INVESTIGATING INFANT CRYING PERSISTENCE AND CRY ACOUSTIC FEATURES AS EARLY RISK INDICATORS FOR SOCIAL ADJUSTMENT: DEVELOPMENTAL ASSOCIATIONS WITH INFANT VAGAL TONE AND ATTACHMENT STRESS

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ABSTRACT

Jacek Kolacz: Investigating Infant Crying Persistence and Cry Acoustic Features as Early Risk Indicators for Social Adjustment: Developmental Associations with Infant Vagal Tone and Attachment Stress
(Under the direction of Jean-Louis Gariépy)

The present pair of studies used hypotheses derived from the Polyvagal Theory (Porges, 2007; 2011) to assess the capacity of infant crying persistence and cry acoustic features to predict social adjustment in toddlerhood, and to elucidate the mechanisms that account for this relation. In view of this theoretical framework, behavioral cry frequency and acoustic features may serve as early markers of the functioning of the neural social engagement system. Importantly, this system integrates the regulation of bodily states, such as cardiac regulation via the vagus nerve, with aspects of social communication such as expressivity in the voice and eyes and the capacity to extract human voices from background noise. Both studies used data from a longitudinal sample of ethnically and economically diverse families with children that were followed from 3 months to 24 months of age. Crying persistence was measured using daily cry diaries at 3 months and retrospective reports. Cardiac vagal tone was indexed using respiratory sinus arrhythmia (RSA). Children's social adjustment was measured by parent reports and observations made in the laboratory. The first study (n = 391) tested a biopsychosocial model of the relation between infant crying and social adjustment as accounted for through two possible paths: the stress imposed on caregivers by persistent crying, and infant vagal activity in free play with the mother at 6 months. Results showed that infant crying persistence negatively predicted social relatedness in toddlerhood and crying persistence was related to higher parent-reported

attachment stress which predicted poorer social relatedness. Crying persistence in infancy did not predict vagal tone in infancy and toddlerhood, but higher vagal tone placed children at risk of poorer social relatedness. A second study, based on a subset of the overall study sample (*n* = 37), assessed infant cries for mean fundamental frequency and its variability, as well as overall cry modulation depth. More smoothly-modulated cries were related to higher RSA during the cry bout as well as higher RSA during a free-play parent-child interaction at 6 months. Behaviorally, children who avoided a stranger in the lab at 24 months had more unstable cries as infants. The results of this pair of studies suggests that infant cry persistence and acoustic cry features may be promising early indices of the developing social engagement system. Examining these cry characteristics at a time when the social behavioral repertoire is still nascent and it organization highly sensitive to the quality of the social context presents a promising new avenue for prospective research on social development.

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CHAPTER 1: INTRODUCTION

Persistent crying – characterized by frequent, extended, inconsolable bouts of distress, often with unusually high and variable acoustic cry features - is among the most common infant problems reported by mothers (Barr et al., 2000; Forsyth et al., 1985). It is estimated to affect between 5 - 19% of infants between 2 weeks and three months of age (Lucassen et al., 2001). In the majority of cases, this condition remises without intervention (Barr, 1998). Despite the transitory time course of persistent crying, a growing body of research points to behavioral frequency of infant crying and acoustic features to have lasting predictive value for children's social adjustment, a propensity for positive social relationships and positive, engaged reactions to novel social situations. (e.g., Papousek & von Hofacker, 1998; Salley, Miller, & Bell, 2013). Given that the social relationships have been found to be of importance for promoting adaptive development and reducing risk of clinical pathology (e.g., Choukas-Bradley & Prinstein, 2014; Bagwell, Newcomb, & Bukowski, 1998; Hartup, 1996; Parker & Asher, 1987), understanding the emergence of individual differences in social development in the first years of life and its developmental origins may lead to greater ability to assess early risk and inform intervention efforts. However, little research has examined the mechanisms that underlie the link between infant crying and social development, perhaps in part because of the limited availability of conceptual models that provide specific testable hypotheses about these relations.

The Polyvagal Theory (Porges, 2007; 2011) provides one such framework. This theoretical model describes the intimate link between the neural regulation of body states with the muscles of the face and head that facilitate or inhibit spontaneous social behavior, forming a neural social engagement system that facilitates affiliative social behaviors and promotes regulation of arousal states through interactions with others (Porges, 2007; see Figure 1). The key functional conduit of this body-face connection is made possible by the activity of the myelinated branch of the vagus nerve. The development of the social engagement system occurs rapidly during the first years of life, both shaping and being shaped by the parent-infant relationship, children's first social context. This provides a developmental biopsychosocial foundation for neural and behavioral organization that contributes to individual differences in children's social adjustment. The following pair of studies aim to examine the biopsychosocial developmental sequence that can account for the prediction of social adjustment from infant crying persistence and cry acoustic features.

The first study focuses on the role of infant crying persistence and social adjustment. Within the framework of the Polyvagal Theory, cry persistence may reflect early neural development of the social engagement system and regulation of distress states. Further, persistent crying may augment parental attachment stress (e.g., Papousek & Hofacker, 1998), reduce maternal sensitivity to infant needs (Mills-Koonce et al., 2007) and limit opportunities for positive social interactions. Thus, in the first study, three potential mediating pathways between infant crying persistence and social adjustment are tested. The first of these is cardiac vagal tone – the tonic influence of vagal activity on the heart - during a calm state of social engagement. The second mediating pathway between infant persistent crying and toddler social adjustment is that of parent attachment stress, which reflects stress on the bond between parent and child. While both these pathways may contribute to the developmental processes that link crying with social adjustment, they may not be independent. Emerging experimental evidence shows that parent stress can have a direct impact on children's vagal regulation of body states (Waters, West, & Mendes, 2014). Thus, a third mediation pathway will be examined wherein the parent attachment stress induced by persistent crying may exacerbate difficulty with the vagal regulation of bodily states and, in turn, lead to problems of social adjustment in toddlerhood.

A second study examines a similar pattern of relations but focuses, instead, on infant cry acoustic features. As part of the social engagement system, the regulation of bodily states by the vagus nerve is anatomically integrated with the muscles of the larynx and pharynx, which affect the modulation of vocalizations (Porges, 2007; Porges & Lewis, 2009). As such, differences in cry pitch and its modulation may also serve as indicators of vagal tone. Emerging evidence shows that high pitch and variability of infant cries are a risk factor for Autism Spectrum Disorders, which are marked by severe social deficits (Esposito & Venuti, 2010; Sheinkopf et al., 2012, Esposito et al., 2014). However, to my knowledge, no previous study has examined the predictive power of cry features for social adjustment within a normative sample. Not only may cries with high and unstable pitch reflect problems with the vagal regulation as part of the neural social engagement system, they may also promote parent stress by virtue of their aversive qualities (Zeskind & Marshall, 1988; Gustafson & Green, 1989). Thus, like cry persistence, these acoustic features may also form part of a biopsychosocial developmental pathway that places infants on a path toward poorer social adjustment in later childhood. The analysis in study 2 examines the relations of infant cry acoustic features with infant vagal tone, parent stress, and social adjustment during toddlerhood.

In the following document, I first review the literature implicating the role of parent stress and neural regulation of bodily states in the link between cry persistence and cry acoustic features with social adjustment. This review is then used to inform a developmental account of these pathways. Finally, two studies are then described, both using data collected from the same datasets. In the first, a developmental model of parent attachment stress and neural regulation mediating the link between cry persistence and social adjustment in toddlerhood is examined using structural equation modeling with latent variables. The second study, focused on cry acoustic features, serves as an exploratory analysis in light of the relatively incomplete status of the extant literature and modest sample for testing hypotheses. This study provides a test of the relations between infant cry acoustic features and social adjustment, parent attachment stress, and neural regulation of bodily states within a normative population in the hope that this exploration will inform future, more comprehensive models.

Infant Cries and Social Adjustment

Crying persistence and social adjustment. Human neonates have a limited repertoire of social behaviors. However, the cry vocalization is a prominent distress signal present from birth and its use as a cue for addressing the infant's needs is key for a neonate's survival (LaGasse, Neal, & Lester, 2005). Infants can differ dramatically in the stimuli that elicit crying, amount of crying, and ease of soothing (Rothbart, 2011). These aspects of crying can be distinguished as continuous measures reflecting a broad range of individual differences. Such continuously-distributed differences are often categorically described, with unusually high levels of crying described as colic, persistent crying, or excessive crying. A widely-used categorical system devised by Wessel and colleagues defines clinically significant crying based on a rule of threes: at least three hours per day, more than three days a week, for more than three consecutive weeks (Wessel et al., 1954). In many studies, this criteria is modified to three or more hours per day for three days over the course of *at least one week* (e.g., Barr et al., 2005, Kirjavainen et al, 2001). Though definitional and measurement variation exists across studies, the study of infant crying and its implications for development features much theoretical and methodological overlap. The term crying persistence will be used throughout this document, though literature summaries will strive to adhere to the source nomenclature as closely as possible. While ease of crying onset, length of crying bouts, and soothability may all contribute to crying persistence, all may reflect an underlying propensity for distress or mobilization states. Thus, the operational definition of crying persistence used in this study is, simply, the quantity of crying time.

Individual differences in infant crying and distress have been widely linked to social adjustment. In one study, four-month old infants were exposed to a series of visual, auditory, and olfactory experiences in a laboratory setting (Kagan & Snidman, 1999). Of these infants, about 20% displayed consistent strong reactions that included crying in response to the stimuli. When these children were compared to their low-reactive peers at age 4.5 years, they showed fewer spontaneous comments and smiles with an unfamiliar adult and were more inhibited with unfamiliar peers. In addition, a recent longitudinal study found that among a broad range of temperamental measures, children's negative affect in response to sensory qualities of stimulation at age two (a measure of the intensity of negative reactions to sensory experiences) was the only child characteristic that predicted social responsiveness at age 4, with higher levels of sensory discomfort predicting higher level of impairments in social cognition, communication, and social motivation (Salley, Miller, & Bell, 2013; however, by age 3 a wider range of temperament characteristics were related to age 4 social responsivity). Such effects have been even tracked to persist into middle childhood for children who cry persistently as infants. For instance, children who had colic in infancy were more aggressive and fussy than their non-colic peers at 10 years of age (Savino et al., 2005). Further, a retrospective study of mothers seeking help for their young children's emotional and behavioral problems revealed that children with high incidence of relationship disorders cried more in infancy than their peers (Papousek & von Hofacker, 1998). In contrast to this positive evidence, one prospective study found that infants who had colic did not differ from non-colic infants in social or peer competence by four years of age (Canivet, Jakobsson, & Hagander, 2000). However, there is a general convergence within the literature that points to long lasting predictive value for social development from infant crying and distress.

Acoustic features of vocalizations. In addition to varying in crying persistence, infants also vary on the acoustic features of cries, such as frequency (i.e., pitch) and its modulation. Although such acoustic features have not, to my knowledge, been studied in relation to social development in normal populations, they have been increasingly used to examine risk for the development of autism spectrum disorders (ASD). Because these disorders are often marked by moderate to severe social deficits, their relation to cry features may shed additional light on the role of neural vocalization regulation on atypical social development.

Elements of infant's cries have been linked to ASD in several studies. Infants who would later receive an ASD diagnosis do not display the typically-developing trajectory of decreasing fundamental frequency (F_0, typically perceived as the pitch) of cry over the first years of life in comparison with those who would go on to develop typically (Esposito & Venuti, 2010). It has also been found that six-month-old infants at risk for autism spectrum disorder, as identified by an older sibling with the diagnosis, produce pain-related cries that are higher and more variable in their fundamental frequency than their low-risk peers (Sheinkopf et al., 2012). Additionally, in this study, children that would go on to receive an ASD diagnosis had cries that were among the most variable in F_0 range as infants. In a study of 15-month old high- and low- ASD risk children's responses to separation from a caregiver, high risk toddlers produced cries with higher

F_0 than their low-risk peers (Esposito et al., 2014). Among these, three toddlers that would later receive an ASD diagnosis produced cries that had among the highest F_0 values. Though based on limited samples, this evidence points to infant crying as a potentially informative tool for predicting the emergence of clinical levels of social adjustment problems.

Overall, there is evidence that both infant cry persistence and vocalization acoustic features are predictive of social adjustment. However, little is known about the developmental mechanisms that account for this link. Part of this gap may be explained by a lack of application of guiding theoretical frameworks for generating hypotheses and examining mechanisms. I now turn to a recent theoretical framework to guide the building of a model that explores potential developmental mechanisms.

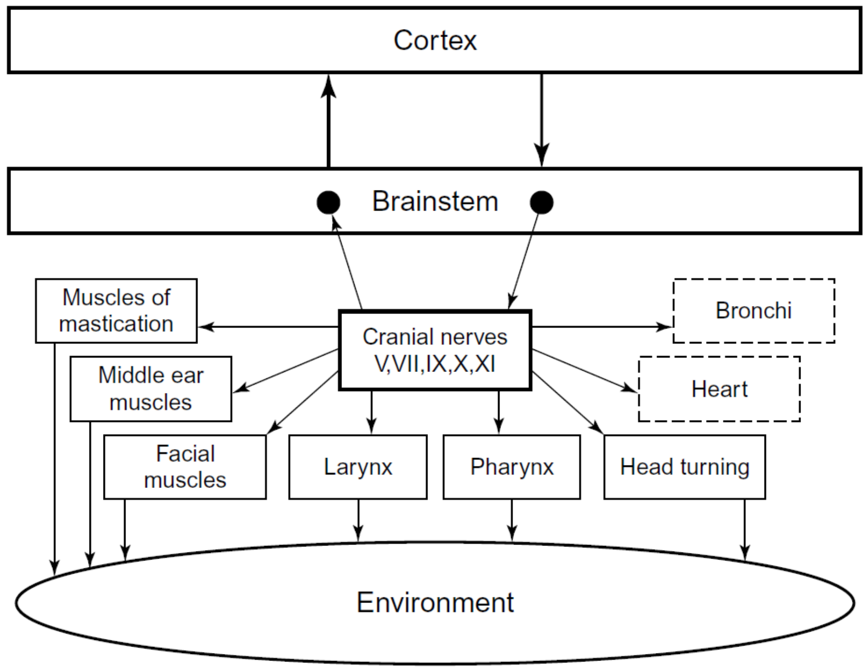
The Polyvagal Theory – A Neural Link between Vocalizations and Social Development

Traditional views of the autonomic nervous system have focused on two major branches – the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). These branches connect the brainstem with target organs throughout the body, with many organs being innervated by both branches. Signals transmitted by the SNS and PNS include both efferent transmission – signals traveling from the brainstem to the organs that influence their function – and afferent transmission – signals traveling from the organs to the brain that provide information about the status of target organs. As recent advances have allowed for disaggregating SNS and PNS contributions to heart regulation (Newlin & Levenson, 1979; Bernston et al., 1997; Granger et al., 2007), we are now in the position to more precisely investigate the role of individual circuits. Such advances have led to the formation of theoretical frameworks with increasing precision regarding the anatomical and functional properties of the autonomic nervous system.

One such framework that has stimulated novel psychophysiological hypotheses is the Polyvagal Theory (Porges, 2007; 2011). This framework focuses on the autonomic nervous system as a key neural regulator of social engagement and distress states that mobilize the body for activation and communication of pain or danger signals to others. The neural structure of the social engagement system is composed of a visceromotor component, the myelinated vagal circuit that innervates the heart and bronchi, and a somatomotor component, which includes neural pathways that innervate the muscles of the face and head (see Figure 1). The somatomotor pathways regulate the facial muscles, which facilitate emotional expression; the middle ear muscles, which aid to extract the human voice from background sounds; the muscles involved with head turning and tilting, which permit social gesturing and orientation; and the laryngeal and pharyngeal muscles that regulate vocal characteristics.

Over the course of evolution, the source nuclei of the myelinated vagus nerve, which originate in the nucleus ambiguous, became integrated with the muscles of the face and head in mammals, forming a bi-directional coupling between spontaneous social engagement behaviors and bodily states. During calm states, this coupling facilitates social interaction through the social engagement system that includes facial and head muscles as well as myelinated vagal activity to the heart and bronchi that promotes relaxed and restorative bodily states. The pathways that regulate the larynx, pharynx, and myelinated vagal influence on the heart are anatomically connected at the level of the brainstem, with their efferent source nuclei originating in the nucleus ambiguus. These social engagement substrates are withdrawn during stress and distress states, decreasing signaling to the heart and bronchi as well regulating the facial and

Figure 1. The neural social engagement system, which integrates body regulation circuits (bronchi and heart; dashed lines) with the muscles of the face and head (solid lines) that include the larynx and pharynx. Reprinted with permission from Porges, 2007.



head muscles to communicate alarm. When myelinated vagal activity is withdrawn during these states, mobilization (fight or flight) states are instigated through increased sympathetic-adrenal activity or immobilization is cued through the unmyelinated dorsal vagal complex (DVC; Porges, 2001).

The Polyvagal Theory provides an evolutionary biopsychosocial approach to the study of social development and opens up opportunities for novel research questions and hypotheses. In this view, social communication is functionally integrated with body states and thus provides an evolutionarily-selected mechanism for relaying and receiving information about internal states between conspecifics (Porges & Lewis, 2009). This view emphasizes the role of infant crying in communicating internal states to caregivers, with internal states being reflected in onset and length of cry, soothability, and the acoustic features of cries. In this framework, ease of cry elicitation reflects autonomic threshold for distress originating from within and without, persistence of crying during soothing efforts reflects openness to social regulation of distress states, and cry acoustic features reflect vagal regulation of the larynx and pharynx. This framework provides a novel approach to studying the role of these features in children's social development, including the parent-child relationship and engagement with peers and strangers. I first turn to a more specific review of the role of the vagal tone as a regulator of cry features before examining its function in social development.

Infant Crying and Vagal Tone

Infant crying is intimately related to physiological states. Behaviorally, crying acts as a signal that communicates the infant's disruption of homeostatic state due to experiences that arise from without or within to caregivers (Zeskind, 2013). Thus, incidents of crying are indicative of distressed states and crying persistence may reflect difficulties with regulation of distress reactions. More generally, the integration of vocal modulation, body states, and the social engagement system points to infant crying as a potential early marker of the functioning of the neural circuits that underlie social communication.

