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Behavioral And Heart-Defined Attention in Infants at High Genetic Risk for Autism

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BEHAVIORAL AND HEART-DEFINED ATTENTION IN INFANTS AT HIGH GENETIC RISK FOR AUTISM

by

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ABSTRACT

Characterizing early predictors of autism facilitates earlier identification, diagnosis and treatment. Although aberrant visual attention is one of the earliest identified predictors of autism and may play an integral role in developmental cascades that contribute to associated impairments, the emergence of atypical attention in infancy is poorly understood. The present dissertation includes three related manuscripts examining early patterns of visual attention in two infant samples at elevated risk for autism: infant siblings of children with autism (ASIBs) and infants with fragile X syndrome (FXS). Together, these manuscripts identify patterns of abnormal heart defined attention among ASIBs (Study 1), investigate the association between abnormal heart defined attention and attention orienting in ASIBs (Study 2), and examine the generalizability of these patterns to infants with FXS (Study 3). Together, findings provide novel evidence of atypical heart-defined and associated behavioral attention in ASIBs and FXS, with abnormalities emerging as early as 6 months of age in ASIBs. Importantly, Study 3 revealed diverging patterns of attention-arousal relationships in infants with FXS, suggesting potentially unique biological pathways subserving similar patterns of abnormal behavior across two infant samples at high risk for autism. These findings provide evidence of both shared and diverging endophenotypic features of autism in infants at high genetic risk, potentially informing early detection and interventions that target mechanisms, rather than symptoms, of impairment.

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List of Abbreviations

ADOS ..... Autism Diagnostic Observation Schedule

AOSI ..... Autism Observation Scale for Infants

ASD ..... Autism spectrum disorder

ASIB ..... Infant sibling of child with autism spectrum disorder

BPM ..... Beats per minute

FXS ..... Fragile X syndrome

HR ..... Heart rate

IBI ..... Interbeat interval

ICD-10 ..... International Classification of Disease – 10th Edition

LR ..... Low risk

MSEL ..... Mullen Scales of Early Learning

RSA ..... Respiratory sinus arrhythmia

SA ..... Sustained attention

SD ..... Standard deviation

CHAPTER 1

BIOBEHAVIORAL ATTENTION IN INFANTS AT RISK FOR AUTISM: AN INTRODUCTION

Over the past decade, over 100 studies have investigated the early emergence of ASD in infant siblings of children with autism (ASIBs) as a means to better understand the genetic and experiential factors associated with autism spectrum disorder (ASD). This high number reflects both the urgency and utility of exploring ASD risk factors in infancy. Rates of ASD in the general population continue to increase, with recent estimates suggesting 1 in 68 children in the United States meet ASD criteria (Centers for Disease Control, 2014). Characterizing ASD emergence in infancy offers opportunity for earlier detection and treatment, potentially altering early brain development and preventing ASD symptoms from emerging (Dawson, 2008; Rogers et al., 2014a). Furthermore, early intervention reduces the public health costs associated with ASD by up to 65% (Järbrink & Knapp, 2001). Thus, understanding early predictors of ASD is of importance for both individual well-being and public health.

The high number of studies investigating prodromal ASD features in ASIBs also reflects increased recognition of the utility of examining prospective ASIB cohorts to better understand ASD. Infant siblings are at 18-20 times higher risk for ASD than the general population (Ozonoff et al., 2011), thus exploring emerging ASD symptomatology in this group offers an efficient framework for engaging in

prospective, theory-driven investigations of ASD. As the natural history of ASD among ASIBs continues to unfold, however, it has become clear that ASIBs face elevated risk for a number of additional developmental and socio-communicative outcomes beyond ASD (Messinger, Young, & Ozonoff, 2013; Ozonoff et al., 2014; Toth, Dawson, & Meltzoff, 2007). In addition, ASIBs and other family members of individuals with ASD exhibit higher rates of subthreshold symptoms, a phenomena termed the *broader autism phenotype* (Baron-Cohen, 2004; Folstein & Rutter, 1977), supporting the modern conceptualization of ASD as a continuum, with subclinical traits extending into the general population (Lord & Jones, 2012; Short & Schopler, 1988; Yoder, Stone, Walden, & Malesa, 2009). Thus, although ASIBs offer an efficient sample for investigating the early emergence of ASD and broader features associated with familial ASD risk, it is possible that early development of ASIBs may differ from infants diagnosed with ASD without a family history.

A promising, complementary model for delineating early risk factors for ASD is to engage in cross-group comparisons across multiple "high risk" samples. These comparisons may inform both converging and diverging patterns of risk that may clarify the complex heterogeneity of ASD. Much like in ASIBs, elevated rates of ASD are reported across a number of genetic syndromes, including fragile X syndrome, a single-gene disorder that affects 1:4000 individuals (Crawford, Acuña, & Sherman, 2001; Hagerman & Hagerman, 2002). Comparing infant development in ASIBs versus infants with FXS may deepen understanding of complex genetic, environmental and developmental interactions not afforded by populations in which specific genetic biomarkers of risk are unknown (Fung, Quintin, Haas, & Reiss, 2012; McCary &

Roberts, 2013; Tonnsen, Malone, Hatton, & Roberts, 2013; Tye & Bolton, 2013). Although few studies have examined ASD symptoms and correlates in infants and toddlers with FXS, extant findings suggest both converging (Roberts, Hatton, Long, Anello, & Colombo, 2011) and diverging (Hazlett et al., 2012; Tonnsen, Malone, et al., 2013) patterns of ASD-related risk factors, supporting FXS as a useful framework for disentangling syndrome-specific and global features of ASD.

The present series of studies focuses on the emergence of ASD-associated abnormalities in infants by focusing specifically on abnormal visual orienting, a commonly identified risk factor for ASD across both ASIBs (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005) and infants with FXS (Roberts et al., 2011). Although a number of studies have described abnormal orienting in high risk infants, the current literature is limited in several ways. First, extant studies have either reported cross-sectional group differences or gross change across wide age categories (e.g. within-individual change between two 2-4 month age ranges; (Elsabbagh et al., 2013; Roberts et al., 2011; Zwaigenbaum et al., 2005), limiting understanding of subtle developmental changes related to the emergence and stability of attention during infancy. Second, extant studies have largely categorized ASD symptoms as present or absent (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005), thus it is unclear how broader spectrum of ASD features are associated with abnormal orienting in infancy. Third, no studies to date have integrated multiple "high risk" comparison groups within a single study to inform divergent and convergent patterns of risk across. These limitations provide a compelling case for systematic, longitudinal surveillance of abnormal orienting

across high risk groups as a means to inform the timing and nature of atypical attention in ASD.

The current literature is also limited by sparse understanding of the neurobiological mechanisms of abnormal orienting in high risk infants. Indeed, only two studies to date have examined neurobiological correlates of abnormal orienting in high-risk infants (Elison et al., 2013; Roberts et al., 2011). In contrast, a number of studies in typically developing infants have characterized physiological processes of attention orienting that sustain behavioral responses. Although measures of *heart-defined sustained attention* have been used extensively in non-clinical infant samples (e.g. Casey & Richards, 1988; Richards & Casey, 1991; Richards, 1997), indicators of global or attention-related physiology have not been examined in ASIBs, and only one study has explored these constructs in infants with FXS (Roberts et al., 2011). Investigating the intersection of ASD risk and sustained attention is warranted given the close association between overt and heart-defined attention in low-risk infants (e.g. Casey & Richards, 1988; Richards, 1987, 1997), abnormal physiological arousal identified in both ASD and FXS (see Klusek, Roberts, & Losh, 2015 for review) and self-regulatory and attentional deficits described in high risk infants (Elison et al., 2013; Elsabbagh et al., 2013; Ozonoff et al., 2010; Ozonoff, Macari, & Young, 2008; Roberts et al., 2011; Zwaigenbaum et al., 2005).

The present series of manuscripts addresses these areas of need by systematically examining both behavioral and heart-defined attention across two infant samples at "high risk" for ASD: ASIBs and infants with FXS. Study 1 examines longitudinal trajectories of behavioral and heart-defined attention during a passive attention task in 5-14 month

ASIBs compared to low risk (LR) controls. The purpose of this study is to inform "resting" patterns of heart activity in ASIBs, correlations between behavioral (e.g. overt looking) and heart-defined attention, and association between these variables and clinical ASD risk, providing an initial conceptualization of heart-defined and behavioral attention, a topic previously unexplored in the ASIB literature. In Study 2, behavioral and heart-defined attention were examined during a gap-overlap task, a commonly used spatial cueing paradigm for examining attention orienting and disengagement in ASIBs (Elison et al., 2013; Elsabbagh, Volein, et al., 2009; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). In contrast to previous studies, we used both individual growth curves and integrated physiology to disentangle developmental processes and biological substrates of abnormal task performance. Thus, the purpose of Study 2 was to examine both nuanced developmental changes in orienting, as well as potential biological mechanisms sustaining abnormal orienting behaviors. To examine the generalizability of abnormal behavior and heart-defined attention to non-ASIB samples, Study 3 repeated Study 2 tasks in a cross-sectional sample of infants with FXS, contrasted to the ASIB and LR participants from Study 2. This study aimed to identify both converging and diverging patterns of risk, informing potentially heterogeneous pathways to ASD. Combined, these studies provide initial evidence of abnormal behavioral and heart defined attention at rest (Study 1), establish the intersection of development, orienting, and physiology (Study 2), and examine the generalizability of abnormal patterns from ASIBs to infants with FXS (Study 3).

The first step to preventing ASD-associated impairments is characterizing the nature, course, and mechanisms of abnormal behaviors in infancy. The present three

studies address this need by elucidating the behavioral and biological scope of abnormal attention in infants at risk for ASD. Although parsing cross-syndrome variability is a complex task, capturing this heterogeneity may pave the way for developmental surveillance that is sensitive to individual differences, as well as targeted treatments that address the mechanisms, rather than symptoms, of impairment.

CHAPTER 2

INFANT HEART-DEFINED SUSTAINED ATTENTION IN THE BROADER AUTISM PHENOTYPE[1]

[1] Tonnsen, B. L., Richards, J. E. & Roberts, J. E. To be submitted to *Child Development*

Visual attention is one of the most robust early indicators of autism spectrum disorder (ASD), with atypical features identified prior to 12 months of age in infants who later meet diagnostic criteria (Adrien et al., 1993; Clifford & Dissanayake, 2008; Holmboe et al., 2010; Jones & Klin, 2013; Swettenham et al., 1998; Zwaigenbaum et al., 2005). The majority of studies on early attention in ASD have been conducted with infant siblings of children with ASD (ASIBs) who exhibit higher rates of ASD diagnoses (19%; Ozonoff et al., 2011) than the general population (1-2%; Centers for Disease Control, 2012, 2014). It is notable that abnormal attention patterns have been observed in ASIBs who do not later meet ASD criteria (Messinger et al., 2013; Ozonoff et al., 2014; Toth et al., 2007), indicating broader patterns of genetic vulnerability associated with familial diagnoses. The recent expansion in ASIB research has been used to inform early identification and treatment protocols, permitting more timely and efficient access to services for affected children. However, although a number of abnormal attention processes have been identified as potential "red flags" for ASD, the emergence and mechanisms of these behaviors remain unclear.

Integrating neurobiological signatures of attention may inform the mechanisms of atypical patterns and, subsequently, early detection and intervention targets. In infants, heart activity may be used to measure the quality of attentional engagement (e.g. Casey & Richards, 1988; Richards & Casey, 1991; Richards, 1997), providing valuable information about the underlying biological systems sustaining attentional responses. Indeed, although measures of *heart-defined sustained attention* have been used extensively in non-clinical infant samples, indicators of global or attention-related heart activity have not been examined in infants at risk for ASD. Investigating the intersection

of ASD risk and sustained attention is warranted given the close association between overt and heart-defined attention in low-risk infants (e.g. Casey & Richards, 1988; Richards, 1987, 1997), abnormal physiological arousal identified in a subset of children with ASD (Anderson & Colombo, 2009; Bal et al., 2010; Kushki et al., 2013; Ming, Julu, Brimacombe, Connor, & Daniels, 2005), and self-regulatory and attentional deficits described in high risk infants (Elison et al., 2013; Elsabbagh et al., 2013; Ozonoff et al., 2010, 2008; Zwaigenbaum et al., 2005). Thus, to inform the emergence and mechanisms of abnormal attention in ASIBs, the present study contrasted longitudinal patterns of behavioral and heart-defined sustained attention in 5-14 month old infants at high- and low-risk for ASD, examining both cross-group differences and clinical correlates of risk.

Attention in Autism Spectrum Disorder

Autism spectrum disorder affects 1:42 males in the United States (Centers for Disease Control, 2014) and is associated with a range of socio-communicative abnormalities and restricted or repetitive behaviors (American Psychiatric Association, 2000). To receive an ASD diagnosis, symptoms must be present in early development and significantly impair the individual's daily functioning. The lifetime costs of ASD are estimated at $2.4 million per child, with a substantial portion of costs – up to $88,000 annually – occurring in adulthood (Buescher, Cidav, Knapp, & Mandell, 2014). Timely and effective interventions may reduce lifetime costs by over $1.3 million per person by altering symptom trajectories (Peters-Scheffer, Didden, Korzilius, & Matson, 2012) and potentially restructuring early neural development (Dawson, 2008). As such, promoting earlier identification and treatment of ASD maximizes positive outcomes for the affected

child and family, as well as substantially reduces public health costs associated with the disorder.

Atypical attention is one of the most commonly reported features in ASD and manifests through a variety of socio-communicative characteristics, including difficulty following and initiating others' attention (Meindl & Cannella-Malone, 2011), failing to orient to one's name (Nadig et al., 2007), and inattention or aloofness during social interactions (Volkmar, 2011). These behaviors, which may be loosely categorized as attention-related in nature, are likely driven by a complex combination of developmental, socio-cognitive, and neurobiological processes. Early visual attention plays a central role in infant socio-communicative development by facilitating leaning and communication (Keehn, Müller, & Townsend, 2013; Mundy & Jarrold, 2010). Effective visual orienting also promotes effective emotional regulation by permitting an individual to adaptively alter sensory input (Landry & Bryson, 2004). As such, impaired attention processes may begin to derail socio-cognitive development early in life, intersecting with additional neurobiological and environmental factors to contribute to the ASD phenotype (Elsabbagh & Johnson, 2007; Keehn et al., 2013).

Attention in Infants at Risk for ASD

Although ASD is often diagnosed clinically as "present" or "absent," it is largely accepted that ASD symptoms are best considered along a continuum, with subclinical traits extending into the general population (Lord & Jones, 2012; Short & Schopler, 1988; Yoder et al., 2009). Complex genetic influences are highly implicated in ASD expression, with sibling recurrence risk estimated at 10-19% (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Ozonoff et al., 2011) and higher levels of subthreshold

symptoms present in family members of affected individuals (Hurley, Losh, Parlier, Reznick, & Piven, 2007; Micali, Chakrabarti, & Fombonne, 2004; Piven, Palmer, Jacobi, Childress, & Arndt, 1997). The presence of these subthreshold symptoms in individuals who do not meet diagnostic criteria is termed the *broader autism phenotype* (Baron-Cohen, 2004; Folstein & Rutter, 1977). The existence of a broader symptom spectrum is supported by evidence that relatives of individuals with ASD present elevated socio-communicative symptoms and repetitive behaviors (Piven et al., 1997), including aloofness, rigidity, and social language deficits (Hurley et al., 2007; Micali et al., 2004). The degree of symptom severity is higher in families with multiple incidences versus single incidences of ASD (Losh, Childress, Lam, & Piven, 2008), further indicating genetic vulnerability for ASD risk. Examining neurobehavioral attention patterns in first-degree relatives of individuals with ASD, therefore, may inform the neurobiological processes related to ASD emergence and expression.

Because ASD is most often diagnosed in toddlerhood or later, the majority of studies investigating attention in ASD have focused on older children and adults, and past infant research largely relied on retrospective reports and home videos solicited after the initial diagnosis (see Rogers, 2009, for review). More recently, longitudinal studies of high-risk infants have begun to inform the natural history of ASD emergence in infancy. Abnormal cognitive and social attentional features have been observed by ASIBs and have been the topic of several reviews (Jones, Gliga, Bedford, Charman, & Johnson, 2014; Rogers, 2009). Identified features include abnormal social attention in the first year of life, reflected by increased gaze toward the mouth versus eyes (Merin, Young, Ozonoff, & Rogers, 2007), diminished attention toward people (Bhat, Galloway, &

Landa, 2010; Chawarska, Klin, & Volkmar, 2003; Ozonoff et al., 2010), reduced initiation and response to joint attention (Rozga et al., 2011), and abnormal social orienting (Jones & Klin, 2013). Atypical non-social visual behaviors, such as abnormal visual inspection of objects (Ozonoff et al., 2008) and impaired attention disengagement (Elison et al., 2013; Elsabbagh, Voleina, et al., 2009; Sacrey, Bryson, & Zwaigenbaum, 2013; Zwaigenbaum et al., 2005), have also been reported. These findings present compelling evidence that aberrant attention is prominent in infants later diagnosed with ASD and – given further characterization – could serve as a candidate marker for early detection and prevention efforts.

Despite the promise of ASIB attention research for translational science, it is difficult to draw clear conclusions from the current literature regarding the primary deficit or deficits that could potentially drive the broad array of attention-related findings. Indeed, although most theories of ASD emergence recognize likely contributions from social-cognitive and self-regulatory domains, various proposals has emphasized different candidate constructs – such as abnormal attentional disengagement (Keehn et al., 2013), social orienting (Jones & Klin, 2013; Klin, Shultz, & Jones, 2014), or parallel social and non-social processes (Bedford et al., 2014) – as primary influences in developmental cascades that produce ASD symptomatology. An alternate and potentially complementary theory is that attentional deficits emerge secondary to abnormal physiological arousal and self-regulation (Porges, 2003, 2004). This framework has been highlighted by several ASD research groups (Bal et al., 2010; Klusek et al., 2015; Quintana, Guastella, Outhred, Hickie, & Kemp, 2012) but has not been applied to studies of ASD in infants. The following sections will examine the applicability of this

framework for characterizing attention deficits in ASIBs, first by describing the theoretical intersection of autonomic functioning in ASD symptomatology, and second by examining this intersection in the context of infant attention.

Autonomic Functioning in ASD

Porges' polyvagal theory posits the human autonomic system has evolved to maintain both behavioral and psychosocial characteristics such as communication abilities, emotional expression, and self-regulation (Porges, 1995). Understanding the association between polyvagal functioning and ASD first requires basic understanding of quantification and processes of autonomic nervous system function. Autonomic functioning is often measured through quantification of electrocardiogram (ECG) signal. The ECG measures activity from several areas of the heart, which generate waves conventionally labeled P, Q, R, S, and T. Heart activity is quantified as the temporal interval between R waves, commonly described as *interbeat interval* (IBI) or *R-R interval. Heart rate* (HR) is the inverse of IBI and may be quantified as beats-per-minute (BPM). Heart rate reflects both sympathetic and parasympathetic processes that are responsible for speeding or slowing HR, respectively. Parasympathetic heart activity is reflected in *respiratory sinus arrhythmia* (RSA), an index of cardiovascular activity associated with respiration. Thus, IBI and RSA are commonly used indicators of combined sympathetic and parasympathetic (IBI) versus parasympathetic (RSA) functioning.

Parasympathetic functioning is regulated by the vagus (cranial nerve X), which influences autonomic functioning through the heart's sinoatrial node. High vagal tone operates on this node as a "brake," inhibiting or slowing HR. When vagal tone relaxes

and lowers, this break is lifted, reducing inhibition and permitting acceleration of HR. Modulating the vagal brake is important to both mobilizing physiological resources during states of acute stress and promoting restoration in the absence of threat. Notably, both cardiac and facial muscles are regulated by fibers that originate from the same nucleus within the vagus. Thus, vagal health affects both effective modulation of heart activity, as well as more complex behaviors such as facial expression and vocalizations. Because the vagus facilitates communication between the brain and a wide number of visceral processes implicated in ASD (e.g. digestion, metabolic functioning, cardiovascular activity, temperature regulation), polyvagal theory has received increased attention as an explanatory framework for the neurobiology of ASD symptoms (Bal et al., 2010; Klusek et al., 2015; Quintana et al., 2012).

Studies of abnormal autonomic functioning in ASD generally support associations between vagal activity and the disorder, although findings vary across samples, ages, and tasks (see Cheshire, 2012; Klusek et al., 2015, for review). In general, individuals with ASD are reported to exhibit higher overall HR (Bal et al., 2010; Kushki et al., 2013; Ming et al., 2005) and larger tonic pupil size (Anderson & Colombo, 2009). Studies of RSA are mixed, with a number reporting lower overall RSA in ASD (Bal et al., 2010; Ming et al., 2005; Vaughan Van Hecke et al., 2009) but others reporting no group differences (e.g. Klusek, Martin, & Losh, 2013; Watson & Roberts, 2012). Autism is also associated with difficulties modulating arousal when task demands change (Althaus, Mulder, Mulder, Aarnoudse, & Minderaa, 1999; Smeekens, Didden, & Verhoeven, 2015; Vaughan Van Hecke et al., 2009). Within ASD samples, lower ASD symptomatology is correlated with lower HR and higher RSA (Bal et al., 2010; Klusek et al., 2013; Vaughan

Van Hecke et al., 2009), suggesting a gradient of within-syndrome variability. Together, these studies implicate abnormal autonomic functioning in ASD, although future work is needed to clarify specific implications of abnormal autonomic functioning and developmental pathways of risk.

Heart-Defined Sustained Attention

In addition to providing a broad biomarker of self-regulation, autonomic functioning is closely associated with behavioral attention. Indeed, patterns of heart activity have been used to index behavioral attention responses for over 50 years (Graham & Clifton, 1966; Lacey, 1959). When the brain's arousal system is activated, cardioinhibitory centers initiate parasympathetic processes to slow HR. In infants, these decelerations may be use to quantify qualities of stimulus engagement that cannot be measured using overt looking patterns alone (Casey & Richards, 1988; Richards & Casey, 1991; Richards, 1997). Richards and colleagues have demonstrated that these patterns of HR decelerations index three primary phases attention in infants: orienting, sustained attention, and attention termination (Casey & Richards, 1991; Richards & Casey, 1991; Richards, 2000). *Orienting* in infants is reflected by a sudden deceleration of 8 to 10 HR beats per minute (BPM; see Richards, 1995, for review). *Sustained attention* (SA) is indexed by maintenance of this decelerated HR, reflecting the exertion of additional cognitive resources to process the stimulus. *Attention termination* occurs when HR begins to return to prestimulus levels, reflecting decreased stimulus engagement despite continued looking. Quantifying duration and magnitude of SA provides useful information about the quality of stimulus processing that may not be detectible from overt behavior alone. Sustained attention may also inform the

mechanisms of behavioral responses. For example, infants in periods of SA are less distractible across a variety of tasks, including computerized orienting paradigms (Casey & Richards, 1988; Richards, 1997) and toy play activities (Lansink & Richards, 1997; Roberts et al., 2011). Thus, SA is a well-established biomarker of attention in infants.

A small number of studies have examined HR deceleration as an index of global attention in older individuals with ASD. Corona and colleagues (1998) reported that children with ASD (ages 3-5 years) demonstrated less change in HR when observing an examiner in distress, using comparisons of average HR across epochs (Corona, Dissanayake, Arbelle, Wellington, & Sigman, 1998). However other studies have reported no group differences in HR decelerations between ASD and non-ASD participants when viewing pictures with varied social and emotional valence (Louwerse et al., 2014; Mathersul, McDonald, & Rushby, 2013). No studies to date have integrated measures of heart-defined SA to index attentional engagement in infants at elevated risk for ASD.

The Present Study

Identifying potential biomarkers of ASD risk may inform earlier and more effective detection and intervention, as well as lend insight into the developmental processes associated with ASD emergence. Although abnormal attention and physiology are well-documented in ASD, the development and intersection of these processes in infancy are poorly understood. Only a handful studies have examined ASIBs' attentional response using psychophysiological techniques (e.g. Elsabbagh et al., 2012; Key & Stone, 2012; McCleery, Akshoomoff, Dobkins, & Carver, 2009), and no studies to date have examined ASIB attention by integrating measures of heart activity, a reliable

biomarker of attention in typically developing infants (e.g. Casey & Richards, 1988; Richards & Casey, 1991; Richards, 1997).

