg, & Zhang, 2018), school-aged children (Asendorpf & Meier, 1993; Marshall, & Stevenson-Hinde, 1998; Poole & Schmidt, 2018; Viana et al., 2017), early adolescents (Dietrich, et al., 2009), or adults (Hofmann, Moscovitch, & Kim, 2006).

Other work has reported that a higher baseline RSA was related to a difficult temperament in toddlers (Porges, Doussard-Roosevelt, Lourdes Portales, & Suess, 1994). Thus, it remains unclear if baseline autonomic measures are accurately and consistently associated with temperamental shyness.

There is another body of work that has aimed to examine whether shyness is related to mean level differences of on-task autonomic indices. Among infants and toddlers, higher on-task RSA during novel social encounters (i.e., approach from an unfamiliar adult) was associated with greater levels of observed fearful behavior (Buss, Davis, Ram, & Coccia, 2018). Among children, it has been found that there were no differences in HR or RSA between shy and non-shy children during the viewing of affective video clips in preschoolers (Theall-Honey & Schmidt, 2006) or during everyday social encounters in a school day (Asendorpf & Meier, 1993).

Relatively fewer studies have charted the temporal dynamics by examining changes in autonomic nervous system activity during social challenges relative to baseline in relation to shyness. In a sample of infants, it was found that lower RSA suppression (i.e., little change in RSA from baseline) to an interaction with an unfamiliar adult at 6 months of age was related to a trajectory of high social fear across toddlerhood (Brooker, Buss, Lemery-Chalfant, Aksan, Davidson, & Goldsmith, 2013). In a study with preschoolers, those who demonstrated decreases in RSA in response to interacting with unfamiliar peers showed higher levels of socio-emotional difficulties (Hastings et al., 2008). An additional study found that temperamental shyness was correlated with higher perceived physiological stress reactivity (but not objective autonomic reactivity) in children in response to a speech task (Evans et al., 2013). A recent study found that a higher HR reactivity and greater vagal withdrawal to a performance task at age four predicted higher levels of observed state shyness (i.e., coy smiling) at age six (Colonnesi et al., 2020). In young adults, shyness has been correlated with increases in HR during an unfamiliar social interaction relative to baseline (Bruch, Gorsky, Collins, & Berger, 1989). Collectively, there exists some evidence that shy individuals show altered autonomic responding during novel social encounters.

We know, however, comparably little about how shy individuals process impending social encounters. In a study with young adults, shy and non-shy undergraduates showed no differences in RSA, HR, or subjective fear from baseline to the anticipation of a speech and singing task (Hofmann, Moscovitch, & Kim, 2006). To our knowledge, only one study has examined changes in autonomic activity level to the anticipation of an impending social event among children. That study found that temperamentally shy seven-year-old children showed significant increases in HR during the anticipation of a self-presentation task relative to non-shy children (Schmidt, Fox, Schulkin, & Gold, 1999). However, the authors did not examine children's subjective affect during the anticipation phase, nor did they examine if patterns of autonomic activity changed during the completion of the speech task relative to the anticipation period.

It is, however, important to examine autonomic and affective changes across distinct phases of processing social threat, including anticipation and participation. This is important to do because it provides a more fulsome understanding of the temporal course of autonomic patterning in response to social stress in shyness and its subjective experience, allowing us to examine convergent evidence across different levels. By middle childhood, children have developed a sense of self and the capacity to understand social-evaluative cues, and there is a marked increase in the development of self-conscious emotions (Crozier & Burnham, 1990; Lagattuta & Thompson, 2007). We therefore hypothesized that a context of self-presentation would be particularly stressful for shy children in middle childhood.

The Current Study

We examined whether temperamentally shy children displayed distinct changes in autonomic activity (i.e., RSA and HR) across three phases: baseline, anticipation of a speech, and delivery of a speech relative to non-shy children. To provide convergent evidence, we also examined whether similar patterns were noted on a self-reported subjective measure of nervous affect across task phases.

Given that shy children typically perceive novel social situations to be threatening (LoBue & Pérez-Edgar, 2014; Pérez-Edgar et al., 2010, 2011), and HR increases and RSA decreases are autonomic responses to perceived threat (Porges, 2007), we hypothesized that shy children would show increases in HR, decreases in RSA, and increases in subjective nervousness across task phases. In contrast, we expected that non-shy children would perceive this social situation as relatively less threatening compared to the shy children and thus would show significantly less arousal across autonomic and affective levels relative to the shy children.

Method

Sample

Participants included 152 children (73 girls) aged 7-to-8 years old (M_{age} = 7.82 years, *SD* = 0.44) and their primary caregivers (M_{age} = 39.95 years, *SD* = 4.27; 90%

mothers, 10% fathers). Children were primarily Caucasian (81.6%), followed by mixed (9.9%), Asian (3.9%), African American (2.6%), and Latin American (2%). Children were primarily from middle to upper socioeconomic class families as indicated by total household income in Canadian dollars (< \$60,000 = 14.5%; \$60,001 to \$100,000 = 19.7%; > \$100,000 = 65.8%). A power analysis was conducted in G*Power (Faul et al., 2007) using the effect size from a previous study that examined autonomic changes to self-presentation in shy children (Schmidt et al., 1999, $d = 1.42$) and confirmed that a minimum sample size of 70 participants would be sufficient to detect significant group differences (Power = 0.80, $\alpha = 0.05$).

Children were recruited from a database containing the birth records of infants whose parents consented for their infant's inclusion in the McMaster Child Database if they were interested in participating in future developmental research studies conducted in the Department of Psychology, Neuroscience, and Behaviour at McMaster University. The children were born at hospitals located in Southern Ontario. All children were typically developing with no significant pre-, peri-, or post-natal health problems.

Procedure

Children and their primary caregivers visited the Child Emotion Laboratory at McMaster University. After obtaining written informed consent from the parent and the child, the child was fitted with ambulatory an electrocardiogram (ECG). The child and the main female experimenter then completed additional activities in a room adjacent to the parent, while the parent completed electronic questionnaires related to the child's socio-emotional development. The parent could watch on a muted, closed-circuit computer monitor his/her child participating in the experimental tasks in an adjacent room. Of particular interest for the current study was children's delivery of a speech (described in detail below), as well as their responses to an interview with the main researcher related to their shyness. The study visit took approximately 75 to 90 minutes to complete. At the end of the visit, the family received a $20 gift card and the child received a Junior Scientist Certificate to compensate them for their participation. All procedures were approved by the McMaster University Research Ethics Board (Protocol Number: 2209, Study Title: Children's Psychophysiology and Social Behaviour).

Overview of Speech Task Phases

Baseline Period. Baseline autonomic activity was collected for two minutes while the child viewed an emotionally neutral computer screen. Because postural differences can influence autonomic measures (Bush, Alkon, Obradović, Stamperdahl, & Boyce, 2011), the child stood during this baseline condition in order to allow for comparability to our speech anticipation and delivery conditions (described below) in which the child was standing. The child was instructed to stand as still as possible and to relax during the baseline condition.

Anticipation Period. Following the baseline condition, the experimenter gave the child the following instructions: "*The next activity you will do is to give a speech to other children about your last birthday. We will video tape this presentation so that other boys and girls can see you and hear all about you! Before we videotape your speech, I want you to think about your last birthday without talking so you know what you want to say in your speech for the other children to see. You will have two minutes to prepare this speech in your head. I will tell you when your time is all up!*". In order to increase the level of stress during the anticipation period, the experimenter gave the child two prompts. The first prompt occurred after 45 seconds: "*Remember that other boys and girls are going to see you and hear your speech, so try to think really hard about what you want to say!*", and the second prompt occurred after 90 seconds: "*Thirty more seconds and then it's time for you to give your speech!*". The child was instructed to stand as still as possible during this anticipation period.

Speech Delivery. The child was then brought to an adjacent room equipped with video and audio recording and asked to stand on a designated spot in front of a video camera and a mirror. The child was given instructions and reminded that the speech would be videotaped and later shown to other children to watch so other children could see and hear all the things the child did during his/her birthday. The female experimenter stood beside the video camera and watched the child during the delivery of the speech. Self-presentation tasks like these pose a potential threat to the social self and have been reliably used in children (Fox et al., 1995; Theall-Honey & Schmidt, 2006). The speech task was video- and audio-taped for later behavioral coding.

Behavioral Coding

We coded children's total time spent speaking (in seconds) and activity level during the speech task to be used as covariates in our analyses as these factors can influence autonomic nervous system activity (Bush, Alkon, Obradović, Stamperdahl, & Boyce, 2011; Grossman, Wilhelm, & Spoerle, 2004; Reilly & Moore, 2003). Total time spent speaking was operationalized as the amount of time in seconds the child spoke during the two-minute speech (M = 55.31 seconds, SD = 32.33, Range: 0 to 120 seconds). Activity level (defined as intensity of bodily movement) was coded on a three-point scale: 0 = *no bodily movement* (e.g., rigid behavior) to 3 = *high bodily movement* (e.g., arms swinging). Activity level was coded in 10-second epochs, and the average score of each behavior was calculated across the two-minute episode (M = 1.59, SD = 0.59, Range: 0 to 3). Excellent inter-rater reliability was established on 10% of the videos for time spent speaking (ICC =.90) and activity level (ICC = .76) by two coders who were not aware of children's level of temperamental shyness.

We also conducted computerized coding in BORIS software to assess non-verbal avoidance during the speech task (Friard & Gamba, 2016). We coded the duration of the child's gaze aversion and head aversion from social stimuli (i.e., camera and/or experimenter). Excellent inter-rater reliability was established on 10% of the videos for gaze aversion (ICC = .95) and head aversion (ICC = .98) by two coders who were not aware of children's level of temperamental shyness. Non-verbal avoidance was operationalized as the proportion of time the child averted their head and/or gaze (M = 64.57%, SD = 28.51, Range: 0 to 100%).

Subjective Nervousness

Children reported on their subjective nervousness using a "*Feelings Thermometer*". The main experimenter first showed the child the *Feelings Thermometer* and explained that it measures how the child is feeling, with 0 reflecting that the child is not at all nervous, and 10 reflecting that the child is extremely nervous. The *Feelings Thermometer* also contained cartoon faces depicting increasing levels of nervousness as a visual representation across the scale. The child was asked to report on his/her nervousness immediately following the baseline condition, speech anticipation period, and speech delivery by pointing to a number between 0 and 10 on the *Feelings Thermometer*.

Children's Temperamental Shyness

Parent- and child-report of the child's shyness was measured using the 11-item *Shyness Situations Questionnaire* (SSQ; Xu & Farver, 2009) which assesses shyness in various social situations. Sample items include: "How likely would your child feel shy when meeting someone for the first time?" for the parent-report, and "How likely would you feel shy when giving a presentation in front of your class" for the child-report. Respondents rate each item on a 5-point scale ranging from 1 = *very unlikely* to 5 = *very likely*. Parent-report was collected using an electronic questionnaire, and child-report was collected as part of a child interview. The main experimenter read aloud each item to the child, and he/she responded by pointing to his/her response on a pictorial response sheet. The child received initial training on using this response format before questions were asked. Both the parent-report ($\alpha = .89$) and child-report ($\alpha = .72$) demonstrated good internal consistency in our sample.

Parent- and child-report of shyness were significantly correlated ($r = .18$, $p = .02$), and a composite score was computed to provide a multi-informant assessment of shyness ($M = 26.61$, $SD = 6.41$, Range: 10 to 45). In order to examine group differences in autonomic and affective response across task phases, we created two groups: shy (upper 20%; $M = 34.89$, $SD = 3.40$; $n = 33$) and non-shy (lower 80%; $M = 24.27$, $SD = 5.00$; $n = 119$). The groups were based on previous work that has found approximately 20% of children are characterized by temperamental shyness (Degnan et al., 2014; Kagan, 1989, 2012; Rubin et al., 1997). Shy and non-shy children did not significantly differ on child sex, age, ethnicity, or familial income level (ps > .21).

Electrocardiogram (ECG) Recording and Reduction

Cardiac and respiratory data were collected using the MindWare Mobile Impedance Cardiograph, Model 50-2303-00. The unit detected R-waves at a sampling rate of 500 Hz and 24-bit ADC digitization. Three ECG electrodes were placed on the child's upper right back and lower left and right sides in the shape of an inverted triangle, and a respiration belt was fastened around the child's chest while the mother was present. The MindWare Mobile Unit was placed inside an age-appropriate backpack that was worn by the child for the duration of the study visit.