The most commonly used indices of autonomic function have focused on the regulation of the heart. The heart is dually innervated at the cardiac sinoatrial (SA) node by both the SNS and the myelinated vagus nerve of the PNS. The effect of these branches on heart rate (or, its inverse, heart period) are mutually antagonistic. Increases in PNS activity slow the timing between heart beats while increases in SNS shorten these intervals. Thus, heart rate can be used as an index of overall autonomic activity on the heart, but lacks specificity about the neural source of such control. An increase in heart rate, for instance, could be caused by a withdrawal of vagal activity, an increase in sympathetic activity, or a combination of both (Berntson, Cacioppo, & Quigley, 1991).

The activity of the myelinated vagus nerve, which is predicted by the Polyvagal Theory to be central to states of social engagement and most intimately linked with the acoustic features of vocalizations, has been found to be largely responsible for heart rate variability related to respiration (Berntson et al., 1997). Robust vagal activity increases heart rate during inspiration and decreases it during expiration. The amplitude of this rhythm, termed respiratory sinus arryhtmia (RSA), serves as an index of cardiac vagal activity (Porges, 1985). Vagal influence on the heart includes both tonic and situational influences. Tonic influence reflects a general strength of regulation by the myelinated vagal system, which is termed vagal tone. Flexibility in withdrawing vagal influence as needed metabolic resource allocation for mobilization or behavioral shut down are reflective of vagal regulation. This modulation of vagal activity can occur rapidly in response to changes in physical demands, such as fight/flight responses, or psychological demands, such as changes in attention (Porges, 2011). Thus, inter-individual and intra-individual variability in the respiratory cardiac rhythm provide a sensitive index of myelinated vagus nerve influence on the heart, allowing a tool for linking the regulation of internal states with external behavior such as crying persistence.

Cry persistence and vagal tone. Though colic or excessive/persistent crying has been described as a medical condition for hundreds of years (von Rosenstein, 1771), only about 5% of persistently crying infants can be diagnosed with an organic cause (Barr, 1998). One widely hypothesized etiology for persistent crying is that it may be the result of low threshold for autonomic arousal or an autonomic regulatory profile characterized by high SNS and low PNS activity (often described as “imbalance”; Lester et al., 1990; Carey, 1984). From a polyvagal perspective, persistent crying may be reflective of low threshold for social engagement system withdrawal and difficulty with the re-engagement of this circuit to promote calm, restorative states. Such difficulties may be reflected in low tonic vagal activity on the heart.

Empirically, little work has directly examined the link between vagal tone and crying persistence. A pair of studies comparing normally crying infants and those with colic did not find these groups to differ in heart period or respiratory sinus arrhythmia measured during sleep at two and eight months of age (Kirjavainen et al., 2001; Kirjavainen et al., 2004). However, these studies were limited by very small sample sizes (n = 12, n = 9 infants with colic, respectively) and respiratory sinus arrhythmia data collection during sleep only. While this pair of studies failed to show this link, some clinical studies that target physiological reactivity and regulation have shown effectiveness in reducing persistent crying. For instance, randomized trials show that colic symptoms can be relieved by anticholingeric medication (Lucassen et al., 1998; Hall, Chesters, & Robinson, 2012), a key neurotransmitter in both sympathetic and parasympathetic signaling. Such findings support the role of autonomic activity in colic but lack specificity about how individual circuits – such as the myelinated vagus nerve - may influence crying persistence.

Although little research has been conducted on the relations between clinical levels of crying (i.e., colic or persistent crying), a parallel line of developmental research has examined crying and soothability variation within normal populations. These normative and clinically-focused lines of research rarely intersect. However, because the clinical status of colic or persistent crying may be merely the high range of a normal distribution of infant crying persistence (Barr, 1991; Lindberg, 2000), studies with normal populations may provide key insights on this problem as well. Colic and persistent crying may reflect both a low threshold for initiating crying and less responsiveness to soothing efforts for reducing distress. Thus, research examining both the threshold for inducing crying and how easily crying can be soothed may reflect key constructs for examining relations between autonomic activity and individual differences in infant crying. In this realm, developmental researchers conceptualize infant crying behavior as reflective of temperamental differences in physiological reactivity and, often, regulation (Rothbart, 2011; Fox, 1989; Kagan, 1982).

Some studies have found support for the hypothesis (derived from clinical observations and reviewed above) that low vagal tone is related to more frequent or prolonged crying bouts and less responsivity to soothing. Such an effect was reported by Huffman and colleagues (1998), who found that infants with higher resting vagal tone required less soothing to complete a laboratory procedure; their findings also showed that those who withdrew vagal tone during a challenge, an indication of adaptive physiological regulation, were rated as more soothable by their mothers than those who did not. This finding is consistent with the role of the myelinated vagus nerve in state regulation (Porges, 2011) as its efficient use protects children from resorting to recruiting more metabolically taxing systems (such as the SNS). Furthermore, vagal tone measured at 9 months of age predicts mother ratings of difficult temperament in toddlerhood, such that children with higher vagal tone in infancy are *less* “difficult” in toddlerhood (Porges et al., 1994). In another study, higher resting vagal tone that was averaged across multiple assessments during the course of a year was related to less parent-rated distress to frustration at 18 months (Stifter & Jain, 1996).

Evidence, however, has also accrued to show that high vagal tone may actually place children at higher risk for triggering more frequent crying and poorer soothability. This evidence includes results from several studies reviewed above. For instance, vagal tone assessed during a rest period and during a developmental assessment in 9-month-old infants was positively associated with mother reports of infant “difficultness” (Porges et al., 1994). Further, infants whose vagal tone was consistently low across infancy responded with less frustration to an arm restraint task than those who were consistently high or unstable between assessments (Stifter & Jain, 1996). Additionally, 5-month olds that cried during an arm restraint task were found to have higher vagal tone at rest than those that did not (Fox, 1989). In 5-month olds, resting vagal tone (but not heart period) was found to be positively related to an index of negative reactivity across a set of lab tasks (Stifter & Fox, 1990). Vagal tone was also higher in children who cried in response to an arm restraint task, suggesting that behavioral distress reactivity may be specifically related to robust function of the myelinated vagus nerve. However, in this study neither vagal tone nor heart period were related to parent ratings of infant distress to limitations or soothability (Stifter & Fox, 1990). Finally, in a study that used a person-centered approach to examine multi-system physiological groupings, children in the group with the lowest resting vagal tone had lower parent ratings of distress to sensory stimulation than the two groups with the highest vagal tone levels (Kolacz et al., 2016).

In sum, substantial research supports both high and low vagal tone may place children at risk of persistent crying. This suggests that this relation may not be linear, but may instead follow a curvilinear pattern, creating an "optimal" relation with moderate levels of vagal tone predicting low persistent crying. Although to my knowledge this relation has not yet been tested, this analysis – as will be detailed later – examines both linear and non-linear links.

Cry acoustic features and vagal tone. The anatomical and functional integration of the myelinated vagus nerve, body states, and the regulation of the pharynx and larynx suggests that vocalizations provide information about mammalian internal states. These expressive features would have been evolutionarily important for social communication in inferring intentions and needs. Indeed, the sound of the cry promotes arousal synchrony between infant and caregiver (Zeskind, 2013) and stimulates action from the caregiver to assist the infant and "turn off" the signal (LaGasse, Neal, & Lester, 2005). The infant cry is frequently compared to a "siren" (Zeskind, 2013; Zeskind & Lester, 2001). This metaphor is not coincidental and likely reflects a set of specific acoustic features that evolved to stimulate alarm states in humans. The features of a siren – a long, slowly modulated wail punctuated by brief pauses – may reflect the mammalian neural appraisal of acoustic properties that are evolutionarily reflective of human infant distress sounds. This section first turns to a review of the anatomy of vocal production before continuing to a discussion of the neural connections between vocalizations and vagal regulation.

Neural projections that are part of the vagal complex from the lower brain stem (cranial nerves IX to XII) regulate muscles of larynx, pharynx, chest, and upper neck (Lester & Boukydis, 1990). These projections coordinate breathing, as well as frequency modulation and temporal patterning of vocalizations (Lester, 1984). The cry sound is part of the expiratory phase of respiration during which sound is produced by the larynx, also known as the “voice box”, which contains the vocal folds and the glottis. During vocalizations, the glottis and vocal folds close. Air pushed through the closed vocal folds results in a pressure drop that causes the folds to rapidly open and close, the speed of which is called the fundamental frequency (F_0) and is typically perceived as the vocalization pitch (LaGasse, Neal, & Lester, 2005). This opening and closing occurs at approximately 250 to 450 cycles per second (Hz) in a typical newborn cry (LaGasse, Neal, & Lester, 2005) and does not differ between males and females (Reby et al., 2016). Vocal frequency (pitch) is modulated via the laryngeal muscles, which alter the shape and tension of the vocal folds. Increased tension in these muscles is accompanied by decreased variability in the modulation of vocalizations (Stevens & Hirano, 1981). In infant cries, variability in pitch suggests instability in the neural control of the vagal cranial complex and is associated with neural damage or elevated risk status (LaGasse, Neal, & Lester, 2005). Thus, a smoothly modulated cry can be used as a marker of well-regulated control of the laryngeal musculature (Lester & Zeskind, 1982).

In addition to modulating pitch, the vagal cranial complex also exerts control on the contour and size of the airway of the supraglottal system, including the pharynx, which modifies the sound emanating from the lower tract and determines the quality of formants (i.e. resonating frequencies; LaGasse, Neal, & Lester, 2005). Formants provide a vocalization with its timbre and occur within frequency ranges that are higher than the fundamental frequency, with the first formant of a typical cry occurring at approximately 1100 Hz and the second formant around approximately 3300 Hz (LaGasse, Neal, & Lester, 2005). Although typically only the first two formants are measured (LaGasse, Neal, & Lester, 2005), the full range of formants covers a much wider frequency band, the qualities of which may influence the effect that cries have on caregivers. Besides these frequency characteristics, neural regulation also influences the temporal patterning of infant cries. Duration of cry utterance, duration of inspiratory period, and number of rapid, unvoiced sounds have all been related to parasympathetic function and the neural control involved in respiration (LaGasse et al., 2005).

Empirical evidence has emerged to support a functional relation between vagal regulation with frequency and temporal cry acoustics. In a study of neonate's responses to circumcision, fundamental frequency was found to be highest and cry duration to be shortest during the most invasive (and presumably most painful) parts of the procedure (Porter, Porges, & Marshall, 1988). This indicates that such acoustic features may be modulated in accordance to differing metabolic demands due to challenges. In this study, infants also showed stable within-person differences in their cry features that were associated with myelinated vagal activity. Infants with the highest levels of vagal tone prior to the procedure had the lowest fundamental frequency of cries and children with the lowest vagal tone had the highest fundamental frequency. Further, cry fundamental frequency was correlated with vagal tone measured during the procedure. In a separate study, reduced fundamental frequency, its variance, and utterance duration were all found to relate to more pronounced reductions in RSA following a social challenge (Stewart et al., 2013). Recently, infant RSA during sleep was found to negatively predict minimum, mean, and maximum cry F_0 for 5-month-old infants that had been born pre-term (Shinya et al., 2016). This finding held even when the infant weight at cry recording – which may influence cry pitch via anatomical differences in overall body size and vocal fold lengths – was taken into account. Taken together, these studies support a functional connection between frequency and modulation of vocalizations and the neural regulation of body states in response to challenges.

Relations between cry behavior and cry acoustic features. The shared neural connections between autonomic regulation and both crying persistence and cry acoustic features suggests that the behavioral and acoustic features of infant cries are related. Indeed, many infants that cry persistently have higher pitch and greater pitch variability than normally crying infants (Lester et al., 1990). Research has repeatedly shown that infants meeting Wessel's criteria for crying amount also demonstrate distinct acoustic features including higher and more variable fundamental or dominant frequency than their peers that were matched on demographics and clinical features (Lester, Boukydis, Garcia-Coll, Hole, & Peucker, 1992; Zeskind & Barr, 1997). In addition, when the mothers of infants with colic were played recordings of infant cries, they reported their infant's cries as more urgent and arousing than mothers of comparison infants. Thus, it is likely that both crying persistence and cry acoustic features may share a common physiological basis.

Vagal Tone and Social Adjustment

The anatomical link between the vagus nerve and the facial, laryngeal, and pharyngeal muscles suggests that the modulation of the myelinated vagus nerve is reflective of the overall state of the broader social engagement system. Difficulties with coordination of the vagal system, reflected in low vagal tone, may result in lower thresholds for distress to negative or ambiguous environmental cues, and thus limit abilities to both self-soothe and to use others as a source of soothing (Porges & Furman, 2011). Differences in myelinated vagal activity may thus be critical to the development of children's social adjustment. Social adjustment can be conceptualized as the quality of social relationships (e.g., Crick & Dodge, 1994). However, because social lives include not only long-term relationships but interactions with strangers, social adjustment will be used in this set of studies to refer to a propensity for positive social relationships as well as positive and engaged reactions to novel social situations.

Empirical evidence has accrued to support the role of vagal tone in children's social adjustment. For example, fourteen-month-olds with high vagal tone approached a strange experimenter more readily and maintained less proximity to their mother when the stranger was present than those with low vagal tone (Fox, 1989). Twelve-month olds who withdrew vagal tone during a cognitive test were rated by observers as more engaged with the experimenter, more emotionally positive, less fearful, and less irritable (Stifter & Corey, 2001). Also, stronger vagal withdrawal in response to a lab assessment at 9 months predicted decreased incidence of mother-reported social withdrawal and aggression when children were 3 years of age (Porges et al., 1996). Further, two- to three-year-old children with higher vagal suppression to challenge spend more time visually orienting to the experimenter (Calkins, 1997). By preschool age, children with higher vagal suppression to challenge are rated as more sociable by their parents (Doussard-Roosevelt, Montgomery, & Porges, 2003).

In addition, as part of a longitudinal study, children between age 1.5 and 5.5 years who were selected for extreme behavioral disinhibition had higher heart rate variability (which can be partly attributed to vagal tone) as well as more consistently dilated pupils (an index of both parasympathetic withdrawal and sympathetic activation) across a series of task than their peers who were selected for extreme inhibition (Kagan, Reznick, & Snidman, 1987). In this same study, inhibited children with the least variable heart rates were more likely to remain inhibited at subsequent lab visits compared to their inhibited peers with relatively more variable heart rates. In addition, children in this study who were inhibited and maintained a high and stable heart rate differed from their uninhibited peers and their inhibited peers with low and variable heart rate. These children observed unfamiliar peers for longer periods of time and stayed closer to their mother during a play session. This evidence supports the notion that the vagal system may be a key factor in the development of stable social adjustment differences across childhood. In sum, empirical evidence supports the notion that higher vagal tone and stronger vagal withdrawal to challenges are related to children's level of sociability and lack of inhibition in the presence of unfamiliar persons.

Crying and Attachment Stress

Whereas internal vagal activity may be one mediator between infant crying and social development, attachment stress - which reflects stress on the bond between parent and child – may also be implicated in this link. Such stress can be detrimental to a parent's sense of closeness with the child and perceived ability to observe and effectively respond to the child's needs (Abidin, 1995). Crying is one of the first social signals used to by the infant to communicate within the parent-child relationship and is utilized often during the first months of life. The persistence and acoustic features of this signal may have effects on the developing infant-parent relationship, and those impacts may have implications for children's social development as their social world begins to grow beyond the immediate family. Thus, for a more complete examination of the links between infant crying and social adjustment, the relationship with the parent must be considered as well.

In the first months of life, infants rely on parents for regulation of their internal states (Hofer, 1987; Tronick, 1989). The neonate's earliest emerging and most reliable signal for distress is the cry. Crying communicates to the caregiver that the infant is in need and motivates action by inducing arousal states in the caregiver. Though the infant cry is a necessary and adaptive aspect of the infant-caregiver bond, crying persistence and unusually arousing acoustic features of the cry can contribute to attachment stress. Persistent and acoustically aversive infant cries may create a stressful social context for the caregiver-child dyad and limit opportunities for positive social interactions between parent and child. These differences in the quality of the parent-child relationships can set the stage for emerging differences in the social behavior of children both within and outside the family system.

Although, to my knowledge, no study has specifically examined the effect of persistent crying on attachment stress, evidence shows that many aspects of the parent-child relationship may be substantially affected by infant cry behaviors. Mothers who are referred for clinical help due to infant persistent crying have high rates of partnership conflicts, low self-efficacy, depression, anxiety, exhaustion, anger, and marital distress (Papousek & Hofacker, 1998). Such disruptions can extend long after the remission of persistent crying or colic. Compared to parents of children who did not have colic, parents of children whose colic went into remission continued to have higher maternal anxiety, depression, and psychological distress (Humphrey & Hock, 1989; Pinyerd, 1992), low feelings of parental competence and more separation anxiety (Stifter & Bono, 1998), higher ratings of being bothered by infant's temperament (Jacobson & Melvin, 1995), and less satisfaction with family functioning (Rautava et al., 1995; Räihä et al., 1996). However, some studies have failed to show a difference in the long-term effects of persistent crying on parents. Parents of children without colic and those whose colic had remitted have not been found to differ in anxiety and postnatal depression (Clifford et al., 2002). In addition, one study failed to find differences in mothers' self-reported responsivity after the resolution of colic in comparison to mothers whose infants did not have colic (Stifter & Braungart, 1992). In spite of these inconsistencies, the majority of studies provide evidence for the detrimental effect of persistent infant crying on maternal mental health, mother-child relationships, and overall family function. These alterations may be especially evident in heightened parental attachment stress, affecting both the bonding and caregiving aspects of the parent-child relationship.

In addition to amount of crying, a range of studies has demonstrated that the acoustic features of infant cries are related to the perceived aversion and salience of the cries in adult raters. Fundamental frequency of infant cries positively predicts adult's ratings of their arousing, urgent, distressing, and aversive responses (Zeskind & Marshall, 1988). Further, relative power concentrated in higher frequency bands (3 kHz or above) is positively related to adult perceptions of urgency, distress, arousal, discomfort, and aversion, while power in the low frequency range (1 kHz and below) is negatively related to these responses (Gustafson & Green, 1989). Adults' affective responses to cries have also been found for temporal features. In a study that featured manual manipulation of the pause and expiratory sound duration of infant cry recordings, increasingly shorter pauses were perceived to be increasingly arousing and aversive, and this effect was enhanced by longer expiration duration for perceived urgency (Zeskind, Klein, & Marshall, 1992). In a separate study, duration of the expiratory sound predicted ratings of urgency, distress, arousal, discomfort, and aversion (Gustafson & Green, 1989). To my knowledge, no studies have directly examined the effects of aversive cries on attachment stress. However, given the wealth of evidence that cry features have substantial effects on adults' subjective experiences based on only brief audio clips, it can be hypothesized that extensive periods of exposure to aversive cries from an infant could augment attachment stress that is induced by infant crying.