To examine the biobehavioral development of abnormal attention in high-risk infants, the present study longitudinally assessed high-risk ASIBs and low-risk controls ranging from 5-14 months of age. This age range was chosen in light of previous studies suggesting abnormal attention in ASIBs emerges between 6 and 12 months of age (Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). The primary goals were to examine (1) correspondence between behavioral and heart-defined attention, (2) group differences in attention patterns across age, and (3) associations between attention indicators and clinical ASD risk among ASIBs. This work aims to identify whether heart-defined attention may serve as a biomarker of ASD risk, potentially informing earlier, targeted, and optimized intervention efforts.

Methods

Participants

Participants were drawn from a study of early development in high risk infants. Infants were required to be born full term (37 weeks or later, >2000 grams) and live with their biological mother. Infant siblings were required to be full biological siblings of a child with a documented ASD diagnosis, verified using medical records. Parents reported no documented developmental delays or diagnosed genetic or medical conditions at study entry. Infants were enrolled between 4.5 and 10.5 months of age and were assessed on up to 3 occasions. These assessments occurred across three age windows, centered around 6, 9 and 12 months: 5-8 months, 8-11 months, and 11-14 months. Nine additional "low risk" (LR) participants were assessed but excluded from the present study due to

developmental or medical concerns (n = 4; e.g. epilepsy, high ASD screening scores at 12 months), a sibling being considered for ASD during the course of the study (n = 1), and chronological age-matching purposes (n = 4).

The final sample included 43 infants (21 ASIBs, 22 LR controls) assessed between 5 and 14 months of age for a total of 77 assessments. Groups exhibited similar numbers of participants with one assessment (ASIB n=8, LR n=10), two assessments (ASIB n=8, LR n=8) and three assessments (ASIB n=5, LR n=4). Each group included 4 female participants. In addition to the 77 assessments included in analyses, 22 additional assessments were conducted but excluded due to greater than 5% error in heart activity data (n = 15; 6 ASIB), technical difficulties (n = 6; 4 ASIB), and infant noncompliance (n = 1; 1 ASIB). Non-compliance was defined as refusing to sit in front of the screen; participants who complied with sitting but did not attend to the screen were included in a subset of analyses to capture variability in overt attention. Proportion of missing data (22.2%) was slightly higher than previous studies in similar samples (Elsabbagh et al., 2009; 16%), likely reflecting physiological data collection not used in prior studies. Number of assessments with missing behavioral or physiological data were equivalent across groups (n = 11 each).

Procedure and Measures

Parents provided consent prior to the onset of the study and were compensated for participation. To minimize family travel, assessments alternated between laboratory and home environments, with assessments in the 6 month assessment window occurring in the laboratory, 9 month in home, and 12 month in laboratory. As part of a larger battery, each assessment included a visual attention task and additional developmental and

clinical evaluations. The present study includes attention and physiological data from all assessments, as well as developmental and clinical testing from the 12 month assessment window only due to increased stability of these measures at older ages. Materials, equipment, and experimental set-up were identical across settings. Order of assessment tasks was standardized across assessments, with the visual attention task attempted prior to other testing to maximize infant engagement. If the infant was fussy or noncompliant, the attention task was reattempted following other components. All testing was conducted with the child's mother present.

Attention Task. During each assessment, participants' looking behavior and heart activity were measured while they viewed an engaging 135-second children's video clip (*Baby Einstein* series). Participants were seated in a darkened room, 10" away from an 11 x 24" LCD monitor. Two video cameras simultaneously recorded stimuli and participants' faces. To minimize distractions and standardize environments, the monitor and infant were surrounded on three sides by a portable, nonreflective black felt shield. Electrocardiogram signal (ECG) was collected using Alive Heart Monitors (Alive Corporation, Gold Coast, Australia), a telemetry based system that directly attached to the infant's chest via two electrodes. Signal was transmitted live to a laptop via Bluetooth technology. If the child did not attend to the video voluntarily, the examiner provided up to 3 prompts to re-engage the child. After 3 prompts, the child was permitted to look toward or away from the screen.

Clinical Autism Risk. The Autism Observation Scale for Infants (AOSI; Bryson et al., 2008) is a semi-structured, interactive assessment that measures risk factors for ASD in infants ages 6-18 months. The AOSI includes various play-based behavioral

presses designed to elicit 18 behaviors empirically related to ASD risk in ASIBs. These items measure a variety of constructs including visual attention (visual tracking, disengagement of attention, orienting to name), social behaviors (response to facial emotion, anticipating social games, imitating, babbling, eye contact, social smile, shared affect), sensory-motor symptoms (coordination of gaze and action, motor control, motor and sensory behaviors), and temperament and reactivity (behavioral reactivity, cuddliness, soothability, transitions). Administration takes approximately 20 minutes and is videotaped for offline coding. Item scores are summed to yield a total score (0-50), and the number of elevated items may also be used to assess whether infants exceed a threshold of clinical risk ("number of markers" $\geq$7). The AOSI has demonstrated good to excellent interrater reliability on individual items (.53-1.0) and acceptable total score test-retest reliability (.61) at 12 months (Bryson et al., 2008). Previous studies have successfully employed the AOSI as indicators of clinical ASD risk in similarly aged ASIB samples (Brian et al., 2008; Rogers et al., 2014b; Zwaigenbaum et al., 2005).The AOSI was administered by staff trained to research reliability through a rigorous series of supervised administrations and reliability coding. All AOSI data were collected during the 12 month assessment window. Assessments were scored by a primary examiner, with 20% of assessments also scored by a second rater for reliability. Interrater reliability at the item level was 89%. Table 1.1 includes descriptive information about AOSI total scores and number of markers. Three of 19 ASIBs with AOSI data exceeded the clinical threshold of 7 elevated AOSI items.

The present study used the AOSI Total Score at the final assessment (e.g. between 11-14 months) as a behavioral indicator of broader autism phenotype symptomatology.

Previous studies suggest the AOSI is sensitive to subthreshold symptoms of the ASD endophenotype in ASIBs, as ASIBs without later ASD diagnoses have exhibited AOSI total scores that generally fall between LR and ASD groups (Gammer et al., 2015a). Although we did not use the AOSI diagnostically, total score on the AOSI at 12-14 months has also been established as a strong predictor of later ASD diagnoses on gold-standard instruments (Gammer et al., 2015b; Zwaigenbaum et al., 2005), suggesting the AOSI is a relatively sensitive clinical tool for emerging ASD features. Thus, we examined AOSI total score as a continuous metric of risk, rather than to inform diagnostic classification. However, future work is underway to also examine the association between early attentional features and gold-standard ASD diagnoses later in development.

Developmental Ability. The Mullen Scales of Early Learning (MSEL, Mullen, 1995) is a standardized measure of cognitive development for children under 68m. The measure includes five developmental domains: gross and fine motor, receptive and expressive language, and visual reception. Scales have demonstrated adequate internal consistency (.75-.83), test-retest reliabilities (.76-.96), and interrater reliabilities (.91-.96). Participants' MSEL Early Learning Composite Standard Score from the 12-month assessment window was used as a covariate in analyses, as previous research indicates a relationship between attention and mental age in ASD (Keehn, Lincoln, Müller, & Townsend, 2010; Leekam, Hunnisett, & Moore, 1998). All administrators underwent rigorous training, which included reading training materials, observing several administrations, completing practice administrations on low-risk children, co-

administering the MSEL on high-risk children alongside trained staff, and independently administering the MSEL under the supervision of trained staff.

Quantification of Behavioral and Physiological Variables

Behavioral data were integrated into Observer XT 10.1 software (Noldus Information Technology, 2010) for offline coding. Proportion of time looking toward the screen ("behavioral looking") was calculated for each participant. Interrater agreement for behavioral looking codes across 20% of files was 83%.

Physiological artifact editing was completed by a coder trained to research-reliability through a standardized training sequence supervised by the Brain-Body Center staff. Files requiring greater than 5% editing were excluded from analyses (*n*=15; 6 ASIB, 9 LR), consistent with previous studies (e.g. Corona et al., 1998). Figure 2.1 provides further justification for using this 5% threshold. This figure depicts the distribution of editing required across participants with data that could be edited (an additional 6 files are not depicted because the proportion edited could not be generated, due to excessive artifacts). As this figure depicts, all data with greater than 5% error fell outside of 1.5 interquartile range, suggesting 5% is a reasonable threshold for considering "outliers" in data quality.

Physiological data were quantified using both summary and heart-defined attention variables. First, data were converted to interbeat interval (IBI), edited for artifacts, and analyzed using the CardioEdit and CardioBatch programs (Brain-Body Center, 2007). Three primary summary variables were generated using CardioBatch: overall IBI, standard deviation (SD) of IBI, and respiratory sinus arrhythmia (RSA). Next, duration and magnitude of HR deceleration during SA were analyzed using mean-

change algorithms in SAS 9.3 (Casey & Richards, 1991; Richards & Casey, 1991). Within the algorithm, behavioral codes were used to isolate portions of the task in which participants looked toward the screen. In each period of looking, numerical algorithms calculated heart-defined attention phases by comparing IBI to baseline, defined as the median IBI of 5 beats preceding gaze toward the screen. Baseline values were reset each time the participant looked away from the screen for more than 1.5 s. *Attention orienting* was defined as IBIs directly following the initiation of behavioral attention, prior to the onset deceleration. The onset of s*ustained attention* (SA) was indexed by 5 successive beats with longer IBIs than baseline. *Attention termination* initiated after 5 successive beats with IBIs shorter than baseline. Figure 2.2 displays individual heart-defined attention phases across these three phases relative to baseline, collapsed across assessments, individuals and groups. Each individual figure line represents a single phase of attention. As expected based on previous use of SA algorithms, infants exhibited variable durations, depths and variability of SA across phases. The present study focused exclusively on SA, which was quantified using three primary dependent variables: proportion of time in SA, average depth of SA, and standard deviation of IBI during SA.

Due to the short duration of the task and necessity of behavioral attention to calculate SA variables, SA variables were not computed for participants who spent less than 30% of the task (~40 seconds) looking toward the screen (n= 9; 6 ASIB, 3 LR). This threshold was selected due to 1) the short duration of the visual attention task, 2) necessity of behavioral looking to code SA variables, and 3) presence of bimodal SA values in participants with low proportion of time in behavioral attention, suggesting brief behavioral attentiveness does not produce SA data representative of longer data

samples. However, given our goal to explore behavioral attentiveness as a primary dependent variable, these participants were retained in behavioral looking analyses to prevent biasing behavioral data

Analyses

Analyses were conducting using SAS 9.3 (Apex, NC) with α set to less than .05. Data were evaluated for analytic assumptions and outliers prior to analyses. Groups did not differ in number of assessments per participant, F (1, 41) = 0.30, p = .59, or mean chronological age across assessments, F (1, 75) = 0.32, p = .57. Normal probability plots of residuals at each analysis level indicated non-normal distribution of the following variables, which were log-transformed prior to analyses: average IBI, IBI SD, RSA, proportion time looking toward screen, proportion time in SA, IBI SD during SA, average IBI deceleration during SA, and total AOSI score. Descriptive statistics for raw data are presented in Table 2.1.

We first examined associations between behavioral looking and physiological variables to test our hypothesis that behavioral attention would positively correlate with lower IBI, higher RSA, and greater SA (higher proportion of time in SA, greater IBI SD during SA, larger IBI deceleration during SA). These analyses were conducted using Spearman partial correlations, controlling mental and chronological age.

Next, a series of multilevel models were constructed to test the hypothesis that the ASIB group would (1) be more behaviorally attentive than LR controls and (2) display higher IBI and lower RSA, and (3) would display greater SA, indexed by greater proportion of time in SA, greater IBI variability during SA, and deeper SA decelerations. To test these hypotheses, a series of multilevel models were fit to test the effect of group

membership (level 2 predictor) on initial level and change of behavioral attention (proportion time looking toward screen), heart activity (IBI, RSA, IBI SD), and heart-defined sustained attention (proportion time in SA, SD of IBI during SA, average IBI deceleration). To test our hypothesis that clinical ASD risk would be predicted by abnormal behavioral and heart-defined attention among ASIBs, we repeated these models in the ASIB group only, using AOSI total score as a continuous level 2 predictor. For each model, continuous age interactions were probed using *post hoc* nonparametric Wilcoxon signed ranked tests that tested group differences in dependent variables across three separate age ranges: 5-8 months, 8-11 months, and 11-14 months. The proportion of variance explained by conditional models was estimated using pseudo R^2, calculated as the proportion of residual variance in the null model reduced by combined explanatory variables (Singer & Willett, 2003).

Multilevel model fit was determined for each dependent variable using a series of model comparisons, which are summarized in Table 2.2. First, intraclass correlation coefficients (ICC) were constructed to determine the proportion of variance accounted for by individuals. Next, AIC and BIC were contrasted across several model types to determine which random effects best characterize data variance and covariance. As detailed in the table, ICCs indicated substantial variance in dependent variables occurring between individuals for three dependent variables: proportion time in behavioral attention, overall IBI, and overall RSA. The ICCs for the remaining variables were 0, indicating that nearly all variance in the dependent variables was accounted for by observation-level data rather than participant-level data. Figure 2.2 depicts high variability of data within and between individuals for IBI SD during SA (ICC=0). Given

the high within-individual variability, data were analyzed using both multilevel and general linear model methods, which produced consistent results. As such, multilevel results are reported for continuity. Across variables, AIC and BIC statistics indicated observations are best modeled as nested within individuals (Model 2). Notably, modeling age as a random effect (Model 3) either worsened or negligibly changed model fit, indicating the association between age and dependent variables was relatively stable across individuals. Thus across analyses, intercepts were modeled as random effects nested within individuals, whereas age was included as a fixed effect only.

For each dependent variable, unconditional means and growth next models estimated level and change in attention over time.

$$\textit{Equation 1.} \quad Y_{ij} = ß_{0j} + ß_{1j}\,(TIME_{ij}) + e_{ij}$$

Y_{ij} indicates the dependent variable (e.g. proportion looking, depth of SA) of observation *i* within individual *j*. $ß_{0j}$ and $ß_{1j}$ represent the true change trajectory intercept and slope, respectively, of individual *j*. e_{ij} represents random error for prediction of the unconditional model.

To test the hypothesis that level and change in attention vary across groups, group and cognitive ability were added to the model as level 2 time-invariant explanatory variables.

$$\textit{Equation 2.} \quad ß_{0j} = g_{00} + g_{01} Z_j + u_{0j} \qquad \textit{Equation 3.} \quad ß_{1j} = g_{10} + g_{11} Z_j$$

Equation 2 predicts overall level of attention (intercept coefficient $ß_{0j}$) from group membership (Z_j). Equation 3 predicts the relationship between attention and age, expressed as the slope coefficient $ß_{1j}$, from group membership (Z_j). g_{00} *and* u_{0j} represent the fixed and random effects of time-invariant Level 2 predictors (group, group x age)

and covariates (cognitive ability) on the intercept of attention ($ß_{0j}$). g_{10} represents the fixed effect of Level 2 predictors on the slope ($ß_{1j}$). Significant group effects indicate mean differences at the centered age of the sample (9 months), and significant age x group interaction indicates group differences in change over time. To examine effects of 12 month AOSI scores on behavioral performance in ASIBs, a second set of models included AOSI scores as time-invariant predictors of level and change in attention, controlling for cognitive ability. The proportion of variance explained by conditional models was estimated using pseudo R^2 (Singer & Willett, 2003).

Results

Biobehavioral Variable Correlations

Consistent with hypotheses that behavioral attention would correspond to greater heart-defined SA, proportion of time in behavioral and heart-defined attention positively correlated (ρ = -.69, p < .001). These effects were maintained in both ASIB and LR groups when examined individually. Contrary to hypotheses, proportion of time in behavioral attention did not correlate with global heart activity (IBI, RSA, IBI variability) or qualities of heart-defined SA (SA IBI variability, SA IBI change; *p*'s > .05). Thus, the association between behavioral attention and physiology was specific to the proportion of time in SA.

Group Differences in Attention

Unconditional models described significant variability in mean levels of variables and characterized general trends in variables across time. Significant variability was observed in mean levels of all dependent variables (p < .001), warranting examination of fixed and random effects in subsequent conditional models. Across the sample,

proportion of time in SA decreased with age (β = -0.02, p = .03), whereas the following variables increased: IBI (β = 0.01, p = .008), IBI SD (β = 0.05, p = .03), RSA (β = 0.03, p = .05) and IBI SD during SA (β = 0.06, p = .05). The remaining unconditional age effects were not significant.

Conditional models indicated group differences in proportion of time spent in both behavioral and heart-defined SA. Fixed effects for these models are listed in Table 1.3.To summarize, ASIB and LR groups exhibited different age-related patterns of proportion time in both behavioral and heart-defined attention. Figure 1.4 depicts these associations, demonstrating individual trajectories of behavioral and heart-defined SA across age (gray lines), as well as average trajectories for each group across all data points (thick black lines). The LR group exhibited age-related decreases in attentiveness across ages, whereas the ASIB group exhibited subtle increases over time. Post-hoc analyses supported this trend, with greater SA in ASIBs (Z = 1.76, p = .04) and behavioral inattention in LR controls (Z = -2.00, p = .02) within the oldest age window and was marginally higher SA in the LR group (Z = -2.47, p = .07; p=.14) within the earliest age window. Additional post-hoc comparisons were not significant, and groups did not differ in other global or heart-defined attention parameters. The conditional models explained 18% and 13% of the variability in the behavioral and heart-defined sustained attention unconditional means models, respectively. Thus, groups were primarily distinguished by distinct age-related patterns of attention, reflected at behavioral and physiological levels, with age-related decreases in attention in the LR but not ASIB groups.

Predictors of Clinical ASD Risk among ASIBs

Within-group conditional models for the ASIB group indicated significant associations between clinical ASD risk and a subset of physiological variables. Fixed effects for these models are presented in Table 2.4. Among ASIBs with available AOSI data (n = 19; behavioral assessment n = 39; physiological assessments n = 37), higher clinical ASD risk scores were associated with abnormal age-related changes in global (overall IBI, overall IBI variability) and SA (IBI change during SA, IBI variability during SA) parameters. For each of these variables, participants with lower clinical risk increased over time, whereas participants with higher clinical risk showed less robust changes across age (lower IBI and IBI variability; less IBI change and IBI variability during SA). In other words, ASIBs with higher clinical risk exhibited less typical patterns of change in each physiological variable over time, increasingly deviating from participants with lower clinical risk. An example of this pattern is depicted in Figure 2.5, with the low risk control data displayed for reference. As this figure depicts, both low clinical risk and LR groups increased in IBI SD, whereas participants with higher clinical risk exhibited more stable levels of IBI SD across age. The predictors in these models accounted for between 19-44% of the variance in unconditional growth models, as detailed in Table 2.4.

Discussion

Physiological self-regulation provides a basis for attention processes by adjusting arousal to most efficiently meet environmental demands (Porges, 1996; Posner & Rothbart, 2000). Within ASD, self-regulatory deficits are well-established and have been posited to relate to emerging ASD features early in development (Klusek et al., 2015).

The present study is the first to examine – and identify -- abnormal patterns of both behavioral and heart-defined attention in ASIBs within the first year of life, suggesting abnormal arousal may contribute to the ASD endophenotype in infants. Furthermore, we identified a subset of variables that predicted clinical markers of ASD risk at 12 months, warranting further investigation of whether infant arousal – particularly as related to visual attention – may operate as biomarkers of clinical risk in this population.

Although identifying biomarkers of ASD offers potential to revolutionize early detection and prevention efforts, it is notable that the vast majority of biomarker research in ASD, including the present study, suggest heterogeneous patterns of risk. We identified a number of attention-related predictors of behavioral ASD features among ASIBs, suggesting physiological patterns may relate to emerging ASD symptomatology. However, similar to previous studies (Messinger et al., 2013; Ozonoff et al., 2014; Toth et al., 2007), we also identified abnormal neurobiological features that distinguished ASIBs from controls but did not predict clinical ASD symptoms. A likely explanation for these patterns is that components of attention represent intermediate endophenotypes of ASD rather than specific, one-to-one clinical biomarkers of risk. An endophenotype is a measurable, heritable trait that associated with a clinical profile (Gottesman & Gould, 2003). Importantly, endophenotypes are present in individuals affected with the clinical profile (e.g. ASD) as well as their relatives, signifying "'downstream' traits or facets of clinical phenotypes, as well as the 'upstream' consequences of genes" (Gottesman & Gould, 2003, p. 637). Heart activity has been endorsed as a potential endophenotype of ASD due to the high heritability of physiological profiles and abnormal autonomic functioning observed in a subset of individuals with ASD (Klusek et al., 2015). Similarly,

a number of researchers have conceptualized atypical profiles in ASIBs and first-degree relatives of individuals with ASD as endophenotypes given clear genetic vulnerabilities and associations with ASD symptoms (Elsabbagh & Johnson, 2010; Klusek et al., 2015; Losh et al., 2008; Walsh, Elsabbagh, Bolton, & Singh, 2011). Our findings contribute to this framework by suggesting that abnormal autonomic and attentional functioning emerges in the first year of life among a subset of infants at genetic risk for ASD.

We also identified both behavioral and physiological patterns that distinguished infants with and without family history of ASD. Notably, gross indicators of behavioral and physiological attention – specifically the proportion of time in both behavioral and sustained attention – distinguished ASIB and LR groups but did not predict clinical ASD risk among ASIBs. Specifically, ASIBs demonstrated more subtle decreases in both behavioral attention and SA over time, relative to low risk controls. This pattern of sustained engagement – rather than typical decreases in engagement with age – parallels clinical observations of attention-related perseveration or "sticky attention" in older children with ASD (Sasson, Turner Brown, Holtzclaw, Lam, & Bodfish, 2008). However, these relatively diffuse temporal variables did not predict within-group variability in clinical ASD risk, suggesting insufficient sensitivity to detect subtle, clinically-relevant changes over time. Thus although it is striking that overt attentional patterns distinguished groups at such a young age, these variables did not predict within-group variability among ASIBs.

In contrast to behavioral markers that distinguished groups but did not predict clinical risk, global indicators of physiology exhibited an opposing pattern; similar patterns were exhibited at the group level, with subtle but significant deviations observed

in ASIBs with higher clinical ASD risk. Group comparisons indicated similar mean levels and age related patterns of global IBI, RSA and IBI variability, with both groups exhibiting age-related increases in IBI typical to infant development. However, ASIBs with higher clinical ASD risk markers in the 12 month assessment window exhibited slower rates of change in IBI and IBI SD across age, suggesting gradual deviations from typical trajectories. These deviations were not robust enough to produce differences between LR and ASIB groups as a whole, potentially reflecting modest sample size or emerging differences in a subset of the ASIB sample. These patterns suggest that autonomic dysfunction observed in children and adults with ASD (see Cheshire, 2012; Klusek et al., 2015, for review) may be detectable during the first year of life. However, higher clinical risk was associated with relative hypoarousal, a pattern that contradicts previous findings that ASD is associated with hyperarousal in older individuals (Bal et al., 2010; Kushki et al., 2013; Ming et al., 2005). Given arousal has been previously unstudied in ASIBs, it is possible that developmental profiles differ from infancy and later development. Indeed, in fragile X syndrome, ASD features are associated with hypoarousal in the first year of life but hyperarousal in later toddlerhood (Roberts, Tonnsen, Robinson, & Shinkareva, 2012), thus it is possible that ASD is associated with shifts in arousal across early development. Further longitudinal work is needed to test this theory in ASIBs, as well as to examine the association between early abnormalities and later ASD diagnoses.

Whereas behavioral attention and global physiology produced group differences (behavioral attention) or predicted clinical ASD risk among ASIBs (global physiology), qualities of heart-defined SA both differentiated groups and predicted clinical ASD risk

in ASIBs, supporting SA as a potentially robust indicator of abnormal development in this sample. Similar to previous samples (Casey & Richards, 1988; Richards & Casey, 1991; Richards, 1997), behavioral attention correlated with measures of SA, supporting the measure as a biomarker of attention in ASIBs. Notably, specific qualities of SA – rather than proportion of time in SA – predicted clinical ASD risk among ASIBs, with higher clinical ASD risk among ASIBs who exhibited slower changes in SA-related IBI variability and IBI change across ages, resulting in lower levels of IBI variability and change at older ages. These emerging group differences may evolve into blunted arousal modulation observed in children and adults with ASD (Althaus et al., 1999), although this theory must be tested empirically. Interestingly, similar patterns of lower IBI variability and less IBI change during SA have been reported in infants with fragile X syndrome, a single-gene disorder highly associated with ASD (Roberts et al., 2011). Together, these data suggest that heart-defined SA may inform the heterogeneity of ASD risk in infancy, although future work is needed to determine the course and implications of these associations.