Cardiac and respiratory data were reduced and analyzed using the Mindware HRV 3.1.1 software package. Signals were edited manually for erroneous or missing beats according to recommendations of Berntson and Stowell (1998). Average RSA (lnms^2) and heart rate (beats per minute) was estimated for one minute epochs and averaged across epochs of interest which included baseline (two minutes), speech anticipation (two minutes), and speech delivery (two minutes) (Caccioppo, 1994). We used a respiratory frequency of 0.15 to 0.40 Hz which is commonly used in middle childhood (Erath & El-Sheikh, 2015; Keller, Kouros, Erath, Dahl, & El-Sheikh, 2014; Schmitz, Krämer, Tuschen-Caffier, Heinrichs, & Blechert, 2011).

Missing Data

There were missing ECG data for two children during the baseline and speech anticipation period due to equipment failure (n = 1) and child refusal to wear electrodes (n = 1). There were missing ECG data for nine children during the speech delivery due to equipment failure (n = 3), child refusal to wear electrodes (n = 1), child refusal to participate in speech task (n = 4), and excessive artifact (n = 1). There was one outlier (> 3SD above the mean) detected and removed for baseline RSA, baseline heart rate, and speech anticipation RSA, and one outlier (< 3*SD* above the mean) detected and removed for RSA during speech delivery. There was missing subjective nervousness for one child for the anticipation period and speech delivery due to experimental error. The subjective nervousness measure during the baseline condition was added to the study part way through data collection, resulting in 86 children having baseline subjective data. Children with missing subjective baseline data did not significantly differ than those without baseline data on child sex, age, familial income, shyness group, or any autonomic measures (*p*s > .08). Missing data across all measures were handled with multiple imputation using 10 generated datasets.

Data Analyses

We used a repeated measures analyses of covariance with task phase (baseline, anticipation period, speech delivery) as the within-subjects factor and group (shy, non-shy) as the between-subjects factor separately for the three dependent measures: RSA, heart rate, and subjective nervousness. When the dependent measure was RSA or heart rate, we statistically controlled for child sex, familial income levels, child's total time speaking (in seconds) and activity level during the speech delivery as these factors have been shown to affect autonomic activity (Bush, Alkon, Obradović, Stamperdahl, & Boyce, 2011; Grossman, Wilhelm, & Spoerle, 2004; Reilly & Moore, 2003). To interpret significant interaction effects between task phase and group, we examined patterns across task phases for the shyness groups separately. All statistical analyses were performed using SPSS Version 24.0, with significance levels set at $\alpha = 0.05$.

Results

Descriptive Statistics

Table 4.1 provides the descriptive statistics for main study measures, including means, standard deviations, and correlations. All variables were normally distributed. RSA and heart rate both demonstrated rank-order stability across task phases across the sample. Girls had a higher absolute heart rate during the speech anticipation, $t(148) = 2.01$, $p = .05$, and speech delivery period, $t(141) = 2.03$, $p = .04$, relative to boys, but did not differ on any other study variables. Child age was not significantly correlated with any main study variables (*p*s > .08).

Autonomic and Affective Responses to Speech Conditions

RSA. There was a significant interaction between shyness group and task phase predicting children's RSA, $F(2, 148) = 6.73$, $p = .002$, $d = 0.52$. Follow up analyses revealed that shy children exhibited a significant decrease in RSA from baseline to speech anticipation $t(32) = 3.84$, $p < 0.001$, followed by a significant increase in RSA from speech anticipation to speech delivery, $t(32) = -2.23$, $p = 0.03$. Conversely, non-shy children showed no change in RSA from baseline to speech anticipation, $t(117) = 0.10$, $p = 0.92$, or from speech anticipation to speech delivery, $t(117) = -1.79$, $p = 0.08$. These patterns of findings are illustrated in Figure 4.1. There were no significant group differences in absolute RSA values for any condition (*p*s > .22).

Table 4.1

Descriptive statistics and correlations for main study measures

	2	3	4	5	6	7	8	9	Mean (*SD*)	Range
1. RSA Baseline	.74**	.70**	-.56**	-.48**	-.38**	.04	.03	-.07	6.35 (0.99)	3.64 – 9.26
2. RSA Speech Anticipation	--	.69**	-.48**	-.57**	-.46**	.07	.04	-.05	6.25 (1.03)	3.41 – 8.68
3. RSA Speech Delivery		--	-.52**	-.49**	-.58**	.09	.03	-.06	6.43 (0.97)	3.97 – 9.14
4. HR Baseline			--	.88**	.79**	-.06	.03	.06	93.13 (9.43)	66.04 – 119.04
5. HR Speech Anticipation				--	.85**	-.14	.06	.07	94.48 (9.77)	70.33 – 117.76
6. HR Speech Delivery					--	-.22*	-.03	-.02	98.18 (9.59)	72.22 – 126.49
7. Baseline Nervousness						--	.28*	.11	1.74 (2.43)	0 – 10
8. Speech Anticipation Nervousness							--	.41**	5.19 (2.99)	0 – 10
9. Speech Delivery Nervousness								--	5.67 (3.25)	0 – 10

**p < .001, *p < .05; HR = heart rate, RSA = respiratory sinus arrhythmia, *SD* = standard deviation

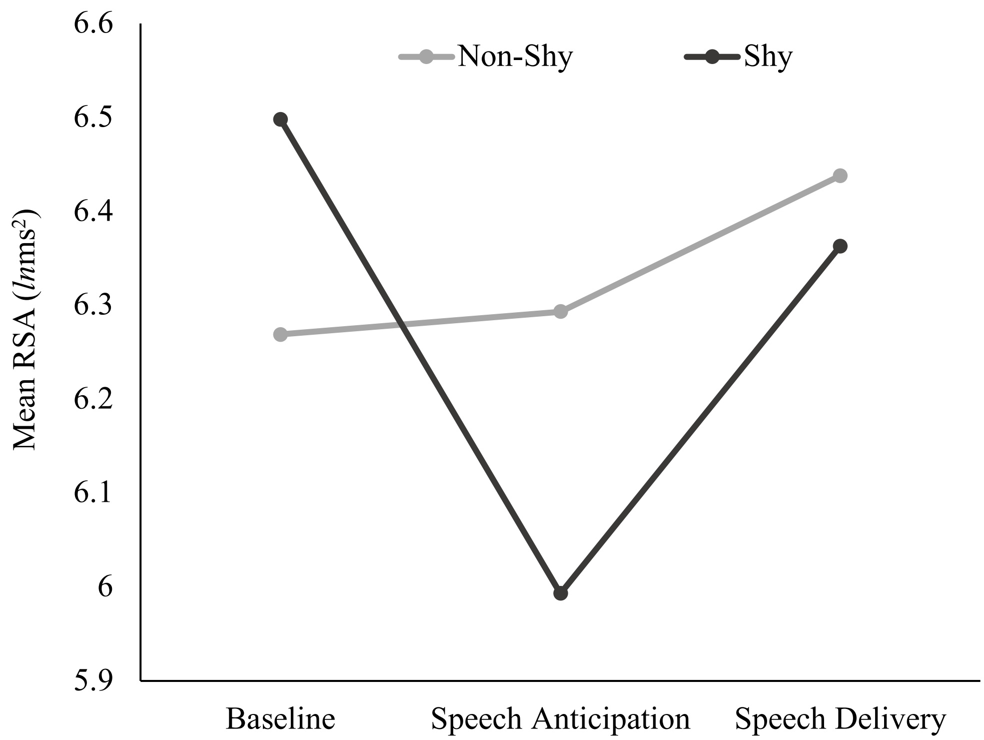

Figure 4.1. Changes in RSA across experimental conditions for shy and non-shy children.

Heart Rate. There was a significant interaction between shyness group and task phase predicting children's heart rate, $F(2, 143) = 3.06$, $p = .05$, $d = 0.35$. Follow up analyses revealed that shy children exhibited a significant increase in heart rate from baseline to speech anticipation, $t(32) = -3.00$, $p = 0.003$, followed by a further increase in heart rate from speech anticipation to speech delivery, $t(32) = -2.61$, $p = 0.01$. Conversely, non-shy children showed no significant change in heart rate from baseline to speech anticipation, $t(117) = -1.69$, $p = 0.09$, but showed an increase in heart rate from speech anticipation to speech delivery, $t(117) = -7.07$, $p < 0.001$. These patterns of findings are illustrated in Figure 4.2. There were no significant group differences in mean heart rate values for any condition (*p*s > .35)

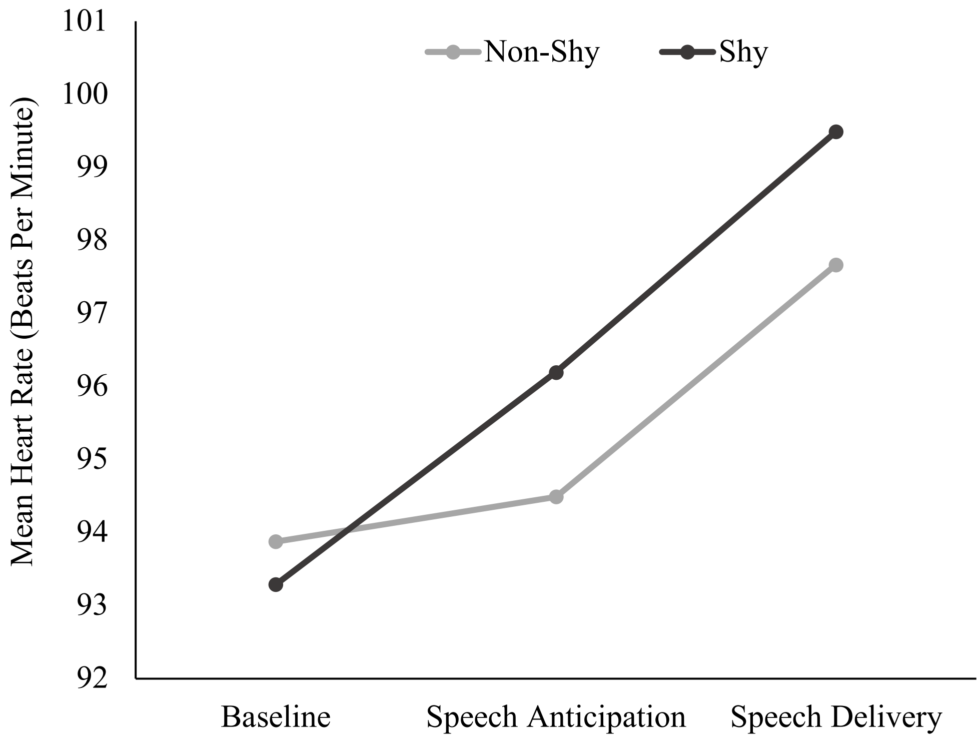

Figure 4.2. Changes in heart rate across experimental conditions for shy and non-shy children.

Subjective Nervousness. There was a significant interaction between shyness group and task phase predicting children's subjective nervousness, $F(2, 148) = 5.07$, $p = .02$, $d = 0.45$. Follow up analyses revealed that shy children exhibited a significant increase in nervousness from baseline to speech anticipation, $t(32) = -8.46$, $p < 0.001$, but no change in nervousness from speech anticipation to speech delivery, $t(32) = 1.52$, $p = 0.77$. Conversely, non-shy children showed an increase in nervousness from baseline to speech anticipation, $t(117) = -8.57$, $p < 0.001$, and a further increase in nervousness from speech anticipation to speech delivery, $t(117) = -2.36$, $p = 0.02$. These patterns of findings are illustrated in Figure 4.3. There were no significant group differences in mean subjective nervousness for the baseline condition ($p = .83$), but shy children rated themselves as being significantly more nervous during the speech anticipation ($p < .001$) and speech delivery phases ($p = .05$) relative to the non-shy children.