Attachment Stress and Child Social Adjustment

The parent-infant relationship is a crucible for early social development. Within these relationships, physiological states are co-regulated and social communication expectancies and skills emerge with both parent and child contributing to the infant's burgeoning capacity for social engagement (Bowlby, 1988). With time, increasingly more complex capacities for social engagement emerge in the dyad, including social referencing, imitation, and joint attention (Carpenter, Nagell, & Tomasello, 1998). These all contribute to a growing repertoire of social skills and expectations which children will bring with them as their social circles grow to include peers and adults within a larger community. Thus, the patterns established within the parent-child relationship in the first years provide a key arena for the development of these expectations and abilities.

Empirical evidence demonstrates that differences in the mother-child relationship during the first years predict social adjustment into preschool entry, middle childhood, adolescence, and even adult relationships (Sroufe et al., 2005). In early childhood, securely attached children – those who explore and socialize when mothers are present and can effectively use the mother as a secure base when distressed - are more active participants in their peer group and spend less time isolated than insecurely attached children (Sroufe, 2005). Toddlers who were securely attached to their mothers are more socially active, positive, popular, and less socially anxious at school age (Bohlin, Hagekull, & Rydell, 2000). However, these effects of positive parent-child relationships may be disrupted by stress placed on the dyad's attachment relationship early years of caregiving. General parenting stress is associated with increased levels of insecure attachment with both mothers and fathers in 18-month-olds (Jarvis & Creasey, 1991; Hadadian & Merbler, 1996). Further, parenting stress has been found to predict increased negative perceptions of the child, hostile attributions about the child's behavior, and harsh discipline practices (Pinderhughes et al., 2000). This effect of stress on the parent-child relationship can be apparent in children's social functioning, even outside the home. For instance, parent-reported stress has been found to be negatively related to children's social competence as rated by their preschool teachers (Anthony et al., 2005). In sum, observed relations between attachment stress, its effects on the quality of the parent-child relationship, and social behaviors point to attachment stress as a potential mediator between infant crying and social adjustment. While such effects may be accounted for via disruptions in the parent-child relationship, it is also possible that attachment stress may directly influence children's vagal regulation.

Maternal Influences on Children's Vagal Tone

The myelinated vagus is immature at birth. In humans, the first months of life are marked by rapid development of this system, with proliferation of myelinated fibers, increasing anatomical linkage with cortical processes, and progressive integration with the circuits that control the muscles of the face and head. This development allows for increasing regulation of autonomic state through social engagement (Porges & Furman, 2010). The greatest surge in myelinated vagal fibers occurs toward the end of the prenatal period and during the first 6 months of life (Sachis et al., 1982). Experimental evidence shows that environmental input affects the development of this neural system. In a randomized study, pre-term infants were assigned to a skin-to-skin contact program (Kangaroo Care) and compared to pre-term infants that received a standard care regimen. Those that received the skin-to-skin contact intervention showed more mature vagal tone and more mature state regulation (longer quiet sleep and alert wakefulness states) compared to their peers (Feldman & Eidelman, 2003). This supports the proposition that social experiences are important contributors to the physiological development that supports social engagement.

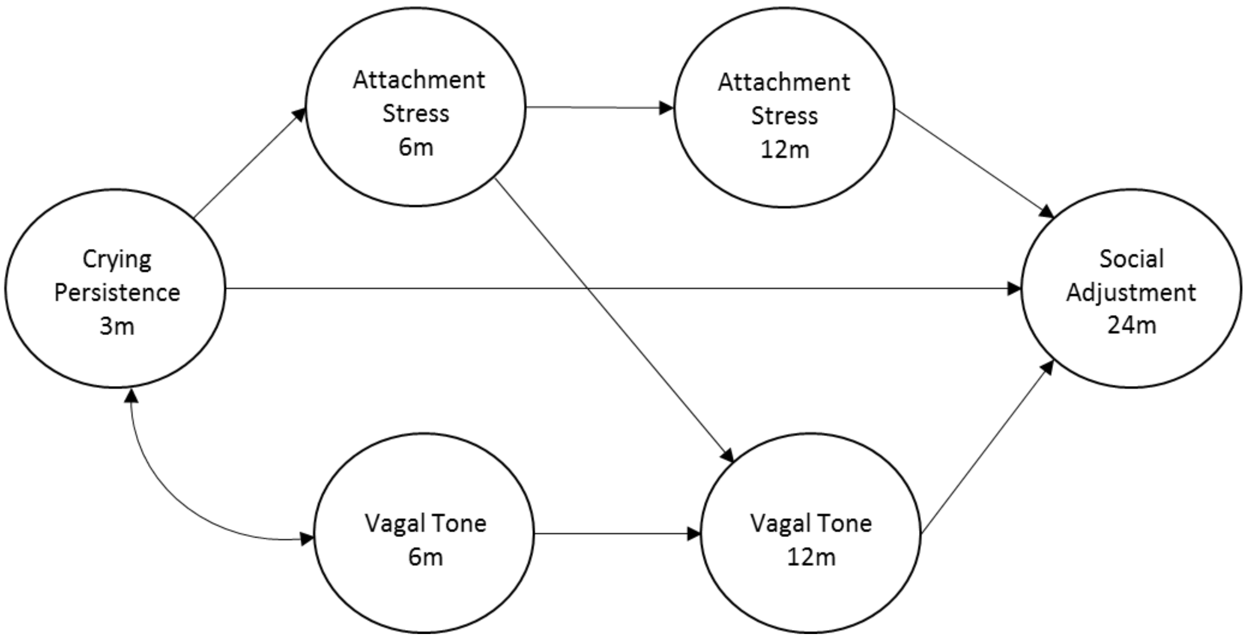
Though the above-described intervention was designed to manipulate touch, emerging research has begun to examine the profound impact that parent's emotional and arousal states can have on their infant's own internal states through non-tactile transmission. In a study that experimentally manipulated mother's stress, infants had an increase in heart rate when reunited with their mothers who were just negatively evaluated (who had an increase in their SNS activity) while infants had a decrease in heart rate when reunited with mothers who were not evaluated (who did not have an increase in SNS activity prior to reunion; Waters, West, & Mendes, 2014). A follow up study that utilized a similar design but included an additional manipulation of mother's ability to have touch contact with the child showed that SNS activity synchronized in infant-mother pairs only when mothers were able to have tactile contact; however, infants' vagal activity was coordinated with their mother whether touch was allowed or not (Waters & Mendes, under review; summarized by Mendes, 2016). These results demonstrate not only the physiological effects that mother's stress states may have on infants but also the profound interpersonal contagion of vagal regulation, which can be coordinated between mother and child even through non-tactile transmission. Given the architecture of the social engagement system, which features intimate connections between the vagus nerve and the cranial nerves that regulate facial features facilitating non-tactile social interactions (e.g., the musculature around the eyes, innervation of the larynx and pharynx, as reviewed above), such results provide a vivid illustration that the coordination of the face and head with the vagus nerve create a dynamic system that promotes social and physiological coordination between interacting partners, even without the use of touch.

While such effects have been studied in short-term experimental manipulations, it is possible that interaction patterns built over time influence the development of vagal tone in the infant. For instance, a mother-infant relationship characterized by high attachment stress may promote dampened vagal tone in the infant. On the contrary, low attachment stress may promote more opportunities for positive interactions between mother and child, scaffolding the child's organization of vagal tone over time in ways that support sociable predispositions. Taken together, this research suggests an indirect mediation between attachment stress and social adjustment via effects on the myelinated vagus nerve that regulates bodily states and conditions the neural social engagement system.

The Present Studies

Study 1. The first study examines a biopsychosocial developmental model of the link between infant cry persistence and social adjustment in childhood using a structural equation model. This pathway is examined for mediation via parent-reported attachment stress and child vagal tone across the latter portion of the first year of life. In addition, this study tests a dual mediation model in which parent attachment stress augmented by persistent crying may exacerbate difficulty with the neural regulation of bodily states and, in turn, lead to problems with social adjustment in toddlerhood. The conceptual model is depicted in Figure 2.

Figure 2. Study 1 conceptual model (depicted here with a 1-factor social adjustment solution)



In order to test the above-described structural model, key construct measurement models are established. First, a cry persistence measurement model is used to assess whether cry data drawn from two samples - which were collected from two nearly identical study designs - as well as two measurement occasions – a cry diary report and a retrospective persistent crying report – reflect a single underlying construct. Cry diary reports collected over the course of three days are also examined for daily reliability in order to support their use in the measurement model. A second measurement model is examined for social adjustment, using an exploratory and confirmatory factor analysis to assess the factor structure of multiple parent reports and lab observations of children's responses to a strange adult. To my knowledge, no study has examined the factor structure of the measures used here. However, given that children may differ in their social relations with familiar peers and adults and in their responses to strangers (Rothbart, 2011), it is tentatively hypothesized that two factors – reflecting social relatedness and shyness – may emerge. Third, a vagal tone measurement model is tested to examine whether the relations among several discrete epochs drawn from a single interaction may reflect an underlying vagal tone construct. The fourth key construct, attachment stress, is measured using a single indicator latent variable with internal consistency estimate drawn from a previous study. Thus, there is no specific aim associated with this construct. In sum, the following measurement and structural aims are examined.

Measurement model specific aims. The following specific aims are examined in regards to key construct measurement models

Specific Aim 1: Examine the cry persistence measurement model by 1) assessing stability of daily cry diary reports, 2) assessing the association between parent-reported crying persistence methods using daily cry diaries and retrospective recalls, and 3) to justify combining observed variable measurement information from two comprable samples.

> Hypothesis: Infant crying persistence measurement will demonstrate day-to-day stability in daily cry diary reports, converge between daily diaries and retrospective measurement methods, and converge between samples.

Specific Aim 2: Examine the structure of social adjustment across multiple parent-report measures and laboratory observations elicited by a stranger's approach.

> Hypothesis: It is tentatively expected that social adjustment may reflect two related constructs of social relatedness with familiar adults and peers as well as shyness or social reticence in interactions with strangers.

Specific Aim 3: Examine whether vagal tone as measured by multiple epochs during a mother-infant interaction can be described as a single latent underlying construct.

> Hypothesis: A single latent variable will adequately describe vagal activity across a set of epochs.

Structural model specific aims. The following specific aims are examined in regards to the structural model.

Specific Aim 4: Examine the association between infant crying persistence and social adjustment in toddlerhood.

Hypothesis: Persistent crying in infancy will predict poorer social adjustment in toddlerhood.

Specific Aim 5: Examine whether the association between infant crying persistence and social adjustment in toddlerhood is mediated by parent attachment stress.

Hypothesis 1: Infant persistent crying will be related to elevated parent attachment stress.

Hypothesis 2: Parent attachment stress during infancy will negatively predict social adjustment in toddlerhood.

Hypothesis 3: Parent attachment stress during infancy will mediate the relation between infant crying persistence and toddler social adjustment.

Specific Aim 6: Examine whether the association between infant crying persistence and social adjustment in toddlerhood is mediated by infant vagal tone.

Hypothesis 1: Infant crying persistence will be associated with vagal tone. Based on the literature above, three possible relations may be observed: Crying persistence may be a) positively related to vagal tone, b) negatively related to vagal tone, or c) exhibit a curvilinear relation with vagal tone with more persistent crying related to both high and low values.

Hypothesis 2: Vagal tone will negatively predict social adjustment in toddlerhood.

Hypothesis 3: Vagal tone in infancy will mediate the relation between infant crying persistence and social adjustment in toddlerhood.

Specific Aim 7: Examine whether a dual mediation model accounts for the relations between infant cry persistence and social adjustment in toddlerhood. This model will test whether persistent infant crying promotes elevated attachment stress, resulting in decreased infant vagal tone, which – in turn – confers risk of social adjustment problems in toddlerhood.

Hypothesis 1: Parent attachment stress during infancy will predict lower infant vagal tone.

Hypothesis 2: The link between infant crying persistence and social adjustment in toddlerhood will be accounted for by a 3-part pathway that includes both parent attachment stress and vagal tone. As such, persistent crying will predict elevated parent attachment stress, parent attachment stress will in turn predict lower infant vagal tone, and low infant vagal tone will in turn predict poorer social adjustment in toddlerhood.

To my knowledge, this study is the first to test a comprehensive mediation model of the developmental mechanisms that link persistent infant crying to social adjustment. In addition, it is among the first large-scale studies to examine vagal tone as a potential underlying correlate of persistent crying. Finally, the examination of mechanisms through which persistent crying, though it may eventually remiss on its own, provide information about future risk for problems of social adjustment and may inform the identification of intervention targets and designs.

Study 2. Study 2 serves as an exploratory study of the relations among infant cry acoustic features, cry persistence, vagal tone, parent attachment stress, and social adjustment in toddlerhood. In service of this goal, a subset of infants that cried during the 6-month Still Face paradigm were drawn from a larger longitudinal sample (n = 37; see below). Study aims are explored using bivariate correlations, scatterplots, and single predictor regressions due to the novelty of many of these questions and the modest sample of infant cry acoustic feature data that were available for analysis. The following specific aims are examined.

Specific aim 1: Examine the relation of cry persistence to cry pitch and modulation.

Hypothesis: Crying persistence will be positively associated with cry pitch and increased modulation of the cry signal.

Specific aim 2: Examine the relation of infant cry pitch and modulation to vagal tone.

Hypothesis: Infant cry pitch and modulation of the cry signal will be negatively related to vagal tone.

Specific aim 3: Examine how infant cry pitch and its modulation are related to attachment stress.

Hypothesis: Infant cry pitch and modulation of the cry will positively predict attachment stress.

Specific aim 4: Examine how infant cry pitch and modulation are related to social adjustment.

Hypothesis: Higher infant cry pitch and greater modulation of the cry will predict poorer social adjustment in toddlerhood.

To my knowledge, this is the first study to examine the predictive utility of cry acoustic features within a normative (non-ASD risk) sample. The above hypotheses are derived from largely untested predictions from the Polyvagal Theory. Their examination may help inform theoretical models of biopsychosocial development and future empirical models of infant risk for deficits in physiological regulation and social development. In addition, this study is - to my knowledge - the first study to utilize the modulation power spectrum (MPS; Singh & Theunissen, 2003; see below) for the quantification of infant cries. This method may serve as a better approximation of neural processing of sounds in the auditory cortex in comparison to more traditional methods that identify fundamental frequency and individual formants (see below). In addition, the utility of cry modulation as measured by the MPS is compared to mean fundamental frequency and fundamental frequency variability measures to determine whether it may provide an alternative that reduces manual burden of these traditional measures.

CHAPTER 2: METHOD

Participants

Both proposed studies use data collected from two longitudinal samples composed of families from a large Midwestern city (total n = 391). Infants in both studies were recruited via local hospitals, clinics, parenting magazines, and internet advertisements. At recruitment, the combined sample was 49% female, 40% Caucasian, 37% African American or Black, 17% Hispanic or Latino/a, 6% Native American, and 4% Asian or Pacific Islander. Participating families were socioeconomically diverse, with 57% of household incomes totaling $50,000 or more, 27% totaling less than $50,000, and 21% of families receiving public assistance. Study 1 utilizes the full combined sample while study 2 is restricted to the subsample of infants who cried during the lab Still Face procedure for the purpose of assessing their cry acoustic features (n = 37; see below). This subsample was 51% female, 61% Caucasian, 29% African American or Black, and 21% Hispanic or Latino/a. Household incomes were $50,000 or greater in 66% of families, less than $50,000 in 34%, and 23% of families received public assistance.

Study design

In sample 1, (Fussy Baby Study; n = 149) primary caregivers tracked their infants' cry behavior using cry diaries during the first months of life. Families then participated in a series of lab visits, of which the 6-, 12- and 24-month time points will be used for the present analysis. At 6 and 12 months, children's cardiac regulation was measured during social interaction (see below). During this session, parents also completed a series of questionnaires that included the Parenting Stress Inventory (PSI). When children were 24 months, they took part in another series of lab tasks that included a play session with a parent and the stranger approach procedure (Lab-TAB; Goldsmith et al., 1999). During this visit, parents completed the Early Child Behavioral Questionnaire (ECBQ) and Infant-Toddler Social Emotional Assessment (ITSEA). Participants in the second sample were recruited between 6 and 24 months (Growing Baby Study; n = 242). In this sample, 32% (n = 76) children had their first lab visit at 6 months, 35% had their first lab visit at 12 months, 33% had their first visit between 12 and 24 months (n = 78). The average age at first lab visit was 13.09 months (SD = 6.21). At this first lab visit, parents completed a retrospective cry diary report. These families then participated in a series of lab visits with the same procedures as those in sample 1, of which the 6-, 12- and 24-month lab visit data were used for the purpose of analysis.

Cry Persistence Measurement

In sample 1, cry diaries were completed by mothers for three consecutive days when children were approximately 3 months of age. The cry diary form was similar to that used by Barr and colleagues (Barr Baby's Daily Diary; Hunziker & Barr, 1986; Barr, Paterson, MacMartin, Lehtonen, & Young, 2005) and assessed amount, frequency, and duration of fussing/crying bouts (see Supplemental Material A1). These diaries consisted of four, six hour "time-ruler" bars, corresponding to the morning (6 a.m. – noon), afternoon (noon – 6 p.m.), evening (6 p.m. – midnight) and night (midnight – 6 a.m.). Mothers were asked to report their children's states in 15-minute blocks using letter codes that represented sleeping, awake and content, fussing, crying, or unsoothable crying. In both study 1 and 2, parents provided retrospective reports of cry patterns using the History of Cry/Reflux (Boukydis, 2008). These reports were obtained from the families shortly after they were recruited. This form asked parents to retrospectively report whether their infants had periods of persistent crying, age when persistent crying began and ended, and report the total number of hours per day that the infant cried, and the month at which the infant stopped crying for more than 3 hours per day. These reports will be combined to create a binary persistent crying variable (1 = persistent crying between birth and 3 months, 0 = no persistent crying between birth and 3 months).