Future Areas of Study

Together, our data suggest ASD-related physiological dysregulation may emerge in infancy, laying the foundation for several future areas of study including (1) the integration of physiology and translational science, (2) longitudinal profiles and consequences of abnormal arousal in ASIBs, and (3) generalizability of these features to other high-risk groups.

First, our data provide initial evidence that physiological profiles may inform the etiology and emergence of ASD-related features, warranting further study of the utility

and scope of integrating physiology in translational science. Next steps will include establishing the specific onset of self-regulatory deficits, given our data suggest emergent group differences between ASIBs and LR controls by 6 months of age. Further work is also needed to clarify whether global atypicalities and attention-specific impairments present as additive or multiplicative components of risk. For example, questions may include whether abnormal SA drives global physiological differences, or whether abnormal SA is a downstream effect of broader self-regulatory deficits. Similarly, the etiology of these self-regulatory deficits – and potential endophenotypes of ASD – must be explored through the integration of clinical science, neuroscience and genetics.

This work may lay foundation for interventions to improve ASD-related outcomes, potentially by targeting infant self-regulation. Although a heart-defined SA predicted clinical ASD risk within our ASIB sample, a number of questions also remain regarding the potential utility and applicability of this biomarker in clinical research. It will be important to determine the incremental validity of employing measures of heart-defined SA beyond simply measuring global indicators of heart activity, as these measures also predicted clinical ASD risk in the present sample. For example, further work may examine whether SA is more sensitive to clinical risk or more closely related to attention-related impairments observed in ASD, as well as study potential directional effects between attention-related physiology and global arousal modulation. These studies may inform whether SA provides an incrementally sensitive marker of developmental change in ASIBs, a first step to potentially incorporating measures of SA into early detection and intervention monitoring protocols.

An important next phase of this work will be to establish long term profiles and developmental cascades associated with abnormal physiology. Although exploring group-level differences in ASIBs – regardless of later ASD status – informs endophenotypes of risk, it will also be important to establish the association between abnormal behavioral and heart-defined SA to later ASD diagnoses and other developmental concerns (e.g. language impairments, mental health concerns) reported in a subset of first-degree relatives of individuals with ASD. As noted by Rogers (2009), unpacking group differences by examining within-group variability and outcomes is critical given (1) a number of studies have reported developmental abnormalities in ASIBs that did not predict specific developmental concerns, and (2) developmental abnormalities do not necessarily follow a linear course, thus developmental differences in infancy may not map onto later developmental atypicalities. Although the present study provides a first step to these investigations by characterizing associations between attention and clinical ASD risk in infancy, longitudinal follow-up of these participants will be critical to informing long-term processes associated with these early impairments. Indeed, this work is currently underway in the present sample and will be the topic of future publication.

The associations we observed between physiological variables and clinical ASD risk also warrant further investigation into the generalizability of these findings to other infant samples at elevated risk for ASD. For example, infants with fragile X syndrome exhibit elevated rates of ASD symptoms (Bailey, Skinner, Davis, Whitmarsh, & Powell, 2008; Kaufmann et al., 2004), and abnormal patterns of heart-defined SA have been associated with ASD symptoms in a small sample of affected infants (Roberts et al.,

2011). Indeed, Roberts et al reported lower IBI variability, less IBI change during SA, and longer behavioral looks in infants with fragile X compared to LR controls, patterns that parallel physiological predictors of clinical ASD risk in our ASIB sample. Given ASIBs have been shown to exhibit abnormal behavioral patterns not associated with later ASD diagnoses (Messinger et al., 2013; Ozonoff et al., 2014; Toth et al., 2007), cross-syndrome comparisons are important to informing the specificity and generalizability of potential ASD precursors detected in ASIB samples.

Limitations

These future directions highlight a number of limitations of the current study, including the relatively small sample, lack of long-term ASD and developmental outcome measures, and lack of non-ASIB high-risk comparison groups. Due to infant noncompliance, our analyses also do not include several assessments in which participants either did comply with the task (*n*=1 ASIB) or provided insufficient behavioral looking to examine physiological data (*n*= 9; 6 ASIB, 3 LR). Although behavioral looking paradigms that included the later 9 participants produced similar patterns to physiological paradigms that excluded these participants, future studies may examine attention during longer paradigms longer paradigms to improve participants' engagement opportunities an increase sample representativeness. Although the present study employed a passive looking paradigm to examine endogenous attentional regulation, gaze-contingent paradigms may also be used by future studies to elicit greater behavioral attention by providing real-time feedback regarding participant compliance. Examining heart-defined SA across multiple contexts and task designs is important to informing the applicability of this method to translational science.

Conclusion

The present study provides initial evidence that infants at elevated genetic risk for ASD, as a group, exhibit abnormal patterns of both behavioral and heart-defined attention, with a subset of physiological markers predicting clinical ASD risk. In addition to informing the early course of ASD emergence by identifying abnormal physiological dysregulation in ASIBs within the first year of life, this work provides a foundation for further study of heart-defined SA as a potential endophenotype of ASD, as well as a potential tool for sensitively monitoring developmental change over time. Given further characterization and study, these biomarkers could both inform etiological processes, targeted detection, and optimized interventions for infants at elevated risk for ASD.

Table 2.1: Descriptions of Primary Variables at both Individual and Assessment Levels

	High Risk Infant Sibling (*n*=21)					Low Risk Control (*n*=22)				
	n	$\bar{X}$	SD	min	max	*n*	$\bar{X}$	SD	min	max
Individual-Level Variables										
n assessments	21	1.86	0.79	1	3	22	1.73	0.77	1	3
MSEL SS	21	97.62	16.26	60	137	22	102.82	10.69	80	117
AOSI Total Score	19	6.47	4.72	1	19					
AOSI N Markers	19	4.32	2.38	1	10					
Assessment-Level Variables										
Age in months	39	9.89	2.42	5.98	13.41	38	10.18	2.17	5.69	13.74
% Inattentive	39	0.40	0.24	0.08	0.95	38	0.44	0.22	0.03	0.81
Heart Activity										
Overall IBI	39	473.90	45.01	382.58	583.41	38	473.19	48.49	396.11	580.71
Overall RSA	39	4.00	1.11	1.93	6.78	38	4.46	1.14	2.59	6.79
Overall IBI SD	39	29.32	15.41	5.63	81.69	38	31.89	12.91	11.48	65.35
Sustained Attention										
Proportion SA	37	0.30	0.21	0.00	0.92	36	0.30	0.21	0.00	0.95
SA IBI SD	35	27.10	16.90	5.45	89.09	35	29.51	14.38	4.47	63.39
SA Mean IBI Δ	35	33.53	22.99	3.96	110.47	35	36.55	22.41	7.97	130.15

Note. Individual-level variables are measured on one occasion per individual (final assessment, during "12 month" assessment window) and assessment-level variables are collapsed across all assessments and individuals in each group. MSEL=Mullen Scales of Early Learning (Mullen, 1995), AOSI=Autism Observation Scale for Infants (Bryson et al., 2008), IBI=interbeat interval, RSA=respiratory sinus arrhythmia, SD=standard deviation, SA=sustained attention

Table 2.2: Model Fit Parameters and R^2, Cross-Group Comparisons

Dependent Variable		**Model 1** No Random Effects	**Model 2 (Null Model)** Fixed: Intercept, Age Random: Intercept	**Model 3** Fixed: Intercept, Age Random: Intercept, Age	**ICC** Level 1	**R^2** Full Model vs. Null Model
Inattentive	AIC	-53.9	-49.8	-47.4	.37	.18
	BIC	-51.5	-46.3	-40.3		
Heart Activity						
Overall IBI	AIC	-118.6	-118.1	-116.1	.26	-.03
	BIC	-116.4	-114.7	-109.4		
Overall RSA	AIC	26.8	31.3	29.3	.06	.05
	BIC	29.0	34.7	36.1		
Overall IBI SD	AIC	92.3	93.1	90.8	0	.003
	BIC	94.5	94.7	97.6		
Sustained Attention						
Proportion SA	AIC	-60.2	-57.3	-53.6	0	.13
	BIC	-58.0	-55.6	-48.5		
SA IBI SD	AIC	118.6	119.8	118.6	0	-.03
	BIC	120.8	121.5	125.3		
SA Mean IBI Δ	AIC	132.6	136.4	141.4	0	-.03
	BIC	134.8	138.1	148.2		

Note. Full Model included main effects of age, diagnostic group, the interaction between age and diagnostic group, mental age (covariate). R^2 = (Residual Variance Null Model – Residual Variance Full Model)/Residual Variance Null Model. Level-1 ICC = Intercept Variance / (Intercept Variance + Residual Variance) when intercept is modeled as a random effect. IBI=interbeat interval, RSA=respiratory sinus arrhythmia, SD=standard deviation, SA=sustained attention

Table 2.3: Fixed Effects of Group Membership on Heart Activity

Model Component	Estimate	*SE*	*df*	*t-value*	*p*
% Time Inattentive					
Intercept	**0.33**	**0.03**	**49**	**9.65**	**<.0001**
Age	**0.02**	**0.01**	**49.2**	**2.31**	**0.03**
Group	0.03	0.05	46.1	0.60	0.55
Group*Age	**-0.04**	**0.01**	**47.9**	**-3.12**	**0.003**
Intellectual Ability	0.00	0.00	37	0.72	0.47
Overall IBI					
Intercept	**6.13**	**0.02**	**47.7**	**311.67**	**<.0001**
Age	**0.02**	**0.01**	**47.1**	**2.55**	**0.01**
Group	0.02	0.03	47.9	0.64	0.53
Group*Age	-0.01	0.01	48.1	-0.99	0.33
Intellectual Ability	0.00	0.00	40.9	-0.61	0.55
Overall RSA					
Intercept	**1.45**	**0.06**	**34.8**	**24.34**	**<.0001**
Age	0.02	0.02	39.1	0.79	0.43
Group	-0.15	0.09	35.3	-1.68	0.10
Group*Age	0.02	0.03	40.8	0.68	0.50
Intellectual Ability	0.00	0.00	27.7	-1.66	0.11
Overall IBI SD					
Intercept	**3.37**	**0.09**	**37.5**	**36.02**	**<.0001**
Age	0.03	0.04	49.9	0.79	0.43
Group	-0.18	0.14	38	-1.32	0.19
Group*Age	0.04	0.05	51.6	0.78	0.44
Intellectual Ability	0.00	0.00	32.8	-0.86	0.40
Proportion SA					
Intercept	**0.29**	**0.03**	**63**	**10.86**	**<.0001**
Age	**-0.04**	**0.01**	**63**	**-3.36**	**0.00**
Group	0.01	0.04	63	0.23	0.82
Group*Age	**0.04**	**0.01**	**63**	**2.49**	**0.02**
Intellectual Ability	0.00	0.00	63	1.82	0.07
Average IBI SD during SA					
Intercept	**3.18**	**0.11**	**62**	**27.74**	**<.0001**
Age	0.07	0.05	62	1.54	0.13
Group	-0.09	0.17	62	-0.52	0.60
Group*Age	-0.02	0.06	62	-0.37	0.71
Intellectual Ability	0.00	0.01	62	-0.71	0.48
Average IBI Change during SA					
Intercept	**3.43**	**0.13**	**62**	**26.40**	**<.0001**
Age	0.03	0.05	62	0.64	0.52
Group	-0.19	0.19	62	-0.98	0.33
Group*Age	0.00	0.07	62	0.04	0.97
Intellectual Ability	0.00	0.01	62	0.01	0.99

Table 2.4: Fixed Effects of Autism Risk on Heart Activity and Heart Defined Attention

Model Component	Estimate	*SE*	*df*	*t-value*	*p*
% Time Inattentive (R^2=.01)					
Intercept	**0.42**	**0.11**	**15.20**	**3.84**	**0.002**
Age	-0.03	0.03	19.40	-1.13	0.27
Autism Risk	-0.03	0.06	14.70	-0.59	0.56
Autism Risk*Age	0.01	0.01	20.90	0.41	0.69
Intellectual Ability	0.00	0.00	15.00	1.32	0.21
Overall IBI (R^2=.22)					
Intercept	**6.16**	**0.06**	**16.30**	**107.36**	**<.0001**
Age	**0.05**	**0.02**	**22.30**	**3.10**	**0.01**
Autism Risk	-0.01	0.03	15.50	-0.25	0.81
Autism Risk*Age	**-0.02**	**0.01**	**24.00**	**-2.59**	**0.02**
Intellectual Ability	0.00	0.00	16.20	-0.12	0.90
Overall RSA (R^2=.13)					
Intercept	**1.06**	**0.16**	**14.40**	**6.86**	**<.0001**
Age	**0.15**	**0.06**	**24.60**	**2.41**	**0.02**
Autism Risk	0.11	0.08	13.40	1.42	0.18
Autism Risk*Age	-0.05	0.03	26.30	-1.69	0.10
Intellectual Ability	0.00	0.00	15.50	-0.93	0.37
Overall IBI SD (R^2=.33)					
Intercept	**2.74**	**0.26**	**13.90**	**10.64**	**<.0001**
Age	**0.41**	**0.09**	**22.70**	**4.46**	**0.0002**
Autism Risk	0.21	0.13	13.00	1.57	0.14
Autism Risk*Age	**-0.17**	**0.05**	**24.60**	**-3.67**	**0.001**
Intellectual Ability	0.00	0.00	14.40	-0.58	0.57
Proportion SA (R^2=-.02)					
Intercept	**0.26**	**0.09**	**32.00**	**2.96**	**0.01**
Age	0.01	0.04	32.00	0.37	0.72
Autism Risk	-0.02	0.04	32.00	-0.34	0.73
Autism Risk*Age	0.00	0.02	32.00	-0.14	0.89
Intellectual Ability	0.00	0.00	32.00	-0.73	0.47
Average IBI Variability during SA(R^2=.44)					
Intercept	**2.58**	**0.33**	**14.20**	**7.89**	**<.0001**
Age	**0.44**	**0.10**	**19.70**	**4.36**	**0.0003**
Autism Risk	0.20	0.17	14.00	1.14	0.27
Autism Risk*Age	**-0.19**	**0.05**	**21.60**	**-3.62**	**0.002**
Intellectual Ability	-0.01	0.01	18.50	-1.15	0.26
Average IBI Change during SA (R^2=.19)					
Intercept	**3.05**	**0.39**	**12.90**	**7.79**	**<.0001**
Age	**0.40**	**0.14**	**21.20**	**2.73**	**0.01**
Autism Risk	0.05	0.20	12.50	0.23	0.82
Autism Risk*Age	**-0.18**	**0.07**	**23.20**	**-2.40**	**0.02**
Intellectual Ability	0.00	0.01	18.00	-0.17	0.87

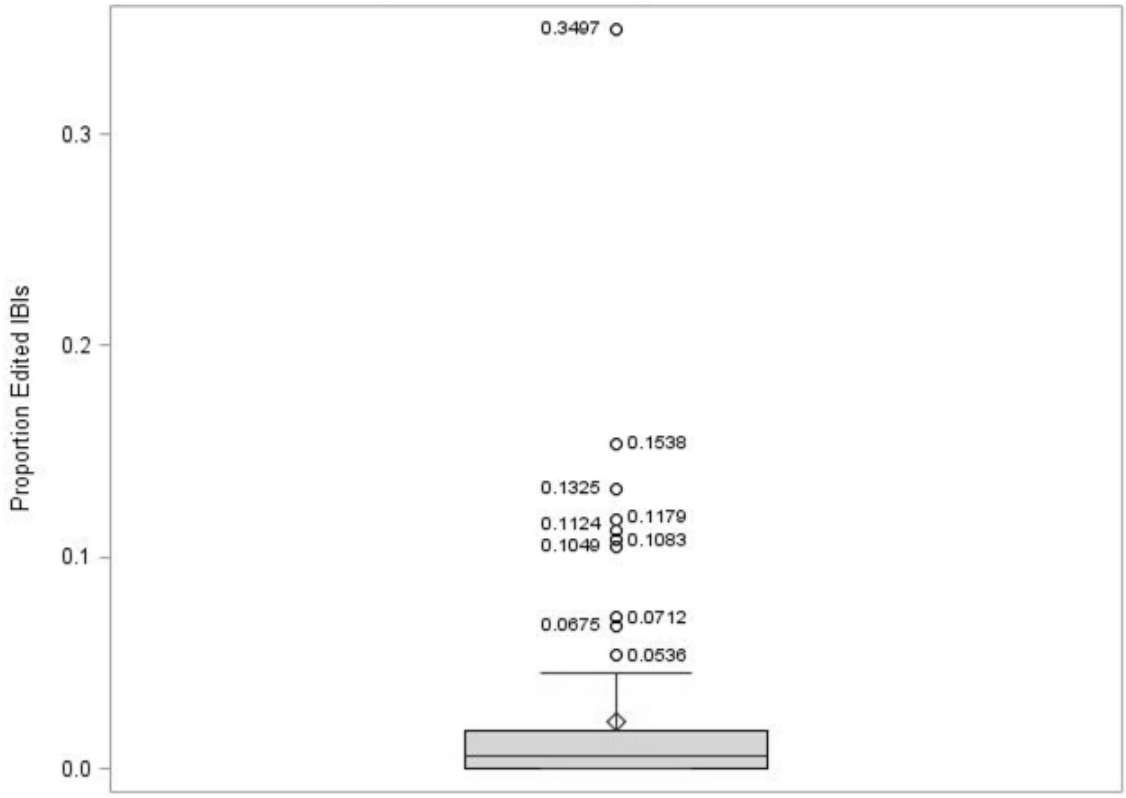

Figure 2.1: Boxplot of Physiological Data Editing, Justifying 5% Cutoff

Note. The upper whisker of the boxplot (0.049) marks 1.5 times the interquartile range (0.018) above the mean (0.022). A total of 15 files were excluded from analyses due to artifact rates exceeding 5% of IBIs. Figure 1.1 includes participants excluded from analyses due to edit rates exceeding 5% (editing rate <.055, n = 9) but does not depict additional participants whose files could not be edited due to excessive artifacts estimated to be 40% or greater (n = 6). These data justify using a 5% cutoff for physiological data inclusion, consistent with prior studies (Corona et al., 1998).

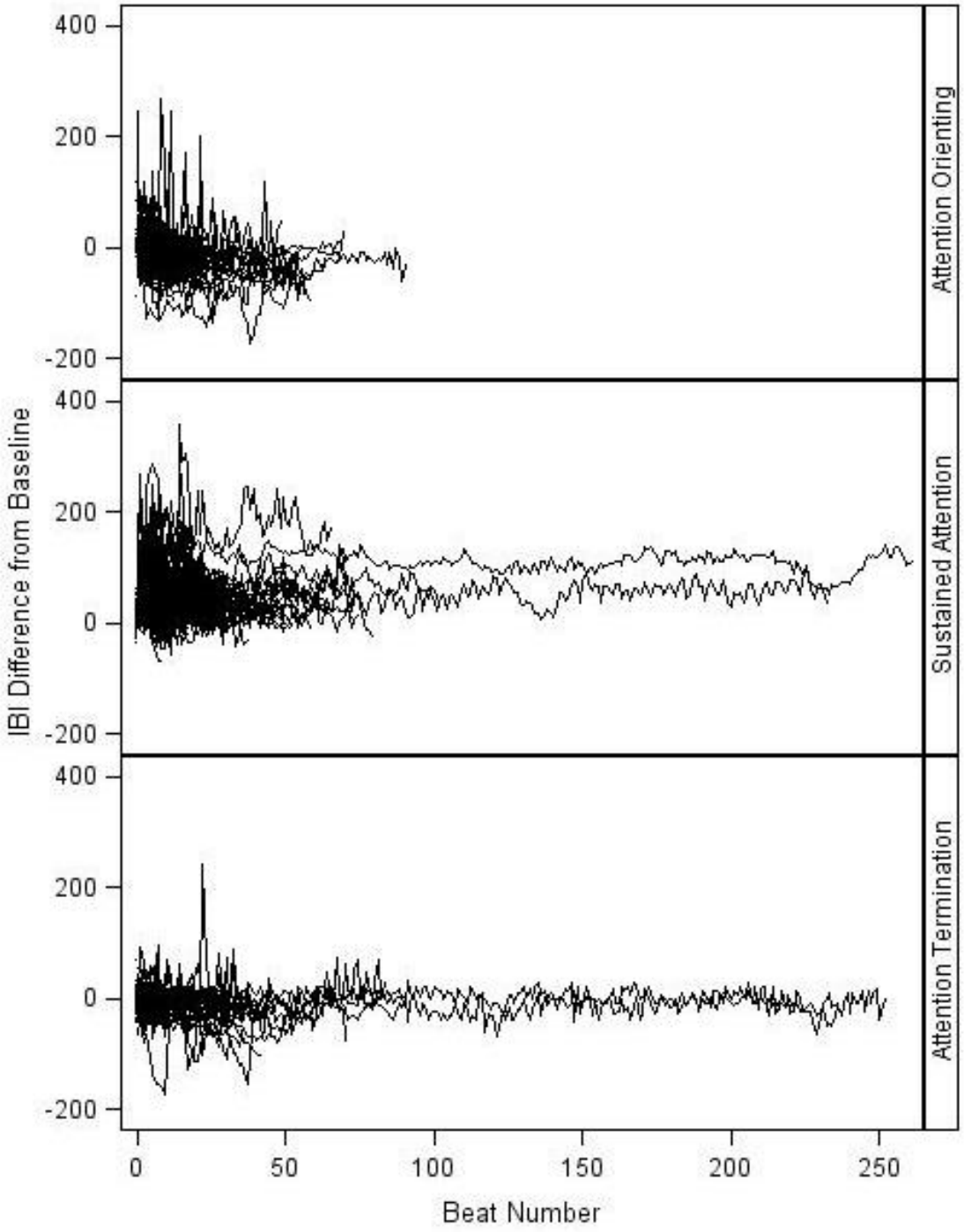

Figure 2.2: IBI Difference from Baseline across Participants and Phases by Phase Type

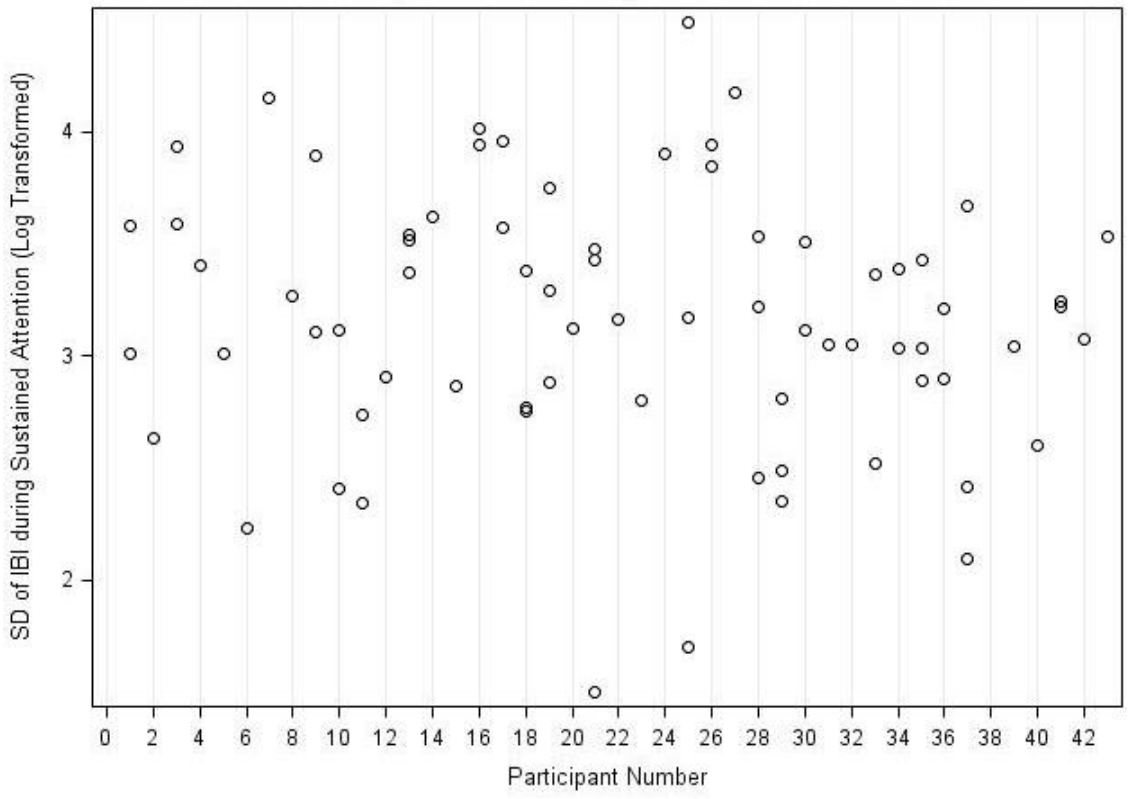

Figure 2.3: High Within- and Between-Person Variability in IBI during Sustained Attention