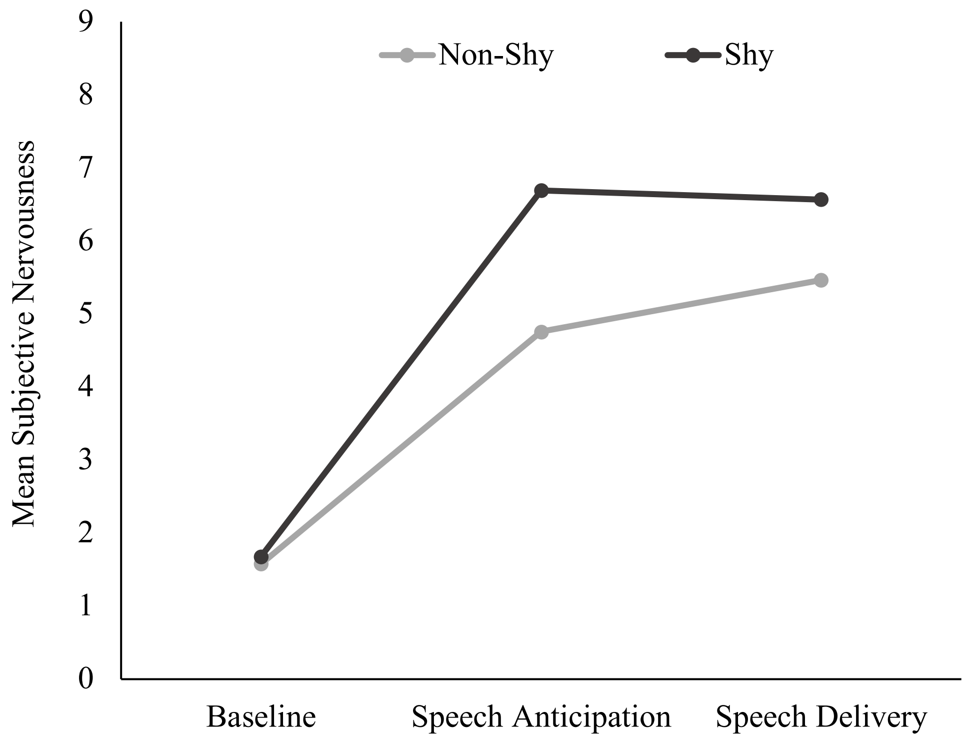

Figure 4.3. Changes in subjective nervousness across experimental conditions for shy and non-shy children.

Post Hoc Relations between Autonomic and Affective Responses and Observed Behavior

Given the differential pattern of autonomic and affective responses during task phases between shy and non-shy children, we wished to examine whether these responses were correlated with observed behavior during the speech task across the sample. When the predictor was subjective nervousness, the avoidance composite comprised both verbal and non-verbal avoidance including child's total time spent speaking (reversed scored) and duration of gaze/head aversion. However, given that autonomic activity is largely influence by speaking, avoidance behavior was operationalized only as non-verbal avoidance (i.e., gaze/head aversion) when predicted by autonomic reactivity.

We found that higher subjective nervous reactivity in response to the speech anticipation from baseline was predictive of greater observed avoidance during the speech delivery ($r = .28$, $p = .01$). Further, we found that increases in RSA during speech delivery relative to the speech anticipation period were related to higher levels of observed avoidance during the speech task ($r = .17$, $p = .04$). Observed avoidance was not significantly related to change in heart rate or RSA from baseline to anticipation, or heart rate or affective change from anticipation to speech delivery (ps > .13).

Discussion

It has been theorized that shyness may manifest due to perturbations in the regulation and perception of fear and higher levels of perceived threat in socially ambiguous or non-threatening environments. In light of this, we expected that shy children would react to an impending novel social event with higher levels of fearful arousal relative to non-shy children. This hypothesis was largely confirmed across both physiological and affective levels of analysis. We found that shy children exhibited significant decreases in RSA from baseline to speech anticipation followed by increases in RSA from speech anticipation to delivery, while non-shy children showed no significant changes in RSA across task phases. Further, shy children showed linear increases in HR across all task phases, while non-shy children only showed increases in HR from speech anticipation to delivery. Finally, shy children showed increases in subjective nervousness from baseline to the anticipation phase, and no further increase in response to speech delivery, while non-shy children showed linear increases in subjective nervousness across all task phases.

According to the polyvagal theory, when an individual encounters a situation of perceived threat, there is decreased activity in the parasympathetic nervous system (i.e., lowering of RSA) and activation of the sympathetic nervous system which results in increased HR and serves to provide an individual with the physiological resources needed to face the stressor (Bush et al., 2011; Porges, 1995, 2007). Given the relative decrease in RSA and increase in HR from baseline to speech anticipation among shy children relative to non-shy children, we believe that this provides evidence that shy children show greater physiological arousal to impending social events. Previous work has likewise found anticipatory worry to be related to cardiac vagal withdrawal (Friedman, 2007) and one study found an increase in shy children's HR from baseline to the anticipation of a self-presentation task (Schmidt, Fox, Schulkin, & Gold, 1999). Our finding of increased autonomic arousal among shy children was accompanied by greater subjective nervous affect during the anticipation period relative to non-shy children, and this heightened nervousness was predictive of more avoidance behavior expressed during the delivery of the speech. These findings suggest that temperamentally shy children may experience greater autonomic and emotion dysregulation particularly during the anticipatory processing of novel social encounters, which may be reflective of their tendency to over react to perceived impending social threat on both physiological and affective levels.

Although some work has suggested that vagal withdrawal is considered to be an adaptive response, it also has been highlighted that it is important to consider context when interpreting the function of RSA change (Hastings et al., 2014). For example, in a study with preschool-aged children, those who showed decreases in RSA in response to interacting with unfamiliar peers showed higher levels of socio-emotional difficulties (Hastings et al., 2008). The authors of this study reasoned that it is important to consider the social context when assessing the relative "adaptiveness" of changes in RSA in response to various task (Hastings et al., 2008). Changes in autonomic nervous system activity during a benign or ambiguous social context could be metabolically costly and unnecessary.

Indeed, other work has proposed that excessive vagal regulation during perceived threat may be linked to poorer emotional functioning as it may interfere with an individual's ability to effectively self-regulate and perform goal-directed behavior (Beauchaine, 2001). There is some evidence to suggest that children with internalizing difficulties show excessive vagal withdrawal in response to cognitive stressors in the laboratory (Calkins, Graziano, & Keane, 2007) and that excessive vagal withdrawal is related to poorer executive function (Marcovitch, Leigh, Calkins, Leerks, O'Brien, & Blankson, 2010). This excessive physiological regulation may impede a child's ability to flexibly process and engage with his/her environment (Calkins, Graziano, & Keane, 2007). Similarly, the excessive physiological regulation (i.e., RSA suppression) manifested by shy children during the social anticipatory period may underlie the inflexible and overcontrolled behavioral style that is characteristic of some inhibited children (Henderson, Pine, & Fox, 2015).

Despite vagal withdrawal during the anticipatory period for shy children, a particularly interesting pattern of findings was that shy children showed a relative increase in RSA from speech anticipation to speech delivery, with levels that were comparable to their baseline RSA. Typically, after an individual experiences a stressful or threatening situation, the autonomic nervous system returns to a state designed for energy efficiency in order to maintain the organism's long-term homeostasis (Porges, 1995, 2007). Thus, shy children tended to show RSA recovery during the speech delivery. We speculate that this may be reflective of the shy child's sense of relief that the anticipation period is now terminated. This hypothesis is further supported by our findings that the shy children peaked in nervous affect during the anticipation of the speech task, with no further increase in nervousness during the speech delivery phase relative to the non-shy children. This provides support that shy children may experience cognitive over arousal and a style of anticipatory processing that has been noted among adults with high levels of social fear (Hinrichsen, & Clark, 2003).

The shy child may perceive that he/she has relatively less control during anticipation phase such that he/she is unable to avoid or employ a behavioral response to allow relief of affective nervousness. It has been noted that when an individual has little control over the situation, there can be exaggerated physiological and affective responses (Dickerson & Kemeny, 2004). In contrast, during the speech delivery period, the shy child has more control over his or her behavioural strategies to deal with arousal. Thus, it may be that shy children were aware of their ability to avoid participation in the speech delivery task, for example, by inhibiting speech or by employing avoidance-related behaviors, such as gaze and/or head aversion. This avoidance may serve as a behavioral regulation strategy to modulate arousal (Doherty-Sneddon, & Phelps, 2005). Other work has noted that increases in RSA may reflect an individual's failure to engage with the environment (Calkins & Dedmon, 2000). In support of this hypothesis, we found that children's avoidance behaviors during the speech delivery phase were related to increases in RSA. It should be noted, however, given that behavior and RSA were concurrently correlated during the same task phase (i.e., speech delivery), the directionality of this relation remains uncertain.

This study has implications for understanding the physiological responding and regulation among shy children during anticipation and participation in social situations. Although we did not find mean-level differences in absolute levels of HR or RSA during baseline or on-task conditions, we nevertheless found differences in the time course pattern of autonomic activity across task phases, highlighting the importance of looking at dynamic changes in physiology in relation to temperamental shyness. The study may also have implications for helping to promote shy children's affective and physiological regulation to impending social events. A recent study demonstrated that as young as five years of age, children are capable of altering thoughts related to negative affect (including fear) and that this may be related to relatively greater RSA regulation during fearful tasks (Davis, Quiñones-Camacho, & Buss, 2016). An interesting avenue for future research is to directly examine if altering shy children's cognitive strategies, particularly during anticipatory processing, influences their physiological regulation and behavioural strategies during social events, such as during self-presentation tasks and interacting with novel social partners.

Limitations

The current study should be interpreted in the context of the following limitations. First, our sample was relatively homogenous in terms of ethnicity and socio-economic status and thus it remains unclear if these findings are generalizable to other cultures and economic backgrounds. Second, we did not have a measure of autonomic recovery while the child was standing to compare to speech task phases, limiting inferences for patterns of autonomic recovery in shy versus non-shy children. Third, our study is cross-sectional in nature, and so we are unable to infer causality, and it remains unknown whether these patterns of findings are generalizable to other age groups. It will be particularly important to examine these patterns using a longitudinal design and also later in development because as children reach adolescence, they may evidence enhanced coordination between their physiological and affective response systems (Hollenstein, McNeely, Eastabrook, Mackey, & Flynn, 2012).

Conclusions

Shy children showed exaggerated autonomic regulation and arousal during the anticipation of a novel social event. We speculate that this provides evidence that shy children may have an innate tendency to overreact to perceived threat in social situations on both physiological and affective levels relative to non-shy children. Future work should continue to investigate shy children's physiological and emotion regulation during different phases of novel social events, and how these processes may contribute to adaptive or maladaptive psychological and behavioral outcomes across development.

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CHAPTER 6

Study 5: Biological and Behavioral Correlates of Subtypes of Shyness in Children

Poole, K.L. & Schmidt, L.A. (2019). Early- and later-developing shyness in children: An investigation of biological and behavioral correlates. *Developmental Psychobiology.* Advance online publication.

Abstract

Early theoretical work by Buss (1986a,b) posited that there is an early-developing fearful shyness that emerges during toddlerhood, and a later-developing self-conscious shyness that emerges during early childhood. It has been theorized that early-developing shyness is related to fear, rooted in inherited biases, and manifests in contexts of social novelty, whereas later-developing shyness is related to self-conscious emotions, may result from social ridicule or poor social skills, and manifests in contexts of social exposure. Despite the hypothesized correlates of these shyness subtypes, this theory has not been empirically tested in children. We tested 96 children aged 5 to 10 years old and classified them into three groups: *early-developing shyness* (n = 28; M_{AgeOnset} = 2.4 years), *later-developing shyness* (n = 19; M_{AgeOnset} = 4.8 years), and *non-shy* (n = 49). Findings revealed that children with later-developing shyness had the highest relative cortisol responses in the context of self-presentation, highest levels of embarrassment, and lowest social skills relative to the other groups, while children with early-developing shyness displayed the highest relative resting right frontal brain asymmetry (a neural correlate of fear) relative to the other groups. These preliminary findings provide partial empirical support for the previously theorized correlates and distinction of early-developing and later-developing shyness in childhood.

Introduction

Shyness is a trait characterized by inhibition in response to social novelty or situations of perceived social evaluation (Melchior & Cheek, 1990). There is substantial heterogeneity in the phenomenon of shyness, and several decades of theoretical writings (Buss, 1986a,b; Buss & Schmidt, 2010; Crozier, 1999; Poole & Schmidt, 2019a; Poole, Tang, & Schmidt, 2018; Schmidt & Fox, 1999; Schmidt & Poole, 2019a) and empirical research (Bruch, Giordano, & Pearl, 1986; Cheek & Buss, 1981; Colonnesi, Napoleone, & Bögels, 2014; Eggum-Wilkens et al., 2015; Poole, Van Lieshout, & Schmidt, 2017; Poole et al., 2019; Poole & Schmidt, 2019b; Schmidt, 1999) have illustrated that shyness is not a unitary construct. For example, social withdrawal in general can result from different social motivations (Asendorpf, 1990; Coplan, Prakash, O'neil, & Armer, 2004), and shyness in particular can have different phenotypic expressions (Colonnesi, et al., 2014; Reddy, 2001).