Cry acoustic feature measurement

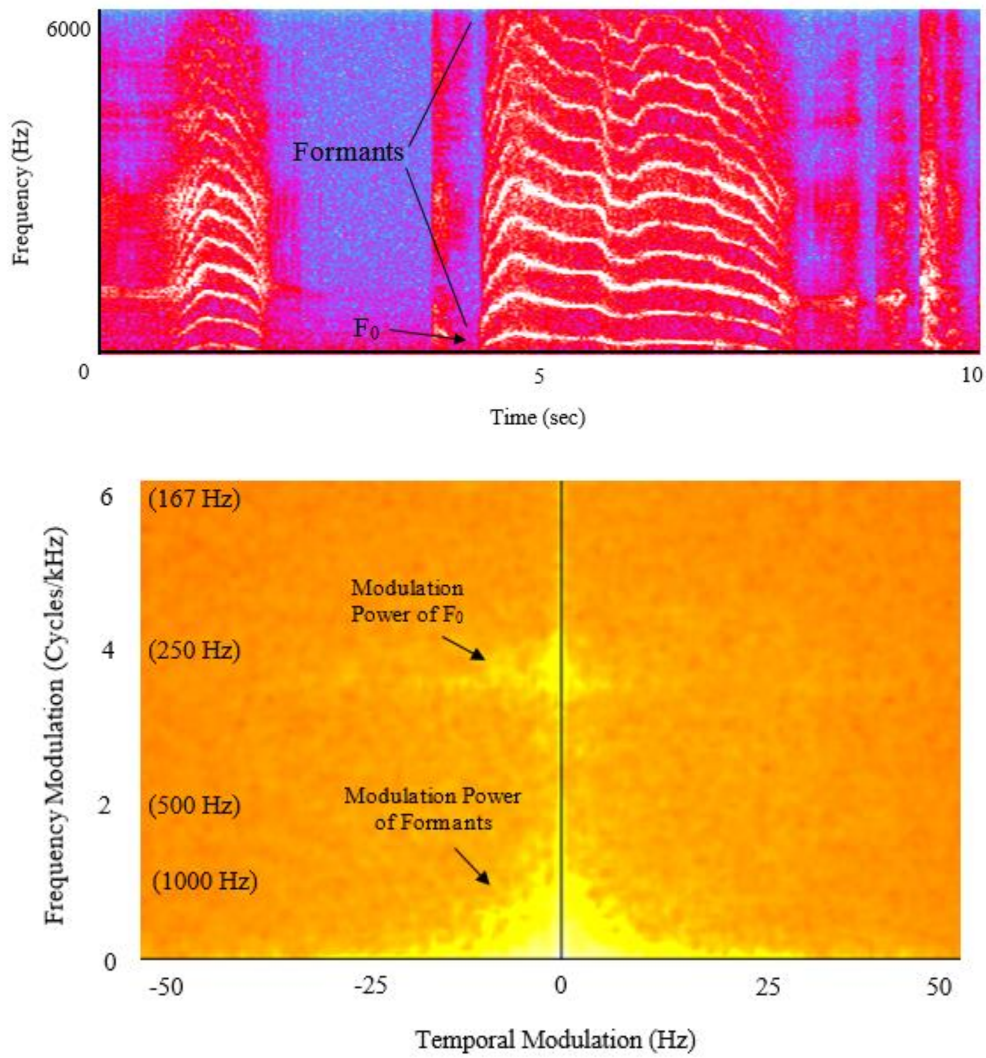
Cry elicitation. At the 6 month lab visit, children took part in a modified Face-to-Face Still Face (FFSF) paradigm (Tronick et al., 1978; Mesman et al., 2011). During the protocol, infants were placed in a high chair with an experimenter seated approximately 50 cm away at the infant's eye level. Mothers were not present. The protocol consisted of three 2-minute conditions: free play, the Still Face episode, and a second free play for recovery. During the first free play, the experimenter playfully interacted with the infant. After 2 minutes, the Still Face condition began. During this time, the experimenter gazed at the infant with a blank expression without moving, touching, or engaging with the infant. This procedure typically induces distress in infants (Mesman et al., 2011). If infants became severely distressed at any point during the procedure, it was terminated and the experimenter resumed interaction with the infant and/or attempted soothing. Once the Still Face episode was completed, the experimenter resumed playful interaction with the infant. Of the full sample, 207 children took part in the Still Face at 6 months (Study 1 n = 127; Study 2 n = 80).

Cry acoustic features. Infant behavior during the Still Face was video recorded and the procedure was coded for infant crying. A total of 49 infants cried during the Still Face. Audio data in the video recordings were processed with a 5500Hz low-pass filter during analog signal acquisition. Audio files were extracted from video files using the Matlab mmread function (Richart, 2005). To examine acoustic features of these vocalizations, audio files were then manually edited to isolate cry vocalizations only. Sounds such as noises associated with infant movement (e.g., kicking), non-cry vocalizations (e.g., fussing, cooing), dysphonation (chaotic vocalizations without harmonic structure), and extended stretches of silence were removed prior to analysis using Audacity software (Audacity Team, 2014). Then, a band-pass filter (0.2 – 6 kHz) was applied prior to acoustic analysis.

The resulting audio files were analyzed for mean fundamental frequency and its variability (see top panel of Figure 3) using the SHRP Matlab function (Sun, 2002-2016). Audio files were first separated into sequential 40ms windows with 30ms overlap. The resulting windows were decomposed using a Fast Fourier transformation. F_0 was identified as the first peak above floor noise within each window and a mean across all windows was computed. Variability was operationalized as the bandwidth in which the fundamental varies between 50% and 100% of its peak energy.

In addition to the fundamental frequency and its variability, relative modulation of fundamental frequencies and formants was assessed using a modified version of the modulation power spectrum Matlab package developed by Singh & Theunissen (2003). The modulation power spectrum (MPS) uses a 2-dimensional Fast Fourier transformation to decompose the time-varying acoustic signals of a spectrogram (Figure 3; top panel) into a two-axis space of spectral (frequency) and temporal (time-related) modulation (Figure 3; bottom panel). Such joint spectro-

Figure 3. Spectrogram and modulation power spectrum example. Top panel: Spectrogram of an infant cry with representations of fundamental frequency (F_0), the lowest band in the vocalization, and formants; Bottom panel: Modulation power spectrum of an infant cry with representations of fundamental frequency (F0) and formants; frequencies coarsely align in decreasing value along the y axis moving away from the origin; temporal changes are represented along the x axis with slower temporal changes near 0 and faster changes to the left and right of the modulation space; frequency down-sweeps are visualized in the right quadrant, up-sweeps in the left



temporal representations of sounds have been found to characterize auditory neuron's higher level processing of isolated natural sounds (Theunissen & Elie, 2014) and organization of the auditory cortex includes features detectors that correspond to specific spectro-temporal patterns (Norman-Haignere, Kanwisher, & McDermott, 2015).Thus, the MPS serves as a functional representation of sound processing that occurs in high level auditory neurons. The MPS was applied to 1.5-second sequential time slices with 50% overlap and averaged over the length of the audio file. Each audio file was assessed for total modulation depth, operationalized using the square root of the ratio between power at origin (0, 0), reflecting unmodulated sound, to power in the rest of the spectrum (Singh & Theunissen, 2003).

Of the 200 children with complete videos of the lab visit, 189 completed the Still Face (*n* = 11 showed distress prior to the Still Face and were deemed too upset to conduct the procedure). Of these, 49 children cried in response (25.93%). Prior to analysis, cry audio files were examined for quality and several files were removed. Reasons for removal included audio files having few cry sounds or total crying time lasting less than 20 seconds (n = 3). In addition, because modulation of the cry exhalation (i.e. wail) was of interest, files were removed if they featured predominantly fussing or choking sounds (n = 8). Finally, one file was removed due to the infant's unusual high-pitched cry (n = 1). This resulted in 37 files for use in the analysis.

Vagal Tone Measurement

Children's cardiac rhythms were monitored using the LifeShirt System (VivoMetrics, San Diego, CA), a non-invasive continuous ambulatory monitor, during the entire lab protocol. Three Ag/AgCl ECG self-adhering electrodes (Covidien, Mansfield, MA) that were used for data collection were applied at the beginning of the session by a female laboratory assistant. Beat-to-beat heart periods were extracted for the segments of interest. At the 6 month visit, these segments were a play session between the mother an infant, a play session with the experimenter that preceded the Still Face, and the Still Face. At the 12 month visit, data was used from the mother-child play session only. Once collected, heart period data derived from the ECG signal were edited for artifacts using CardioEdit software (Brain-Body Center, Chicago, IL). The resulting files were analyzed for respiratory sinus arrhythmia (RSA) magnitude, the variance in the beat-to-beat heart rate pattern in the frequency band associated with spontaneous breathing (.24 to 1.04 Hz in young children). RSA was computed for 30-second epochs using algorithms developed by Porges and Bohrer (1990; Porges, 1985) and implemented in CardioBatch software (Brain-Body Center, Chicago, IL). In study 2, the means of the 30 second epochs for each segmented were calculated.

Attachment stress measurement

At the 6- and 12- month lab visits, primary caregivers completed a set of questionnaires that included the Parenting Stress Index (PSI-3; Abidin, 1995), a 101-item instrument. Most items are scored on a 5-point Likert-type scale (1 = strongly agree, 5 = strongly disagree). This analysis uses the Attachment Stress subscale, which can reflect two related sources of stress. These sources may be lack of emotional closeness with the child or a perceived inability to observe and understand the child’s feelings and/or needs accurately. Items include “How easy is it for you to understand what your child wants or needs?” and “I expected to have closer and warmer feelings for my child than I do and this bothers me”. Coherence of this subscale has been supported by factor analysis. Internal consistency has been reported to be acceptable ($\alpha = .75$; Abidin, 1995; although more appropriate methods for measuring internal consistency are available, Cronbach's alpha is reported for parent reports due to their use by the measures' original authors). PSI data are available for 289 families (6 months n = 189, 12 months n = 242).

Social adjustment measurement

Stranger Approach. At the 24 month lab visit children participated in a series of laboratory tasks that included the Stranger Approach procedure, which was developed as part of the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith et al., 1999; Gagne et al., 2011). During this task, children are allowed to play with a set of toys while the mother is seated in a far corner of the room. The experimenter asks the child to wait while she gets the next activity and leaves the room. After several seconds, there is a knock on the door. A male stranger enters and attempts to engage the child in conversation and play using a friendly voice while gradually moving toward a chair near the child and sitting down. After several attempts at interaction the stranger leaves and the familiar experimenter returns. The procedure was terminated if children showed extreme distress, which resulted in truncated episodes for some children. For the present purpose, the periods of interest are the first three 10-sec blocks following the stranger's entry, which a majority of children completed. Children's behavior during these segments was coded for the intensity of avoidance and degree of verbal hesitancy displayed to the stranger. Avoidance represents the peak intensity of behaviors initiated to increase or maintain distance to the stranger. Examples of avoidance include moving to the far corner of the room. Verbal hesitancy reflecting verbal responses and conversation initiation with the stranger. Lack of hesitancy describes neutral or eager responses to stranger's questions and initiation of conversation. Hesitancy describes no responses to the stranger's questions and no initiation of conversation. A total of 111 children have complete data for this procedure.

Early Childhood Behavioral Questionnaire (ECBQ). When children were 24 months old, parents completed the ECBQ (Putnam, Gartstein, & Rothbart, 2006), a 201-item measure designed to assess temperament characteristics in children between ages 18 and 36 months. Items are on a 7-point Likert-type scale reflecting the typicality of toddler's behavior during the two weeks immediately prior to the assessment (1 = never, 7 = always). Subscales are designed to aggregate behavior across a variety of contexts and eliciting events, demonstrating moderate to strong longitudinal stability correlations between 18 and 36 months (Putnam, Gartstein, & Rothbart, 2006). The present analysis will utilize the Sociability and Shyness subscales, which have been found to reflect distinct aspects of social adjustment in children (Putnam, Gartstein, & Rothbart, 2006). Sociability assesses the extent to which children seek and take pleasure in interactions with familiar others. Items in this subscale include "When around large gatherings of familiar adults or children, how often did your child want to be involved in a group activity?" and "When a familiar child came to your home, how often did your child seek out the company of the child?". Internal consistency for this subscale has been found to be high for children aged 24-months (α = .89; Putnam, Gartstein, & Rothbart, 2006). The shyness subscale assesses the extent to which children show slow or inhibited approach and/or discomfort in social situations that involve novelty or uncertainty. Items in this subscale include "When approaching unfamiliar children while playing, how often did your child watch rather than join?" and "In situations where s/he is meeting new people, how often did your child become quiet?". Internal consistency for this subscale has also been reported to be very good (24 months: α = .82; Putnam, Gartstein, & Rothbart, 2006). ECBQ data are available for 219 children.

Infant-Toddler Social and Emotional Assessment (ITSEA). The ITSEA is a 166-item questionnaire designed to assess multiple aspects of young children's social emotional development (Carter et al., 2003). Items range on a 3-point scale based on how well the item describes the target child or how often the target child engages in a type of behavior: 1) Not true/rarely; 2) Somewhat true/sometimes, 3) Very true/often. Test-retest scores over a 1.5 month interval were moderate to high for individual subscales (r range = .69 - .85). The prosocial peer relations and social relatedness index will be utilized from this study. The prosocial peer relations is a 5-item subscale that includes items such as “Plays well with other children” and “Takes turns when playing with others”. Internal consistency for this subscale has been found to approach acceptability (α = .66; Carter et al., 2003). The social relatedness index is composed of 10 items and includes items such as “Likes being cuddled, hugged, or kissed by loved ones”. Internal reliability for this subscale has been found to be α = .56 (Carter et al., 2003). ITSEA data are available for 239 children at age 24 months.

Analytic Plan

Descriptive and exploratory data analysis was conducted using SAS 9.4 (SAS Institute, Cary NC). Latent variable extraction and structural equation modeling was conducted using Mplus version 7.2 (Muthén & Muthén, 1998-2014). Figures were created using R software version 3.1.2 (R Core Team, 2014) and RStudio version 0.98.1091 (RStudio, Inc., 2009-2014). Prior to analysis, data were examined for completeness and systematic patterns of missingness. Structural equation models were estimated using WLSMV in Mplus (Muthén & Muthén, 1998-2014). This method provides weighted least square parameter using a diagonal weight matrix with standard errors. The mean- and variance-adjusted chi-square test statistic of this method uses a full weight matrix. Where possible, model results were compared to full information maximum likelihood (FIML; also known as “direct ML” and “casewise ML”). However, several models estimated using FIML did not converge. WLSMV estimates, which did not substantially differ from FIML results in compared models, are reported for consistency.

Study 1. The first study, using the total combined sample (*n* = 391), examines the role of infant crying persistence in the development of individual differences in social adjustment. Further, the potential mediating roles of vagal tone and parent attachment stress in this link are examined (see conceptual model in Figure 2). Measurement models are first examined, followed by structural models.

To assess the cry persistence measurement model, reliability of cry diary data was assessed using a simplex model. This method entails using longitudinal structural equation modeling to estimate a latent variable for each observed measure that represents a “true” score taking into account measurement error (Heise, 1969; 1970; Joreskog, 1979; Wiley & Wiley, 1970). In this application, day 1 daily crying true scores was used to estimate day 2 true scores, which, in turn, was used to estimate day 3 true scores. The prediction of each day’s score from the previous day’s score was used to assess reliability. In order to permit model identifiability, the model was specified with a stationary error variance assumption (Biemer, Christ, & Wiesen, 2009). Once reliability of cry diary data was assessed, a latent factor measurement model for cry persistence was examined. In sample 1, data from cry diaries was used to extract a latent crying persistence factor. Then, this factor was used to predict retrospective parent reports of cry persistence in the first 3 months of life.

Next, the social adjustment measurement model was assessed using a combination of exploratory and confirmatory factor analysis. Avoidance and verbal hesitancy to the stranger approach lab task, the sociability and shyness subscales of the ECBQ, and the prosocial peer relations and social relatedness subscales of the ITSEA were examined for inter-correlations and measures with substantial convergence were fit to a latent factor model. For this purpose, all participants (n = 391) were randomly divided into an exploratory and confirmatory group. In the exploratory group, an exploratory factor analysis (EFA) was conducted. Factor retention was guided by examination of the scree plot (Cattell, 1966), model fit, and simple structure among factor loadings. Model fit was evaluated using the root mean squared error of approximation and its 90% confidence intervals (*RMSEA*; Steiger & Lind, 1980; Steiger, 1990), the Tucker-Lewis Index (*TLI*; Tucker & Lewis, 1973); the Relative Noncentrality Index (*RNI*; McDonald & Marsh, 1990), and the Bayesian Information Criterion (*BIC*, calculated as $\chi 2\text{-}[df*(\ln(n))]$; Schwartz, 1978; Bollen et al., 2014). As suggested by Hu and Bentler (1999), good fit to the data was assessed by an *RMSEA* value near .06 or lower as well as *TLI* and *RNI* values near .95 or greater. The *BIC* fit index was used to examine whether the tested model was superior to the full saturated one (reflected by negative values). The resultant factor structure was then tested on the other half of the sample using a confirmatory factor analysis, using the model fit criteria described above. The two samples were then recombined for confirmatory factor extraction in order to assess relations with predictors in all further modeling, ensuring maximum power for detecting effects. The final measurement model for latent vagal tone was assessed at 6 and 12 months using the above-described model fit criteria.

To test the structural models, the resulting social adjustment factor was regressed on the cry persistence factor. Mediating variables were added cumulatively to allow for close examination of potential model misspecification problems. Note that typically paths indicated by one-headed arrows in structural equation modeling are substantively interpreted as causal. In the tested model, the path predicting 6 month vagal tone from cry persistence is strictly predictive. As outlined in the theoretical review above, vagal tone is expected to influence the propensity for crying persistence. However, given the nature of the study aim to assess whether infant crying can be used as a risk indicator and the timing of cry persistence which precedes the 6 month vagal tone collection, cry persistence was included as a predictor of 6-month latent vagal tone when assessing indirect effects. Otherwise, the two variables are specified as covarying. The indirect effects of the mediation paths were examined as compound parameters (Bollen, 1987).

Study 2. Study 2 examines cry acoustic features of children who cried during the Still Face elicitation and their relation to infant crying persistence, vagal tone, parent attachment stress, social adjustment. The goal of this analysis is exploratory and results are intended to inform future model building. Due to the limited sample size (n = 37), only bivariate associations are examined. To examine whether children that cried during the Still Face were at heightened risk for unusual cry features, low vagal tone, heightened attachment stress, and social adjustment deficits (see above), all these key variables were examined for mean and variance differences between children who cried and those who did not.

CHAPTER 3: RESULTS

Study 1

*Missingness analysis***.** Predictive patterns of missing values were examined for all study variables using logistic regressions. Six-month attachment stress percentile score negatively predicted missing values on 24-month parent-reported sociability (B = -0.418, SE = .1742, p = .017, OR = .659). Parent-reported sociability could thus be considered missing at random and ignorable in models where 6-month attachment stress is included (Allison, 2001). Missing values on all other variables were unrelated to the values on any previous time point.