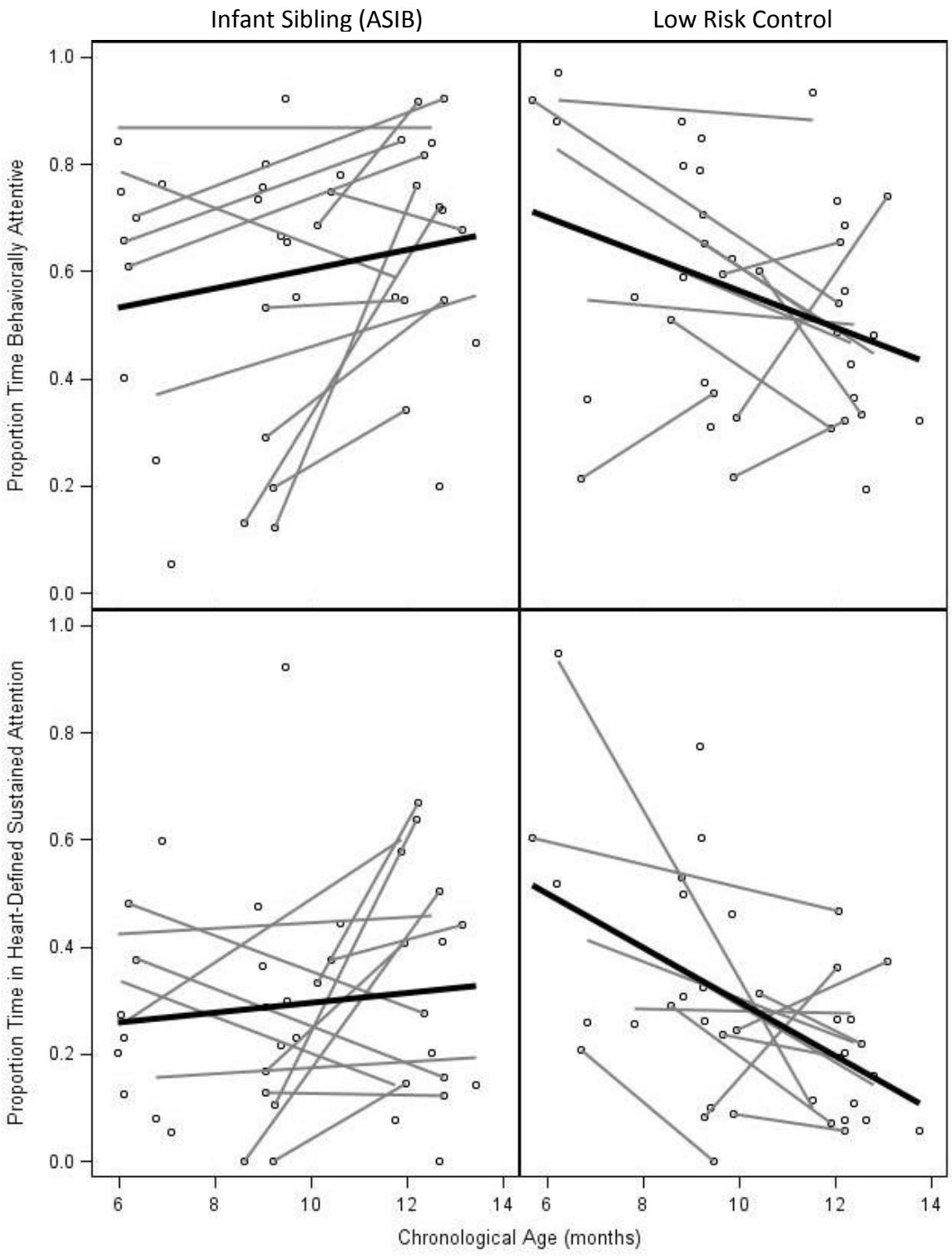

Figure 2.4: Group Difference in Behavioral and Heart-Defined Attention across Age

Note: Gray = average trajectories within individuals; black = overall regression lines by group

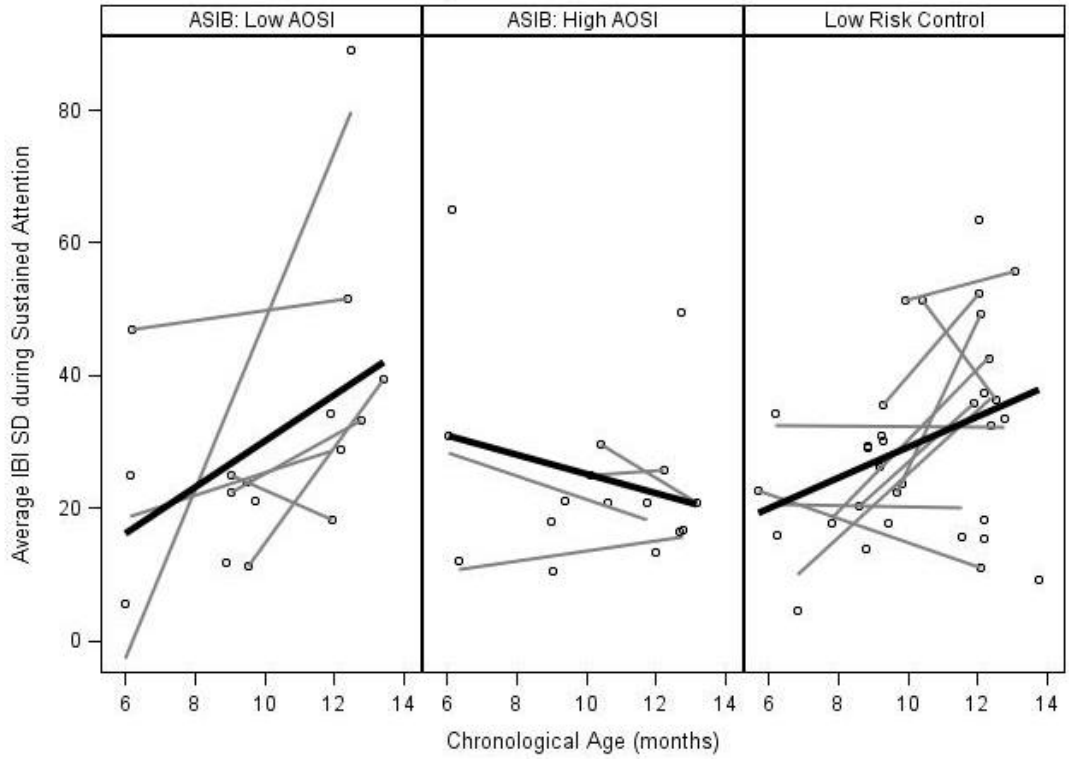

Figure 2.5: IBI Variability during SA across Age, Separated by Clinical Autism Risk

Note. Autism Observation Scale for Infants (AOSI) dichotomized using the median (Total Score > 5 = "High AOSI") for display purposes only. Average IBI SD in the LR group is displayed for reference. Gray = average trajectories within individuals; black = overall regression lines by group

CHAPTER 3

BIOBEHAVIORAL SIGNATURES OF VISUAL ATTENTION IN THE BROADER AUTISM PHENOTYPE[2]

[2] Tonnsen, B. L., Richards, J. E. & Roberts, J. E. To be submitted to *Journal of the American Academy of Child and Adolescent Psychiatry*

The ability to flexibly orient attentional processes in response to environmental stimuli is critical for optimal learning and development. Children with autism spectrum disorder (ASD) often exhibit abnormal patterns of attention disengagement, the component of orienting that involves separating attention from an ongoing stimulus, a deficit that has been posited to contribute to later symptom expression (Elsabbagh & Johnson, 2007; Keehn et al., 2013; Zwaigenbaum et al., 2005). A number of studies suggest this abnormal process emerges early in development, prior to the age of ASD diagnosis, and thus may serve as a robust indicator of later ASD risk. For example, "high risk" infants with a family history of ASD display abnormal orienting toward social and nonsocial stimuli (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Landry & Bryson, 2004; Swettenham et al., 1998) and slower saccade latencies on computerized orienting tasks (Elison et al., 2013; Elsabbagh et al., 2009, 2013; Zwaigenbaum et al., 2005), compared to "low risk" controls. Although these patterns of abnormal attention disengagement have been identified across multiple high-risk samples and measures, the developmental course and biological underpinnings of these deficits are unclear, limiting knowledge about potential developmental cascades that could be minimized through early detection and prevention efforts. The present study examined behavioral and physiological patterns of attention orienting in a longitudinal sample of infants at elevated risk for ASD due to having an older sibling with an ASD diagnosis ("infant siblings"). This work aims to establish the longitudinal course of abnormal attention orienting in infant siblings, potential physiological mechanisms subserving this process, and clinical implications of risk.

Autism Precursors in Infants

Characterizing the natural history of autism precursors in infancy permits implementation of targeted interventions that may alter aberrant behavioral and neural development (Dawson, 2008). Indeed, early ASD treatment has been linked to substantial improvements in language development, adaptive behaviors, social skills, and intellectual functioning (Dawson et al., 2010; Eldevik et al., 2009). In addition to benefitting the affected child, early detection also contributes to family well-being by permitting early access to community resources and genetic counseling. These resources are particularly important in light of high stress and depression reported in parents of children with disabilities (Dabrowska & Pisula, 2010; Estes et al., 2009). Early intervention also reduces the public health costs associated with ASD by up to 65% (Järbrink & Knapp, 2001), which is particularly important given ASD is the third most expensive diagnosis in special education (Ganz, 2007) and requires lifetime treatment costs estimated as $2.4 million per child (Buescher et al., 2014). Despite the individual and community-level benefits of early intervention, the average age of ASD diagnosis remains at 4.4 years (Centers for Disease Control, 2014), resulting in treatment delays. Thus, understanding early predictors of ASD is a concern of public health importance.

Prospectively studying infant samples at elevated risk for ASD facilitates these translational efforts by permitting active surveillance of aberrant symptom profiles as they emerge. Infant siblings of children with autism (ASIBs) exhibit 10-20 times higher rates of autism diagnoses (19%; (Ozonoff et al., 2011) than the general population (1-2%; (Centers for Disease Control, 2014) and comprise the most commonly studied "high-risk" ASD sample. Research on ASIB development can be categorized by two approaches. The

first seeks to evaluate the unique characteristics of ASIBs that distinguish them from infants without a family history of ASD. This approach parallels an extensive body of adult-focused literature documenting subthreshold ASD symptoms that extend to the general population as part of the broader autism phenotype (Bolton et al., 1994; Dawson et al., 2002; Ozonoff et al., 2014; Pickles et al., 2000). Identifying these broader phenotypic features associated with genetic ASD heritability – sometimes referred to as the ASD endophenotype (Elsabbagh & Johnson, 2007; Walsh et al., 2011) – may inform the genetic architecture of ASD and risk factors for symptom expression. A second, complementary approach to ASIB research investigates specific associations between early abnormalities and later ASD diagnoses. This approach recognizes the utility of differentiating between ASIBs who demonstrate subtle atypicalities from those who ultimately receive an ASD diagnosis. Examining multiple outcomes is particularly warranted given ASIBs without ASD are also at risk for other developmental delays or disorders (Messinger et al., 2013; Ozonoff et al., 2014; Toth et al., 2007), and a subset display abnormal developmental trajectories not associated with ASD by 12 months of age (Ozonoff et al., 2014). These two approaches – examining the ASD endophenotype, as well as specific predictors of ASD diagnoses – provide complementary information about genetic vulnerability for ASD and potential "red flags" for individual development.

Attention and the ASD Endophenotype

Abnormal attention has been described as a central feature of the ASD endophenotype due to consistent, clinically-relevant abnormalities in observed in ASD (see Keehn et al., 2013, for review) and early-emerging differences in ASIBs as early as two months of age (Jones & Klin, 2013). As originally proposed by Posner and

colleagues (Posner & Petersen, 1990; Posner, 1980), attention is commonly conceived as interconnected networks of orienting, alerting, and executive control; which interact to produce stimulus response (Posner & Fan, 2008). According to Posner's model, the orienting network facilitates movement of attention toward sensory events; the alerting network is responsible for maintaining vigilance and sustaining attention; and the executive control network regulates and resolves conflict between anticipated and observed thoughts, feelings, and occurrences (Macleod et al., 2010; Posner & Rothbart, 2007). Two components of these attentional networks – orienting and arousal – are of particular relevance for understanding attention in infants at risk for autism. Here, we review the neurobiology and development of orienting and arousal, measurement of each construct, and current knowledge of their functioning within ASD.

Orienting

Attention orienting involves aligning attention with sensory or memory input by disengaging, shifting, and reengaging attention (Posner & Cohen, 1984; Posner, 1980) and is generally associated with neural activity in the ventral frontal-parietal brain regions (Corbetta & Shulman, 2002). Sokolov (1963) first described orienting as a novelty-sensitive reflex that enhances stimulus processing. Orienting may occur overtly, with accompanying head and eye movements, or covertly, without behavioral indicators (Posner, 1980). Similarly, orienting may be exogenously driven by individual intention or exogenously drive by stimulus input.

The ability to flexibly orient attention emerges and strengthens across the first year of life. Although the emergence of orienting in infants was originally attributed to shifts from subcortical to cortical processing (Bronson, 1974), more recent models

describe interactions among multiple developing pathways. Around 1 month of age, infants are able to produce saccades but display difficulty disengaging from stimuli, resulting in *obligatory looking* (see Johnson, 1989, for review). According to Johnson (1989), obligatory looking emerges due to the onset of an inhibitory pathway that prevents orienting. The maturation of additional cortical layers from age 2 months onward overrides this inhibitory pathway, permitting increased visual flexibility. Thus, the ability to disengage attention from visual stimuli, which enables attention orienting to occur, improves from 2 to 6 months of age (Frick, Colombo, & Saxon, 1999) and continues to develop until pre-adolescence or later (Wainwright & Bryson, 2002).

Measuring orienting. The gap-overlap task is a commonly used paradigm to measure flexibility of attention orienting, particularly the process of attention disengagement (e.g. (Hood & Atkinson, 1993). An extension of spatial cueing tasks developed by Posner and colleagues (e.g. Posner & Petersen, 1990; Posner, 1980), gap-overlap paradigms require a participant to shift attention from a central stimulus to a subsequently presented peripheral stimulus. Reaction times (RT) are compared across experimental manipulations, which may include three trial types: baseline, gap, and overlap. During baseline trials, the central stimulus is extinguished simultaneously with peripheral stimulus onset. During gap trials, a temporal gap separates the offset of the central stimulus and onset of peripheral stimulus, providing an exogenous cue for orienting to occur. During overlap trials, the peripheral stimulus appears during presentation of the central stimulus, requiring the participant to disengage attention from the central stimulus prior to shifting attention to the peripheral stimulus. Reaction times across conditions are then compared to assess *disengagement*, the relative increase in RT

in overlap versus baseline trials, and *facilitation*, the relative reduction in RT in gap versus baseline trials. This paradigm has been used to examine attention orienting in clinical and non-clinical samples from infancy to adulthood (e.g. Hood & Atkinson, 1993; Kikuchi et al., 2011; Van Der Geest, Kemner, Camfferman, Verbaten, & Van Engeland, 2001; Zwaigenbaum et al., 2005).

Orienting in ASD and ASIBs. Abnormal orienting has been extensively documented in ASD. In fact, individuals with ASD are often described as displaying *sticky attention* (Kawakubo et al., 2007; Keehn et al., 2013) due to persistent orienting deficits observed toward social and nonsocial stimuli (Dawson et al., 1998; Landry & Bryson, 2004; Swettenham et al., 1998). The term *sticky attention* is distinguished from the terms *sticky fixation* or *obligatory looking*, which are terms used to describe young infants who are unable to disengage visual attention due to immature cortical development (Johnson, 1989). Characteristics of sticky attention in ASD appear to emerge during infancy, prior to the full presentation of symptoms required for ASD diagnosis. Behavioral studies have reported that ASIBs who later meet ASD criteria attend less toward an examiner in distress (Hutman et al., 2010) and display atypical joint attention and requesting behaviors (Rozga et al., 2011). Similarly, cognitive paradigms indicate that between 6-12 months of age, a subset of ASIBs begin to exhibit abnormal attention orienting, particularly in their ability to disengage attention from to attend to competing stimuli (Elison et al., 2013; Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009; Zwaigenbaum et al., 2005). Although the neurodevelopmental cause of sticky attention in ASD is unclear, slower attention disengagement has been posited to restrict early learning and social opportunities essential to typical development, potentially

intensifying ASD symptom trajectories (Elsabbagh & Johnson, 2007; Keehn et al., 2013; Zwaigenbaum et al., 2005).

To date, four primary studies have employed spatial cuing "gap-overlap" tasks to investigate attention disengagement in ASIB samples. Key findings are summarized in Table 3.1. Results from these studies generally suggest that slower orienting – particularly during attention disengagement – emerges within the first year of life in a subset of ASIBs, although specific findings vary across samples and methodologies. In one of the first prospective ASIB studies, Zwaigenbaum and colleagues (2005) reported that change in smoothness of visual tracking and speed of disengagement between 6 and 12m, but not group differences at 6m, distinguished ASIBs from low risk (LR) controls, and slower disengagement at 12m correlated with 24m algorithm scores on the Autism Diagnostic Observation Schedule (ADOS). Notably, based on descriptive statistics provided by the authors, group differences in disengagement at 6m produced a moderately sized effect (*d*=.47;Cohen, 1988), suggesting emerging differences that may have been detectable in a larger sample. Longer latencies on Overlap trials have been similarly reported 8-12m ASIBs (Elsabbagh, Volein, et al., 2009), as well as in 6-8m ASIBs who later meet ASD criteria (Elison et al., 2013). These data suggest that slower disengagement is a relatively consistent construct in ASIBs and may indicate risk for later ASD symptomatology.

Zwaigenbaum's initial (2005) study highlights the utility of longitudinal designs to detect developmental processes – such as within-individual change – that cannot be detected through static, cross-sectional designs. In a longitudinal sample of infants at approximately 7 (range 6-10) and 14 (range 12-15) months, Elsabbagh and colleagues

(2013) similarly reported that slower speed of disengagement in older infants, but not younger infants, related to later ASD outcomes in ASIBs. Again, ASIBs who later exhibited ASD failed to show typical improvements in overlap latencies over time, with 40% of ASIBs later diagnosed with ASD displaying slower overlap latencies at 14m. However, the authors also reported that ASIBs defined as "atypical" – either because they did not meet full ASD criteria or displayed developmental impairments – similarly did not improve over time, and slower latencies were observed across trial types in children with lower intellectual abilities. This finding suggests that blunted improvement in the orienting network may relate to the broader ASD endophenotype rather than ASD-specific risk factors, although further work is needed to replicate and explain these findings.

Arousal

Given accumulating evidence of abnormal orienting in the first year of life among ASIBs, an important next step is to examine potential mechanisms of these impairments. It is possible that abnormal orienting in ASD may reflect simultaneous abnormalities in the alerting network, particularly in the domain of physical arousal, which has been described extensively in ASD (see Keehn et al., 2013, for review) and ASIBs (Study 1). Alertness– often defined as a state of readiness to process information – was originally investigated in 1949 when Moruzzi and Magoun directly stimulated the reticular formation of cats to produce sleep and wakefulness (Moruzzi & Magoun, 1949). Since this classic experiment, our conceptualization of alerting has evolved to include both tonic and phasic components (Porges, 1976, 1980). Tonic alertness refers to general levels of arousal and wakefulness, whereas phasic alertness describes the reactive,

transient process of increasing arousal in response to an external stimulus (Sturm & Willmes, 2001). These processes are closely linked to the reticular activating system, thalamus, and neurochemical pathways that connect the brainstem and neocortical areas (see Colombo, 2001; Reynolds & Richards, 2007; Richards, 2008, for review).

Measuring alerting. Voluntary control of tonic alertness, termed *sustained attention,* is reflected by bodily, physiological, and brain changes indicative of increased processing (Jennings, 1986; Porges, 1976; Richards, Reynolds, & Courage, 2010). As attention increases, parasympathetic terminals release acetylcholine at the heart's sinoatrial node, slowing depolarization of synapses and resulting in HR deceleration. As attention decreases, sympathetic terminals release norepinepherine to speed depolarization, resulting in increased HR. Decades of research have examined these decelerative patterns of HR as biomarkers of sustained attention (Graham & Clifton, 1966; Lacey, 1959). In infants, these attention-related HR fluctuations have been quantified as three phases: orienting, sustained attention, and attention termination (Casey & Richards, 1991; Richards & Casey, 1991; Richards, 2000). *Attention orienting* is marked by deceleration of HR by 8-10 beats per minute (BPM), s*ustained attention* is the maintenance of this decelerated HR as stimulus details are processed, and *attention termination* is the return of heart-rate to prestimulus levels. Importantly, this progression through attention phases occurs during continued behavioral looking, thus heart-defined measures capture qualities of attention not detectable using overt looking alone.

Infants' behavioral performance on orienting tasks has been closely associated with heart-defined sustained attention. Richards (1987) presented 14 to 26 week infants with central stimuli that were interrupted by peripheral stimuli on a subset of trials.

Infants who were in periods of heart-defined sustained attention were less distractible than infants in periods of attention termination. In subsequent studies, same-aged infants were less likely to orient to a briefly-presented peripheral stimulus during heart-defined orienting and sustained attention compared to pre-attention and attention termination (Casey & Richards, 1988; Richards, 1997). These findings have been replicated across a variety of stimuli types, including children's videos (Richards & Casey, 1991) and toy play (Lansink & Richards, 1997; Roberts, Hatton, Long, Anello, & Colombo, 2011). Although the majority of sustained attention studies have examined non-clinical samples, sustained attention has also been used to index attention in infants with fragile X syndrome, a single-gene disorder highly associated with intellectual disability and ASD (Roberts et al., 2011). Thus, sustained attention is a valid and reliable biomarker of attention engagement in infants and is closely associated with behavioral performance on orienting tasks.

Arousal in ASD and ASIBs. Abnormal autonomic functioning has been posited to contribute to the emergence and expression of numerous psychological conditions (Beauchaine, 2001; Clark & Watson, 1991; Porges, 1976) and is well-documented in children and adults with ASD (see Cheshire, 2012; Keehn et al., 2013; Klusek et al., 2015, for review). Although findings vary across studies, ASD is generally associated with faster HR (Bal et al., 2010; Kushki et al., 2013; Ming et al., 2005) and difficulty modulating arousal during changing task demands (Althaus et al., 1999; Smeekens et al., 2015; Vaughan Van Hecke et al., 2009). In general, respiratory sinus arrhythmia (RSA), HR variability associated with respiration, is lower at rest in ASD (Bal et al., 2010; Ming et al., 2005) but may increase abnormally during cognitive stress (Porges, 2013),

suggesting inability to modulate vagal functioning. It has also been proposed that ASD includes both hyper- and hyporesponsive subtypes, which may be associated with increased self-soothing and self-stimulatory behaviors, respectively (Hirstein, Iversen, & Ramachandran, 2001). In older participants with ASD, shallower HR decelerations have been reported in response to an emotional event (Corona et al., 1998), although other studies have reported similar HR patterns across groups (Louwerse et al., 2014; Mathersul et al., 2013). Faster HR and lower respiratory sinus arrhythmia also correspond to greater ASD symptomatology within ASD samples (Bal et al., 2010; Klusek et al., 2013; Vaughan Van Hecke et al., 2009), supporting autonomic functioning as a potential index of symptom severity. Interestingly, developmental shifts from hypoarousal to hyperarousal have been linked to later ASD symptoms in infants and toddlers with fragile X syndrome (Roberts et al., 2012), suggesting abnormal heart activity may relate to ASD precursors in infancy. Given these well-documented abnormalities, it is possible that abnormal autonomic functioning may index – and possibly contribute to -- abnormal orienting observed in ASD.