Despite the known heterogeneity in shyness, relatively little work has been conducted to understand whether there are distinct behavioral and biological correlates associated with this heterogeneity. One reason for this lack of work is that there have been few theoretical models on the heterogeneity of shyness that can help guide research questions. It is, however, important to identify heterogeneity in shyness, as this allows for greater precision in understanding the social, emotional, and biological foundations and consequences of different shy subtypes (Poole & Schmidt, 2019).

Buss' Theory of Fearful and Self-Conscious Shyness

One particularly relevant theory on the heterogeneity of shyness is the work by Arnold Buss (1986a,b). Over three decades ago, Buss argued that there are at least two types of shyness that differ in age of developmental onset, contextual elicitors, and may be related to distinct types of fear responses.

The first type is an early-developing shyness appears during infancy or toddlerhood and is presumed to be rooted in early innate biological biases underlying a basic fear response (Buss, 1986a,b). Buss coined the term *fearful shyness* to describe this early-developing shyness. This type of shyness is elicited in contexts of social novelty and intrusiveness (e.g., close proximity of a stranger) which may manifest as inhibition, freezing, or escape behaviors (Buss, 1986a,b). It has been proposed that early-developing, fearful shyness is an evolutionarily older form of shyness that likely evolved from a basic fear system to protect individuals from possible physical harm by unfamiliar conspecifics (Schmidt & Poole, 2019). Conceptually, early-developing shyness is similar to the temperamental construct of behavioral inhibition (wariness in the face of novelty) which emerges during infancy and toddlerhood (e.g., Garcia-Coll & Kagan, 1984; Kagan, Reznick, & Snidman, 1987, 1988). Both behavioral inhibition and fearful shyness are likely linked to a dominant avoidance motivation. Indeed, behavioral inhibition has been conceptually and empirically related to biological correlates underlying fear sensitivity and avoidance, such as right frontal brain alpha asymmetry (Calkins, Fox & Marshall, 1996; Davidson, & Fox, 1989; Fox & Davidson, 1987).

The second type is a later-developing shyness which emerges during early to middle childhood (Buss, 1986a; Crozier, 1999), coinciding with the development of more advanced social cognitive capacities and the social self. Buss coined the term *self-conscious shyness* to describe this later-developing shyness. Buss speculated that self-conscious shyness may develop due to environmental and socialization factors such as parental criticism, social ridicule, or children's lack of social skills, which can contribute to feelings of low self-perceived social efficacy and further perpetuate self-consciousness (Buss, 1986a; Schmidt & Buss, 2010). The contexts that elicit this later-developing self-conscious shyness are situations in which an individual is socially exposed and/or the object of social attention, as well as being available to possible social-evaluation and scrutiny (Buss 1986a,b). One common affective reaction that has been proposed for self-conscious shyness is embarrassment, and physiologically, self-conscious shyness has been hypothesized to be associated with autonomic arousal such as blushing (i.e., reddening of the face) and increased heart rate during situations of perceived social evaluation (Buss, 1986a).

It should be noted, despite the use of term "fearful" to describe early-developing shyness by Buss (1986), this does not preclude the idea that later-developing (self-conscious) shyness cannot also be related to processes of fear. However, the type of fear and contexts that elicit the experience of fear are likely to differ among subtypes. Specifically, later-developing (self-conscious) shyness is more closely related to fear of negative social evaluation and threat to the ego during situations of social exposure (Schmidt & Poole, 2019a). We have further proffered that self-conscious shyness may be an evolutionary more recent form of shyness that evolved as complex social interactions became an increasing important part of the human condition (Schmidt & Poole, 2019).

Empirical Distinctions between Fearful and Self-Conscious Shyness

Although Buss (1986a,b) highlighted the importance of distinguishing early-developing fearful shyness from later-developing self-conscious shyness due to proposed differences in origins, elicitors, and correlates of each, relatively little empirical research exists on testing his theory. Some early work administered self-report measures of trait fearfulness, trait self-consciousness, and trait shyness in order to derive groups of fearful shyness (i.e., high fearfulness, high shyness) and self-conscious shyness (i.e., high self-consciousness, high shyness) in a sample of adults (Bruch, Giordano, & Pearl, 1986). In line with Buss' hypothesis, Bruch and colleagues (1986) found that fearful shy adults retrospectively reported that their shyness had an earlier developmental onset than self-conscious shy adults. Additionally, Bruch and colleagues (1986) reported that fearful shy adults self-reported more symptoms of physiological anxiety and behavioral inhibition compared to self-conscious shy adults.

Another study with undergraduates used a similar methodological design to Bruch and colleagues (1986) and found that fearful shy adults self-reported lower self-esteem relative to self-conscious shy adults (Schmidt & Robinson, 1992). Finally, a third study using the same methodological design examined whether the two shyness subtypes were distinguishable on a psychophysiological level using regional EEG alpha measures at rest and in response to affective musical stimuli in undergraduates, and found limited separation between the groups (Santesso, Lewandowski, Davis, & Schmidt, 2006). Although informative, these three studies were limited in that they relied on self-report measures of traits thought to underlie fearful and self-conscious shyness (i.e., fearfulness, self-consciousness, and shyness), and none of the studies examined these shyness subtypes in childhood, the developmental period in which they are hypothesized to emerge.

To our knowledge, only one study has examined the development of fearful and self-conscious shyness in young children. Nearly three decades after initial work with adults, Eggum-Wilkens et al. (2015) revisited fearful and self-conscious shyness using a longitudinal, twin study design and observed children in the laboratory during contexts thought to elicit fearful shyness (i.e., context of social novelty) from 6 months to 22 months of age, and self-conscious shyness (i.e., context of social exposure) from 19 to 28 months of age. The authors found that the two shyness subtypes were not significantly related, self-conscious shyness increased across toddlerhood, and there were strong genetic contributions to both types of shyness (Eggum-Wilkens, Lemery-Chalfant, Aksan, & Goldsmith, 2015).

Despite long-standing theoretical distinctions between early-developing (fearful) shyness and later-developing (self-conscious) shyness and some limited empirical work, there remain several important gaps that remain to be addressed. First, to our knowledge, no studies have been conducted on these shyness subtypes in children beyond age 2 years. Studying these shyness subtypes in early to middle childhood is particularly important because during this developmental period children undergo further socio-cognitive development for the understanding of social-evaluation and continue to development self-conscious emotions, which are hallmarks of self-conscious shyness (Crozier & Burnham, 1990; Lagattuta & Thompson, 2007; Piaget, 1970; Younger, Schneider, & Guirgius-Younger, 2008; Yuill, & Banerjee, 2001). Second, although some work in adults has examined *self-report* of physiological symptoms of anxiety (Bruch, Giordano, & Pearl, 1986), only one study to date has examined *objective* physiological correlates that may distinguish these shyness subtypes, and this study was with adults (Santesso et al., 2006); no work has examined these measures in childhood. By utilizing biological measures, we may further our understanding of possible putative biological mechanisms implicated in the development and maintenance of these shyness subtypes.

Two biological correlates that have frequently been correlated with shyness and related constructs are salivary cortisol (Buss et al., 2003; Kagan et al. 1987; Schmidt et al. 1997) and resting frontal EEG alpha asymmetry (Calkins et al., 1996; Fox et al., 1996; Schmidt, 1999; Theall-Honey & Schmidt, 2006). Cortisol is a primary hormone of the hypothalamic-pituitary-adrenal (HPA) axis and situations that are perceived to be threatening can activate the HPA axis, and subsequently involves the release of cortisol from the adrenal cortex (Schulkin et al. 2005). For example, increases in salivary cortisol have been documented in relation to behavioral inhibition and shyness in both human and nonhuman samples (e.g., Buss et al., 2003; Kagan et al. 1987; Kalin et al., 1998; Schmidt et al. 1997) at baseline and in response to social novelty.

Frontal brain EEG alpha asymmetry also has been frequently been used to index motivation and emotion. Greater relative left frontal brain activity at rest has been linked to approach-related motivations and positive emotions such as joy, whereas greater relative right frontal brain activity at rest has been linked to withdrawal-related motivations and negative emotions such as fear (see Davidson, 2000; Fox, 1991; see also Harmon-Jones & Gable, 2018; Reznik & Allen, 2018, for recent reviews). In particular, greater relative right frontal brain activity has been correlated with shyness and behavioral inhibition across development (e.g., Calkins, Fox & Marshall, 1996; Davidson, & Fox, 1989; Fox & Davidson, 1987). An examination of salivary cortisol and resting frontal EEG alpha asymmetry in relation to early- and later-developing shyness may further our understanding of putative biological mechanisms underlying motivation and emotions in early- and later-developing shyness.

The Present Study

The objective of the current study was to describe the behavioral and biological correlates of early-developing shyness and later-developing shyness in children between the ages of 5 and 10 years. We classified children into three groups, including early-developing shy (shyness onset in infancy to toddlerhood), later-developing shy (shyness onset in early to middle childhood), and non-shy children. Parents and teachers reported on children's embarrassment and social skills, children completed a self-presentation task (i.e., context of social exposure), and we coded observed shy behaviors and assessed salivary cortisol responses to this stressor. Finally, a subset of children had their resting state frontal EEG activity collected to measure patterns of resting frontal brain asymmetry.

We made several predictions based on Buss' theory. First, we hypothesized separate statistically significant linear trends for the three groups on the behavioral measures. Given that later-developing shyness is presumed to be closely related to embarrassment as well as fear of social evaluation/sensitivity to social exposure, and may develop due to poor social skills (Buss, 1986a; Schmidt & Poole, 2019), we predicted that children classified with later-developing shyness would experience the highest levels of embarrassment (according to parent- and teacher-report) and observed behavioral shyness during the self-presentation task, and the lowest social skills (according to parent- and teacher-report); children classified as non-shy would exhibit the lowest levels of embarrassment and observed behavioral shyness, and highest social skills; and children classified with early-developing shyness would fall in between the later-developing and non-shy groups on these behavioral measures.

Second, we hypothesized that there also would be separate statistically significant linear trends for the three groups on the two biological measures. Based on Buss' (1986a,b) proposal that children with later-developing (self-conscious) shyness would regard self-presentation contexts to be particularly threatening and salivary cortisol is released in response to situations perceived as stressful (Schulkin et al., 2005), we predicted that children classified with later-developing shyness would show the greatest cortisol response to the self-presentation task; children classified as non-shy would exhibit the lowest salivary cortisol response; and children classified with early-developing shyness would fall in between the later-developing and non-shy groups on salivary cortisol response. Finally, because greater relative right frontal EEG asymmetry at rest is associated with a disposition to avoidance and a basic fear response and has been evidenced among children with conceptually similar temperaments to early-emerging (fearful) shyness (Calkins, et al., 1996; Davidson, & Fox, 1989; Fox & Davidson, 1987; Fox et al., 2001), we predicted that children classified with early-developing shyness would exhibit greater relative right frontal EEG activity at rest; children classified as non-shy would show greater relative left frontal EEG brain activity at rest; and children classified with later-developing shyness children would fall in between the early-developing and non-shy groups on resting frontal EEG asymmetry.

Method

Sample Overview

Participants were selected from a larger sample of 135 children (M_{age} = 8.17 years, *SD* = 1.63 years) who were between the ages of 5 and 10 years and were recruited into a study examining the development of social fearfulness. These 135 children were recruited using two strategies. First, a subsample (*n* = 58; 26 boys, 32 girls) was selected for scoring high on social fear. This was achieved by having clinicians at four children's mental health agencies in Southern Ontario administering the 8-item Managing Social Anxiety scale as part of the Brief Child and Family Phone Interview (BCFPI) at the point of intake prior to clinical assessment to all parents of children (Cunningham et al., 2009). If the child scored ≥8 on this measure, families received information about the study. Interested parents were contacted via telephone by research staff at McMaster University and enrolled in the project.

Second, children were also recruited from the McMaster Child Database (*n* = 77; 46 boys, 31 girls) and were not selected for social fear, thus this sample represents normal variations in social anxiety among the general community. This database contains the birth records of children whose mothers were recruited upon their child's birth for future developmental research studies conducted in the Department of Psychology, Neuroscience, & Behaviour at McMaster University. All children were typically developing with no pre-, peri-, or post-natal health problems and were primarily Caucasian and from middle-class backgrounds. Children recruited from the two different methods did not significantly differ on sex, age, primary caregiver education, secondary caregiver education, or primary caregiver age (*p*s > .13).