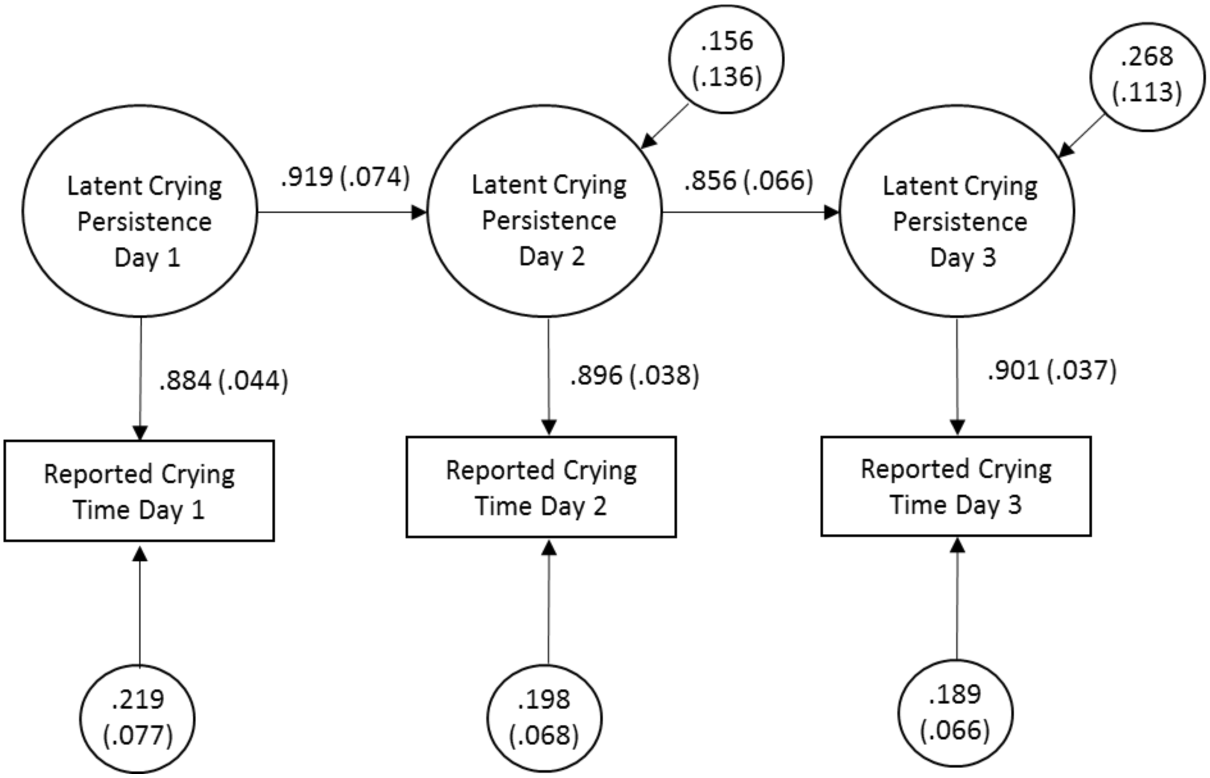
Reliability of cry diary data. Parent reports of infant crying time were positively skewed and day 2 and 3 reports were highly kurtotic. Thus, these variables were transformed to adhere to normal distribution assumptions using the equation y = (ln(x + 60)). Raw and adjusted variable descriptive statistics are presented in Table 1. The transformed variables were used for all further analysis. Next, daily cry diary reports were assessed for day-to-day reliability using a simplex model. The results showed that parent reports of daily cry hours were highly stable (Day 1/Day 2 β = .919, SE = .074; Day 2/Day 3 β = .856, SE = .066; see Figure 4). These reliability estimates did not significantly differ from one another (Wald's χ^2 = 0.00, df = 1, p = .998). Thus, cry persistence over the 3 measurement days was found to be stable.

Convergence of retrospective reports. According to retrospective reports, 21.09% (N = 66) of parents rated their infant as persistently crying around the age of 3 months. However, parents in the fussy baby study sample – who also completed cry diaries at 3 months of age were

Table 1. 3-month cry diary variable descriptive statistics

Variable	***N***		***Mean***	***SD***	***Min***	***Max***	***Skew***	***Kurtosis***
Day 1 Crying (Minutes)	125	Raw	131.90	98.04	0.00	420.00	0.99	0.56
		Adjusted	5.13	0.51	4.09	6.17	-0.05	-0.58
Day 2 Crying (Minutes)	119	Raw	134.21	115.06	0.00	735.00	1.92	5.86
		Adjusted	5.13	0.53	4.09	6.68	0.25	-0.24
Day 3 Crying (Minutes)	117	Raw	135.37	108.07	0.00	525.00	1.16	1.36
		Adjusted	5.13	0.55	4.09	6.37	-0.01	-0.70

Figure 4. Simplex model assessing prospective cry diary reliability over three days; all latent variables have mean of 0, day 1 latent variable variance set to 1



less likely to retrospectively report their infants as having persistently cried (5.57%, $n = 5$) than those parents in the growing baby sample, who did not complete cry diaries (27.0%, $n = 61$; $\chi^2 = 17.04$, $df = 1$, $p < .001$). This discrepancy in rates of reporting persistent crying was examined in relation to age of retrospective reports. All families in the fussy baby sample – who had cry diary data – completed retrospective cry reports when children were 24 months. However, families in the growing baby sample completed retrospective reports at their first visit, which for the majority of families was prior to 24 months and, for some families, as early as 6 months. To test whether report timing was responsible for the reporting discrepancy, the retrospective persistent crying designation was regressed on age of retrospective report collection in the growing baby sample. The results did not support an age-related difference in probability of retrospectively reporting a child as having cried persistently at age 3 months ($B = .026$, $SE = .024$, $p = .293$, $OR = 1.026$). Thus, the reason for the discrepancy in categorizing persistent crying by study remains unknown. Though it was decided that the convergence of retrospective reports and cry diaries would be assessed as planned, this surprising result highlighted the need to compare all future models for consistency between a cry diary-only measurement model and that which includes retrospective reports as well.

Combined cry diary and retrospective report measurement model. The three days of cry diary reports were well described by a single latent factor. Standardized factor loadings were .812, .896, and .771, for day 1, day 2, and day 3, respectively. The variance explained by the latent cry persistence factor ranged from $R^2 = .59$ to .80. Next, this model was expanded to include the retrospective persistent crying report. Addition of the retrospective report did not substantially alter the factor structure contribution of the cry persistence diary reports and this model performed about as well at explaining the variance of the observed variables as the cry diary report only (R^2 range = .62 to .82; for more information see Supplemental Material Figure A2). The crying persistence latent variable was strongly associated with the retrospective cry report, with a 1 *SD* increase in the cry persistence latent variable increasing the odds of a retrospective report of persistent crying by 8.53 times (B = 2.144, SE = .513, p < .001). Because the cry diary data may have contributed unique shared method variance, a model was tested that allowed the 3 cry diary observed reports to covary beyond their association with the latent factor. The resulting covariances were not significant and their inclusion yielded a model with very large standard errors for all estimates. Thus, these were not included in the final measurement model.

Social adjustment measurement model. Measured social adjustment variable descriptive statistics are presented in Table 2. Raw values for sociability and social relatedness showed substantial skew and kurtosis. Sociability was transformed using $e^{(x/2)}$ and social relatedness was transformed using $e^{(x*2)}$. The resulting transformed distributions improved skewness in both measures and kurtosis in sociability. Sex was unrelated to differences in all social adjustment variables (all $p > .05$).

Correlations among continuous social adjustment variables are presented in Table 3. Sociability was negatively related to shyness and positively related to peer relations and social relatedness. In addition, peer relations was positively related to social relatedness. There was no apparent relation between shyness and peer relations or social relatedness. All significant correlations were low. ANOVA models were used to assess differences in continuous variables between children who showed avoidance and verbal hesitancy during the stranger approach and those who did not. Children who showed avoidance during the stranger approach did not differ from those that didn't show avoidance on any parent-reported social adjustment variables (all p >

Table 2. Social adjustment measured variable descriptive statistics.

Parent Reports	***N***	***M***	***SD***	**Min**	**Max**	**Skew**	**Kurtosis**
Sociability	219	5.57	0.92	2.13	7.00	-0.82	0.62
Sociability (adjusted)	219	17.78	7.10	2.89	33.12	0.23	-0.58
Shyness	219	3.30	0.88	1.44	6.00	0.42	-0.04
Peer Relations	212	1.15	0.41	0.20	2.00	-0.06	-0.38
Social Relatedness	239	1.71	0.21	0.90	2.00	-0.88	0.77
Social Relatedness (adjusted)	239	32.89	11.91	6.05	54.60	0.04	-0.80

Stranger Approach Observation	***N***	**Freq Yes**	**% Yes**
Avoidance	111	69.00	62.16
Verbal Hesitancy	111	64.00	57.66

Table 3. Social adjustment continuous variable Pearson correlations

	Sociability	**Shyness**	**Peer Relations**	**Social Relatedness**
Sociability (adjusted)		-0.22*	0.17*	0.26*
Shyness			0.00	-0.01
Peer Relations				0.18*
Social Relatedness (adjusted)				

* $p < .05$

.05). Children that showed verbal hesitancy during the stranger approach were rated by parents as more shy (F (1, 98) = 4.00, p = .048, R^2 = .040) and as having poorer peer relations (F (1, 92) = 4.64, p = .034, R^2 = .049). No differences between these two groups of children were found on sociability (F (1, 98) = 0.40, p = .529, R^2 = .004) or social relatedness (F (1, 102) = 0.04, p = .839, R^2 < .001; see Supplemental Materials Figure A3). Finally, the relation between avoidance and verbal hesitancy during the stranger approach was examined using a Chi-Square test. These two variables were unrelated χ^2 (1, N = 111) = .096, p = .756. Given the lack of relation of observed stranger avoidance to other social adjustment indicators, avoidance was not included in the factor analysis.

To prepare for factor analysis, the variables were randomly separated into two datasets for EFA and CFA. The resulting datasets did not differ in values on any indicator variables, as assessed by ANOVA comparisons and a Chi-Square test for verbal hesitancy (all p > .05). The exploratory factor analysis sample consisted of 195 cases. Based on fit indices, the 1-factor solution fit the data better than the 2-factor solution (see Table 4). For this solution, the RMSEA 90% confidence interval was below .06, the TLI and RNI were above .95, and the negative BIC value demonstrated that this model was superior to one that is fully saturated. The two-factor model loadings did not have a simple structure and some indicators did not have substantial loadings on either factor; thus, the one-factor structure was retained given its parsimony and adequate fit to the data. Loadings for the 1-factor model are presented in Table 5. As suggested by the data exploration of indicator correlations, the observed sociability, peer relations, and social relatedness variables were all related to the single factor but loadings for shyness and hesitancy were non-significant. Only significantly-loading variables were included in the confirmatory factor analysis for the remaining half of the sample (these three variables also

Table 4. Exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) model fit statistics

Factors	χ^2	***df***	***RMSEA***	***RMSEA* 90% Confidence Intervals**		***TLI***	***RNI***	***BIC***
				5-Indicator EFA				
1	1.322	5	.000	.000	.035	5.183	3.091	-22.574
2	0.785	1	.000	.000	.159	6.263	1.526	-4.705
				3-Indicator CFA				
1	0.000	0	.000	.000	.000	1.000	1.000	0.000

RMSEA = root mean square error of approximation; *TLI* = Tucker-Lewis Index; *RNI* = Relative Noncentrality Index; *BIC* = Bayesian Information Criterion

Table 5. Standardized exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) factor loadings

Indicator	**EFA**	**CFA**	
		Loading	R^2
Sociability	.447*	.538*	.290
Shyness	-.002		
Peer Relations	.346*	.367*	.135
Social Relatedness	.442*	.573*	.329
Verbal Hesitancy	-.291		

* $p < .05$

reflected a clearer structure when compared to the full five-factor variables in EFA scree plots; for more information see Figure A4 in the Supplementary Material). The CFA factor loadings for these three indicators were similar to those observed in the EFA, suggesting a convergence between the two methods (see Table 5). The explained variance of the indicators by the factor was low but non-trivial (R^2 range = .135 - .329). Thus, the single factor as measured by the sociability, peer relations, and social relatedness parent reports was retained for full model testing. This factor captures relatively long term social relations aspects of children's social adjustment, as opposed to interactions with unfamiliar persons. Interestingly, the variables measuring responses to unfamiliar persons – parent-reported shyness, verbal hesitancy and avoidance during the stranger approach – did not converge on a single factor. Thus, the identified factor was interpreted as reflecting social relatedness.

Vagal tone measurement model. Vagal tone was indexed using RSA quantified from alternating 30-second blocks drawn from the first five minutes of an unstructured parent-child interaction at age 6 months and 12 months. 30-second epochs are widely used for RSA measurement as they provide a long enough time window to estimate variance of heart period variability in the respiratory frequency. Descriptive statistics for these blocks are presented in Table 6. A latent variable was used to isolate the underlying vagal tone during the mother-child social interaction from attention or engagement influences that may be specific to any particular epoch. The indicator variables were all normally distributed (skew and kurtosis were less than .80 or greater than -.80). Correlations between epoch values were moderate to high within each time point and low but significant between 6- and 12-month time points (see Supplementary Material Table A1). There was no evidence of systematic differences in RSA as a function of epoch at either 6 months (F (4, 886) = 0.86, p = .485) or 12 months (F (4, 1074) = 1.78, p =

Table 6. Vagal tone indicator descriptive statistics

Age (months)	**Epoch**	***N***	***M***	***SD***	**Min**	**Max**	**Skew**	**Kurtosis**
6	1	184	3.413	1.126	0.744	6.367	0.178	0.066
	3	183	3.449	0.987	0.785	6.404	0.254	0.359
	5	179	3.327	0.915	0.742	5.585	-0.109	0.133
	7	176	3.328	0.883	0.592	5.607	-0.100	0.212
	9	169	3.289	0.873	1.030	5.536	-0.142	-0.264
12	1	216	4.288	1.191	1.155	8.156	0.402	0.189
	3	216	4.173	1.112	1.232	7.407	0.398	0.165
	5	216	4.096	1.072	1.658	7.403	0.553	0.422
	7	216	4.111	1.164	1.075	8.221	0.404	0.498
	9	215	4.008	1.152	1.659	8.362	0.607	0.334

.130). The latent vagal tone model fit the observed data well at both time points (see Table 7). Factor loadings were scaled by the first epoch within each time point (i.e., this loading was set to one) and the remaining loadings ranged from .747 to .938 at 6 months and .968 to 1.005 at 12 months. A between-person difference of 1 *SD* in latent vagal tone at 6 months predicted a .493 *SD* between-person difference at 12 months, indicating moderate stability in latent vagal tone over time ($SE = .082$, $p < .001$).

Study one aimed to examine the relations of cry persistence around 3 months of age with social relatedness in toddlerhood and explore the potential mediators of attachment stress and vagal tone in this relation. The cry persistence, social relatedness, and vagal tone measurement models were utilized as described above. Attachment stress percentile was measured as a single indicator latent variable with error variance specified according to the internal consistency of the scale, which was found to be $\alpha = .75$ in a large, normative sample (Abidin, 1995). Thus, the error variance at each time point was set to .25 (1 - .75). Prior to standardization, a paired t-test showed that attachment stress percentile at 6 months ($M = 34.79$, $SD = 28.11$) did not systematically differ from stress percentile at 12 months ($M = 35.78$, $SD = 28.89$; t (153) = - 1.16, $p = .248$). Thus, attachment stress percentile scores were not found to systematically increase or decrease over time and were standardized within time point to allow ease of interpretation. Descriptive statistics for continuous variables are presented in Table 8. On average, children cried 135 minutes per day and mean daily crying time ranged from 0 to over 500 minutes. Single day crying time measures were adjusted for normality using a ln (x + 60) transformation (see Chapter 3). As expected, RSA increased from 6 months of age ($M = 3.37$, $SD = 0.80$) to 12 months of age ($M = 4.10$, $SD = .97$; t (112) = - 8.75, $p < .0001$).

Table 7. Latent vagal tone measurement model fit statistics

Model	***N***	χ^2	***df***	***RMSEA***	***RMSEA* 90% Confidence Intervals**		***TLI***	***RNI***	***BIC***	**MV Mean R^2**
6 Month	184	7.013	5	.047	.000	.120	.992	.996	-19.062	.652
12 Month	216	13.115	5	.087	.030	.146	.984	.992	-13.761	.791
Combined	287	72.302	34	.063	.042	.083	.968	.976	-120.12	.720

MV = Measured variable

Table 8. Study 1 measured variable descriptive statistics; RSA = respiratory sinus arrhythmia; STD = Standardized

Variable	**Age (months)**	***N***	***M***	***SD***	***Min***	***Max***	***Skew***	***Kurtosis***
Mean Daily Crying (min)	3	125	134.780	97.721	0.000	515.000	1.276	1.644
Mean Daily Crying (ln)	3	125	5.132	0.476	4.094	6.298	0.227	-0.499
Mean RSA (ln msec^2)	6	184	3.376	0.805	0.960	5.547	0.015	0.162
	12	216	4.101	0.972	1.893	8.225	0.610	1.019
Attachment Stress (STD)	6	189	0.000	1.000	-1.202	2.248	0.649	-0.855
	12	242	0.000	1.000	-1.204	2.188	0.718	-0.787
Peer Relations (STD)	24	212	0.000	1.000	-2.313	2.073	-0.060	-0.377
Sociability (STD)	24	219	0.000	1.000	-2.098	2.160	0.227	-0.583
Social Relatedness (STD)	24	239	0.000	1.000	-2.253	1.822	0.041	-0.803

Correlations among the measured variables are presented in Table 9. Mean daily crying at 3 months was negatively related to peer relations and sociability at the trend level ($p < .10$). Mean daily crying at 3 months was unrelated to mean RSA during a mother-child interaction at 6 months of age. This association was examined for non-linear associations by examination of a scatterplot and a regression with mean daily crying entered as a quadratic predictor (inclusion of daily crying and daily crying squared terms). The squared parameter was non-significant (p = .531), suggesting that the relation between daily crying and mean RSA at six months was not curvilinear. Mean daily crying was, however, positively related to mean RSA during a mother child interaction at the 12-month assessment at the trend level. The use of a quadratic parameter as a predictor in a multiple regression equation was non-significant (p = .224) and thus provided no evidence that this relation was better described as a curvilinear relation. Mean daily crying was significantly related to 6- and 12-month mother-reported attachment stress. Mean RSA demonstrated moderate stability between 6 and 12 months. However, there were no significant relations between mean RSA and attachment stress at any time point. Finally, mean RSA at 12 months had a trending negative correlation with toddler social relatedness. Attachment stress demonstrated moderate stability between time points. Six-month attachment stress was negatively correlated with peer relations and social relatedness at 24 months. Twelve-month attachment stress was negatively related with peer relations and sociability at 24 months. Finally, as seen in the previous chapter, measured social relatedness variables all demonstrated low correlations.