Although a number of studies have documented abnormal autonomic functioning in ASD, this topic has been largely unstudied in "high risk" infants. We previously examined patterns of global autonomic functioning and heart-defined SA in a longitudinal sample of 5-14m ASIBs and LR controls (Study 1). During passive viewing of a brief children's video, ASIBs demonstrated atypical maintenance of greater behavioral and heart-defined SA across age, despite age-related reductions in these markers (e.g. increased inattention) in LR controls. The quality of SA during the task predicted clinical ASD risk at the latest time point. Specifically, ASIBs with higher

clinical risk markers exhibited slower developmental changes in both global (overall IBI, overall IBI variability) and sustained-attention related (IBI change during SA, IBI variable during SA) physiological markers, relative to ASIBs with lower clinical risk scores. Thus, higher ASD clinical risk was predicted by increasingly abnormal physiological trajectories across age. To our knowledge, Study 1 is the first paper to document abnormal autonomic functioning in ASIBs and suggests that abnormally elevated behavioral and heart-defined attention, as well as increasingly deviant physiological profiles across time, may characterize the ASD endophenotype in infants. However, additional work is needed to examine these constructs in different experimental contexts. For example, it is likely that physiological profiles will differ substantially in response to passive viewing paradigm versus gaze-contingent tasks, such as gap-overlap paradigms that require participant looking. Studying sustained attention in different contexts may also inform whether atypical patterns we previously documented in ASIBs relate to abnormal attention orienting. With further study, examining the intersection of physiological functioning and attention orienting may inform whether abnormal autonomic functioning serves as a biological indicator, and potential contributing factor, to aberrant behavioral profiles in ASIBs.

Unpacking ASIB Orienting: An Integrated Perspective

From the previous literature, it is clear that abnormal attention orienting, particularly attention disengagement, is present early in development in a subset of infants at risk for ASD. It is also possible that autonomic functioning may index these early abnormalities, potentially providing a sensitive biomarker of risk and developmental change. A critical next step will be to unpack the developmental course

and mechanisms of aberrant attention disengagement during the first year of life, both through longitudinal surveillance and diversified methodologies. This work will inform the applicability of aberrant disengagement to characterizing the ASD endophenotype and identifying early ASD risk.

It is increasingly recognized that longitudinal surveillance is critical to delineating abnormal trajectories in neurodevelopmental disorders (Cornish, Scerif, & Karmiloff-Smith, 2007; Karmiloff-Smith, 2009; Tonnsen, Grefer, Hatton, & Roberts, 2014; Tonnsen, Malone, et al., 2013).Indeed, the two prior longitudinal studies of attention disengagement in ASIBs (Elsabbagh et al., 2013; Zwaigenbaum et al., 2005) have revealed within-individual patterns of change that were not detectable in cross-sectional, static designs. However, to date, the majority of ASIB attention studies, including the two longitudinal studies of gap-overlap performance in ASIBs, have examined associations between clinical outcomes and 1-2 behavioral time points across the first 24 months of life (see Jones et al., 2014, for detailed review of studies). These studies have used categorical models to examine age effects (e.g. repeated measure analysis of variance), often collapsing participants in age "bins" to examine developmental change. For example, all previous ASIB gap-overlap studies have employed categorical age groups, some collapsing participants across age ranges as large as 4 months (Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009). Given the rapid maturation of attention orienting during the first year of life, this categorical approach gives a valuable estimate of gross developmental change but may ultimately limit our conceptualization of nuanced age-related changes relevant to ASD emergence. In recognition of this limitation, a number of ASIB studies have begun employing more developmentally sensitive surveillance

strategies using multiple time points within the first year of life (Jones & Klin, 2013; Ozonoff et al., 2010, 2014; Sacrey et al., 2013), although this approach has yet to be applied to examine attention orienting in ASIBs.

Further studies are also needed to clarify the neurobiological processes sustaining abnormal attention orienting in ASIBs. Measuring these biological processes, in addition to their behavioral artifacts, would likely permit more sensitive measurement of individual differences, increasing our capacity to identify etiological processes, inform treatment targets, and measure developmental change (Walsh et al., 2011). However to date, only one study of attention disengagement in ASIBs has employed neurobiological methods to inform behavioral differences. Elison and colleagues (2013) reported that group differences in disengagement were differentially related to white matter radial diffusivity, measured by diffusor tensor imaging. In LR controls, slower disengagement related to white matter immaturity in the splenium, a region of the corpus callosum that undergoes substantial postnatal development and has been associated with attention disengagement. This association was not found in the ASIB group, suggesting a potential neurobiological atypicality underlying orienting deficits in ASIBs. This study begins to inform the neurobiology of abnormal orienting in ASIBs, although a number of questions remain, including the developmental course of abnormal development, additional neurobiological processes implicated in atypical orienting, and whether additional vulnerabilities – such as self-regulatory deficits reported in ASD – may relate to disengagement abnormalities. For example, although arousal is associated with attention disengagement in typically developing infants (Casey & Richards, 1988; Richards, 1997) and has been implicated in aberrant attention in children with ASD (Corona et al., 1998),

the association between behavioral orienting and physiological arousal has not been studied in ASIBs.

The Current Study

Examining early patterns of abnormalities in ASIBs may inform both the ASD endophenotype and specific predictors of clinical risk. Although a handful of studies have reported abnormal attention disengagement – or "sticky attention" – in ASIBs, the specific developmental course and neurobiological underpinnings of this aberrant process are unclear. Examining these points of ambiguity is an important next step to informing translational efforts that may facilitate earlier identification and treatments, potentially preventing maladaptive outcomes associated with ASD (Dawson, 2008).

To characterize the course and mechanisms of aberrant attention disengagement, the present study longitudinally examined visual and heart-defined sustained attention (SA) in 5-14m ASIBs and low-risk (LR) controls. Using a gap-overlap task with concurrently measured heart activity, we aimed to inform both the nature of behavioral abnormalities, as well as the physiological patterns sub serving these behaviors. We specifically examined (1) developmental differences in behavioral and heart-defined attention across groups, (2) associations between behavioral and heart-defined attention, and (3) the association between these variables and clinical autism risk among ASIBs. We hypothesized that relative to LR controls, ASIBs would exhibit increasingly longer latencies to disengage attention across age. Longer latencies would be indexed physiologically by longer proportion of time in SA, as well as increased depths and stability of SA decelerations. Abnormal behavioral and physiological trajectories would predict higher clinical risk symptoms around 12 months of age.

Methods

Participants

Participants included 46 infants (23 ASIB, 23 LR) assessed on 1-3 occasions each for a total of 99 assessments (ASIB one assessment *n*=7, two *n*=5, three *n*=11; LR one *n*=6, two *n*=8, three *n*=9). Participants for the present study largely overlapped with Study 1, with slight variability due to missing data and the later initiation of data collection for Study 1's passive viewing task. Infant sibling and LR groups contained similar numbers of females (3 ASIBs; 5 LR; Table 3.1). Recruitment procedures and exclusionary criteria were identical to Study 1, and participants overlapped across studies. Additional assessments were conducted but excluded due technology problems (*n* = 13; 4 ASIB) and noncompliance (*n* = 2 ASIBs). Physiological data were excluded for additional 18 behavioral assessments (11 ASIB) due to high artifacts, were not collected from 5 participants (2 ASIB) during the initial battery implementation, and were not collected for 1 LR participant due to parental preference. Seven additional LR participants were assessed but excluded from the present study due to matching (*n* = 2) and other developmental or medical concerns (*n* = 5; 1 epilepsy, 3 suspected ASD, 1 suspected ASD in sibling during study course). Proportion of missing behavioral (13%) and physiological data (18%) was similar to previous studies in similar samples (Elsabbagh et al., 2009; 16%).

Procedures and Measures

Procedures paralleled Study 1. To review, the present study includes attention data from all assessments, as well as developmental and clinical testing from the 12 month assessment window. Materials, equipment, and experimental set-up were identical

across settings. The following measures from Study 1 were collected for Study 2 using identical procedures and are therefore not reviewed in detail: ECG and heart-defined sustained attention, AOSI Total Score (ASD symptoms in 12 month window), and Mullen Scales of Early Learning Standard Score (developmental abilities in 12 month window). Within the present sample, 3 of the 21 ASIBs with AOSI data exceeded the clinical risk threshold of 7 items.

Attention orienting. Behavioral attention was measured using a gap-overlap task. Participants were seated in a darkened room, 10" away from an 11 x 24" LCD monitor. Two video cameras simultaneously recorded stimuli and participants' faces. To minimize distractions and standardize environments, the monitor and infant were surrounded on three sides by a portable, nonreflective black felt shield. During the video, electrocardiogram signal (ECG) was collected using Alive Heart Monitors (Alive Corporation, Gold Coast, Australia) and was transmitted live to a laptop via Bluetooth technology.

The gap-overlap task used in the present study was provided courtesy of the Centre for Brain & Cognitive Development, Birbeck College, University of London. The task includes three phases: baseline, gap, and overlap. During each trial, an engaging animated sun or clown hat spun in the center of the screen at a 12^{o} by 12^{o} visual angle, attracting the infant's attention. The peripheral target, an animated animal accompanied by a consistent sound effect (e.g. cow presented with "moo" sound), then randomly presented either to the left or right of the central stimulus at the eccentricity of 13^{o}. The target was manually triggered by the examiner, ensuring the participant was attending at the start of the trial. This stimulus remained on the screen until the infant shifted gaze or

3 s elapsed. During baseline trials, the peripheral stimulus appeared simultaneously with the central stimulus disappearance. During gap trials, a 200 ms gap occurred between central stimulus disappearance and the peripheral stimulus appearance. During the overlap trials, the peripheral target appeared while the central stimulus remained on the screen. The three conditions were randomly presented across 36 trials for each block. Trials continued until infants become fussy or the maximum 72 trials were reached. Primary dependent variables included (1) baseline saccade latency, (2) overlap and gap saccade latencies, controlling for baseline latencies, and (3) proportion of "failed" trials in which a saccade did not occur.

Quantification of Behavioral and Physiological Variables

Attention orienting. Gap-overlap task coding procedures were selected to parallel previously published analyses of this task in a same-aged ASIB sample (Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009). During the first portion of coding, each trial was evaluated for acceptability using the following criteria: (1) infant attends to the central stimulus directly prior to the onset of the peripheral stimulus, (2) infant does not blink or look away while the peripheral stimulus is being displayed, (3) infant attends to the screen throughout the trial (spinning stimulus through balloon appearance). During the second phase, three series of variables were coded: (1) participants' task engagement, (2) trial validity, and (3) reaction times. Saccade latencies for each valid trial were calculated as the difference in time (ms) between the appearance of the peripheral stimulus and the infant's saccade. Consistent with previous studies (Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009), latencies <100ms or >1200 ms were determined to be invalid. For each assessment, 5 valid trials per trial type were

required for inclusion in analyses, resulting in partial missing data for 12 trial-level data points: gap (*n* = 1 ASIB), baseline (*n* = 1 LR), or overlap (*n* = 10, 9 ASIBs). Notably, failed trials contributed to higher proportions of missing overlap data compared to the other trial types and were therefore analyzed as a separate variable (proportion failed trials).

Data were coded offline by the first author (primary coder) and other trained research staff (secondary coders). Training consisted of reading training documentation, co-coding 2 files with the primary examiner, then coding independently until the secondary coder was reliable with the primary examiner on 3 consecutive files. Twenty percent of the secondary coders files were co-coded for reliability, and interrater saccade latencies correlated at *r* = .99. Interrater agreement for looking codes (looking versus not looking at screen) was 83%.

Heart activity and heart-defined sustained attention. Procedures for editing heart activity and quantifying heart-defined SA were similar to Study 1. Files requiring greater than 5% editing were excluded from analyses (*n*=18; 11 ASIB). Behavioral codes were used to isolate portions of the task in which participants were looking toward the screen. These procedures are detailed in Study 1. In brief, numerical algorithms were then used to extract phases of heart-defined attention by comparing IBIs to the "Baseline IBI" for each look, calculated as the median of five IBIs preceding the participant looking toward the screen. Baseline IBI was reset when the participant looked away for 1.5 s. Unlike Study 1, participants were not required to meet a minimum looking threshold for SA data to be included in analyses, as the gap-overlap task required participants look for

stimuli to be activated. Thus, participants were engaged for a sufficient amount of time to calculate SA phases.

Analyses

Analyses were conducting using SAS 9.3 (Apex, NC) with α set to less than .05. Infant sibling and LR groups did not differ in number of assessments per participant, F (1, 44) = 0.03, p = .86, age across assessments, F (1, 97) = 0.03, p = .85, or number of acceptable trials, F (1, 97) = 0.95, p = .33. Normal probability plots of residuals at each analysis level indicated non-normal distribution of the following variables, which were log-transformed prior to analyses: saccade latencies (baseline, overlap, gap), overall IBI, overall RSA, proportion time looking toward screen, proportion time in SA, IBI SD during SA, average IBI deceleration during SA, and total AOSI score.

Group Differences in Behavioral and Heart-Defined Attention

Group differences in behavioral and heart-defined SA were examined using multilevel models, which were constructed using parallel procedures to Study 1. For each dependent variable, a series of models were constructed to determine optimal model fit, using (1) ICC to determine whether observations should be nested within individuals and (2) AIC and BIC to determine which random effects best characterize data variance and covariance. As detailed in Table 3.2, ICCs indicated substantial variance in dependent variables occurring between individuals (19-70%), and AIC and BIC statistics indicated observations are best modeled as nested within individuals (Model 2). Notably, modeling age as a random effect (Model 3) either worsened or negligibly changed model fit, indicating the association between age and dependent variables was relatively stable

across individuals. Thus across analyses, intercepts were modeled as random effects nested within individuals, whereas age was included as a fixed effect only.

Analytic procedures paralleled Study 1. First, unconditional means and growth models were constructed to estimate level and change in attention over time. Next, group and cognitive ability were added to the model as time-invariant level 2 explanatory variables. Significant group effects indicate mean differences at the centered age of the sample (9 months), and significant age x group interaction indicates group differences in change over time. Significant age x group interactions were probed using nonparametric Wilcoxon signed ranked tests that tested group differences in dependent variables across three separate age ranges: 5-8 months, 8-11 months, and 11-14 months. The proportion of variance explained by conditional models was estimated using pseudo R^2 (Table 3.2), calculated as the proportion of residual variance in the null model reduced by combined explanatory variables (Singer & Willett, 2003). To facilitate comparisons with previous cross-sectional studies, effect sizes for group differences were also calculated for each age range using Cohen's *d* (Cohen, 1988).

Correspondence between Behavioral and Heart-Defined Attention

To determine whether cross-group and within-group differences in behavioral attention were associated with heart-defined attention parameters, partial Spearman correlations were calculated between each behavioral predictor and overall IBI, IBI SD, RSA, and parameters of heart-defined SA (proportion of time in SA, SD of IBI during SA, mean IBI change during SA). Chronological age and developmental ability were included as covariates in all models, and baseline latency was included as a covariate in overlap and gap latency models. Observations were collapsed across individuals.

Predictors of Clinical Autism Risk among ASIBs

To examine whether clinical ASD risk related to abnormal behavioral and heart-defined SA among ASIBs, multilevel models were constructed with clinical ASD risk included as a time-invariate predictor of each dependent variable. Model fit was tested for ASIB-only models and were determined to follow a similar pattern to models examining group differences. Thus, parallel models (intercept-only random effects) were used for ASIB-only analyses. Similar to cross-group models, unconditional means and growth models were first constructed to estimate within-group levels and change in attention over time. Next, clinical autism risk (AOSI total score), the interaction between age x autism risk, and cognitive ability were included as time-invariant explanatory variables. Significant interactions were probed using Wilcoxon signed rank tests across three age categories (5-8 months, 8-11 months, 11-14 months).

Results

Descriptive data are detailed in Table 3.3 (behavioral and clinical data) and Table 3.4 (physiological data). Although all statistical models included age as a continuous variable, descriptive statistics were reported across three age categories (5-8 months, 8-11 months, 11-14 months) to inform age-related changes and facilitate comparisons with previous cross-sectional studies.

Group Differences in Behavioral and Heart Defined Attention

Multilevel models revealed group differences in both behavioral and physiological variables. Unconditional models indicated significant variability in the mean levels of all variables except proportion of time in SA (p = .18) and average IBI SD

during SA (p = .10), warranting further examination of predictors through conditional modeling.

Groups differed in trajectories of saccade latencies for each trial type. These differences are reported in Table 3.6 and depicted in Figure 3.2, which displays individual trajectories (light gray lines) as well as average trajectories by group (thick black lines) for each group and trial type. As depicted in this graph, ASIBs demonstrated initially slower baseline latencies that improved more rapidly with age, controlling for developmental abilities. Post-hoc analyses indicated significant group differences in the youngest age group only (<8 months; Z = -2.36, p = .009). Age related patterns of overlap and gap latencies also differed by group, controlling for baseline latencies and developmental abilities. As a group, ASIBs demonstrated initially similar overlap latencies that improved less rapidly with age, compared to accelerated improvements in overlap latencies in the LR group. This pattern was distinct from gap trials, in which the ASIB group demonstrated initially slower gap latencies that improved with age, whereas the LR group exhibited relatively stable gap latencies over time. Post-hoc cross sectional analyses generally corroborated these trends, with longer gap latencies in the youngest group only (Z = -2.47, p = .007) but non-distinct overlap latencies (p's > .15). Interestingly, mean latencies generally shortened over time in all groups and trial types, with the exception of overlap latencies among ASIBs, which decreased between 5-8 and 8-11 months (Z = 2.41, p = .02) but then remained relatively stable, with a slight but nonsignificant increase between 8-11 and 11-14 months (Z = 1.00, p = .32). Groups did not differ in behavioral attentiveness or proportion of stuck trials. Developmental ability did not relate to behavioral attention variables.

Group differences in physiological variables were less robust. Table 3.7 reports fixed effects for physiological models. To summarize, age-related patterns of IBI varied across groups, with initially longer IBI in ASIBs that generally decreased toward typical levels across age. These group differences are depicted in Figure 3.3. Post-hoc Wilcoxon analyses indicated significantly longer IBI in ASIBs in the youngest age group (Z = -1.86, p = .03). Groups did not differ in other physiological variables. Across the sample, lower developmental abilities were associated with higher RSA and greater proportion of time in SA.

Association between Behavioral and Heart-Defined Attention

Table 3.5 includes the full correlation matrix for the full sample, as well as ASIB and LR groups. Across behavioral and physiological variables, overlap latencies positive correlated with proportion of time in SA across participants (partial ρ = .40; p = 002), controlling chronological age and developmental ability. When correlations were examined within LR and ASIB groups, this effect was only statistically significant within the LR group (partial ρ = .40; p = .03; ASIB partial ρ = .30; p =.16). Greater proportion of overall attentiveness correlated with higher RSA in the ASIB group only (ASIB partial ρ = -.37; p = .04; LR partial ρ = .07; p =.70) No other biobehavioral associations were significant.

Attentional Predictors of Clinical Autism Risk

Among ASIBs, higher clinical ASD risk factors were associated with greater proportion of stuck trials and marginally slower baseline latencies. Figure 2.4 depicts individual and group-level trajectories of proportion stuck trials across age, separated by

median AOSI score (5). Data from the LR group is included for reference. These effects were stable across age.

To quantify the relative change in number of stuck trials over time among ASIBs with high AOSI scores, rates of change in proportion of stuck trials were calculated for each participant [(final proportion-initial proportion)/(final age-initial age)] and ranked. Sixteen participants had available AOSI data and at least two assessments. The 3 ASIBs who received scores above the clinical cut-off for risk ($\geq$7 elevated markers) ranked 2, 14, and 16 in rate of change over time, with a rank of 1 indicating the greatest reduction in stuck trials (improvement) and rank of 16 indicating some of the greatest increases in stuck trials. Thus, although two participants exhibited significant increases in stuck trials compared to other ASIBs, the third exhibited robust improvements over time, relative to other ASIBs.

Discussion

It is increasingly recognized that longitudinal surveillance is critical to mapping early trajectories of risk among neurodevelopmental disorders. Indeed, the two previous studies to longitudinally examine orienting among ASIBs identified patterns of risk that were not characterized in cross-sectional comparisons alone (Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). We, too, identified abnormal age-related changes in overlap, gap, and baseline saccade latencies among ASIBs, which could be conceptualized as increased "disengagement difficulties" over time. Our physiological data support this finding, with evidence of hypoarousal in our youngest participants that potentially relates to longer saccade latencies. However, our use of longitudinal models also unveiled associations that were unexpected given previous reports. Within our sample, abnormal

disengagement appears to be driven by group differences in general orienting – across trial types – that are present in our youngest participants and improve at different rates over time. In other words, increasing "disengagement" deficits emerged in the context of dampened "normalizing" of orienting latencies over time. This conceptualization provides an alternate interpretation to prior assumptions that aberrant disengagement among ASIBs emerges from overlap latencies that increasingly deviate from "typical" baseline trajectories. These data may suggest that group differences may be present – not simply emerging – around 6 months of age in ASIBs.

Notably, this interpretation deviates somewhat from the few previous studies of disengagement in ASIBs, although differences likely relate, in part, to methodological differences across studies. Including the present study, five primary studies have examined gap-overlap task performance among ASIBs, each using different stimuli and operationalization of "disengagement." For example, although stimuli across Elsabbagh's two studies (2009, 2013) and our study used cartoons of objects and animals, Elison's group (2013) used pictures of faces and objects, and Zwaigenbuam (2005) used geometric shapes. Task design also varied by group; Elsabbagh et al 2009 and the present study employed baseline, gap (200 ms) and overlap trial types, whereas Elison and Zwaigenbaum examined gap (250 ms) and overlap trial types only, and Elsabbagh's 2013 study exclusively examined overlap and baseline trial types. "Disengagement" has also been quantified differently across groups. Studies have examined performance across trial types separately (Zwaigenbaum et al., 2005), incorporated multiple trial types as multivariate dependent variables (Elison et al., 2013; overlap and gap only), or examined "disengagement effects" by controlling for baseline latencies when interpreting overlap

latencies (Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009; the present study). Thus although each study has described abnormal attention "disengagement," broadly defined, the actual stimuli and analytic approaches used by each group reflect different interpretations of this construct.

The following discussion describes the present findings in relation to the prior literature, within the context of these cross-study differences. To facilitate cross-study comparisons, Table 3.10 summarizes current studies of gap-overlap tasks in ASIBs using calculated standardized mean group differences (Cohen's d) for latencies across each trial type and age group. When not supplied by the original authors (Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009; Zwaigenbaum et al., 2005), effect sizes were calculated using means and standard deviations reported by the original authors. Comparisons included ASIB versus LR groups at each age interval, as well as for ASIBs later diagnosed with ASD (ASIB+ASD) versus LR controls, where applicable. Although these effect sizes do not account for covariates (e.g. developmental abilities, age at assessment), they provide an approximated landscape of the current published gap-overlap data in infants.

Development of "Sticky Attention" in ASIBs

Our identification of atypical orienting in very young (5-8 month) ASIBs is consistent with a subset of previous studies. In a cross-sectional comparison of ASIBs later diagnosed with ASD (ASIB+ASD) versus LR controls, Elison and colleagues (2013) reported longer overlap and gap latencies (baseline latencies not measured) in the ASIB+ASD versus LR groups. Group differences in this 6-8 month sample produced large effects (d=.71 for each trial type). Similarly, although Zwaigenbaum and colleagues

(2005) reported nonsignificant group differences overlap and gap latencies within their 6-7 month ASIB sample (*n*=25), group differences in overlap latencies produced a large effect (*d*=.47), suggesting these differences may have been statistically significant with a larger cohort. Together, these studies suggest that group differences may be present by around 6 months of age in ASIBs.

However, not all studies have reported consistent patterns across early development. Although Elsabbagh and colleagues (2009, 2013) report similarly longer gap and overlap latencies in ASIBs relative to LR controls, they suggest these differences emerge later in infancy. The authors also report that baseline latencies are generally *longer* in LR controls at younger ages. In Elsabbagh and colleagues' (2009) initial cross-sectional report, 8-12 month ASIBs were reported to exhibit longer gap (*d*=.86) and overlap (*d*=.41) latencies than LR controls, with similar baseline latencies across groups (LR>ASIB *d*=.07). Similarly, the follow-up longitudinal study (Elsabbagh et al., 2013) reported group differences in overlap latencies relative to baseline latencies in 12-15 month ASIBs, but not 6-10 month ASIBs. Again, within the 6-10 month sample, the LR group actually exhibited *longer* baseline latencies than ASIBs (*d*=.44), opposing our finding of longer baseline latencies in ASIBs (*d*=.96). Although this baseline effect becomes negligible in this sample at older ages (Elsabbagh et al., 2009, ages 8.5-12.3 months, *d*=.07; Elsabbagh et al., 2013, ages 12-15 months: *d*=.01), the absence – and counterevidence – of longer baseline latencies in young ASIBs is striking, particularly in light of the similar stimuli and procedures used across Elsabbagh's reports and the present study.