Derivation of Shyness Groups

From the larger sample of children (N = 135), we created three groups of children (i.e., early-developing shy, later-developing shy, and non-shy) based on two criteria: 1) their current levels of shyness, and 2) developmental onset of shyness. Individuals in the non-shy group scored extremely low on current levels of shyness (i.e., below the median) and on the measure of past shyness parents indicated that their child was not shy (thus, there was no developmental onset information provided). Children classified into one of the shyness groups were rated as high on current levels of shyness (i.e., above the median), and parents also indicated that their child was previously shy (thus, both shy groups have developmental onset information provided).

Current levels of child shyness were assessed using a shyness composite from items on the parent-report screen for child-anxiety related disorders (SCARED) that are rated on a three-point scale ranging from 0 = *not true* to 2 = *very true* (Birmaher et al., 1997). The five items for this shyness composite included: “My child is shy”; “My child feels shy with people he/she doesn’t know well”; “My child doesn’t like to be with people he/she doesn’t know well”; “My child feels nervous with people he/she doesn’t know well”; and “It is hard for my child to talk to people he/she doesn’t know well”. This measure demonstrated excellent internal reliability in our sample ($\alpha = .91$). A total of 132/135 parents completed this measure of shyness. Because there is no determined cut point to reflect “shy”, we opted to use a median split on this shyness measure (median = 5.6), to create two groups that classified children as shy ($n = 56$, $M = 8.56$, $SD = 1.47$) and non-shy ($n = 76$; $M = 2.22$, $SD = 1.78$).

We then examined developmental onset of shyness among the shy group. As part of a demographic questionnaire, parents were asked the following question: “Was there a time when your child was extremely nervous about meeting, being with, or being embarrassed in front of other people?”. If the parent answered yes to this question, they were asked to report at what age their child first began displaying this shyness. Of the 56 selected shy children, 47 parents reported yes. The range of developmental onset of shyness ranged from 1 to 9 years (M = 3.97 years, SD = 1.81 years). Using these data, we then created two shyness groups reflecting *early-developing shyness* (age of shyness onset: 1 – 3 years; n = 28), and *later-developing shyness* (age of shyness onset ≥ 4 years; n = 19; Buss, 1986a; Crozier, 1999). Finally, a *non-shy* comparison group (n = 49) was formed which included children who were currently and previously not shy. Thus, the final sample for the current study included 96 children (M_{age} = 7.96 years, SD = 1.71 years). Sample characteristics are presented in Table 5.1. Please see Supplementary Material for information pertaining to Total SCARED scores among groups.

Table 5.1

Sample characteristics

Measure	Early-Developing Shy (n = 28)	Later-Developing Shy (n = 19)	Non-Shy Controls (n = 49)	F-Ratio / X^2	P-value
Child Age in Years, Mean (S.D.)	6.93 (1.65)	7.38 (1.80)	7.52 (1.97)	1.59	.21
Child Sex, M/F	12/16	9/10	32/17	4.22	.12
Development Onset of Shyness, in Years	2.39 (0.59)[a]	4.79 (1.65)[a]	N/A	49.73	<.001
Current Shyness, Mean (S.D.)	8.57 (1.47)[a]	8.52 (1.50)[b]	2.22 (1.78)[a,b]	177.21	<.001
Primary Caregiver Age				3.15	.53
18-39 years, n (%)	14 (50)	11 (58)	23 (47)		
40-64 years, n (%)	14 (50)	8 (42)	26 (53)		
Family Income				6.03	.20
< $60,000 CAN, n (%)	9 (32)	7 (37)	8 (17)		
$60,000 to $100,000 CAN, n (%)	14 (50)	9 (47)	23 (48)		
> $100,000 CAN, n (%)	5 (18)	3 (16)	17 (35)		

Note. Identical superscripts indicate statistical significance between those groups. Income data missing on n = 1 non-shy participant; Family income in Canadian dollars

Procedure

Children and their mothers visited the Child Emotion Laboratory at McMaster University. After obtaining parental consent and assent, the child expectorated 0.5 to 1.5 ml of saliva into a small tube (saliva sample 1). At the time of the first saliva sample, the child was not aware that they would be doing a social speech task. Following this, a subset of the sample (n = 36) had baseline EEG recordings collected.[5] The child then participated in a social stressor (a videotaped self-presentation task; described below). Approximately 15 to 20 minutes after the social stressor, the child provided a second saliva sample (saliva sample 2). The children then completed additional tasks as part of the larger study (academic assessments, not reported here), following which the child provided a third and final saliva sample (saliva sample 3). The average time of collection for sample 1 was 12:07 pm; the average time of collection for sample 2 was 1:28 pm; and the average time of collection for sample 3 was 2:29 pm. Time of saliva sample collection did not significantly differ as a function of shyness group (p = .50). Parents were informed that children should refrain from eating, drinking, or participating in extensive physical activity one hour prior to the laboratory visit as these factors can affect cortisol levels. Parents completed questionnaires pertaining to their child's socio-emotional adjustment, and children's teachers were mailed questionnaire packages related to the child's socio-emotional functioning. At the end of the visit, the parent was debriefed, and the child received a small toy or gift certificate depending on age to compensate him/her for his/her participation. All procedures received ethics approval.

[5] The reason that we were only able to collect EEG from a subset of participants was due to equipment problems and significant delays with repairs to the EEG system during the study.

Electroencephalogram (EEG) Data Collection and Reduction

EEG Recording. Resting EEG recordings were obtained for approximately two minutes alternating with the child's eyes open and eyes closed while the child was seated. Two minutes of continuous EEG data has been shown to provide a reliable estimate of individual differences in resting brain activity (e.g., Allen et al., 2004; Theall-Honey & Schmidt, 2006). The child was instructed to simply relax during the baseline testing and to try stay as still as possible. EEG was recorded using a lycra stretch cap (Electro-Cap, Inc.) with electrodes positioned according to the international 10/20 Electrode Placement System (Jasper, 1958). Electrode impedances below 10 K ohms per site were considered acceptable. EEG was recorded from the frontal (F3, F4), central (C3, C4), parietal (P3, P4), occipital (O1, O2) sites. These sites represent the left and right hemispheres and anterior and posterior regions of the brain. All electrodes were referenced to the central vertex (Cz). The channels were amplified by individual SA Instrument Bioamplifiers. The filter settings for the channels were set at .1 Hz (high pass) and 100 Hz (low pass). The data from all channels were digitized on-line at a sampling rate of 512 Hz.

EEG Data Reduction and Analysis. The EEG data were visually scored for artifacts due to eye blinks, eye movements, and other motor movements using software developed by James Long Company (EEG Analysis Program, Caroga Lake, NY). This program removes data from all channels if artifact is present on any one channel. Regional absolute EEG power (in μV^2) was derived in the alpha range (8 to 13 Hz) which has been used in similarly aged samples to examine differences in affective style (e.g., Gao, Tuvblad, Raine, Lozano, & Baker, 2009; Light et al., 2009). A natural log (*ln*) transformation was performed on all EEG power data to reduce skewness. The number of

1-second artifact-free [i.e., discrete Fourier transform (DFT)] EEG windows was not significantly correlated with any study measures. The eyes-open and eyes-closed conditions were highly correlated (F3 alpha: $r = .94$, $p < .001$; F4 alpha: $r = .94$, $p < .001$), and thus were combined into a common baseline condition to provide a more reliable and stable estimate (see Tomarken et al., 1992). The mean number of DFT windows was 132.56 ($SD = 15.24$) for the early-developing shy group, 156.25 ($SD = 13.20$) for the later-developing shy group, and 150.00 ($SD = 15.24$) for the non-shy group.

Social Stressor: Self-Presentation Task

Children participated in a social speech where they were asked to deliver a speech to a video camera about their last birthday for three minutes. Children were told that their speech would be videotaped and later shown to other children their own age to watch so other children can see and hear all the things the child did during his/her birthday. Self-presentation tasks like these pose a potential threat to the social self and are likely to elicit increases in social stress and have been used in children as young as 4 years of age (Fox et al., 1995; Theall-Honey & Schmidt, 2006). We used this task to observe shy behaviors and also measured salivary cortisol responses to this social stressor.

Salivary Cortisol Assaying

Cortisol levels can be reliably determined from saliva assays and correlate highly with those derived from plasma (Walker, 1984). All saliva samples were transported on ice and stored at -80 °C until assayed. Saliva was centrifuged at 3000 X g for 15 minutes and the supernatant was assayed. All enzyme immunoassays were carried out on NUNC Maxisorb plates. Cortisol antibodies (R4866) and corresponding horseradish peroxidase conjugate were obtained from C. Munro (Clinical Endocrinology Laboratory, University of California, Davis). Steroid standards were obtained from Steraloids, Inc. (Newport, RI). Each sample was assayed in duplicate and averages reported. Two quality control salivary samples were prepared at 30% and 70% binding. A regression line was fit to the sensitive range of the standard curve (typically 40% to 60% binding). Interplate variation (the coefficient of variation) was 6.45% and intraplate variation was 6.51%.

Behavioral Coding and Measures

Three behaviors coded from the delivery of the speech were used to derive a composite of observed shyness which captured three conceptual distinct behaviors characteristic of shyness (i.e., avoidance, activity level, and latency to speak). Behaviors were coded in 10-second epochs, and the average score of each behavior was calculated across the birthday speech episode. The majority of children participated in the speech for the full three minutes. The mean task length was 172 seconds (Range: 40 to 180 seconds).

We used behaviors from the Laboratory Temperament Assessment Battery (Lab-TAB) manual for coding shyness (Goldsmith, et al., 2001). First, intensity of avoidance behavior was coded on a four-point scale: 0 = *no avoidance behavior* (e.g., child stands on designated place) to 3 = *high avoidance behavior* (e.g., child sits down, refuses to stand on designated place). Second, activity level (defined as intensity of bodily movement) was coded on a three-point scale: 0 = *no bodily movement* (e.g., rigid behavior) to 2 = *high bodily movement* (e.g., big arm swinging). We chose to examine activity level because some children have the tendency to reduce activity in response to perceived threat (Buss et al., 2004; Frazier-Wood, & Saudino, 2017; Kagan, Snidman, & Arcus, 1992). Third, latency to speak was measured by recording the time (in seconds) elapsed between indication that the child may begin their speech and the child's first spontaneous utterance. Factor analysis with varimax rotation confirmed that our three behaviors converged on to one factor which we called observed shyness (eigenvalue = 1.36). The factor accounted for 45.47% of the variance. All factor loadings were ≥ .62. Accordingly, we created a composite score using *z*-scores of intensity of avoidance behavior, activity level (reverse coded), and latency to speak (*log* transformed to reduce skewness).

Coding reliability was established on 20% of the participants' videos by coders who were not informed of the purpose and hypotheses of the present study. Coding discrepancies were resolved through discussion. Adequate inter-rater reliability was achieved for each behavior (latency to speak: $r = .94$; avoidance: $\kappa = .70$, activity level: $\kappa = .73$).

Parent- and Teacher-Report Measures

Child's Social Skills. Parents and teachers reported on the child's social skills using the Social Skills Rating System (SSRS; Gresham & Elliot, 1990). The social skills total score is comprised of four subscales including: social assertiveness, social cooperation, social responsibility, and social control. The parent-report measure is 38 items; the teacher-report measure is 30 items; and respondents' rate how often the child demonstrates the social behavior on a scale from 0 = *never* to 2 = *very often*. This scale demonstrated good internal reliability in our sample according to parent-report ($\alpha = .89$) and teacher-report ($\alpha = .92$). Parent- and teacher-report of social skills were correlated ($r = .30$, $p = .02$) and because the scales had a different number of items, the *z*-score of each scale was aggregated into a composite social skills score.

Child's Embarrassment. As part of the SSRS, both parents and teachers reported on the following item: "Child is easily embarrassed", using a three-point scale from 0 = *never* to 2 = *very often*. This item was not part of the social skills composite score reported above. Parent- and teacher-report of embarrassment were correlated (r = .27, p = .04) and aggregated into a composite embarrassment score.

Psychophysiological Measures

Salivary Cortisol Response. We considered two measures when examining the child's cortisol response to the self-presentation task. First, we created a cortisol reactivity score, which was the difference between the child's baseline cortisol sample (saliva sample 1), and the child's post-stressor cortisol sample (saliva sample 2). This measure of salivary cortisol provides an estimate of the relative change in cortisol production that an individual experiences from a baseline state to a stressful state, with increases in cortisol conceptualized as a stress response. Second, we created a ratio score that reflected the proportion of the post-stressor cortisol sample (saliva sample 2) relative to the total cortisol output across the visit (the sum of saliva sample 1, 2, and 3). Unlike the cortisol reactivity score which is a difference score and may be influenced strongly by baseline states, the ratio score provides information regarding how much cortisol was produced during the stressor relative to the total cortisol production. This can provide a proxy for how stressful this task may have been relative to other study components.