Final model examination. The final model was constructed iteratively, to allow for assessment of sources of model misfit. No such indication was found. The final model, with standardized estimates, is presented in Figure 5. This model fit the data well (see insert in

Table 9. Study 1 measured variable correlations; RSA = respiratory sinus arrhythmia

Age (months)	Variable	2	3	4	5	6	7	8
3	1. Mean Daily Crying (ln)	.01	.22†	.21*	.20*	-.21†	-.19†	-.15
6	2. Mean RSA		.54*	-.07	-.05	-.01	-.05	-.03
12	3. Mean RSA			-.04	.06	-.12	-.07	-.15†
6	4. Attachment Stress				.59*	-.20*	-.11	-.21*
12	5. Attachment Stress					-.16*	-.15*	-.10
24	6. Peer Relations						.17*	.18*
24	7. Sociability							.26*
24	8. Social Relatedness							

†*p* < .10; * *p* < .05

Figure 5. Final study 1 model with standardized estimates; solid lines represent parameters significant at p < .05; dashed lines represent parameters significant at p >/= .05; RSA = respiratory sinus arrhythmia

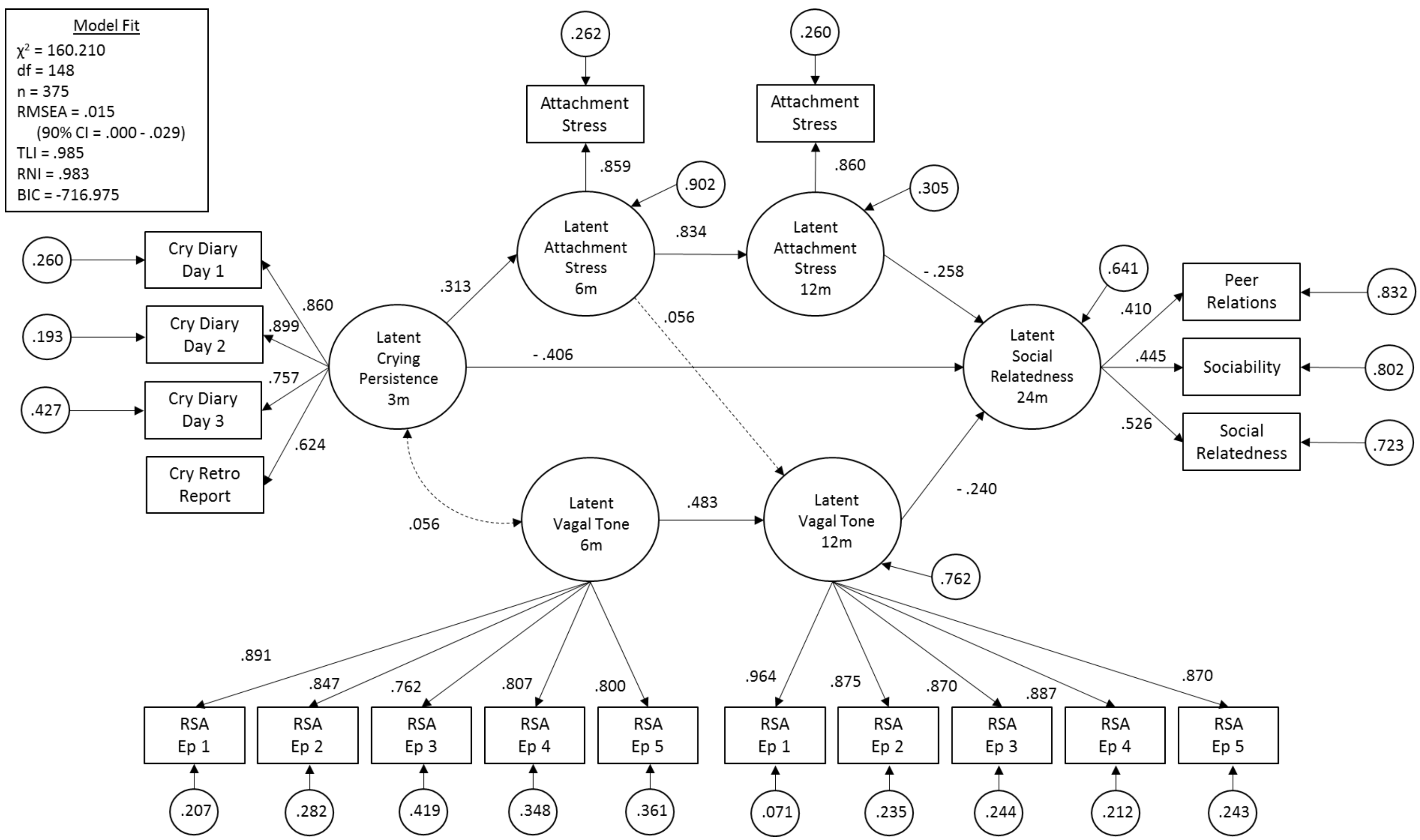


figure). Latent crying persistence negatively predicted social relatedness. In addition, latent crying persistence positively predicted 6-month attachment stress. 12-month attachment stress was, in turn, predicted by 6-month attachment stress. Finally, 12-month attachment stress negatively predicted social relatedness. Latent cry persistence did not covary with 6-month vagal tone. However, 6 month vagal tone did predict 12-month vagal tone, which – in turn – negatively predicted social relatedness in toddlerhood. There was no effect of 6-month attachment stress on 12-month child vagal tone. No substantial differences were observed when the model was re-run using the maximum likelihood estimator with standard errors computed via a sandwich estimator (MLR in Mplus). This alternate estimator, however, resulted in several instances of model non-convergence and thus WLSMV results are reported.

Indirect effect testing. Several planned indirect effects were tested to examine mediation pathways. Standard errors were computed using bias-corrected bootstrapped confidence intervals (as recommended by MacKinnon, Lockwood, & Williams, 2004). First, the path between crying persistence and social relatedness through vagal tone was examined. In order to test this indirect pathway, the relation between latent cry persistence and 6 month vagal tone was specified as a regression, rather than covariance, which did not alter the model estimates substantially. The mediating path was non-significant ($\beta = -.006$, *95% CI*: -.036, .024, $p > .100$). Next, the mediating path through attachment stress was examined. This path was also non-significant ($\beta = -.067$, *95% CI:* -.172, .037, $p > .100$). Finally, a joint-mediation indirect effect of crying persistence on social relatedness through 6-month attachment stress and 12-month vagal tone was examined. It, too, was non-significant ($\beta = -.004$, *95% CI:* -.036, .027, $p > .100$). To examine the potential long-term effects of crying persistence on attachment stress, a post-hoc test was used to examine the path between 3-month crying persistence to 12-month attachment stress via 6-month attachment stress. This path was significant ($\beta = -.261$, *95% CI:* .042, .484, $p < .050$), indicating that high crying persistence at 3 months may have long term effects on the parent-child relationship that extend at least as far as 12 months of age.

Planned comparisons with alternate models. To examine whether potential measurement error caused by different retrospective reporting of persistent crying may have affected the results, a model with the retrospective report removed was estimated. The resulting model fit the data well and the magnitude of effects in this model were substantially comparable to the model with retrospective reports included. Thus, there was no apparent evidence that measurement bias caused by retrospective reporting affected the model results.

Ad-hoc comparisons with alternate models. To examine the potential effect of different retrospective reporting levels of persistent crying between samples (see Chapter 3), a model that included the effect of sample on the retrospective report was tested. While the dichotomous sample variable did predict the rate of retrospective persistent cry reporting, subsequent model testing showed that it did not affect any other latent variable and the resulting model interpretation was unchanged. Thus, the previously reported final model, sans dichotomous sample variable, was used for ease of interpretation and parsimony.

Second, a 12-month only mediator model was also examined to assess whether evidence for mediation via 12-month attachment stress could be observed. This variation was examined in case the 2-path mediation model may result in increased power to detect mediation over a 3-path mediation model. This model also examined whether latent cry persistence may be a predictor of 12-month latent vagal tone, as suggested by the correlation table. This alternative model did not result in better support for the theoretical model and thus the initial model was retained.

Study 2

Study 2 examined the relation between children's cry acoustic features and vagal tone, parent-reported attachment stress, and social adjustment in toddlerhood. Cry acoustic features were assessed in children that cried during the Still Face procedure.

Differences in key variables as a function of inclusion in study 2. All key variables were examined for differences among children that were included in study 2 (i.e., those who cried during the Still Face) and those that were not (i.e., did not cry). First, these group were compared on vagal tone during the Still Face. As expected, children that cried had lower vagal tone (M = 2.823, SD = .943) than those that did not cry (M = 3.411, SD = .914, t (169) = 3.57, p < .001), suggesting a higher level of social engagement system withdrawal during crying and recruitment of metabolic resources for mobilization. The variance of vagal tone did not differ between the crying and non-crying groups, as assessed by Browne-Forsythe tests (all p > .05). Next, all other key analysis variables were analyzed for mean and variance differences between children that cried during the Still Face and those that did not. These key variables were mean crying time at 3 months, parent-reported attachment stress at 6 and 12 months, vagal tone during the mother-infant interaction at 6 and 12 months, as well as peer relations, sociability, and social relatedness at 24 months. A series of independent samples t-tests showed that there were no significant mean differences between these groups on all key variables (all p > .05). Browne-Forsythe tests showed that the variances of these key variables did not differ between groups (all p > .05). These results were substantially consistent when all children that cried during the Still Face were considered (n = 49) or when this group was reduced to only those used in the analysis (n = 37; see above). These results support the hypothesis that children who cried during the Still Face were representative of the full sample on all study variables and that the crying group did not have less variability on study variables than the children that did not cry.

Fundamental frequency cry variables. Fundamental frequency and 50% fundamental frequency bandwidth variables were natural log transformed to adjust for skewness and kurtosis (see Table 10 for descriptive statistics). These aspects of fundamental frequency were uncorrelated and did not correlate with modulation depth (all $p > .10$). Cry fundamental frequency was unrelated to vagal tone, attachment stress, or social adjustment outcomes (all $p > .10$). The 50% bandwidth of the fundamental frequency positively predicted attachment stress at 6 months ($\beta = .405$, $SE = .160$, $p = .027$, $R^2 = .167$; see Supplementary Material Figure A5).

Cry persistence and cry modulation depth. Cry persistence at 3 months was trending to predict higher cry modulation depth at 6 months ($\beta = .437$, $SE = .238$, $p = .084$, $R^2 = .166$; see top left panel of Figure 6). Examination of residuals suggested the possibility of a curvilinear relation. However, addition of a curvilinear parameter (i.e., squared cry persistence) to the regression equation did not result in a significant parameter ($p = .604$), nor a substantial increase in explained variance ($\Delta R^2 = .014$).

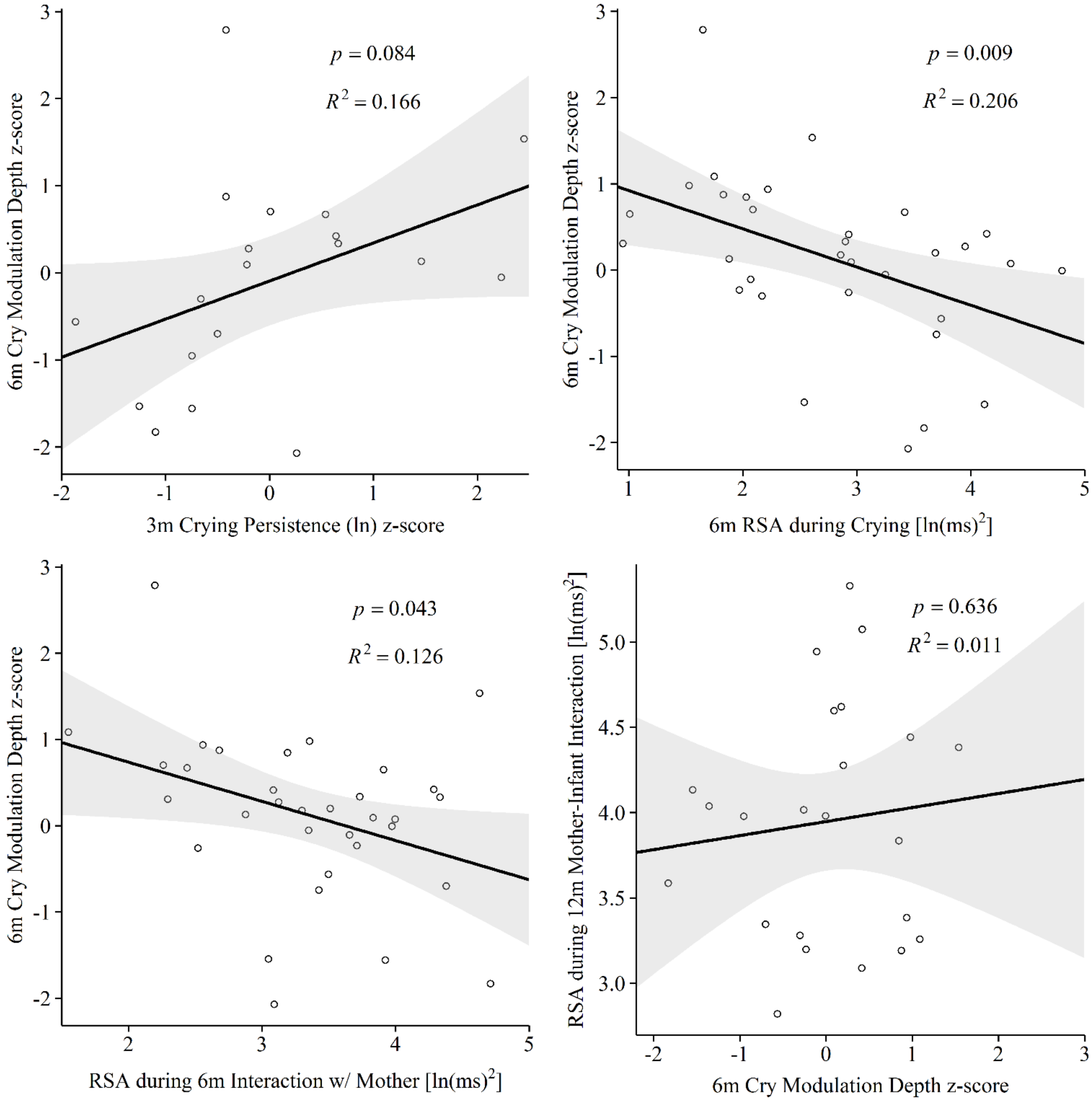
Cry modulation depth and vagal tone. Vagal tone during crying was negatively related to modulation depth, with a 1 standard deviation increase in vagal tone corresponding to a .421 standard deviation decrease in modulation depth ($SE = .151$; see top right panel of Figure 6). In addition, vagal tone during the 6 month mother-infant interaction was negatively related to cry modulation depth, with a 1 standard deviation increase in vagal tone during the interaction predicting a .366 standard deviation decrease in cry modulation depth ($SE = .173$; see bottom left panel of Figure 6). However, there was no evidence that 6 month cry modulation depth was predictive of vagal tone during the parent-child interaction at 12 months of age (see bottom right panel of Figure 6).

Table 10. Study 2 cry acoustic features descriptive statistics

Variable	***N***	***M***	***SD***	***Min***	***Max***	***Skew***	***Kurtosis***
Modulation Depth	37	1.303	0.252	0.781	2.004	0.009	0.757
F_0	37	510.732	94.449	396.503	835.292	1.583	2.834
F_0 (ln)	37	6.221	0.168	5.983	6.728	1.131	1.072
F_0 50% Bandwidth	37	49.342	9.929	33.805	76.824	1.288	1.900
F_0 50% Bandwidth (ln)	37	3.881	0.188	3.521	4.342	0.686	0.881

F_0 = Fundamental frequency

Figure 6. Results of regression models for 6m cry modulation depth predicted by cry persistence at age 3m, 6m respiratory sinus arrhythmia (RSA) during crying, 6m RSA during interaction with mother, and predicting 12m RSA during interaction with mother

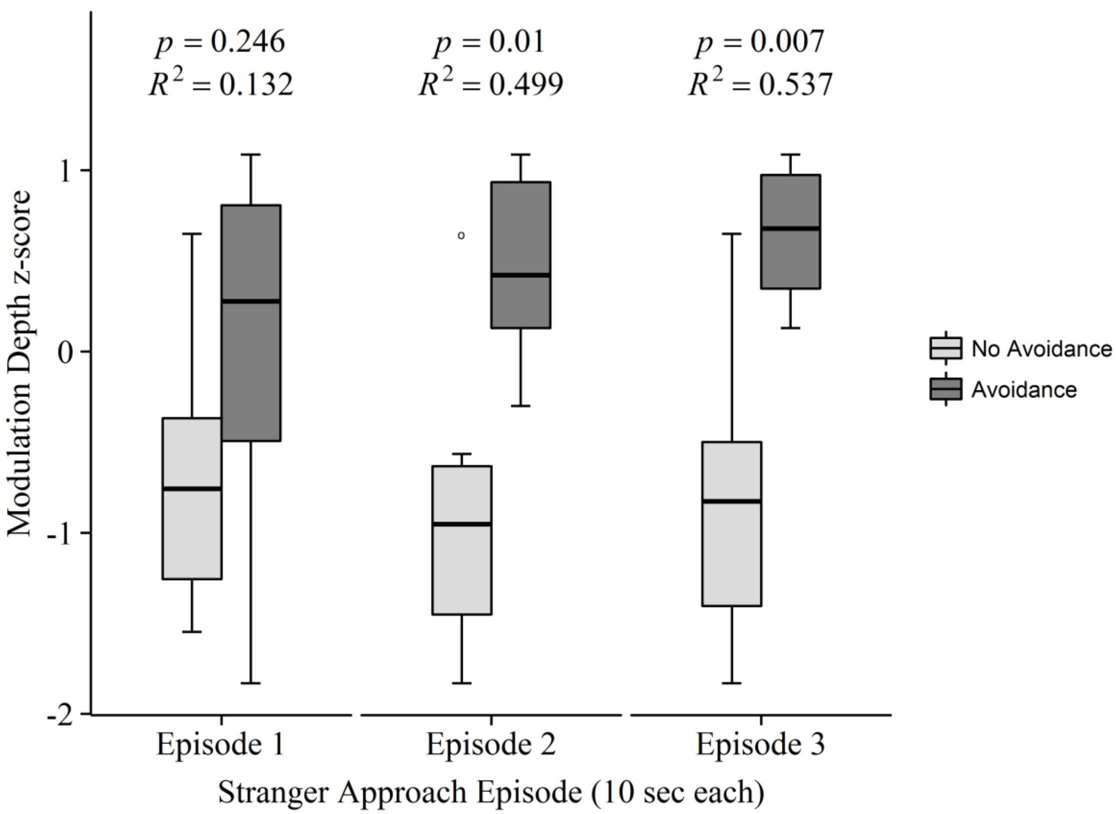


Cry modulation depth and attachment stress. There was a trend level prediction of 6-month mother-reported attachment stress on infant cry modulation depth, with decreased attachment stress relating to higher modulation cry depth (β = -.312, *SE* = .183, *p* = .098, R^2 = .084). There was no evidence of 6 month cry modulation depth predicting 12-month attachment stress (β = .229, *SE* = .222, *p* = .312, R^2 = .043).