Several sampling and statistical considerations may have contributed, in part, to these divergent findings. First, each study has defined and modeled developmental change differently. Prior to the present study, studies of gap-overlap tasks in AISBs have employed categorical modeling of age groups, despite the possibility – and likelihood – that rates of change in orienting changes during the first year of life. Indeed, between the 5-8 month and 8-11 month age windows in our own sample, the average baseline latency changed by 70.42 ms in ASIBs (16.99 ms in LR), compared to only 16.78ms (10.95 ms in LR) between the 8-11 month and 11-14 month age groups. As such, collapsing across this earlier age window in previous studies may have produced more modest, or even absent age effects. It is also likely that differences also emerged due to modeling choices. The present study employed individual growth trajectories based on exact age at assessment, whereas previous longitudinal reports have used categorical approaches, comparing two assessments nested within broad age ranges (e.g. performance at 12-15 months compared to 6-10 months). Although our multilevel models accounted for individual differences in performance over time, the linear models we employed may still oversimplify developmental trajectories, smoothing over brief periods of rapid, non-linear change that may be detectable in larger, more complex models. For example, our within-group contrasts suggest that overlap latencies within ASIB shortened between 5-8 month and 8-11 month groups but were stable – and even exhibited slight but nonsignificant increases – between 8-11 and 11-14 months. This potential nonlinear pattern may explain why some studies have reported longer overlap latencies among ASIBs across two time points (Elsabbagh et al., 2013; Zwaigenbaum et al., 2005), whereas we observed shorter overlap latencies across a broader age span of up to three

time points. This nonlinear pattern may also indicate a period of vulnerability in which ASIBs begin to exhibit more heterogeneous profiles, with some increasing and some decreasing between 8-11 and 11-14 months, resulting in nonsignificant group-level change. The later scenario is supported by previous reports that ASIBs who later meet ASD criteria exhibit longer overlap latencies over time compared to general shortening among ASIBs who do not meet for ASD (Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). Our data complement these previous studies by suggesting that group-level differences are apparent prior to this developmental juncture, warranting further study of these complex trajectories and the potential neurobiological substrates that sustain both initial group differences and potential age-related increases in heterogeneity among ASIBs.

It is likely that variations in sample composition, such as ASD symptoms and sex, have also contributed to variations across studies. When conceptualizing participant groups, some have reported ASIB data regardless of outcome (Elsabbagh, Volein, et al., 2009), others used ADOS scores alone to quantify ASD symptoms (Elison et al., 2013; Zwaigenbaum et al., 2005) and the remaining study interpreted ADOS scores within broader diagnostic criteria (Elsabbagh et al., 2013). This use of both diagnostic and symptom-based outcome measures reflects complementary approaches of examining both broader endophenotypic features – similar to the extensive adult literature on the broader ASD phenotype (Bolton et al., 1994; Dawson et al., 2002; Pickles et al., 2000) – as well as specific risk factors for ASD among ASIBs. However, due to the presence of subthreshold ASD features in ASIBs without ASD (Elsabbagh et al., 2013), studies that strictly categorize ASIBs based on the presence or absence of ASD diagnoses may

restrict the variability of their "clinical" sample, reducing power to detect variation relevant to the ASD endophenotype. Similarly, different sex distributions across studies likely also affect the distribution of ASD symptoms, given ASD is 4.5 times more common in males (Centers for Disease Control, 2014). Proportion of males in previous studies ranges from 40% (Elsabbagh et al., 2013) to 75% (Elsabbagh et al., 2009; Elison et al., 2013 = 56%; Zwaigenbaum et al., 2005 sex not reported). Thus, classification systems and sex distributions may cause fluctuations in findings across groups. For example, our predominantly male sample (82% male) may have produced more robust group differences than previous studies with predominantly female samples (Elsabbagh et al., 2013). Teasing apart these patterns of sampling differences across studies may contribute to our conceptualization of heterogeneous symptom emergence in ASD.

Despite these differences across studies, our data generally converged with previous reports that atypical orienting may be a marker of ASD risk among ASIBS. In our sample, higher ASD clinical risk – as measured by a behavioral screening tool between 11-14 months, correlated with higher proportion of stuck trials and moderately longer baseline latencies (p=.06), consistent with previous findings of "sticky attention" in ASIBs who later meet ASD criteria (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). Interestingly, both effects were stable across ages (e.g. age interactions were not significant), supporting that core vulnerabilities are detectable among young participants. Future studies are certainly needed to examine the long-term outcomes associated with infant orienting in this sample, particularly to examine whether these markers specifically predict ASD versus other developmental concerns (Elsabbagh et al., 2013). However, in the interim, our findings support that (1) sticky attention

remains a salient feature of the ASD endophenotype, and (2) these effects may be present earlier than previously reported.

Autonomic Processes in ASIBs

In addition to clarifying the behavioral emergence of abnormal orienting in ASIBs, the present study contributes novel information regarding the autonomic correlates of these aberrant behavioral profiles. In the present sample, ASIBs demonstrated slower IBI at younger ages, perhaps indicating global hypoarousal that may relate to slowed attention shifting and disengagement. Although correlations between overlap latencies and overall IBI did not reach statistical significance, the ASIB group exhibited subthreshold associations between longer IBI and slower latencies (n=29, ρ =.22, p=.29) that were not present in the LR group (n=38, ρ=-.05, p=.78). Although RSA did not differ across groups, ASIBs with greater behavioral attention exhibited higher RSA, potentially indicating that more abnormal visual attention is associated with poorer parasympathetic regulation in ASIBs, consistent with studies of reduced vagal suppression during cognitive stressors in older children with ASD (Porges, 2013). Interestingly, although RSA was negatively associated with developmental ability (p=.01), this effect was not significant in our previous study of an overlapping ASIB sample during a passive viewing task (Study 1; p=.11). These differences suggest the association between poorer RSA suppression and lower developmental ability was more apparent during a more cognitively taxing task, although it is also possible that slight differences in samples and missing data contributed to study differences. Paired with the behavioral patterns of slower latencies in ASIBs versus LR groups at younger ages, these data support the theory that attention disengagement in ASIBs is indexed by an

exaggerated biobehavioral attentional profile, with generally consistent data across behavioral and physiological measures. Interestingly, this pattern of hypoarousal and poor regulation in young ASIBs parallels associations between hypoarousal and higher ASD symptomatology previously in young infants with fragile X syndrome, a single-gene disorder highly associated with ASD (Roberts et al., 2012). Together, these studies suggest that abnormal arousal may be characteristic of a subset of infants at risk for ASD.

Consistent with previous studies in low-risk samples (Casey & Richards, 1988; Richards, 1997), behavioral orienting was grossly associated with heart-defined SA in infants who are typically developing. This association was less robust – and dropped below statistical significance – in our ASIB sample, despite similar proportions of time spent in SA across ASIB (sample-wide mean = 23%, SD=11%) and LR (mean =20%, SD=11%) groups. On the surface, this finding somewhat contradicts our hypothesis that abnormal visual orienting would be indexed physiologically by greater SA. However, it is notable that overlap latencies and proportion of stuck trials were more closely associated with properties of SA – such as IBI SD --- in ASIBS (ρ=.23, p=.28) compared to nearly no association in controls (ρ=.00, p=.99). Thus, it is possible that the properties of SA, rather than the overall proportion of time in SA, more closely index abnormal orienting in this group. Indeed, in our previous study of heart-defined SA in an overlapping sample, we found that qualities of SA during a passive viewing task predicted within-group variability in ASD symptoms among ASIBs (Study 1), suggesting qualities of SA may more closely relate to ASD symptomatology in this population.

Interestingly, although our previous study (Study 1) identified group differences in heart-defined SA across overlapping ASIB and LR samples, SA did not distinguish

groups in the present study. In addition, heart activity and heart-defined SA also did not predict clinical autism risk among ASIBs, unlike the robust associations between ASD clinical risk and numerous physiological variables (IBI, IBI SD, IBI change during SA, IBI SD during SA) in Study 1.We propose these divergent findings likely reflect task-related differences. Study 1 examined a passive viewing task that involves minimal examiner prompts to re-engage the child, thus capturing "resting" heart activity in a non-demanding environment. Study 2, in contrast, examined active manipulation of attention (e.g. the examiner was directing the child's attention to the task and did not initiate trials until fixation occurred). Furthermore, trials were separated by distinct inter-trial breaks in which no stimuli were on the screen, potentially disrupting endogenous physiological patterns related to attention. These preliminary studies suggest that measures of "resting state" physiology and heart-defined SA may be most informative of clinical ASD risk among ASIBs, although it is also possible that more fluid contingent looking paradigms may similarly reveal more nuanced patterns.

Limitations and Future Directions

Despite providing novel evidence that both attentional and physiological differences may be detectable around 6 months of age or earlier among ASIBs, our findings are limited in a number of ways. First, although similar in size to previous studies of attention in ASIBs (Elison et al., 2013; Elsabbagh, Volein, et al., 2009; Zwaigenbaum et al., 2005), our sample is insufficient to model complex, non-linear changes that are likely present in the attentional trajectories in young infants. Capturing these trends is important to identifying potential "critical periods" (Rice & Jr, 2000) of abnormal development that could both (1) inform neurobiological processes sustaining

aberrant attention in ASIBs and (2) serve as points of prevention and intervention. Indeed, recent concentrated longitudinal assessments of visual attention in ASIBs have suggested abnormalities as early as 2 month of age (Jones & Klin, 2013), supporting the utility and importance of integrating repeated assessments to capture developmental change. Although the present study focused on continuous ASD risk factors as a measure of the ASD endophenotype, it will also be important to continue to follow this cohort of children to assess long-term "endpoints" of these early, aberrant processes. Although a number of previous studies have associated abnormal orienting with ASD risk (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005), abnormal orienting has also been identified among ASIBs with non-ASD developmental abnormalities (Elsabbagh et al., 2013). Establishing the specificity of these associations will define the clinical scope of aberrant visual orienting as a marker of ASD risk.

As we continue to document the role of abnormal orienting in the ASD endophenotype, another important next step will be to tease apart how sampling and methodological differences affect findings across studies. This question is important to both clarifying the aforementioned inconsistencies in the ASIB literature, as well as informing translational efforts such as early intervention protocols. Because the sex distribution in our study was designed to parallel rates of ASD in the general population (4.5 males to 1 female; Centers for Disease Control, 2014), our sample is underpowered to tease apart whether abnormal orienting is similarly apparent across male and female ASIBs. It is likely that our capacity to detect group differences between ASIB and LR samples was also enhanced by the skewed sample of males, although testing this possibility statistically will be important for understanding sex-specific risk patterns.

Similarly, additional work is needed to clarify the specific variables that affect efficient orienting, particularly given the different stimuli and presentation timing used in current studies. For example, in both our and Elsabbagh's studies (2009, 2013), the central stimulus during overlap trials changes properties from an animated spinning stimulus to a static image. Although this change was designed to increase similarity between the central and peripheral stimuli (a balloon that alternates between smaller and larger sizes), the changed central stimulus may operate as a cue that another stimulus is about to appear. Our own data across Studies 1 and 2 also demonstrate the capacity of different tasks to elicit varied performance within an overlapping sample. For example, abnormal looking patterns were observed during passive viewing (Study1) but not a the gaze-contingent task (Study 2), suggesting typical gaze patterns in the presence of examiner and task-related prompts, but not in the absence of these supports. Continuing to examine the scope of orienting deficits – such as whether effects vary across social versus nonsocial stimuli, static versus dynamic stimuli, and cued versus non-cued stimuli – will provide important clues as to the attentional processes subserving abnormal orienting and ASD risk.

This study also paves the way for new research investigating autonomic functioning among ASIBs. It has been suggested that psychophysiological variables, such as abnormal arousal, may provide sensitive information that may eventually result in biological rather than behavioral categorization of ASD (Cuthbert & Insel, 2013; McPartland, Bernier, & South, 2015). Our finding of hypoarousal in young ASIBs parallels one of the few studies to examine heart activity as a biomarker of ASD risk in "high risk" infants with fragile X syndrome (Roberts et al., 2012), warranting further

research into whether early autonomic profile may index ASD risk among ASIBs. Our findings also suggest that although heart-defined SA may serve as an index of attentional processes, the utility of this method likely varies across task designs, as associations emerged in our previous passive viewing study (Study 1) that were not present in the present gaze-contingent task. Further studies of computerized visual attention tasks may be designed to answer these questions. However, it will be important to examine the association between ASD risk and heart-defined SA during naturalistic activities, such as play-based assessments, as a means to inform the clinical utility of heart-defined SA as a marker of the ASD endophenotype.

Conclusion

The present study identified abnormal visual orienting and co-occurring physiological functioning within the first year of life in infant siblings of children with ASD. Importantly, our longitudinal models suggest that commonly reported "disengagement deficits" observed among ASIBs may emerge due to initially abnormal patterns of general orienting (across trial types) that improves at varied rates over time. This interpretation, supported by aberrant heart activity observed at younger ages among ASIBs, suggests persistent orienting deficits that are present, rather than beginning to emerge, around 6 months of age. These deficits also relate to later behavioral markers of clinical ASD risk, supporting further investigation as to whether biobehavioral orienting patterns may index ASD vulnerability. Although additional work is needed to clarify the long-term clinical implications of these findings, our results suggest that abnormal orienting is apparent within the infant ASD phenotype and may co-occur with aberrant physiological functioning early in development.

Table 3.1. Previous Gap-Overlap Task Studies in Infant Siblings of Children with Autism

Authors and Tasks	Analyses/Comparisons	Key Findings
Zwaigenbaum et al., 2005 **Sample:** ASIBs assessed at 6m (n=25) and 12m (n=27; 20 with 2 assessments); 25 LR assessed at 6-7m. Sex not reported. **Outcome:** At 24m, 6/25 6m ASIBs and 10/27 12m ASIBs scored ADOS+ for Autism/ASD.	Cross-sectional group differences in Overlap and Gap at 6m Paired *t* test to examine 6 vs. 12m Overlap and Gap within ASIBs ASIBs categorized by whether Overlap latency increased by 1500ms+ Correlation between ADOS algorithm score and 12m Overlap and Gap	Gap (p=.78; *d=0.08) and Overlap (p=.12; *d=0.47) ns at 6m From 6-12m, ASIB Overlap increased (p=.01), Gap ns (p=.78) All ASIBs with 1500ms+ increases in latencies were ADOS+ at 24m ADOS correlated with Overlap (r=.42, p <.05) but not Gap (r= -0.04, p =.85)
Elsabbagh et al., 2009 **Sample:** 16 ASIBs (4 male) and 16 LR (4 male), assessed at 8-12m (1 assessment each). **Outcome:** No outcome data	Group differences in Baseline, Overlap and Gap latencies Group differences in likelihood to orient on Overlap trials Group (2) x Condition (2) ANOVA - Model 1: Overlap vs Baseline - Model 2: Gap vs. Baseline	Baseline (p>.05, *d=.07) and Overlap (p>.05, *d=.41) ns, ASIB Gap latencies slower (p=.02; d=.86). Group differences ns (p=.24, *d=.36; ASIB=91%, LR=84%) Relative to LR, ASIBs showed longer Overlap (p=.02, n_p^2=.17) and Gap (p=.02, n_p^2=.17) latencies vs. Baseline
Elsabbagh et al., 2013 **Sample:** 54 ASIBs (21 male) and 50 LR (21 male), assessed at 6-10m ("7m") and 12-15m ("14m"; 2 assessments each). **Outcome:** 16 ASIBs met ASD ICD-10 criteria at 36m; 12 ASIBs "atypical" due to ASD symptoms or low IQ	Group (4) x Condition (2) repeated measure (Age) GLM, controlling developmental abilities (DA) - Groups=LR, ASIB+ASD, ASIB-Atypical, ASIB-Typical - Conditions=Overlap, Baseline	Three-way interaction (p=.008) in which ASIB+ASD group exhibited longer latencies than other 3 groups at 14m, but not 7m. LR and ASIB-Typical groups exhibited shorter Overlap latencies relative to Baseline from 7 to 14m, whereas 40% of ASIB+ASD group exhibited longer latencies from 7 to 14m. Slower latencies in lower DA (p=.01)
Elison et al., 2013 **Sample:** 56 ASIBs (31 male) and 41 LR (24 male), assessed at 6-8m (1 assessment each) **Outcome:** 16 ASIBs scored ADOS+ for ASD at 25m	Multivariate ANOVA predicting Overlap and Gap latencies by Group(3) - Groups: LR, ASIB+ASD, ASIB-Typical	ASIB+ASD group exhibited longer Overlap than LR group (d=.71, p=.03) and ASIB-Typical groups (d=.73, p=.01). ASIB+ASD group exhibited longer Gap than LR group only (d=.71, p=.03) ASIB+ASD group did not show typical associations between saccade latencies and radial diffusivity of splenium.

Table 3.2: Model Fit Parameters for Fixed and Random Effects, Cross- Group Comparisons

Dependent Variable		**Model 1** No Random Effects	**Model 2 (Null Model)** Fixed: Intercept, Age Random: Intercept	**Model 3** Fixed: Intercept, Age Random: Intercept, Age	**ICC** Level 1	**R^2** Full Model vs. Null Model
Behavior						
Baseline Latency	AIC	10.5	-10.8	-9.5	.52	.11
	BIC	13.1	-7.2	-2.1		
Overlap Latency	AIC	14.5	11.7	13.4	.35	.13
	BIC	16.9	15.3	18.9		
Gap Latency	AIC	22.3	15.4	16.3	.33	.18
	BIC	24.8	19.1	23.7		
% Failed Trials	AIC	-80.0	-77.1	-73.9	.31	.00
	BIC	-77.5	-73.5	-66.6		
Prop. Inattentive	AIC	-92.3	-87.5	-84.1	.33	.01
	BIC	-89.9	-83.8	-76.8		
Overall Heart Activity						
Overall IBI	AIC	33.1	22.3	24.1	.70	.11
	BIC	35.4	25.9	31.4		
Overall RSA	AIC	61.3	62.0	59.5	.52	-.02
	BIC	63.6	65.7	66.8		
Overall IBI SD	AIC	98.8	94.5	86.0	.59	-.04
	BIC	98.9	98.1	94.6		
Sustained Attention						
Proportion SA	AIC	-129.7	-123.2	-124.5	.19	.08
	BIC	-127.5	-119.5	-117.2		
SA IBI SD	AIC	79.5	84.4	87.4	.26	-.003
	BIC	81.7	88.0	94.7		
SA Mean IBI Δ	AIC	89.0	86.6	87.4	.52	-.02
	BIC	91.2	90.3	94.7		

Table 3.3. Clinical and Behavioral Descriptive Statistics by Group and Chronological Age

	Infant Sibling (*n*=23)					**Low Risk Control (*n*=23)**				
	n	$\bar{X}$	SD	min	max	*n*	$\bar{X}$	SD	min	max
Individual-Level Variables										
n assessments	23	2.17	0.89	1	3	23	2.13	0.81	1	3
MSEL SS	23	97.87	14.29	60	121	23	102.48	10.68	83	119
AOSI Total Score	21	6.14	4.69	1	19	--	--	--	--	--
AOSI N Markers	21	4.00	2.37	1	10	--	--	--	--	--
Assessments between 5-8 months										
Age in months	13	6.43	0.49	5.95	7.53	12	6.38	0.63	5.65	7.82
Baseline Latency	13	437.49	103.05	212.5	601.95	12	349.28	79.3	241.66	513.88
Overlap Latency	12	578.57	121.38	385.71	747.61	11	561.13	149.82	290.47	805.55
Gap Latency	13	368.01	85.9	215.38	510.25	12	282.59	85.32	186.66	495.83
% Failed Trials	13	0.07	0.06	0	0.15	12	0.07	0.07	0	0.24
Prop. Inattentive	11	0.46	0.17	0.16	0.71	6	0.41	0.23	0.1	0.74
Assessments between 8-11 months										
Age in months	19	9.45	0.55	8.88	10.62	17	9.44	0.38	8.84	10.42
Baseline Latency	19	367.07	86.56	249.27	540.74	17	332.29	78.12	225	485.71
Overlap Latency	17	467.12	124.8	354.16	885.75	17	468.92	126.42	329.63	816.66
Gap Latency	19	311.73	87.21	197.5	514.81	17	288	91.16	198.48	497.22
% Failed Trials	19	0.08	0.07	0	0.2	17	0.06	0.04	0	0.14
Prop. Inattentive	16	0.49	0.18	0.09	0.8	15	0.54	0.18	0.3	0.89
Assessments between 8-11 months										
Age in months	18	12.49	0.58	11.7	13.78	20	12.28	0.49	11.54	13.74
Baseline Latency	18	350.29	81.4	229.41	526.66	19	321.34	69.6	180.57	475.43
Overlap Latency	12	489.36	83.98	383.33	657.14	20	461.2	132.58	287.1	799.99
Gap Latency	18	275.5	53.68	208.33	372.22	19	278.82	74.02	186.12	490.42
% Failed Trials	18	0.1	0.08	0	0.26	20	0.08	0.06	0	0.24
Prop. Inattentive	17	0.46	0.19	0.06	0.70	16	0.52	0.20	0.07	0.80

Table 3.4. Physiological Descriptive Statistics, by Group and Chronological Age

	Infant Sibling (*n*=23)					Low Risk Control (*n*=23)				
	n	$\bar{X}$	SD	min	max	*n*	$\bar{X}$	SD	min	max
Assessments between 5-8 months										
Overall IBI	11	336.36	113.80	214.58	515.21	8	239.75	78.65	190.96	430.40
Overall RSA	11	2.71	1.37	1.31	6.13	8	2.05	0.71	1.05	3.57
Overall IBI SD	11	19.90	15.80	7.29	59.96	8	12.51	4.00	5.55	16.42
Proportion SA	9	0.27	0.12	0.06	0.41	5	0.31	0.18	0.12	0.55
SA IBI SD	9	27.20	17.33	9.27	62.99	5	28.63	5.96	21.70	37.32
SA Mean IBI Δ	9	26.30	18.54	5.77	62.76	5	25.78	6.11	17.62	31.66
Assessments between 8-11 months										
Overall IBI	13	281.62	103.48	211.70	486.34	15	241.55	46.36	205.41	397.34
Overall RSA	13	2.34	1.05	1.23	5.33	15	2.07	0.38	1.42	2.76
Overall IBI SD	13	19.04	11.46	8.58	51.76	15	14.59	4.41	10.37	25.48
Proportion SA	13	0.19	0.11	0.06	0.42	14	0.19	0.12	0.01	0.46
SA IBI SD	13	25.59	7.04	13.80	38.24	14	28.38	12.77	16.37	62.94
SA Mean IBI Δ	13	24.16	8.15	8.18	34.47	14	23.75	7.80	12.06	37.82
Assessments between 11-14 months										
Overall IBI	13	283.16	85.30	214.97	440.62	16	280.36	107.63	209.12	561.19
Overall RSA	13	2.53	0.91	1.35	4.65	16	2.57	1.30	1.64	5.82
Overall IBI SD	13	19.01	8.28	9.17	39.24	16	17.89	10.25	9.90	43.51
Proportion SA	13	0.24	0.09	0.13	0.37	15	0.17	0.06	0.03	0.28
SA IBI SD	13	32.06	15.13	6.89	65.32	15	28.16	11.00	15.11	57.25
SA Mean IBI Δ	13	26.58	10.57	5.55	43.58	15	26.30	8.42	13.42	50.69

Table 3.5. Partial Spearman Correlations between Behavioral and Physiological Variables, Controlling for Chronological Age, Developmental Ability, and Baseline Latency (Overlap and Gap Only)