Frontal Alpha EEG Asymmetry. An EEG alpha asymmetry score was computed using the frontal data (i.e., *ln* right EEG alpha power *minus ln* left EEG alpha power). Because EEG alpha power is inversely related to activity, negative scores reflect greater relative right activity, and positive scores reflect greater relative left activity (Tomarken et al., 1992).

Missing Data

An overview of missing data is presented in Table 5.2. Of the 96 children in the study, a total of 81 had parent-report social skills, 63 had teacher-report of social skills, 80 had parent-report of embarrassment, 71 had teacher-report of embarrassment, 84 had observed behavioral data, 64 children provided saliva sample 1 and 2 and thus had cortisol reactivity data, whereas a total of 62 children provided all three saliva samples and thus had a cortisol ratio score. The patterns of missing data did not violate the assumption that data were missing completely at random (Little's MCAR test, X^2 = 87.03, p = .25). As well, children with and without social skills or embarrassment data for either parent- or teacher-report did not differ on child sex, child age, familial income, or shyness group (ps > .05). Likewise, children with complete and missing behavioral data did not significantly differ on age, sex, shyness group, or familial income (ps > .05). Children with missing cortisol data were younger (M = 6.82 years, SD = 1.51) than children with complete cortisol data (M = 7.74 years, SD = 1.96; F(1,94) = 5.73, p = .02, d = 0.49), but did not differ on child sex, familial income, or shyness group (ps > .17). Therefore, we controlled for age when examining salivary cortisol response.

Multiple imputation was used to impute missing data across 10 imputations. All analyses reported below present results from the imputed dataset, with results using pairwise deletion presented in Supplementary Material. Findings using imputation and pairwise deletion produced statistically similar results for all dependent measures, with the exception of frontal EEG asymmetry. As mentioned previously, EEG was collected on a subset of approximately one third of the sample in the current study (n = 36). There were additional missing EEG data due to excessive noise or equipment failure (n = 5) and extreme values (3 SD ± mean; n = 3), resulting in a subsample of 28 children (9 early-emerging shy, 10 later-emerging shy, and 9 non-shy) who had complete frontal alpha EEG asymmetry. Because of the high levels of missing data (70%) on this dependent measure, we present both the pairwise deletion and imputed results below. The subset of children with EEG data were older (M = 7.97 years, SD = 1.71) than children without EEG data (M = 7.08 years, SD = 1.88; $F(1,94) = 5.42$, $p = .02$, $d = 0.48$), but did not differ on child sex, familial income, or shyness group (ps > .49). Therefore, we controlled for age when examining frontal EEG asymmetry.

Data Analyses

We used a one-way analysis of variance (ANOVA) to compare group differences (i.e., early-developing shy, later-developing shy, and non-shy) on dependent measures. When our dependent measure was cortisol response, we controlled for time of day for saliva collection as cortisol is known to have a diurnal rhythm. Finally, we included age a covariate for each biological measure as mentioned above. All significant ANOVAs were tested for significant linear contrasts. All statistical analyses were performed using SPSS Version 24.0, with significance levels set at $\alpha = 0.05$.

Results

Descriptive Statistics

Descriptive statistics were computed prior to imputation and are presented in Table 5.2. All variables were normally distributed as indicated by kurtosis and skewness statistics. There were no significant gender differences on measures (ps > .52), with the exception that girls (*M* = 0.27, *SD* = 0.73) had higher observed shyness relative to boys (*M* = -0.13, *SD* = 0.56; $F(1,82) = 7.77$, $p = .01$, $d = 0.62$). Therefore, gender was included as a covariate when examining this dependent measure. Age was not correlated with any study measures (*p*s > .19), with the exception of being negatively correlated with observed shyness ($r = -.21$, $p = .05$), and age was therefore included as a covariate for this measure.

Table 5.2

Descriptive statistics and correlations for main study variables

Variable	*n*	1	2	3	4	5	Mean (SD)	Range	Kurtosis
1. Social Skills (z-scored)	87	--					0.02 (0.86)	-2.25 to 1.81	-0.50
2. Embarrassment	89	-.54**	--				0.93 (0.64)	0 to 2	-0.90
3. Observed Shyness	84	-.27*	.12	--			0.05 (0.67)	-1.39 to 2.06	0.38
4. Cortisol Reactivity	64	.04	.17	.10	--		0.11 (1.63)	-3.95 to 5.02	1.79
5. Cortisol Ratio Score	62	-.17	.24	.17	.67**	--	0.31 (0.20)	.02 to .92	1.81
6. Frontal EEG Alpha Asymmetry	28	-.09	.07	.07	-.56*	-.42	-0.01 (0.18)	-.40 to .25	0.59

SD = standard deviation, *Note:* the *n* reflects the sample size prior to imputation.
** $p < .001$; * $p < .05$

Behavioral Correlates

Social Skills. There was a significant main effect of shyness group on child's social skills, $F(2, 92) = 9.26$, $p < .001$, $d = 0.92$ (see Figure 5.1A). As predicted, there was a significant linear association among groups ($p < .001$) with the later-developing shy group having the lowest social skills (*M* = -0.53, *SE* = .19), followed by the early-developing shy group (M = -0.25, SE = 0.16), followed by the non-shy group (M = 0.32, SE = 0.12). Pairwise comparisons revealed that the non-shy group was significantly different than the later-developing and early-developing shy groups (ps < .001).

Embarrassment. There was a significant main effect of shyness group on child's embarrassment levels, $F(2, 92) = 21.00$, $p < .001$, $d = 1.44$ (see Figure 5.1B). As predicted, there was a significant linear association among groups ($p < .001$) with the later-developing shy group having the highest levels of embarrassment (M = 1.50, SE = 0.13), followed by the early-developing shy group (M = 1.09, SE = 0.10), followed by the non-shy group (M = 0.59, SE = 0.08). Pairwise comparisons revealed all three groups were significantly different from one another (ps < .05).

Observed Shyness. Contrary to our prediction, there was no significant effect of shyness group on child's observed shyness during the self-presentation task, $F(2, 92) = 1.86$, $p = .16$.

Biological Correlates

Salivary Cortisol Stress Response. The main effect of shyness group on cortisol reactivity did not reach significance, $F(2, 92) = 1.90$, $p = .15$, while the effect of shyness group on cortisol ratio score was statistically significant, $F(2, 92) = 6.17$, $p = .003$, $d = 0.68$ (see Figure 5.2A). As predicted, there was a significant linear association among groups ($p = .04$), with the later-developing shy group showing the greatest relative cortisol release to the self-presentation task (M = 0.42, SE = 0.06), followed by the early-developing shy group (M = 0.38, SE = 0.05), followed by the non-shy group (M = 0.27, SE = 0.03). The later-developing shy and non-shy groups were significantly different from one another (p = .01). There were no group differences in baseline levels of cortisol (p = .20).

Frontal EEG Asymmetry. Findings using pairwise deletion revealed a significant main effect of shyness group on resting frontal alpha EEG asymmetry, $F(2, 24) = 3.60$, p = .05, d = 1.14 (see Figure 5.2B). As predicted, there was a significant linear association among groups (p = .04) with the early-developing shy group having the highest relative right frontal EEG asymmetry score (M = -1.24, SE = 0.06), followed by the later-developing shy group (M = -0.01, SE = 0.06), and finally the non-shy group showing greater relative left frontal EEG activity (M = 1.11, SE = 0.06). The early-developing shy and non-shy groups were significantly different from one another (p = .02).

When using multiple imputation, the results did not reach statistical significance, $F(2, 92) = 2.10$, p = .12. However, the pattern was similar such that the early-emerging group had the greatest relative right frontal activity (M = -0.01, SD = 0.04), the later-emerging was intermediate (M = 0.01, SE = 0.05), and the non-shy had the greatest relative left frontal activity (M = 0.04, SE = 0.04).

(A)

(B)

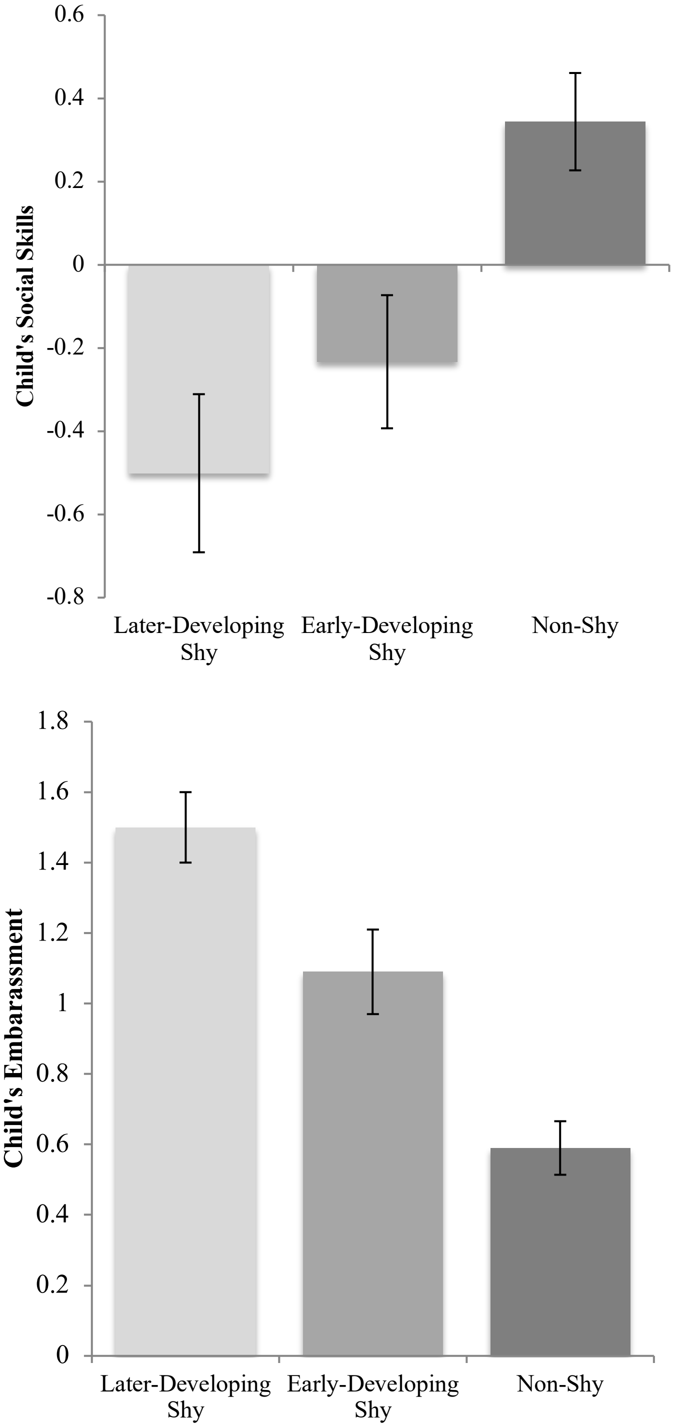
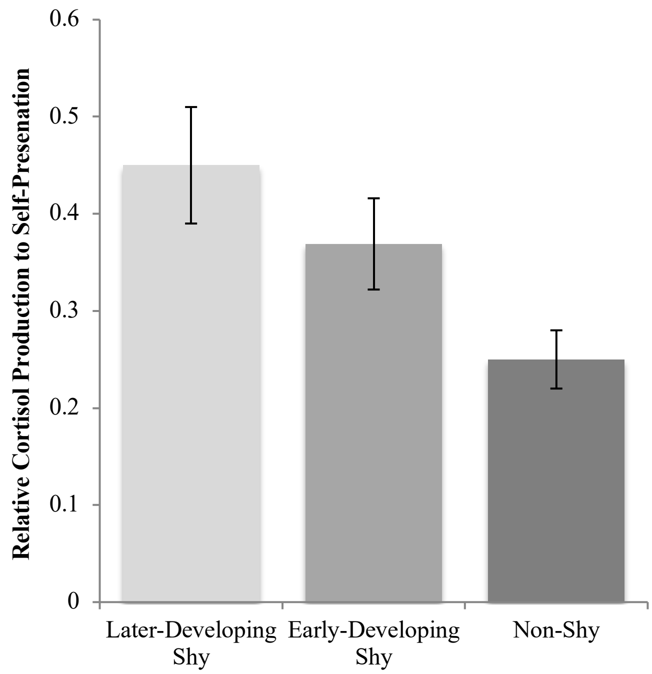


Figure 5.1. Behavioral correlates: Differences among shy groups on measures of (a) social skills and (b) embarrassment. Error bars reflect standard error of the means.