Cry modulation depth and social adjustment. There was no relation between cry modulation depth and parent-reported peer relations, sociability, or social relatedness (all p > .10). When examining children's behavior during the stranger approach, no relation between cry modulation depth and verbal hesitancy emerged (all p > .10). However, cry modulation depth differed in children that avoided the stranger in comparison to those that did not (see Figure 7). This was not evident in the first 10 seconds of the interaction (episode 1; *F* (11) = 1.52, *p* = .246, R^2 = .132). However, children's responses during the next two sets of conversation initiation efforts from the stranger were related to cry modulation depth. In both of these episodes, children who avoided the stranger had higher levels of cry modulation depth at 6 months (episode 2: *F* (11) = 9.99, *p* = .010, R^2 = .499; episode 3: *F* (11) = 11.61, *p* = .007, R^2 = .537).

Figure 7. 6-month cry modulation depth differences between children based on avoidance during the 24-month Stranger Approach; Top panel: example of 24-month Stranger Approach procedure with stranger script; Bottom panel: 6m cry modulation depth in 24-month old children who avoided and didn't avoid stranger during episodes of the Stranger Approach

Episode 1	Episode 2	Episode 3
Stranger enters	Stranger walks toward chair	Stranger sits
"Hi! Have you ever been here before?"	"Are you having a good time today?"	"Are you playing with lots of toys?"



CHAPTER 4: DISCUSSION

The two studies described here focused on two aspects of infant crying – behavioral persistence and acoustic features – and their relation to social development, vagal tone, and parent-child attachment stress. The first study used a mediational model to test the hypothesis that crying persistence predicts social relatedness via altered child vagal tone and heightened levels of parent-child attachment stress. The second study was an exploratory study that examined the relations between infant cry acoustic features and infant vagal tone, parent-child attachment stress, and social adjustment outcomes.

Study 1

Study 1 aimed to examine whether infant crying persistence at 3 months of age – a time when colic tends to peak – is a predictor of social adjustment in toddlerhood. Its main goal was to assess whether this relation can be explained using a biopsychosocial model. The mediating pathways proposed in this study involved three variables: high crying persistence, the stress it imposes on the nascent mother-infant relationship, and emerging myelinated vagal activity whose physiological organization supports the social engagement system and regulation of distress.

Prior to assessing the structural model of relations among variables, this study first examined the measurement of key constructs. In service of this goal, the first aim was the assessment of the cry persistence measurement model, which required the integration of two measurement methods from two samples (the ‘fussy baby’ and ‘growing baby’ samples). First, a simplex model was used to establish reliability of daily cry diary reports, which were collected as part of sample 1 (‘fussy baby’) over a span of three days. These reports were found to be highly stable over the course of all measurement days, demonstrating the stability of daily crying persistence around 3 months of age. Next, retrospective parent crying reports, which were collected at 24 months in sample 1 (‘fussy baby’) and between 6 and 24 months in sample 2 (‘growing baby’), were examined. The rates of reported retrospective persistent crying varied considerably between these two samples. Rates of infant colic vary widely between other published studies, with common rates being estimated to range between 5 – 19% (Lucassen et al., 2001). In light of this, the 5.75% rate of parent persistent reporting in sample 1 (‘fussy baby’) falls within the typical range. The 27.0% rate reported in sample 2 (‘growing baby’) is substantially higher but consistent with retrospective colic reports that reached rates as high as 40% in one study (Stahlberg, 1984). These differences could not be explained by the timing of the retrospective reports, which may have affected memory recall in sample 2 (‘fussy baby’) due to the variable range of assessment between 6 and 24 months. Other sources of variability unmeasured in this study may also have contributed to those differences.

The full cry persistence measurement model assessment was then examined to determine if cry diaries collected in sample 1 (‘fussy baby’) could be integrated with retrospective parent recalls of persistent crying that were collected at 24 months in sample 1 (‘fussy baby’) and between 6 and 24 months in sample 2 (‘growing baby’). Results were consistent when both measurement types were included as well as when only daily cry diaries were used. Further, the latent factor was a strong predictor of both types of cry reports. Thus, there was no evidence that the two approaches used to obtain cry data affected the model results and the combined cry diary and retrospective report model was used to measure crying persistence.

The second aim of study 1, in service of establishing measurement models, was to examine the structure of social adjustment using a combination of exploratory and confirmatory factor analysis. The results showed that a single latent factor could be used to describe the underlying structure of parent reports of sociability, peer relations, and social relatedness. These measured indicators were exclusively based on long-term relationships with familiar adults and peers, and were in contrast to the parent-reported shyness and lab-observed responses to an adult stranger that did not converge on a factor. Thus, this factor was interpreted as reflecting social relatedness and was used for all subsequent models.

The final measurement model assessed in this study was that of vagal tone. A key assumption of the factor analytic approach used in this study is that each sampled epoch occurs independently. When examining the data, it was notable that error variance at both 6 and 12 month time points increased after the first epoch, which reflects poorer prediction of measured vagal tone values after the initial epoch. These patterns may reflect stochastic effects in vagal activity as the interaction progresses. While a time series approach may best describe such dynamic effects over time, the latent factor approach was utilized for its optimal reflection of vagal tone, the construct of interest, which represents an underlying propensity regardless of momentary, situation-related effects. Empirically, the latent factor model fit the data well and standardized loadings on the latent vagal tone factors were high (range = .76 - .96), indicating that a latent factor approach provided an adequate fit to the data and supported this statistical approach to measuring the underlying construct of interest.

With the establishment of the above-described measurement models, the analysis then examined the structure relations among the key constructs. In service of this, the fourth aim of study 1 was to examine whether infant crying persistence at 3 months predicted social adjustment in toddlerhood. This hypothesis was supported by the model, with crying persistence predicting maternal reports of poor social relations at 24 months of age. That similar predictions were verified in studies of infant responses to experimentally-induced stressors and retrospective reports of colic (see introduction), supports the utility of persistent crying in infancy as a predictor of early social development in toddlerhood. Thus, aims 5 and 6 examined potential mediators of this relation, testing if it could be explained via infant-parent attachment stress or myelinated vagal regulation.

The fifth aim of Study 1 was to examine whether the link between infant crying persistence and toddler social relations could be partly explained by the stress that frequent crying places on the parent-child attachment relationship, as attachment stress may negatively impact early social development. The model showed that infant crying persistence had long-term predictive value on maternal attachment stress that persisted up to the end of the first year, a finding that resonates with previous longitudinal studies that examined those same relations (e.g., Papousek & Hofacker, 1998). Further, attachment stress at 12 months of age predicted poorer child social relatedness at 24 months. This effect on child development may reflect the cumulative burden mothers may experience as they raise a persistently crying infant (e.g., Bowlby, 1988) However, an empirical test of this mediational pathway did not reach significance and, thus, direct evidence linking early crying persistence to social relatedness via increased attachment stress was not supported. However, every link specified in this model was significant, supporting a causal relationship between crying persistence and attachment stress and another between attachment stress and children's social relatedness.

The role of parent attachment stress, as induced by infant persistent crying in the prediction of social relations in late toddlerhood, remains to be fully examined. It is possible that heightened attachment stress may be expressed behaviorally in reduced warmth and sensitivity. Indeed, observational studies have shown that maternal sensitivity decreases during infant-mother interactions characterized by high child negative affectivity (Mills-Koonce et al., 2007). Further, maternal quality has been demonstrated to be a strong and robust influence on child social outcomes. For instance, recent studies have shown that children who received sensitive parenting tend to be less shy, more socially competent with peers, establish warm and affectionate relations with teachers, and have higher peer status ratings in early childhood than children reared by less sensitive mothers (Grady, Karraker, & Metzger, 2012; Stright et al., 2008). Thus, direct observational measures are needed to examine whether such alterations in maternal sensitivity can explain the role of parent-reported attachment stress on social relatedness that were found in the present study.

The sixth aim of study 1 was to examine whether the link between crying persistence and social relatedness could be explained by differences in regulation of the neural social engagement system, as reflected in the control of cardiac rhythms by the myelinated vagus nerve. Model testing, however, showed no evidence of this pathway. This may be partly explained by the fact that, contrary to hypothesis, infant crying persistence at 3 months did not predict vagal tone at 6 months. It is possible that the detection of this relation may have been affected by the measurement context, in which infants interacted with their mothers. Given that the activity of the myelinated vagus nerve is sensitive to situational social influence (Waters, West, & Mendes, 2014), it is possible that unmeasured effects of maternal sensitivity or scaffolding affected child vagal activity during free play. Future directions could focus on measurement of vagal tone in the absence of social scaffolding or aggregate vagal tone across a series of social, solitary, and distress-inducing tasks to derive an underlying latent vagal tone independent of situational effects.

Nonetheless, vagal tone did predict social relatedness, albeit not in the hypothesized direction. High vagal tone during the mother-infant interaction at 12 months predicted poorer social relatedness at 24 months. Although it was unexpected, this finding does not stand alone as other studies have shown that high vagal tone may serve as a developmental risk factor. For instance, infants with regulatory disorders – marked by hypersensitivities and difficulties with self-soothing and behavior regulation – had higher vagal tone during a laboratory testing session than their peers without difficulties (Dale et al., 2011). Further, infants with high vagal tone during an interaction with their mother were at increased risk of attachment disorganization when their mothers were negative-intrusive (Holochwost et al., 2014). These results highlight the need to examine the interactions between the child's social context and vagal regulation more closely. In this regard, it has been observed that vagal flexibility in response to challenges, more than the tonic values, may be most predictive of positive developmental outcomes (e.g., Stifter & Corey, 2001; Calkins, 1997; Porges et al., 1996). Thus, future studies examining the development of the social engagement system would benefit from examining individual differences in the capacity for vagal regulation.

The final aim of this study was to examine whether a dual bio-social pathway may explain the relation between infant crying persistence and social relatedness in toddlerhood. However, attachment stress at 6 months did not predict vagal tone during the mother child interaction at 12 months. Contemporaneous relations between these variables were not apparent in the correlation table either. One explanation for the lack of evidence for this pathway may reside in the fact like most similar instruments (see Cairns & Green, 1979) the attachment stress questionnaire captures perceptions that are typically averaged by respondents over time and contexts. Indeed, this study found a high stability between 6 and 12-month in parent reports, with a 1 standard deviation difference at 6 months reports predicting a .84 standard deviation difference at 12 months, reflecting a relatively stable construct that likely acts in aggregate across situations. Predictions about the effect of attachment stress on child vagal tone, which was not supported by the data in the present analysis, were based on studies that induced parent stress prior to interaction with their infant (Waters, West, & Mendes, 2014). As vagal activity is highly responsive to moment-to-moment changes in the quality of the social context, a global measure of parental stress may not be representative of the interpersonal dynamic that affected vagal activity in a brief free-play interaction with the child at 12 months. Studies that disentangle state-based attachment stress during interactions with the child from more long-term (trait-like) global perceptions of attachment stress may be necessary to capture parental effects on children's vagal regulation, as each may bring a unique contribution to the organization of this regulation.

Once attachment stress and vagal tone were controlled for, the direct prediction of social relatedness by crying persistence remained significant and substantial. Indeed, a 1 standard deviation increase in crying persistence was related to a .41 standard deviation decrease in social relatedness, demonstrating that a large portion of this relation remains unexplained by the proposed mediators. Future research should be designed to examine the mechanisms that underlie prediction.

Limitations. Several limitations of the study must be taken into account. First, attachment stress and social relatedness outcomes were measured using only parent reports. It's been demonstrated that parent reports frequently diverge from observational measures (e.g., Kagan, 1994; Saudino, 2009). This may reflect parenting bias caused by a desire to be perceived in a positive light by the researchers, differences in use of item scales, differences in references for "typical" child behavior, or personal factors such as depression. However, parent reports provide insight into children's behavior across a wide variety of contexts and eliciting situations that may not be captured by relatively brief researcher opportunities for observation. This is especially true of this study, which used several measures of social relatedness with familiar family members and peers. Future studies would be well served to examine the convergence of observed and parent-reported measures.

Retrospective reporting of crying persistence was also limited by the different interpretations that parents may have had of this term. The persistent crying item asked parents "Was there a time when your baby cried persistently?" Without an operational definition, parents were free to interpret this construct. Despite this open-endedness, these retrospective reports converged substantially with daily cry diaries, and measured rates were within the range of colic rates reported in previous studies (see above).

Furthermore, measurement issues in vagal tone estimates must be considered. First, child vagal tone was measured during a mother-child interaction. As discussed above, this situation likely impacted vagal activity during measurement. Second, vagal regulation is a process that occurs dynamically over time such that activity through the course of the interaction may be influenced by earlier-occurring events. Thus, infant vagal activity is reflective of the unique patterns of interaction that take place over stochastically over the course of the interaction.

However, as noted above, the single-factor model fit the data well and closely reflected the theoretical construct of vagal tone as a “trait-like” factor that reflects a latent propensity for vagal regulation that is relatively free of momentary changes in attention or fleeting physiological perturbations.

In addition, the social relatedness outcomes were measured only at 24 months of age. This represents an early period for social development, with many skills still left to acquire. While early social development provides insight into the emerging lives of children, it remains possible that the effects of early cry persistence would be more clearly discernable when individual differences in social development are more firmly consolidated. However, studying children at this young age provides insight into their emerging social lives. Future studies could inform further knowledge by examining social development into preschool and school age children.

Furthermore, attachment stress was included in the model at a later time point than the measurement of crying persistence. Thus, it is not possible to determine whether attachment stress was elevated from lower levels by children who cried persistently. Additional measures of attachment stress prior to the onset of peak crying persistence would be needed to examine whether the trajectory of attachment stress of parents of persistently crying infants is elevated above normal situations with the onset of persistent crying.

Despite these limitations, this study demonstrates that cry persistence in infancy predicts social relatedness in toddlerhood in a large and diverse sample. Though much of this link remains unexplained, parent-child attachment stress and vagal regulation, which reflects a component of the neural social engagement system, appear to contribute to variability in social relatedness. This study opens the potential for further research to examine this link more closely.

Social relatedness in toddlerhood reflects a burgeoning capacity for enacting an increasingly complex set of social skills and thus may be an important marker of development within this domain. Additional studies are needed to examine whether the predictive power of infant crying persistence extends beyond toddlerhood, including the putative mediators postulated to explain this prediction. If infant crying is a strong risk factor for emerging social engagement, understanding the underlying causes of this relation may point to early intervention targets for scaffolding positive social development and prevent social adjustment problems.

Study 2

Study 2 was an exploratory examination of infant cry acoustic features and their relation to cry persistence, vagal tone, parent stress, and social adjustment. The measurement of cry acoustic features was based on both traditional methods that focused on the fundamental frequency of the cry (mean fundamental frequency and its variability) as well as a novel method that included data from the entire vocalization frequency range.

The first aim of this study was to examine the association of cry persistence at 3 months of age and cry acoustic features at 6 months. Cry acoustic feature measures derived from the fundamental frequency were unrelated to crying persistence. Thus, there was no evidence of infants with higher cry persistence in infancy to have higher cry pitch at 6 months, as was hypothesized. This discrepancy from previous findings of higher pitch cries in infants with colic (e.g., Lester et al., 1992; Zeskind & Barr, 1997) may be a reflection of timing of assessment. Previous studies examined cry pitch in concurrence with colic symptoms. Thus, failure to predict 6-month cry pitch from 3-month cry persistence – the typical time of colic peak – may reflect a lack of continuity of cry pitch after the window of colic has passed. However, this analysis found a trending effect of 3-month cry persistence positively predicting 6 month cry modulation depth. The parameter estimate for this model was substantial (β = .437), but was flanked by large confidence intervals. This variability may reflect poor estimate precision due to the modest sample size of this exploratory study. The collection of additional data is needed to better assess the relation between these aspects of infant cries. Given that some researchers have called for the use of cry modulation as a diagnostic aspect of colic (Lester et al., 1990), better understanding of this relation could facilitate the development of improved clinical models of infant cry dysregulation. Further, given the anatomical connections of the social engagement system that link modulation of the larynx and pharynx with internal distress states, additional research could help clarify the functional integration of these systems.

The second aim of this study was to examine the relation of infant cry acoustic features to vagal tone. Neither fundamental frequency nor its variability were related to vagal tone. However, modulation depth of the entire cry was negatively predicted by cardiac vagal tone during crying such that higher cardiac vagal tone was related to a more smoothly modulated cry. This provides support for the predicted dependencies between cardiac regulation and control of laryngeal and pharyngeal muscular activity that give rise to vocal acoustic features. Thus, the results of this study suggest that poor vagal regulation may affect not only the heart, but also affect control of the larynx and pharynx in ways that compromise the ability to stabilize vocalizations. In addition, previous studies have found that hyperphonated cries, which are reflective of unstable pitch, induce more perceptions of aversion and higher increases in sympathetic activity than normally phonated cries (Crowe & Zeskind, 1992). When viewed in light of the effects of cry acoustic features on listeners, this study provides support for the theoretical model suggested by the Polyvagal Theory, which argues that the regulation of the larynx and pharynx evolved within social mammals to communicate internal states to conspecifics (Porges, 2001). However, it is important to note that this study examined between-person differences only. Emerging evidence from animal models suggests that vocalization acoustic features may serve as an index of within-individual vagal state changes (Kolacz, Lewis, & Porges, in press). Further work is needed to ascertain whether changes in infant cry acoustic features – or, more broadly, vocalizations - are functionally related to changes in cardiac vagal tone.