	Baseline Latency		Overlap Latency		Gap Latency		% Stuck Trials		% Inattention	
	n	*partial ρ*	*n*	*partial ρ*	*n*	*partial ρ*	*n*	*partial ρ*	*n*	*partial ρ*
Full Sample										
Overall IBI	78	.14	70	-.02	77	.18	79	.14	69	-.17
Overall IBI SD	78	.06	70	.03	77	.08	79	.10	69	-.07
Overall RSA	78	.06	70	-.01	77	.03	79	.12	69	-.23^
Proportion SA	71	-.08	**63**	**.40***	70	-.16	72	.00	69	-.77*
SA IBI SD	71	-.04	63	.11	70	.09	72	.07	69	.03
SA Mean IBI Δ	71	-.03	63	.04	70	-.12	72	-.05	69	-.09
ASIB Group										
Overall IBI	37	.03	29	.22	37	-.03	37	.19	35	-.24
Overall IBI SD	37	.12	29	.29	37	.05	37	.12	35	.07
Overall RSA	37	.01	29	.23	37	-.04	37	.13	35	-.37*
Proportion SA	35	-.17	27	.30	35	-.27	35	.04	35	-.74*
SA IBI SD	35	-.02	27	.23	35	.05	35	.05	35	.15
SA Mean IBI Δ	35	-.03	27	.11	35	-.08	35	-.08	35	.04
LR Group										
Overall IBI	41	.08	41	-.06	40	.20	42	.05	34	.03
Overall IBI SD	41	-.03	41	-.09	40	.06	42	.05	34	.07
Overall RSA	41	-.00	41	-.07	40	-.01	42	.07	34	.07
Proportion SA	36	.02	**36**	**.40***	35	-.15	37	-.03	34	-.79*
SA IBI SD	36	.03	36	.01	35	.15	37	.12	34	.02
SA Mean IBI Δ	36	.12	36	.04	35	-.14	37	.12	34	-.06

*Significant at $p \leq .05$ ^$p = .06$

Table 3.6: Fixed Effects of Group on Behavioral Attention

Model Component	Estimate	*SE*	*df*	*t-value*	*p*
Baseline Saccade Latency					
Intercept	**-1.13**	**0.05**	**44.20**	**-24.14**	**<.0001**
Age	-0.01	0.01	61.60	-0.93	0.35
Group	**0.17**	**0.07**	**43.70**	**2.56**	**0.01**
Group*Age	**-0.03**	**0.01**	**60.00**	**-2.37**	**0.02**
Developmental ability	0.00	0.00	39.50	0.79	0.44
Overlap Saccade Latency					
Intercept	**-0.03**	**0.11**	**56.80**	**-0.27**	**0.78**
Age	**-0.03**	**0.01**	**71.80**	**-2.37**	**0.02**
Group	-0.02	0.05	38.10	-0.35	0.73
Group*Age	**0.04**	**0.02**	**71.80**	**2.10**	**0.04**
Developmental ability	0.00	0.00	35.50	1.34	0.19
Baseline Saccade Latency	**0.63**	**0.10**	**60.80**	**6.46**	**<.0001**
Gap Saccade Latency					
Intercept	**-0.53**	**0.10**	**47.50**	**-5.15**	**<.0001**
Age	0.01	0.01	74.10	0.55	0.58
Group	0.04	0.04	28.90	0.83	0.41
Group*Age	**-0.03**	**0.02**	**72.50**	**-1.98**	**0.05**
Developmental ability	0.00	0.00	24.50	-0.52	0.61
Baseline Saccade Latency	**0.68**	**0.09**	**52.20**	**7.79**	**<.0001**
% Stuck Trials					
Intercept	**0.15**	**0.03**	**46.70**	**5.36**	**<.0001**
Age	0.00	0.01	76.70	0.04	0.97
Group	0.05	0.04	46.80	1.22	0.23
Group*Age	0.01	0.01	74.00	0.71	0.48
Developmental ability	0.00	0.00	41.50	0.96	0.34
Proportion Time Inattentive					
Intercept	**0.39**	**0.03**	**47.40**	**13.62**	**<.0001**
Age	0.01	0.01	57.40	1.22	0.23
Group	-0.01	0.04	43.40	-0.32	0.75
Group*Age	-0.01	0.01	54.90	-1.06	0.29
Developmental ability	0.00	0.00	34.10	-0.39	0.70

Table 3.7: Fixed Effects of Group on Physiological Variables

Model Component	Estimate	*SE*	*df*	*t-value*	*p*
Overall IBI					
Intercept	**5.55**	**0.06**	**38.90**	**87.91**	**<.0001**
Age	0.01	0.01	40.90	1.08	0.29
Group	0.11	0.09	37.00	1.21	0.23
Group*Age	**-0.04**	**0.02**	**38.80**	**-2.53**	**0.02**
Developmental ability	-0.01	0.00	36.30	-1.54	0.13
Overall RSA					
Intercept	**2.69**	**0.09**	**37.50**	**29.75**	**<.0001**
Age	0.03	0.03	48.00	1.33	0.19
Group	0.13	0.13	35.00	1.03	0.31
Group*Age	-0.02	0.03	44.10	-0.46	0.65
Developmental ability	**-0.01**	**0.00**	**34.30**	**-2.44**	**0.02**
Overall IBI SD					
Intercept	**0.79**	**0.07**	**36.50**	**10.74**	**<.0001**
Age	0.02	0.02	48.40	1.14	0.26
Group	0.07	0.10	33.90	0.65	0.52
Group*Age	-0.03	0.03	44.10	-0.97	0.34
Developmental ability	-0.01	0.00	33.20	-1.72	0.09
Proportion SA					
Intercept	**0.19**	**0.02**	**40.10**	**10.08**	**<.0001**
Age	**-0.01**	**0.01**	**49.80**	**-2.02**	**0.05**
Group	0.02	0.03	37.60	0.79	0.43
Group*Age	0.01	0.01	48.10	1.19	0.24
Developmental ability	**0.00**	**0.00**	**33.20**	**2.18**	**0.04**
Average IBI Variability during SA					
Intercept	**3.18**	**0.11**	**43.50**	**29.45**	**<.0001**
Age	0.01	0.03	38.10	0.43	0.67
Group	-0.06	0.15	40.00	-0.40	0.69
Group*Age	0.02	0.04	37.60	0.51	0.61
Developmental ability	0.00	0.01	35.80	-0.06	0.95
Average IBI Change during SA					
Intercept	**3.31**	**0.10**	**40.10**	**34.71**	**<.0001**
Age	0.00	0.03	45.60	-0.14	0.89
Group	-0.10	0.13	37.10	-0.79	0.43
Group*Age	0.04	0.04	44.20	1.02	0.31
Developmental ability	0.00	0.00	32.60	-0.75	0.46

Table 3.8: Fixed Effects of Clinical ASD Risk on Behavioral Attention among ASIBs

Model Component	Estimate	*SE*	*df*	*t-value*	*p*
Baseline Saccade Latency (R^2=.28)					
Intercept	**-1.17**	**0.12**	**17.40**	**-9.58**	**<.0001**
Age	0.01	0.04	33.00	0.18	0.86
Autism Risk	0.13	0.07	17.00	2.02	0.06
Autism Risk*Age	-0.03	0.02	32.90	-1.41	0.17
Developmental ability	**0.01**	**0.00**	**17.40**	**3.00**	**0.01**
Overlap Saccade Latency(R^2=.18)					
Intercept	-0.37	0.21	28.90	-1.79	0.08
Age	0.04	0.04	21.20	1.07	0.30
Autism Risk	0.03	0.06	11.30	0.55	0.60
Autism Risk*Age	-0.02	0.02	24.60	-1.16	0.26
Developmental ability	0.00	0.00	13.80	1.53	0.15
Baseline Latency	**0.37**	**0.16**	**33.00**	**2.36**	**0.02**
Gap Saccade Latency(R^2=-.01)					
Intercept	**-0.49**	**0.16**	**35.20**	**-3.11**	**0.00**
Age	-0.04	0.03	34.70	-1.38	0.18
Autism Risk	0.00	0.04	19.40	0.01	0.99
Autism Risk*Age	0.01	0.02	34.90	0.36	0.72
Developmental ability	0.00	0.00	19.90	0.20	0.85
Baseline Latency	**0.67**	**0.12**	**39.20**	**5.72**	**<.0001**
% Stuck Trials (R^2=-.07)					
Intercept	0.02	0.08	14.60	0.29	0.77
Age	0.02	0.03	33.80	0.80	0.43
Autism Risk	**0.11**	**0.04**	**14.30**	**2.55**	**0.02**
Autism Risk*Age	-0.01	0.02	34.20	-0.58	0.57
Developmental ability	**0.005**	**0.002**	**15.20**	**2.82**	**0.01**
Proportion Inattentive (R^2=.01)					
Intercept	**0.46**	**0.08**	**16.30**	**5.89**	**<.0001**
Age	0.03	0.02	25.20	1.15	0.26
Autism Risk	-0.04	0.04	15.60	-1.03	0.32
Autism Risk*Age	-0.02	0.01	25.60	-1.27	0.22
Developmental ability	0.00	0.00	16.60	0.51	0.62

Table 3.9: Fixed Effects of Clinical ASD Risk on Heart Activity among ASIBs

Model Component	Estimate	*SE*	*df*	*t-value*	*p*
Overall IBI (R^2=.12)					
Intercept	5.83	0.22	14.70	26.11	<.0001
Age	-0.10	0.05	18.00	-2.01	0.06
Autism Risk	-0.08	0.12	14.30	-0.68	0.51
Autism Risk*Age	0.04	0.03	18.80	1.38	0.18
Developmental ability	-0.01	0.01	15.40	-1.77	0.10
Overall RSA (R^2=.09)					
Intercept	0.82	0.25	15.50	3.31	0.00
Age	-0.07	0.07	21.10	-1.01	0.33
Autism Risk	0.02	0.13	14.90	0.14	0.89
Autism Risk*Age	0.04	0.04	22.00	0.98	0.34
Developmental ability	-0.01	0.01	16.30	-2.01	0.06
Overall IBI SD (R^2=.06)					
Intercept	2.66	0.30	15.80	8.83	<.0001
Age	0.07	0.08	20.70	0.92	0.37
Autism Risk	0.07	0.16	15.30	0.44	0.66
Autism Risk*Age	-0.03	0.04	21.60	-0.72	0.48
Developmental ability	**-0.02**	**0.01**	**16.70**	**-2.88**	**0.01**
Proportion SA (R^2=.004)					
Intercept	0.22	0.06	15.80	3.54	0.00
Age	0.00	0.02	19.70	0.05	0.96
Autism Risk	-0.01	0.03	14.10	-0.36	0.72
Autism Risk*Age	0.00	0.01	20.20	-0.10	0.92
Developmental ability	0.00	0.00	16.50	0.23	0.82
Average IBI Variability during SA (R^2=-.004)					
Intercept	3.03	0.32	16.10	9.51	<.0001
Age	0.14	0.11	19.30	1.33	0.20
Autism Risk	0.07	0.17	14.50	0.40	0.69
Autism Risk*Age	-0.05	0.06	19.90	-0.93	0.37
Developmental ability	-0.01	0.01	16.80	-0.97	0.35
Average IBI Change during SA (R^2=-.12)					
Intercept	2.96	0.43	13.80	6.85	<.0001
Age	0.07	0.10	14.20	0.70	0.50
Autism Risk	0.05	0.23	12.90	0.20	0.85
Autism Risk*Age	-0.02	0.05	14.80	-0.30	0.77
Developmental ability	-0.01	0.01	14.20	-0.63	0.54

Table 3.10. Standardized Mean Group Differences (d) of Raw Latencies and Proportion Stuck Trials in Gap-Overlap Studies in ASIBs

Study	Groups	Age		Sample Size		Cohen's *d*			
		Mean(SD)	Range	ASIB	LR	Baseline	Gap	Overlap	% Stuck
Zwaigenbaum et al. 2005	LR vs. ASIB	^ 6.36 (0.48)	~ 6-7m	25	25	--	0.08 [a] ASIB>LR	0.47 ASIB>LR	--
Tonnsen et al (current)	LR vs. ASIB	6.40 (0.54)	5.65-7.82	13	12	0.96 ASIB>LR	1.00 [b] ASIB>LR	0.13 ASIB>LR	0.07 LR>ASIB
Elison et al., 2013	LR vs. ASD	7.04 (.95)	~6-8m	16	41	--	0.71 [a] ASD>LR	0.71 ASD>LR	0.55 ASD>LR
Elsabbagh et al. 2013	LR vs. ASIB	7.35 (1.20)	~ 6-10m	52	48	0.44 LR>ASIB	--	0.13 LR>ASIB	0.19 LR>ASIB
Elsabbagh et al. 2013	LR vs. ASD	7.35 (1.20)	~ 6-10m	16	48	0.37 LR>ASD	--	0.10 LR>ASD	0 ASD=LR
Tonnsen et al (current)	LR vs. ASIB	9.45 (0.47)	8.84-10.61	19	17	0.42 ASIB>LR	0.27 [b] ASIB>LR	0.01 LR>ASIB	0.26 ASIB>LR
Elsabbagh et al. 2009	LR vs. ASIB	9.75m (2.73)	8.58-12.32m	16	16	0.07 LR>ASIB	0.86 [b] ASIB>LR	0.41 ASIB>LR	0.36 ASIB>LR
Tonnsen et al (current)	LR vs. ASIB	12.37 (0.54)	11.54-13.78	18	20	0.38 ASIB>LR	0.05 [b] LR>ASIB	0.25 ASIB>LR	0.32 ASIB>LR
Elsabbagh et al. 2013	LR vs. ASIB	13.79 (1.46)	~12-15m	52	46	0.01 ASIB>LR	--	0.30 ASIB>LR	0.10 ASIB>LR
Elsabbagh et al. 2013	LR vs. ASD	13.79 (1.46)	~12-15m	16	46	0.01 ASD>LR	--	1.00 ASD>LR	0.32 ASD>LR

Note: LR=low risk; ASIB = All infant siblings, regardless of outcome; ASD = infant siblings later diagnosed with ASD
^Mean for broader study sample; ages for participants with attention data not reported
[a]200ms gap, [b]250ms gap

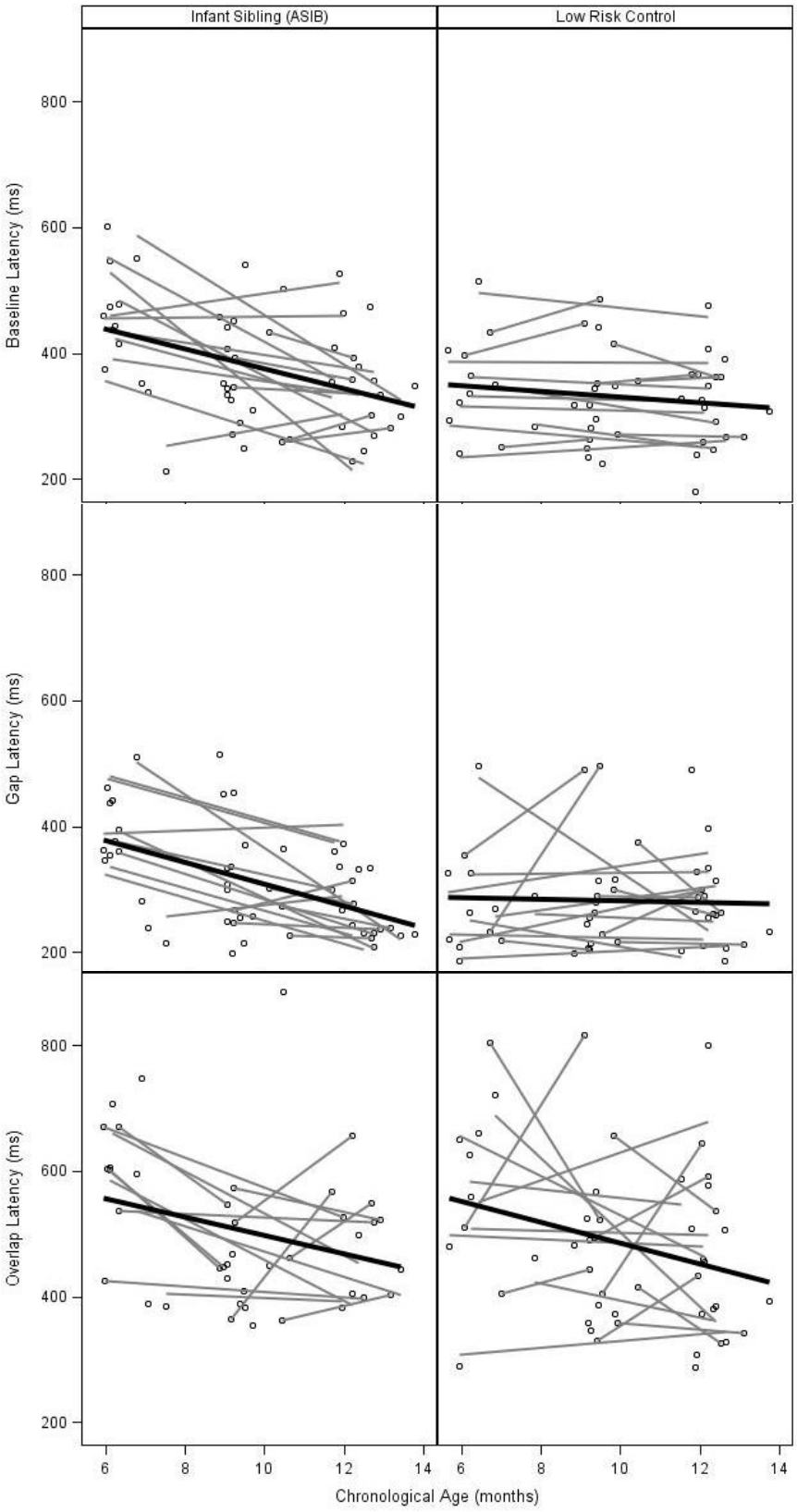

Figure 3.1: Group Differences in Saccade Latencies across Age, by Trial Type

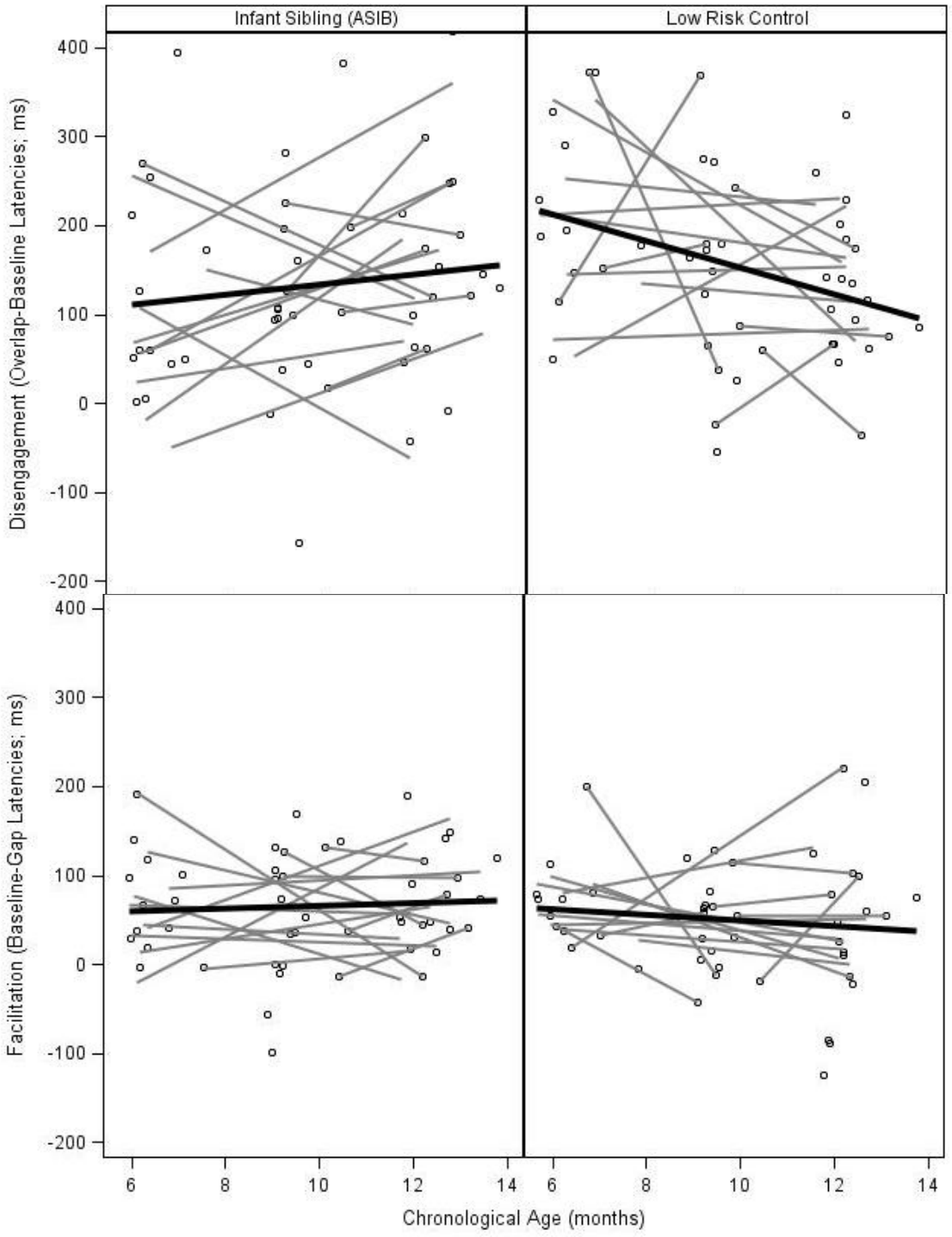

Figure 3.2: Group Differences in Disengagement and Facilitation across Age

Note: Difference scores used for display purposes only. Statistical models were constructed for Overlap and Gap latencies, controlling for Baseline latency. Compared to the LR group, ASIBs exhibited less improvement in Overlap and Gap latencies over time, relative to Baseline latencies.