(A)



(B)

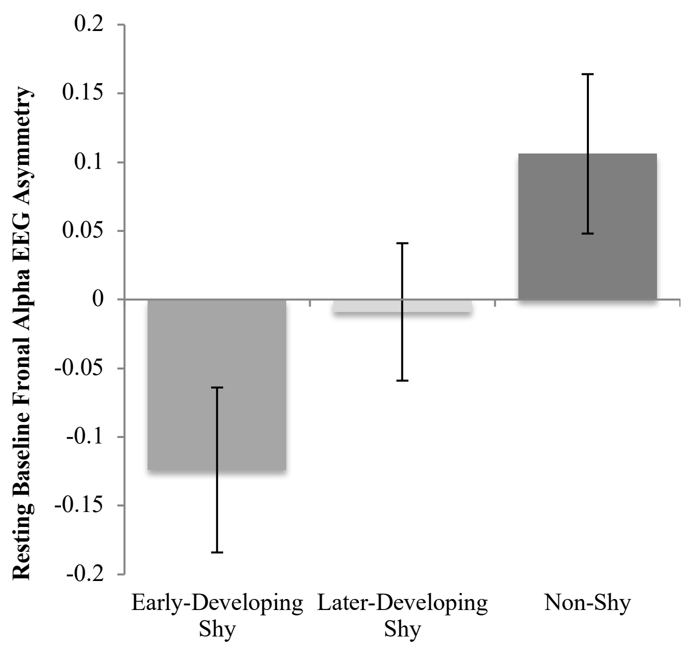


Figure 5.2. Biological correlates: Differences among shy groups on (a) cortisol response and (b) resting frontal EEG asymmetry. Error bars reflect standard error of the means.

Discussion

We sought to empirically test the previously theorized (Buss, 1986a,b) correlates and distinction of early- and later-developing shyness in children. We found preliminary evidence that children classified with later-developing shyness had a greater cortisol response to a context of social exposure, and were also rated as having relatively higher levels of embarrassment and poorer relative social skills according to teacher- and parent-report compared to other children in the study, whereas children classified with early-developing shyness tended to exhibit a pattern of greater relative right frontal brain activity (a neural correlate of fear and avoidance). These preliminary findings will be discussed in the context of Buss' theory (1986a,b) to describe different types of children's shyness.

Buss (1986a,b) proposed that there were at least two types of shyness, including an early-developing (fearful) shyness, and a later-developing (self-conscious) shyness which differed not only in developmental onset, but also may be related different underlying affective, behavioral, and physiological mechanisms. It has been further hypothesized that later-developing shyness is closely related to the affective experience of self-conscious emotions such as embarrassment (Buss, 1986a). We found support for this hypothesis, such that children with later-developing shyness had the highest relative levels of embarrassment according to parent- and teacher-report. We know of no studies that have previously tested differences among self-conscious emotions between these two shyness subtypes, however, given the developmental age of onset of later-developing being early to middle childhood (Buss, 1986a; Crozier, 1999), it is possible that the development of more advanced social cognitive capacities and the social self during this developmental period may underlie the experience and expression of self-conscious emotions that contribute to some types of shyness.

In addition, it has been proposed that later-developing (self-conscious) shyness also may be related to fear processes, but particularly in situations where there is a threat to one's ego, resulting in fear of negative of social evaluation by social partners (Schmidt & Poole, 2019). Indeed, the contexts that have been proposed as eliciting later-developing self-conscious shyness are situations in which an individual is socially exposed, as well as being available to possible social-evaluation (Buss 1986a,b). Despite this, children with later-developing (self-conscious) shyness *did not* exhibit more observed shyness during the self-presentation task relative to other children in our study. In fact, all three groups of children (i.e., early-developing shy, later-developing-shy, and non-shy) exhibited similar levels of observed shyness during the self-presentation task. This may indicate that irrespective of temperament, self-presentation tasks are a stressful situation for all children in early to middle childhood. The lack of distinguishing on this behavioral measure may be explained in part by the fact that, after the developmental period of toddlerhood, fearfully shy children also may be sensitive to situations of perceived social evaluation (Buss, 1986a). It is possible that as children with early-developing shyness enter later childhood, their shyness becomes generalizable to additional contexts including social exposure.

Although the early- and later-developing shyness groups were not distinguishable on observed behavior in our study, children classified with later-developing shyness showed the greatest relative production of cortisol in response to the self-presentation task compared to the other children in the study. Increases in cortisol release from the

HPA axis are thought to reflect a physiological response to threatening situations (Schulkin et al., 2005). Despite there being no observed behavioral differences during the self-presentation context, children with later-developing shyness may *perceive* this context as a threatening situation, and consequently their body produces higher level of cortisol. It may be the case that all children in the study had similar levels of behavioral regulation (or arousal), but the later-developing shy children nevertheless exhibit greater *physiological* arousal during social exposure contexts.

Buss (1986a,b) further speculated that some children may develop shyness during early to middle childhood due to environmental and socialization factors such as parental criticism, social ridicule, or children's lack of social skills. While we did not have data on each of these measures, we found that children with later-developing shyness exhibited poorer social skills relative to other children in the study. Although we are cautious in interpreting this finding as causal given the concurrent relation, if we interpret this finding in the context of Buss' theory, this may suggest that children with poorer social skills may have a lower self-perceived social efficacy, and this may contribute to feelings of self-consciousness, particularly in situations in which the child is the object of social attention and available to evaluation by peers (Buss, 1986a; Schmidt & Buss, 2010). However, given that we did not collect data on children's self-perceived social efficacy, this should be considered speculative.

Finally, Buss proposed that early-developing shyness was closely linked to inherited predispositions towards a dysregulated fear system. This conceptualization of fearful shyness shares overlap with the temperamental style of behavioral inhibition, in that each of the phenomena are characterized by wariness in the context of novelty, with fearful shyness being specific to *social* novelty, whereas behavioral inhibition encompasses wariness to social and/or non-social stimuli (Garcia-Coll, Kagan & Reznick, 1984). Indeed, some previous work in adults has found that fearful shy adults had higher levels of behavioral inhibition relative to self-conscious shy adults (Bruch et al., 1986).

We found preliminary evidence that children classified with early-developing shyness may exhibit greater relative right frontal brain activity at rest relative to the other children in the study, which is similar to work that has likewise found that behavioral inhibition was related to greater relative right frontal brain activity (e.g., Calkins, et al., 1996; Davidson, & Fox, 1989; Fox & Davidson, 1987; Fox et al., 2001; Hane, Fox, Henderson, & Marshall, 2008; Howarth, Fettig, Curby, & Bell, 2016; McManis, Kagan, Snidman, & Woodward, 2002). Motivational models of frontal brain activity posit that greater relative resting right frontal brain activity may reflect a trait-like measure of avoidance-related motivations and emotions such as fear (Davidson, & Fox, 1989; Fox & Davidson, 1987). In line with Buss' (1986a,b) hypotheses, this provides partial support that early-developing shyness may be maintained by a dominant avoidance-related motivation and a sensitivity towards experiencing fear. However, we acknowledge that, although the frontal EEG findings were in the predicted direction, this finding did not reach conventional levels of significance when using an imputed dataset, and therefore should be interpreted with appropriate caution. We also acknowledge that this preliminary finding should be interpreted as correlational in nature, and a longitudinal study design would be necessary to tease apart possible bidirectional brain-behavior relations (e.g., Howarth, Fettig, Curby, & Bell, 2016).

Limitations, Considerations, and Future Directions

There are several limitations that should be noted in the present study. First, our sample size was relatively small when examining resting frontal alpha EEG asymmetry, given this measure was only collected on a subset of the sample. Although we yielded large effect sizes in the hypothesized directions, the relatively small sample size should be replicated using larger sample sizes, particularly given that findings for the frontal EEG asymmetry measure did not reach conventional levels of statistical significance when using an imputed dataset. Second, the current study was cross-sectional, and the developmental onset of shyness was retrospectively reported by parents using a single question. Although retrospective report of shyness has been previously used (Bruch et al., 1986), it would be methodologically stronger to examine the developmental onset and stability of shyness using a longitudinal prospective study design (e.g., Eggum-Wilkens et al., 2015). Our use of retrospective parent-report of shyness onset should be interpreted with appropriate caution and replicated and validated in future work. Third, our measure of embarrassment was based on one item, and thus future work should replicate using longer versions of validated questionnaires. As well, although not uncommon in multi-informant developmental studies (De Los Reyes & Kazdin, 2005; Kiel, Buss, & Molitor, 2015), the correlations between our parent- and teacher-report measures were relatively low. This is likely due to differences in the contexts in which each informant views the child. Fourth, our sample was relatively homogenous, possibly affecting the generalizability of the findings.

It also should be noted that in the current study, due to our methodological design, we were required to operationalize two mutually exclusive shyness “groups” based on developmental onset of shyness and median levels of current shyness which was guided by previous work theorizing when these two shyness subtypes are most likely to develop (Buss, 1986a; Crozier, 1999). However, it has been theorized that is indeed possible after the developmental period of toddlerhood, fearfully shy children also may be sensitive to situations of perceived social evaluation, which may be akin to self-conscious shyness (Buss, 1986a). This also may be one reason that the majority of our findings yielded significant linear trends among groups, as opposed to differences among shyness groups in particular. To our knowledge, only one study has examined the relation between continuous scores of fearful and self-conscious shyness in toddlers, and reported that the two were not significantly related (Eggum-Wilkens et al., 2015). It remains a challenge to accurately examine the relative co-occurrence of fearful and self-conscious shyness; however, one strategy would be to observe children's shy-related behavior in contexts of social novelty (to elicit fearful shyness) and social attention (to elicit self-conscious shyness) in order to see if there are overlap in these shyness subtypes. Relatedly, our use of groups only captured children with early-emerging *stable* shyness in that they were shy at the time of assessment, and did not capture children with early-emerging shyness who outgrew their shyness. It will be important for future research to examine how stability of shyness influences behavioral and biological functioning.

Conclusion

We provide preliminary evidence that age of developmental onset of shyness may be related to distinct biological, affective, and behavioral correlates in children. In line with Buss' (1986a,b) theoretical model of early- and later-developing shyness, we provide partial support that later-developing shyness may be more closely tied to self-conscious emotions and be sensitive to contexts of social exposure while early-developing shyness may be linked to biological systems regulating basic fear responses. We recommend that future work continues to examine the developmental course, biological origins, and psychosocial correlates of subtypes of shyness.

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Supplementary Material

Total SCARED Scores among Shyness Groups

On the SCARED, a total score of 25 or more indicates the possibility of an anxiety disorder (Birmaher et al., 1997). When examining how many children exceed this cut-point, overall, 34 children scored 25 or higher. The distribution by group was as follows: 16/26 in early-developing shy group, 13/19 in later-developing shy group, and 5/48 in non-shy group. The early-developing and later-developing shyness groups did not significantly differ from one another, but the non-shy group was significantly different than each shyness group. The relatively high proportion of children meeting this cut-off likely reflects the fact that the shyness items that we used to select the children were included in the total SCARED score.

Results without Multiple Imputation

Behavioral Correlates

Social Skills. There was a significant main effect of shyness group on child's social skills, $F(2, 84) = 8.76$, $p < .001$, $d = 1.12$ (see Figure 1A). As predicted, there was a significant linear association among groups ($p = .005$), with the later-developing shy group having the lowest social skills ($M = -0.50$, $SE = .19$), followed by the early-developing shy group ($M = -0.23$, $SE = 0.16$), followed by the non-shy group ($M = 0.34$, $SE = 0.12$). Pairwise comparisons revealed that the non-shy group was significantly different than the later-developing and early-developing shy groups (ps < .01).

Embarrassment. There was a significant main effect of shyness group on child's embarrassment levels, $F(2, 86) = 24.71$, $p < .001$, $d = 1.53$. As predicted, there was a significant linear association among groups ($p < .001$), with the later-developing shy group having the highest levels of embarrassment (M = 1.50, SE = 0.12), followed by the early-developing shy group (M = 1.18, SE = 0.10), followed by the non-shy group (M = 0.58, SE = 0.07). Pairwise comparisons revealed all three groups were significantly different from one another (ps < .05).

Observed Shyness. Contrary to our prediction, there was no significant effect of shyness group on child's observed shyness during the self-presentation task, $F(2, 80) = 1.55$, $p = .22$.