In addition to the negative relation between cry modulation depth and concurrent vagal tone, the modulation depth of infant cries was also negatively related to vagal tone during a social interaction with the mother. As such, cry modulation depth may reflect not only vagal regulation during states of distress but also index the regulation of the social engagement system during social interactions. However, while these relations were observed when children were 6 months of age, 6-month cry modulation depth was not predictive of vagal tone during an interaction with the mother at 12 months of age. This may be due to the large changes that occur in the development of the vagal system during the first month of life (Porges & Furman, 2011). Further work is needed to examine whether vocal modulation depth becomes less coupled with cardiac vagal tone during a social interaction at later ages and if individual trajectories of cardiac vagal regulation development make their prediction from cry modulation over the first 6 months of life unlikely.

The third aim of this study was to examine the relation between cry acoustic features and attachment stress. The results showed that greater variability of cry fundamental frequency was related to elevated attachment stress at 6 months. This may reflect the particularly aversive responses that infant cry pitch instability elicit from adult listeners (Crowe & Zeskind, 1992).

Aversive infant cries may place particular stress on the parent-infant relationship in the early months when crying bouts are frequent. However, this effect did not carry over to attachment stress at 12 months, suggesting that maternal perceptions of that stress may change as their child mature out the colic-prone period. While there was no relation between mean infant cry pitch and attachment stress, a trend-level negative association was observed for cry modulation depth and attachment stress at 6 months. The direction of the effect was contrary to that seen in previous studies and the model-estimated effect was weak ($R^2 < .10$), suggesting that this may be a type one error. Larger samples could be used to examine this effect more closely.

The final aim was to examine whether 6-month cry acoustic features have predictive value for social adjustment in toddlerhood. Neither mean fundamental frequency nor its variability were related to 24-month social outcomes. However, toddlers who avoided an adult stranger that attempted to engage them had more modulated cries in infancy. Importantly, this effect was dependent on time spent with the stranger. The initial reaction to a stranger's approach was unrelated to crying. However, children's avoidance behaviors showed a relation to crying after the stranger had spent at least 20 sec. attempting to interact with the child. This lack of effect at the initial stranger's approach may reflect temperamental differences in fear and inhibited approach tendencies at this age (Rothbart, 1988). However, the stranger's extended attempts at interaction and the mother's presence during this situation may ease this fear and promote reciprocated social interaction for some children. It is at this point in the interaction with a stranger that the early development of the neural social engagement system, as reflected in infant cry modulation, may be more predictive of child behavior. Children with a compromised or poorly regulated neural social engagement system may develop poorer social skills or reticence for interacting with strangers. Infants with more modulated cries have been found to be at risk for Autism Spectrum Disorders, which are marked by intense social deficits. This would be the first study, to my knowledge, to demonstrate that cry modulation may predict poorer social relations within normal populations. However, given the small sample of this study, these results should be interpreted with caution.

Limitations. Several limitations of this study need to be considered. First, as in study 1, measurement was limited by use of parent reports for attachment stress and measurement of vagal tone during a mother-child interaction (see study 1 limitations for discussion of these methods). In addition, this analysis was conducted with a subset of the full sample used in study 1. This may have impacted the results in two ways. First, the truncated sample reduced power for detecting effects. Given the exploratory nature of this study, these results are encouraging as they provide evidence for the potential utility of cry acoustic features as an index of the developing neural social engagement system at an age when social abilities are nascent. It is early, however, to conclude that the relations found in this study are broadly generalizable, as its sample was limited to infants who cried during a laboratory-based social stressor. An analysis that compared all key study variables between infants who cried during this stressor to those that did not showed that the two groups were largely indistinguishable in both the means and variances of these variables. However, it is still possible that these infants varied on unmeasured variables that may have impacted the results in unexpected ways. Thus, prospective longitudinal studies using representative samples will be needed to identify person and contextual factors that may either exacerbate the predictions made by the model tested here, or foster de-escalation of cascade effects and promote reorganization over the course of early childhood.

Furthermore, to my knowledge, the intra-individual stability of cry acoustic features is unknown. One key component of such stability is vocal fold size, which can influence vocalization fundamental frequency and that supraglottal tract length can alter the power of formants. One recent study used overall infant size as a crude index of these features but did not find that controlling for this variable affected the relations between RSA and cry fundamental frequency (Shinya et al., 2016). However, future research designs could be well served to measure anatomical features of infants' vocal tracts to improve vocalization models. Overall, research on the intra-individual stability of infant cry features over time is lacking. The measurement of acoustic features in this study was limited to one laboratory visit. It is unclear whether aspects of the cry signal can be decomposed into those reflecting a trait-type characteristic and those that reflect dynamic physiological regulation in response to context. Further research is needed to address this question.

Lastly, the ANOVA models linking stranger avoidance with persistent crying make predictions of variables measured in infancy from those measured in toddlerhood. This temporally reversed prediction was necessitated by the small sample. Forward predictions using logistic regressions resulted in unusually high parameter estimates with unusually large standard errors, reflecting questionable results and likely due to a limited sample size. ANOVA modeling was instead chosen for its superior performance with very small samples. Future studies utilizing larger samples are needed to examine predictions forward in time.

Despite these limitations, this exploratory study opens several directions for research. First, it demonstrates that the novel application of modulation depth derived from the modulation power spectrum (MPS) is a promising tool for assessing infant cry acoustic features. Unlike traditional measures of mean pitch and pitch variability derived from the fundamental frequency, the MPS modulation depth was correlated with an index of cardiac vagal tone during the cry as well as during a social interaction. Further, MPS modulation depth in infancy was also related to children's elicited social responses to a stranger in toddlerhood. Furthermore, traditional fundamental frequency measures examine – by definition – only the fundamental frequency. The MPS, on the other hand, exploits the full spectral range of the vocalization, providing a more accurate representation of the cry signal and likely a closer representation of acoustic auditory processing in the brain compared to measures of pitch alone (Theunissen & Elie, 2014). In addition, this study demonstrates that infant cry acoustic features may constitute a new point of entry for studying the early developmental stages of the neural social engagement system. The relation of cry modulation to both concurrent vagal tone and vagal tone during a social encounter as well as its prediction of social response to a stranger in toddlerhood is encouraging. This exploratory study demonstrates that such a research strategy may be promising.

Conclusion

Taken together, these studies suggest that both infant crying persistence and acoustic features reflect abilities for the regulation of internal states and potentially the function of the neural social engagement system. Both aspects of crying were related to social behavior in toddlerhood and cry modulation depth was related to both vagal tone during the cry and vagal tone during a social interaction with the mother. These results point to the usefulness of parent cry diaries, which are common in pediatric studies but relatively underused by developmentalists, and of the modulation power spectrum, a novel application for studying variability in vocalizations that, as of yet, has been rarely utilized for studying early social development. The study of development may be broadened by information tapped from infant cries, one of the first and most potent social signals in the human repertoire.

APPENDIX A: SUPPLAMENTAL MATERIAL

Figure A1. Daily cry diary form

Fussy Baby Cry Diary

Please record your baby's activity during the following times for today, ______________________ (date)

Please note the main activity of your baby during each 15-minute period. The activities are the following:

F=Fussing**, C**=Crying**, U**=Unsoothable Crying, **S**=Sleeping, **E**=Eating, **A**=Awake & Content, **X**=Cannot remember

6am				**7am**				**8am**				**9am**				**10am**				**11am**			
12noon				**1pm**				**2pm**				**3pm**				**4pm**				**5pm**			
6pm				**7pm**				**8pm**				**9pm**				**10pm**				**11pm**			
12midnight				**1am**				**2am**				**3am**				**4am**				**5am**			

Circle One Number

How frustrating to you was your baby's crying today?

0 1 2 3 4 5

Was this a typical day? (*Circle One*) **YES** **NO**

If no, please explain ____________________________

____

Adapted from Barr Baby Day Diary (Barr et al., 1988) and Baby's Day Diary from the Women & Infants' Hospital Cry and Sleep Center

Figure A2. Cry persistence measurement model; OR = odds ratio

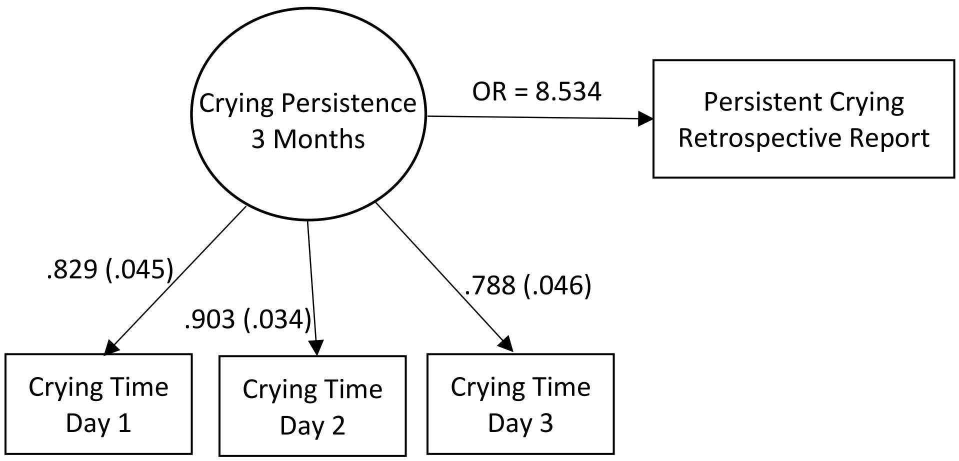


Figure A3. ANOVA model results for parent-reported social adjustment variables as a function of verbal hesitancy during stranger approach observation.

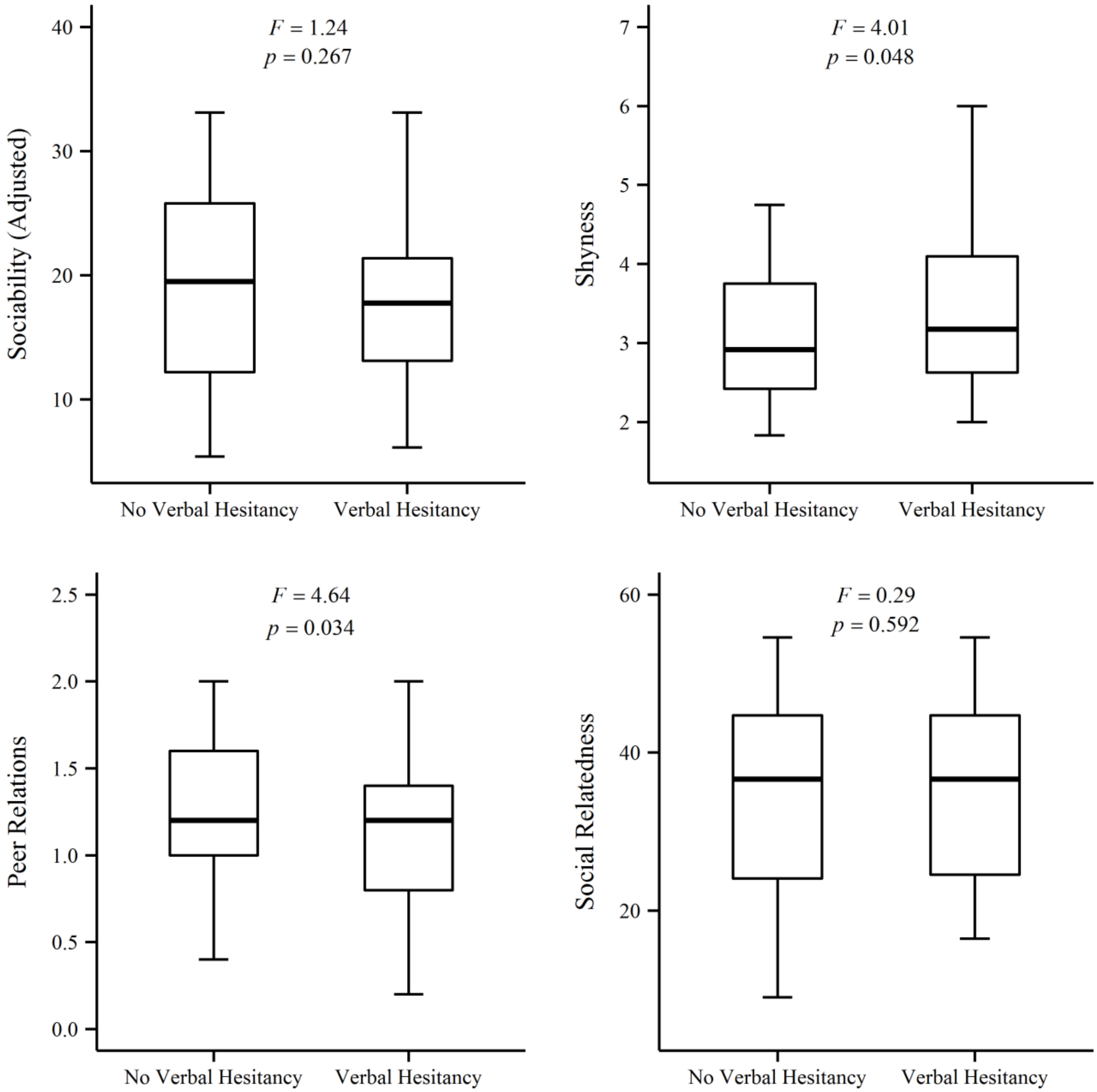


Figure A4. Social adjustment exploratory factor analysis (EFA) scree plot for the five-indicator model (dashed line) and three-indicator model (solid line); note the more pronounced “bend” for the three-variable EFA

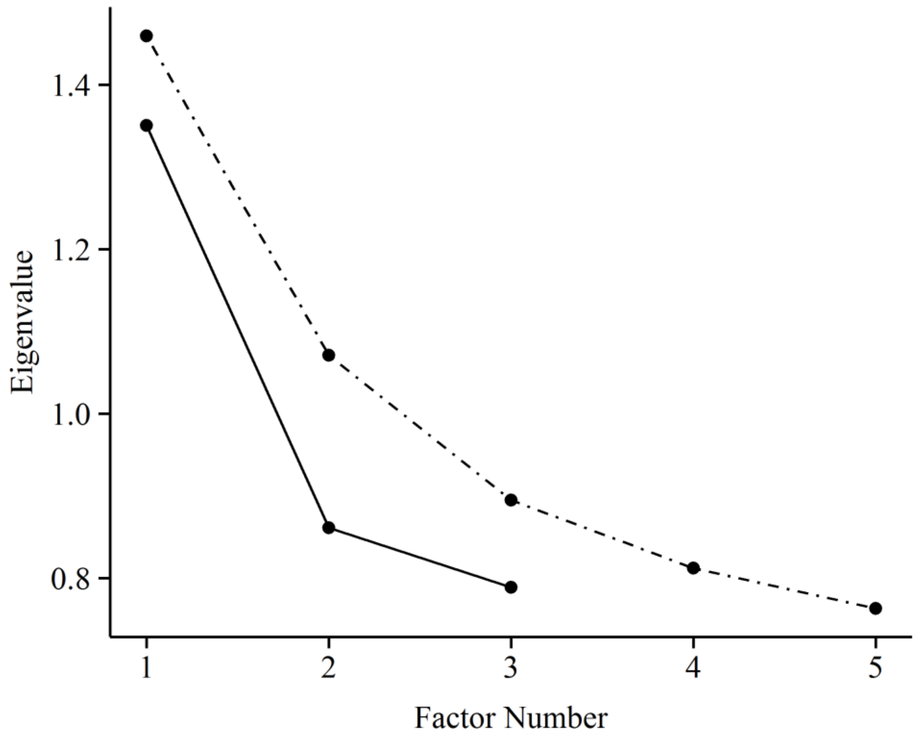
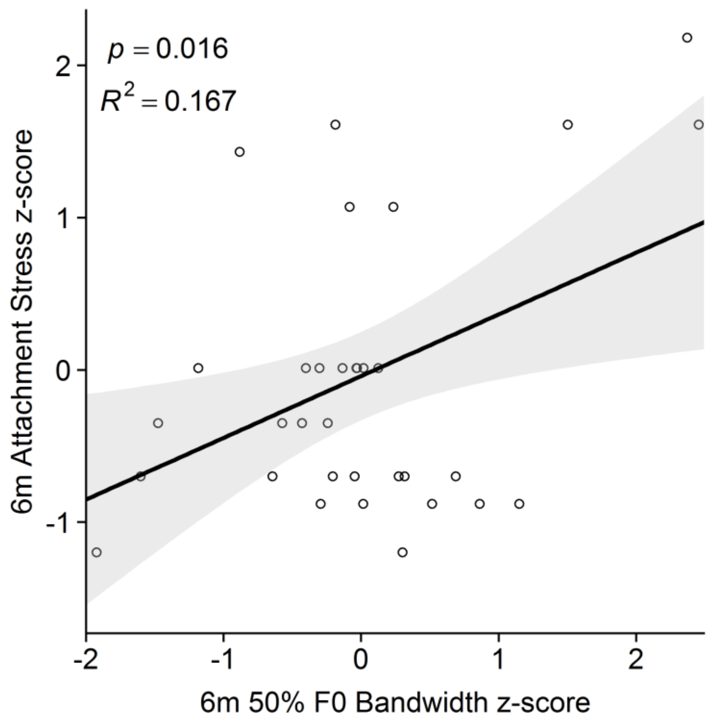


Table A1. Respiratory sinus arrhythmia measured variable correlations (all $p < .05$); Ep = Epoch

		6 Months				**12 Months**				
	Ep	**3**	**4**	**7**	**9**	**1**	**3**	**5**	**7**	**9**
6 Months	**1**	.70	.61	.67	.57	.39	.28	.24	.39	.26
	3		.67	.69	.64	.43	.34	.27	.33	.35
	5			.69	.55	.39	.29	.29	.32	.30
	7				.66	.37	.31	.27	.40	.31
	9					.52	.41	.32	.49	.41
12 Months	**1**						.78	.79	.71	.74
	3							.82	.77	.78
	5								.81	.80
	7									.81

Figure A5. Results of regression model for 6m attachment stress predicted by fundamental frequency (F0) 50% bandwidth at age 6m



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