Biological Correlates

Cortisol Stress Response. Although the main effect of shyness group on cortisol reactivity only approached significance, $F(2, 57) = 2.76$, $p = .07$, $d = 0.62$, the main effect of shyness group on cortisol ratio score was statistically significant, $F(2, 57) = 4.63$, $p = .01$, $d = 0.81$. As predicted, there was a significant linear association among groups ($p = .04$), with the later-developing shy group showing the greatest relative cortisol release to the self-presentation task (M = 0.42, SE = 0.07), followed by the early-developing shy group (M = 0.37, SE = 0.05), followed by the non-shy group (M = 0.26, SE = 0.03). The later-developing shy and non-shy groups were significantly different from one another ($p = .01$). There were no group differences in baseline levels of cortisol.

CHAPTER 7

General Discussion

Summary of Findings

This dissertation examined putative origins and heterogeneity in shyness from a developmental and biological perspective. I first examined how exposure to early stressors in the prenatal period can shape the development of shyness from childhood through to adulthood using a longitudinal prospective study design over four decades. Subsequently, I then examined biological correlates of fear across central, autonomic, and neuroendocrine levels in relation to shyness in general and shyness subtypes in particular during early to middle childhood. The investigation of these biological processes included resting, baseline physiology, as well as in response to social threat manipulations in the laboratory. Collectively, findings from this dissertation provide evidence that shyness is related to distinct developmental and biological indices of threat sensitivity and stress reactivity related to social threat. These developmental and biological processes may have an underlying influence on the expression and maintenance of shyness across development, possibly placing some shy children at risk for psychological problems.

Chapter 2 tested the hypothesis that shyness may have its earliest origins in utero for some individuals who are exposed to threatening prenatal environments. To my knowledge, this study is the first to examine the influence of multiple pre- and postnatal stressors (i.e., extreme prematurity and exogenous corticosteroids) on the developmental trajectory of shyness from childhood to adulthood. I found that infants who were born at extremely low birth weight (ELBW) and also exposed to synthetic corticosteroids prenatally exhibited high levels of shyness, which demonstrated stability from childhood through adulthood. The findings from this study are in line with a developmental programming hypothesis, which posits that the prenatal and early postnatal environments can exert long-term influence on social-emotional and physical development (Gluckman, Hanson, & Buklijas, 2010; Harris & Seckl, 2011).

As a result of early threatening prenatal environments, some infants may be developmentally programmed to detect postnatal threat, which is reflected in a behavioral pattern of hypervigilance and inhibition towards novelty during the postnatal period. This may be maintained in part by biological influences that are thought to mediate one's ability to detect threat and regulate the emotion of fear in the face of perceived threat. Previous empirical work has demonstrated that the typical biological modifications that occur in response to pre- and postnatal stress include central and peripheral physiological systems responsible for the regulation of fear and the detection of threat, such as altered activity in the prefrontal brain, autonomic nervous system, and sensitivity of the HPA axis (Gluckman, Hanson, & Buklijas, 2010; Harris & Seckl, 2011).

In Chapters 3 to 6, I extended and complemented the findings from Chapter 2 to directly test biological substrates related to a fear regulation and threat processing in relation to children's shyness across multiple levels of analysis using samples of typically developing children. In Chapter 3, I examined if patterns of frontal brain activity were related to the developmental course of shyness in early childhood. I found that children who had greater relative right frontal brain activity at baseline showed a pattern of increasing shyness from age 6 to 8, relative to children with greater relative left frontal brain activity. Motivational models of frontal brain activation have posited that greater relative right frontal brain activity at rest may reflect a biological predisposition towards avoidance and fear-related behavior and emotion (see Reznik & Allen, 2018, for a review). While the data did not support this hypothesis concurrently, I found support for this hypothesis longitudinally. It is possible that resting right frontal brain activity may reflect a biological diathesis to the growth of shyness as a child encounters normative developmental stresses across the early school years. For example, the neural circuits involved with the stress response, changes in social environment, peer comparisons, and social-cognitive development occur during middle childhood (Crozier & Burnham, 1990; Lagattuta & Thompson, 2007; Piaget, 1970). Thus, right frontal brain activity may reflect a biological diathesis towards withdrawal and avoidance of novel social stimuli, that in concert with normative development across the early school years may result in continual priming of the brain circuits regulating normal fear responses (Rosen & Schulkin, 1998), lower the threshold for arousal to social stressors, and place a child on a pathway towards shyness (Crozier & Burnham, 1990; Schmidt, Polak, & Spooner, 2005).

In Chapters 4 and 5, I examined shy children's patterns of autonomic activity in response to socio-affective threat and socio-evaluative threat in two separate experiments. First, in Chapter 4, I found that shy children demonstrated a pattern of stable, heightened autonomic arousal while viewing threat-related video stimuli across four repeated assessments from age 6 to 7.5 years in the laboratory. In the reported literature, this is the first study to investigate the longitudinal trajectories of the psychophysiological processes that had been previously long proposed to underlie the development of shyness in typical development. The stable pattern of autonomic arousal to socio-affective threat may reflect a characteristic way of responding and an innate bias towards threat-related information, possibly a basic mechanism subserving shyness.

The longitudinal pattern of autonomic functioning among shy children in Chapter 4 was assessed in response to the viewing of socio-affective threat, which can be considered a *passive* task. In Chapter 5, to extend the findings in Chapter 4, I examined shy children's autonomic and affective responses during an *active* task in response to socio-evaluative threat while they were anticipating and delivering a speech in front of peers. Consistent with findings from Chapter 4, in Chapter 5, I found that shy children exhibited autonomic arousal while they were anticipating a social speech relative to non-shy children. I also reported that shy children showed cardiac vagal withdrawal during the anticipation of the speech, which is support for the notion that shy children perceive this social context as threatening in nature (Porges, 1995, 2007). This was paralleled by increases of self-reported nervous affect. These findings provide support that temperamentally shy children experience greater autonomic and emotion dysregulation particularly during the anticipatory processing of novel social encounters, which may be reflective of their tendency to over react to perceived impending social threat on both physiological and affective levels.

Finally, in Chapter 6, I examined if individual differences in the developmental onset of shyness was related to distinct patterns of behavior and biology among children. I found that children with an early-developing shyness (i.e., onset in toddlerhood) had greater relative right frontal brain activity at rest. This provides partial support for previous theoretical work proposing that shyness with an early developmental onset may be rooted in early temperamental and biological biases (e.g., behavioral inhibition and right frontal brain activity) underlying a basic fear response (Buss, 1986a,b). I further found that children with a later-developing shyness (i.e., onset in early to middle childhood) had greater salivary cortisol levels in response to a self-presentation task, and higher levels of embarrassment and lower social skills as rated by parents and teachers. This also provides support for Arnold Buss' theory on the heterogeneity of shyness (Buss, 1986a,b), such that later-developing shyness may coincide with the development of more advanced social cognitive capacities and the social self, and may develop due to environmental and socialization factors such as parental criticism, social ridicule, or children's lack of social skills, which can contribute to feelings of self-consciousness (Buss, 1986a; Schmidt & Buss, 2010). Later-developing shyness may be more closely related to fear of negative social evaluation and threat to the ego during situations of social exposure (Schmidt & Poole, 2019), which is supported by the finding that they had increases in salivary cortisol to a socio-evaluative task.

Limitations, Considerations, and Future Directions

There are several limitations of the research in this dissertation which highlight important areas for future research. First, although Study 1 was longitudinal and spanned four decades, the remaining studies were short-term longitudinal or cross-sectional study designs. In order to precisely determine directionality of findings, it is important to examine the relation between biology and behavior using larger scale, long-term prospective studies across development.

Further, although this dissertation provides convergent and complementary evidence across multiple levels of analyses in a series of studies, an area for future research is to examine multiple biological processes within a single study across development. This would allow for the investigation of how different biological systems may be coordinated or reinforce each other among shy children and if this is this pattern of physiological (de)coupling is related to different developmental outcomes. This integrated approach can help us to approximate mechanism(s) of action and help to inform brain-body-behavior models of socio-emotional development (see, e.g., Miskovic & Schmidt, 2012). Further, although I examined neural correlates of shyness using EEG, this methodology has poor spatial resolution and it remains uncertain where the brain electrical activity is originating from (Pizzagalli, 2007). Future work should integrate measures of structural and functional brain activity using additional methodologies such as MRI (see Schwartz et al., 2003; Yang et al., 2013, for example) in order to better understand the neural bases of temperamental shyness. Relatedly, a challenge when collecting psychophysiological data from children is a relatively high rate of missing data due to child refusal to wear recording equipment or excessive artifact due to the child moving around during recording (see Bell & Cuevas, 2012, for a review).

It is also important to note that although the focus of my dissertation was on the biological correlates of shyness, the origins of shyness are indeed multifaceted, with both biological and contextual influences affecting its developmental course (e.g., Poole, Tang & Schmidt, 2018; Schmidt, Polak, & Spooner, 2005; Stevenson-Hinde, 2002). Beyond biological influences on the development of shyness, additional developmental models of shyness highlight the important role of social influences and context, such as peer acceptance and rejection, parenting styles, as well as parent-child and teacher-child interactions (Coplan, Arbeau, & Armer, 2008; Gazelle, & Ladd, 2003; Hastings et al., 2010; Rubin, Bowker, & Gazelle, 2010; Schmidt, Polak, & Spooner, 2005; Stevenson-

Hinde, 2002). Indeed, the development and maintenance of shyness is likely a result of the transactional nature of a child's biological predispositions towards shyness, in dynamic interaction with his or her social world. Although beyond the scope and objective of the current dissertation, future work should examine the complex interaction of biological and environmental influences on the emergence of shyness across development (Poole et al., 2018; Schmidt, Polak, & Spooner, 2005).

Implications and Conclusion

Findings from this dissertation have both theoretical and methodological implications for the understanding and study of shyness. First, this dissertation provides evidence that the origins and maintenance of shyness may be rooted in developmental and biological processes associated with threat and fear processing and regulation (LeDoux, 1996; Miskovic & Schmidt, 2012; Schmidt, Polak, & Spooner, 2005). The collection of findings also illustrates that there is heterogeneity in the developmental origins, onset, and course of shyness. Although shyness may be relatively stable in some children, there are individual differences in the onset and growth of shyness across development, which may be influenced by unique developmental stressors and biological diathesis towards fear and avoidance.

Findings suggest that the two developmental periods that may be relevant to the growth of shyness include middle childhood (Chapter 3, 5) and adolescence (Chapter 2). Middle childhood may be particularly salient to shyness because at approximately age 7-8 years, social comparison become increasingly salient and plays a large role in social-evaluation (Harter, 1986). It is also during this time that we see marked increases in socio-cognitive development responsible for self-conscious emotions which may contribute to feelings and expressions of shyness (Crozier & Burnham, 1990; Lagattuta & Thompson, 2007). Likewise, adolescence is another developmental period marked by the onset of puberty, increases in socio-cognitive development, and an increased reliance on peers and need for social acceptance (Cheek, Carpentieri, Smith, Rierdran & Koff, 1986). These hormonal, neural, and social changes can affect the development, expression, and regulation of emotions (Del Piero, Saxbe, & Margolin, 2016), and result in the onset or increases of shyness during adolescence (Cheek et al., 1986; Cheek & Krasnoperova, 1999; Tang et al., 2017; Westenberg, Drewes, Goedhart, Siebelink & Treffers, 2004).

Further, the findings of this dissertation highlight the importance and utility of collecting and examining shyness and its psychophysiological correlates within a developmental framework. Such longitudinal frameworks have been integral to my dissertation work, and have allowed me to begin charting the trajectory of shyness across developmental periods and investigating developmental and biological processes that influence different pathways of shyness. Importantly, these longitudinal data analytic techniques have allowed for the investigation of the longitudinal trajectories of the psychophysiological processes that had been previously long proposed to underlie the development of shyness and related phenotypes in typical development in earlier seminal work (e.g., Fox et al., 1995, 2001; Kagan et al., 1987, 1988; Schmidt, et al., 1997, 1999).

In summary, this dissertation aimed to highlight developmental and biological mechanisms underlying the origins and heterogeneity in children's shyness. It appears that there are individual differences in the developmental onset and trajectory of shyness. As well, there is evidence that shy children may have underlying developmental and biological predispositions towards avoidance of novel social stimuli. These predispositions may result in shy children to be more likely to perceive threat in social situations, and may influence the regulation and experience of fear, ultimately playing a role in the expression and maintenance of shyness across development.

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