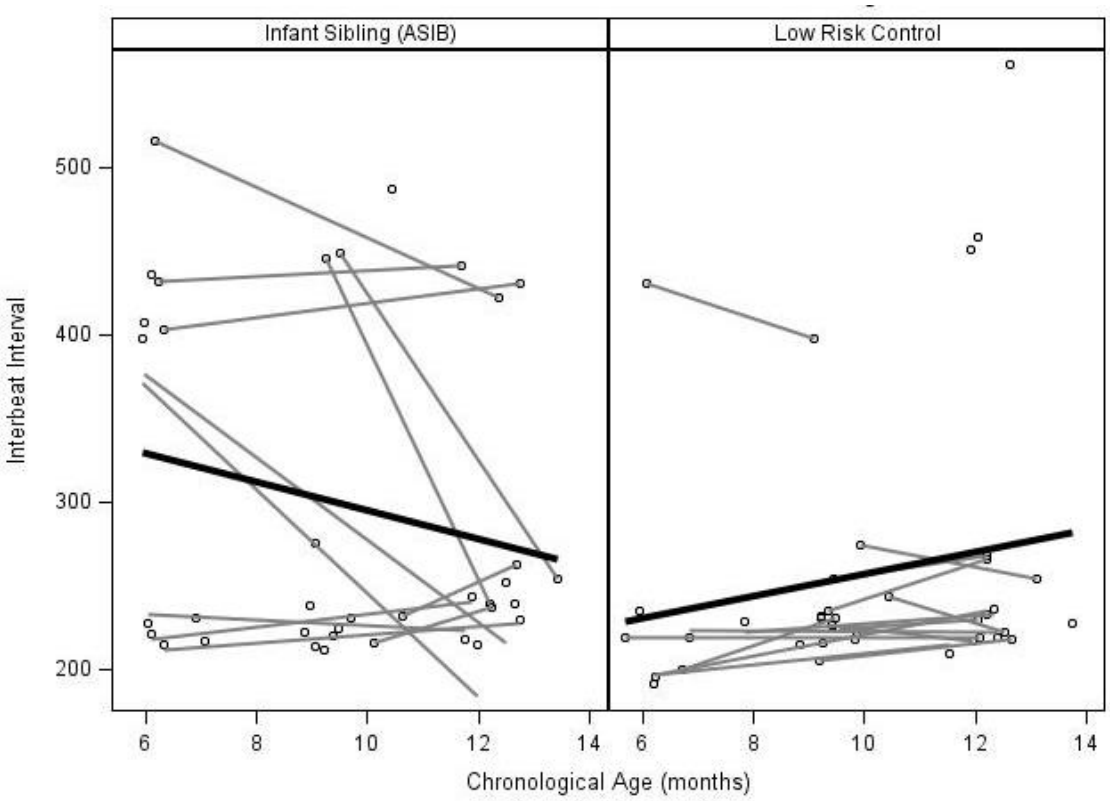

Figure 3.3: Group Differences in Trajectories of Interbeat Interval across Age

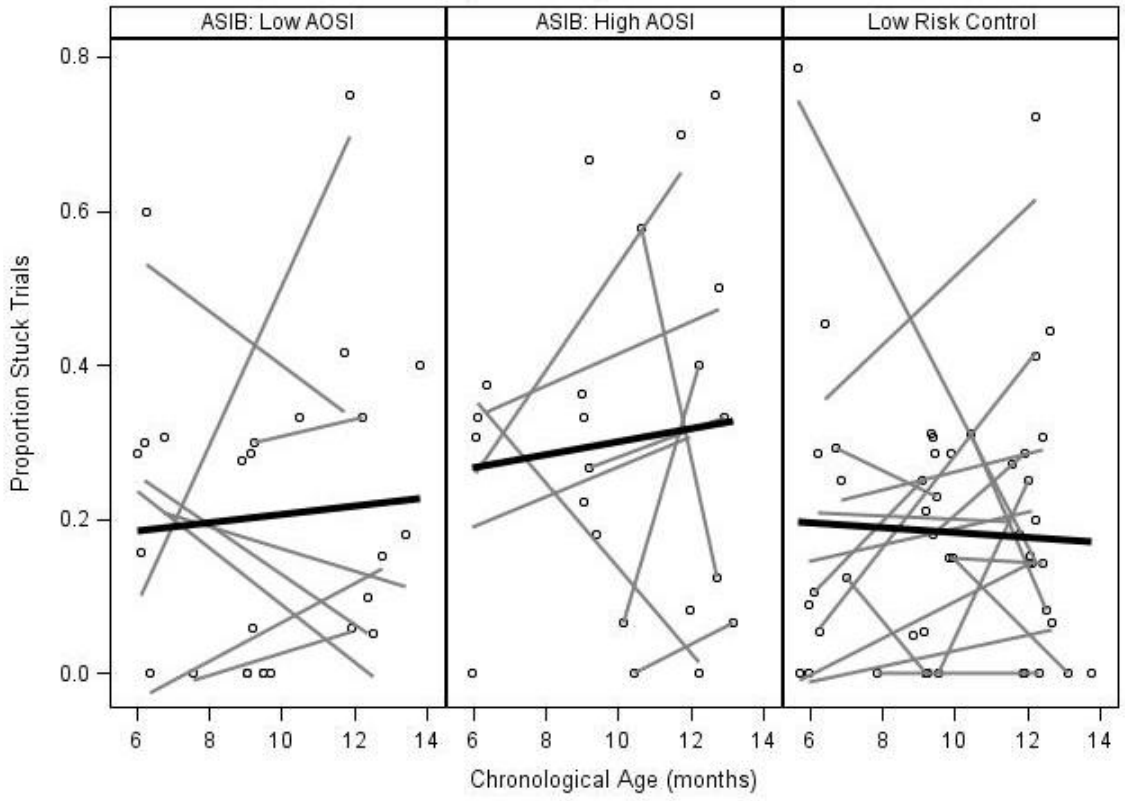

Figure 3.4: Proportion of Stuck Overlap Trials across Age, by Clinical ASD Risk

Note: Proportion of stuck trials across ASIBs with AOSI Total Score less or greater than the group median (<5 = "Low AOSI"; ≥ 5 = "High AOSI") versus LR controls.

CHAPTER 4

CROSS-SYNDROME ATTENTION IN INFANTS AT RISK FOR AUTISM[3]

[3] Tonnsen, B. L., Richards, J. E. & Roberts, J. E. To be submitted to *American Journal of Psychiatry*

After decades of research seeking to characterize the developmental course of autism spectrum disorder (ASD), our field is beginning to complement *reactive* approaches – reducing symptoms that cause impairment – with *proactive* approaches – preventing debilitating symptoms before they emerge (Dawson, 2008). The first step to prevention, however, is fine-tuned characterization of the nature and course of atypical development. One of the most common techniques for identifying prodromal indicators of ASD risk is to prospectively examine cohorts of "high risk" infants over time. A number of longitudinally studies have followed infant siblings of children with autism (ASIBs), who exhibit 10-20 times higher rates of autism diagnoses (e.g. 19%; (Ozonoff et al., 2011) than the general population (1-2%; Centers for Disease Control, 2014) to establish endophenotypes of ASD and potential predictors of later ASD risk. However, far fewer studies have examined emerging autism symptoms and risk factors in other infants at elevated risk for ASD, such as infants with fragile X syndrome (FXS), a single-gene disorder highly associated with autism (Bailey, Raspa, Olmsted, & Holiday, 2008). Comparing aberrant pathways identified in ASIBs across multiple high-risk groups may establish convergent pathways of risk that indicate generalizable targets for population-based surveillance. These comparisons may also identify sources of syndrome-specific

variability that could clarify etiological pathways of ASD symptoms and mechanism-specific treatments.

The present study employs a cross-syndrome approach to investigate attention orienting in infants at risk for ASD. Aberrant orienting is one of the most commonly reported abnormalities in ASIBs (Study 2; Elison et al., 2013; Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009; Zwaigenbaum et al., 2005) and has been identified in a small sample of infants with FXS, as well (Roberts et al., 2011). We previously reported atypical patterns of attention orienting in 5-14 months ASIBs compared to low risk controls using a computerized gap-overlap task (Study 2). In the present study, we investigated cross-sectional profiles of attention orienting, as well as concurrent physiological arousal and heart-defined sustained attention, in 9 and 12-month infants with FXS contrasted our previously reported ASIB and low risk (LR) data (Study 2). The purpose of this work is twofold. First, we aimed to establish whether abnormalities we previously reported in ASIBs similarly manifest in infants with FXS, informing potential heterogeneity in symptom expression. Second, we sought to determine whether converging cross-syndrome behavioral profiles are associated with similar physiological substrates, informing whether shared phenotypes are potentially sustained by distinct biological mechanisms. By examining both shared and heterogeneous pathways of ASD risk across groups, this work may characterize both global and syndrome-specific information about the etiology and emergence of ASD risk in infants.

A cross-syndrome approach to ASD

Between 2010 and 2014, over 100 peer-reviewed papers were published on the early development of infant siblings of children with ASD (ASIBs). This body of

literature has identified a number of abnormal features in ASIBs, ranging from socio-communicative symptoms to visual attentional abnormalities, which have paved the way for earlier ASD detection and treatment (see Jones et al., 2014; Rogers, 2009; Zwaigenbaum, Bryson, & Garon, 2013, for review). A notable limitation of the "infant sibling" approach, however, is the likelihood that ASIBs exhibit different patterns of prodromal autism risk than infants diagnosed without a family history of ASD. For example, compared *simplex* families with one child affected with ASD, *multiplex* families (more than one child with ASD) exhibit less robust elevations in ASD rates in males versus females (Ozonoff et al., 2011; Zwaigenbaum et al., 2012), suggesting differing profiles of genetic risk. Similarly, sub-clinical autism features are more common in multiplex versus simplex families (Constantino et al., 2010; Szatmari et al., 2000), as well as in and simplex versus adoptive families (Szatmari et al., 2000), indicating genetically-mediated gradients of symptoms in non-ASD samples. Infant siblings who do not meet ASD criteria also exhibit higher rates of subthreshold ASD symptoms and elevated risk of developmental and language impairments compared to low-risk controls (Messinger et al., 2013; Ozonoff et al., 2014; Toth et al., 2007), suggesting complex risk profiles among ASIBs that may not extend to non-ASIB groups. Thus, although the ASIB literature has been central to defining infant predictors of multiplex ASD and genetically-mediated symptom profiles, a critical next phase will be disentangling the generalizability of ASIB profiles to the majority of autism cases diagnosed *without* a family history.

A promising, complementary model for delineating early risk factors for ASD is to engage in cross-group comparisons across multiple "high risk" samples. Much like in

ASIBs, elevated rates of autism are reported across a number of genetic syndromes, including fragile X syndrome (Bailey, Skinner, et al., 2008; Farzin et al., 2006; Kaufmann et al., 2004; Rogers, Wehner, Hagerman, & Wehner, 2001a), tuberous sclerosis (Tye & Bolton, 2013), Smith Lemli Opitz syndrome (Sikora, Pettit-Kekel, Penfield, Merkens, & Steiner, 2006), and Down syndrome (Kent, Evans, Paul, & Sharp, 1999). Similar to the "infant sibling" model, prospectively following children with these disorders from infancy through toddlerhood may inform developmental trajectories of risk and resilience (McCary & Roberts, 2013; Tonnsen, Malone, et al., 2013). Importantly, studying populations with known genetic vulnerabilities also permits conceptualization of complex genetic, environmental and developmental interactions not afforded by populations in which specific genetic biomarkers of risk are unknown (Fung et al., 2012; McCary & Roberts, 2013; Tonnsen, Malone, et al., 2013; Tye & Bolton, 2013), deepening our understanding of complex genetic and epigenetic risk factors for ASD.

Autism in Fragile X Syndrome

Although the cross-syndrome approach is applicable to a number of genetic syndromes associated with ASD, the present study focuses on fragile X syndrome (FXS), a single-gene disorder that affects 1:4000 individuals (Crawford et al., 2001; Hagerman & Hagerman, 2002) and is highly associated with ASD (Bailey, Raspa, et al., 2008; Farzin et al., 2006; Kaufmann et al., 2004; Rogers, Wehner, Hagerman, & Wehner, 2001b). "Fragile X" is caused by a CGG triplet repeat mutation on the X chromosome that results in methylation of the *FMR*1 gene and subsequent absence of fragile X mental retardation protein (FMRP), a protein that contributes to synaptic plasticity in typical

neural development (Fernández, Rajan, & Bagni, 2013). The behavioral phenotype of FXS is well-defined and includes elevated anxiety, inattention, hyperarousal, autistic symptoms, and intellectual disability (Bailey, Raspa, et al., 2008; Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Tonnsen et al., 2014; Tonnsen, Shinkareva, Deal, Hatton, & Roberts, 2013). Cognitive delays in FXS generally emerge by 9 months of age in males (Roberts, Mankowski, et al., 2009), and females generally exhibit less severe symptoms due to random X-inactivation (Sobesky et al., 1996).

Relevant to the present study, between 25% and 60% of children with FXS also meet diagnostic criteria for ASD, and up to 90% display ASD symptoms (Farzin et al., 2006; Kaufmann et al., 2004; Rogers et al., 2001b). In general, co-occurring ASD ("FXS+ASD") is associated with more severe outcomes including behavior problems (Hatton et al., 2002), receptive language delays (Rogers et al., 2001b), more severely impaired cognitive profiles (Bailey, Hatton, Skinner, & Mesibov, 2001; Roberts, Mankowski, et al., 2009), and abnromal approach and socialization (Hernandez et al., 2009; Roberts et al., 2007; Roberts, Clarke, et al., 2009).Given the well-defined genetic mechanisms and behavioral profile of FXS, as well as high rates of co-occurring ASD symptoms, FXS may serve as a salient model for disentangling early profiles of ASD risk (McCary & Roberts, 2013; Tonnsen, Malone, Hatton, & Roberts, 2013b).

The elevated co-occurrence of ASD in FXS has been conceptualized through a number of theoretical lenses. One perspective is that ASD features in FXS emerge from abnormal function of the *FMR*1 gene and compounding intellectual disability, thus ASD within FXS is quantitatively different from "true" ASD and may be considered largely redundant with the broader FXS phenotype (Abbeduto, McDuffie, & Thurman, 2014; S.

Hall, Lightbody, & Hirt, 2010). An alternate interpretation is that a number of factors relevant to FXS – including *FMR*1 gene function, environmental contexts, phenotypic features, developmental change, and ASD-specific vulnerabilities – may produce divergent patterns of ASD within FXS that inform *meaningful variability* in ASD. This dynamic framework reflects the notion that developmental phenotypes emerge probabilistically rather than deterministically (Gottlieb, 2007), with genetic syndromes representing complex, interactive sequelae of development rather than static, deterministic end products (Karmiloff-Smith, 2009). Although these perspectives are not mutually exclusive, conceptualizing the FXS phenotype as a dynamic process treats variability in ASD symptom profiles as *expected* reflections of the complex heterogeneity of ASD rather than an artifact of phenotypic miscategorization or error. Within this framework, cross-syndrome comparisons of ASD in FXS produce a number of meaningful benefits, such as informing convergent risk factors, differentiating subgroups, and characterizing the developmental emergence of ASD. Here, we briefly summarize literature in the context of these benefits, focusing our discussion on infants at risk.

Expected Outcomes of Cross-Syndrome Comparisons

Identifying *convergent* patterns of risk across multiple high-risk groups such as FXS may inform ASD etiology and candidate mechanisms for targeted intervention. For example, similar patterns of "sticky" visual attention have been identified in a small sample of infants with FXS with elevated ASD symptoms (Roberts et al., 2011) and ASIBs who later meet ASD criteria (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005), suggesting a potential common risk factor for later ASD symptomatology across groups. Similar trends have also emerged related to physiological

arousal. We previously identified higher autism symptoms associated with hypoarousal during toy play in young infants with FXS (Roberts et al., 2012), as well as hypoarousal in similarly aged ASIBs during an active attention task (Study 2). Thus, hypoarousal in young infants may be a shared vulnerability for ASD symptoms across groups. However given the limited extant literature on ASD emergence in FXS, extracting these types of similarities is complicated by a number of developmental and contextual factors, such as age-related shifts from hypo- to hyper-arousal across early childhood in FXS (Roberts et al., 2012), and apparent specificity of hypoarousal to active (Study 2) versus passive (Study 1) attention tasks in ASIBs. Thus, although emerging trends across studies suggest converging features across FXS and ASIBs, within-study, cross-group comparisons are needed to clarify the developmental processes and contexts in which these shared patterns are expressed.

A second meaningful outcome of cross-syndrome comparisons is the likelihood of revealing *divergent* patterns of risk. Indeed, because ASD is defined by behavioral rather than biological symptoms, it is likely that autistic symptoms emerge secondary to a variety of genetic and neurodevelopmental processes (Abrahams & Geschwind, 2008; Pinto, Pagnamenta, Klei, & Anney, 2010). Establishing connections between specific phenotypic features and multiple layers of measurement – in essence, attending to symptoms rather than categories – is consistent with recent impetus from the National Institute of Mental Health that psychopathology be conceptualized across multiple layers and dimensions, rather than through arbitrary diagnostic categories (Cuthbert & Insel, 2013). Symptom-based approaches have been similarly advocated to improve conceptualization of ASD in FXS (Abbeduto et al., 2014). From this transdiagnostic

perspective, profiles of symptom expression in genetic disorders such as FXS provide a rich context for exploring complex interactions among multiple levels of risk. Indeed, divergent patterns of early temperament have been identified in infants with FXS compared to patterns reported in ASIBs, with markers of ASD in ASIBs predicting anxiety, not autistic symptoms, in FXS (Tonnsen, Malone, et al., 2013). In early childhood, structural brain differences have also been observed in FXS+ASD versus ASD-only, with FXS+ASD exhibiting enlarged caudate and smaller amygdala volume compared to substantially enlarged amygdala volume in ASD-only (Hazlett et al., 2012). These behavioral and neurobiological differences in FXS+ASD may inform genetic pathways of symptom expression specific to the developmental neurobiology of FXS. Although parsing cross-syndrome variability is a complex task, capturing this heterogeneity may pave the way for developmental surveillance that is sensitive to individual differences, as well as targeted treatments that address the mechanisms, rather than symptoms, of impairment.

These convergent and divergent patterns of ASD-associated features in FXS highlight a third meaningful outcome of cross-syndrome comparisons: clarifying the developmental emergence of ASD. From a practical standpoint, static, categorical diagnoses – such as those present in the Diagnostic and Statistical Manual for Mental Disorders (DSM-V (Association, 2013) – provide an organized nomenclature for comparing research and translating science relevant to clinical subgroups (First, 2005). However, current theoretical positions increasingly recognize that developmental disabilities do not emerge from static, modular deficits that persist over time, but instead evolve through complex interactions across multiple layers of development (Cornish et

al., 2007; Karmiloff-Smith, 1998, 2009). This perspective is apparent in ASD research, as a number of ASD theories specify complex interactions among genetic and neurobiological sequelae over time (Abrahams & Geschwind, 2008; Johnson et al., 2005; Keehn et al., 2013; Pinto et al., 2010), resulting in a continuous spectrum of risk rather than categorical impairments. Layering developmental theories with cross-syndrome comparisons may disentangle these pathways by holding constant a portion of genetic variability, as well as by revealing shared biological pathways that emerge downstream from initial genetic differences (Abrahams & Geschwind, 2008). These comparisons are particularly critical in ASD given the multitude of genetic risk factors for the disorder and heterogeneous presentation of symptoms across affected individuals, even among siblings diagnosed with ASD within the same family (Yuen et al., 2015).

Despite this emerging popularity of a dynamic, developmental approach to ASD, empirical studies of ASD in FXS have largely focused on static, cross-sectional comparisons of symptom profiles in childhood and adulthood (McDuffie, Thurman, Hagerman, & Abbeduto, 2014; Roberts et al., 2007; Roberts, Clarke, et al., 2009; Sally J Rogers et al., 2001), likely due to the limited information about emerging ASD symptoms in infants (McCary & Roberts, 2013). However, a handful of repeated-measure studies have begun to index dynamic temporal changes that clarify complex presentations of ASD symptoms within FXS. For example, ASD symptoms are associated with short-term patterns of sustained withdrawal across the duration of an assessment (Roberts, Clarke, et al., 2009), as well as long-term deficits in peer relationships and adaptive socialization across multiple annual evaluations (Hernandez et al., 2009). Our emerging work in infants also suggests that longitudinal trajectories provide unique information about

emergent ASD predictors in FXS that is not detectable from cross-sectional profiles (Roberts et al., 2011) and may distinguish FXS from other high risk groups (Tonnsen, Malone, et al., 2013). Further clarifying these early pathways of ASD risk in FXS may contribute to broader understanding of ASD emergence and stability, informing questions of ASD expression within FXS and its generalizability to non-FXS samples.

Taken together, these multiple benefits of cross-syndrome comparisons – including identifying commonalities, disentangling differences, and clarifying developmental profiles – attest to the utility of examining ASD profiles in FXS. However, few studies to date have examined prospective emergence of ASD symptoms in infants with FXS (Roberts et al., 2011; Tonnsen, Malone, et al., 2013). The present study applies this framework to examine one of the most robust predictors of ASD, aberrant attention orienting, in infants with FXS compared to ASIBs and low-risk controls. This work aims to both examine shared pathways of risk FXS and ASIB groups in early development, as well as disentangle potential cross-group differences in underlying mechanisms that may inform pathways to ASD risk.

The Case of Attention in "High Risk" Infants

Aberrant attention orienting is well-defined as a predictive feature of ASD in ASIBs within the first year of life. Previous studies on attention in ASIBs, as well as methods for measuring constructs such as orienting and heart-defined attention, are reviewed in Studies 1 and 2. In brief, previous work suggests ASIBs display abnormal orienting toward social and nonsocial stimuli (Dawson et al., 1998; Landry & Bryson, 2004; Swettenham et al., 1998) and slower saccade latencies on computerized orienting tasks (Elison et al., 2013; Elsabbagh et al., 2009, 2013; Zwaigenbaum et al., 2005). In

Study 2, we used a previously-published version of the gap-overlap task (Elsabbagh et al., 2013; Elsabbagh, Volein, et al., 2009) to examine both behavioral orienting and heart-defined sustained attention in ASIBs contrasted to LR controls. We identified abnormal orienting in ASIBs around 6 months of age, with blunted improvement in disengagement latencies among ASIBs. We also identified patterns of hypoarousal in young ASIBs, which may be reflective of slower saccade latencies in this sample. Importantly, the proportion of "stuck" trials, those in which participants failed to disengage attention, was associated with higher clinical autism risk symptoms around 12 months of age, supporting previous reports that abnormal orienting predicts ASD risk among ASIBs (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). Together, these data support further investigation into the role of aberrant attention orienting in ASD symptom emergence, as well as whether orienting deficits are similarly present in other high-risk infant groups such as FXS.

Orienting in FXS. Abnormal orienting is one of many attentional impairments reported in FXS, with others including poor inhibitory control (Cornish, Cole, Longhi, Karmiloff-Smith, & Scerif, 2012; Tonnsen et al., 2014), inattention (Bailey, Raspa, et al., 2008; Cornish et al., 2012), and hyperactivity (Bailey, Raspa, et al., 2008; Wheeler et al., 2014). Due to the early and pervasive nature of these attention problems, it has been suggested that early attention patterns "constrain" later developmental trajectories within FXS (Cornish et al., 2012). Indeed, early attention abnormalities within FXS have been associated with a variety of clinical outcomes, including lower intellectual abilities, poorer classroom behavior, and higher autistic symptoms (Cornish et al., 2012; Roberts et

al., 2011; Tonnsen et al., 2014), suggesting early abnormal attention may index risk and warrant intervention.

Similar to the orienting deficits reported in ASD, orienting deficits have been reported in FXS across the lifespan. Using a series of ocular motor paradigms, Lasker and colleagues (2007) identified longer latencies to disengage attention from competing stimuli in 7-22 year old females with FXS (n=17) and Turner syndrome (n=19), suggesting similarly impaired attention orienting to previous reports in ASD (Dawson et al., 1998; Landry & Bryson, 2004; Swettenham et al., 1998). In a small sample of infants with FXS (n=13), Roberts and colleagues (Roberts et al., 2011) reported higher rates of ASD symptoms in infants who spent more time looking at a toy at age 12 months and exhibited increased latency to disengage attention between 9 and 12 months. These results parallel findings of increased look duration and atypical disengagement in ASIBs (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005)Study 2). Cornish and colleagues (Cornish et al., 2007) also examined orienting toward targets in response to cues that were either valid (cue same side as target) or invalid (cue opposite side from target). In this small sample of 9 toddlers with FXS (ages 14-55 months) compared to infants with Williams syndrome (n=8) and mental age-matched controls (n=20), similar saccade latencies were observed among groups during validly-cued trials, with slower performance on invalid trials in the Williams syndrome group only. However, the authors caution that nonsignificant findings in the FXS likely relate small sample size; a conclusion that is supported by evidence of a moderate effect (Cohen's d=.50, (Cohen, 1988) between validly cued trials across FXS and mental-age matched groups (calculated for the present study using published means and standard deviations). Thus, orienting

deficits appear to be present from infancy through adolescence in FXS, although small samples and varied experimental designs warrant further replication and clarification of the nature of these deficits.

Mechanisms for abnormal orienting in FXS. The neurobiological mechanisms and longitudinal emergence of orienting deficits in FXS are also unclear, although both self-regulatory and visual processes have been posited to sustain these behaviors. Abnormal autonomic functioning is well-documented in FXS. In general, children and adults with FXS exhibit hyperarousal (Hall, Lightbody, Huffman, Lazzeroni, & Reiss, 2009; Heilman, Harden, Zageris, Berry-Kravis, & Porges, 2011; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001; Roberts et al., 2012; Tonnsen, Shinkareva, et al., 2013), which appears to be a chronic state in FXS rather than a state-dependent feature (Klusek et al., 2015).

Given these well-documented abnormalities in autonomic functioning in FXS, it is possible that abnormal arousal contributes to orienting deficits observed in this population. In typically developing samples, behavioral orienting is closely associated with patterns of physiological arousal that index the attentional response (Casey & Richards, 1988; Richards, 1987, 1997). As attention increases, parasympathetic processes activate, producing HR decelerations that serve as biomarkers of attention engagement (Graham & Clifton, 1966; Lacey, 1959). In infants, s*ustained attention* is the maintenance of decelerated HR as stimulus details are processed (Casey & Richards, 1991; Richards & Casey, 1991; Richards, 2000). During sustained attention, typically developing infants are less likely to orient to peripheral stimuli, both during computerized attention tasks (Casey & Richards, 1988; Richards, 1997) and toy play (Lansink &

Richards, 1997). In the only study to examine heart-defined sustained attention in FXS, Roberts and colleagues (2011) identified shallower and less variable sustained attention during toy play in a small sample (n=12) of infants with FXS. Thus, integrating measures of heart-defined sustained attention may inform whether abnormal orienting reported in FXS is rooted in atypical physiological functioning.

However, it is also possible that aberrant orienting in FXS is sustained or compounded by abnormal visual processes apparent in the disorder. Similar to a number of other developmental disabilities, FXS is associated with "dorsal stream deficits" related to processing dynamic visual information (Kogan, Bertone, & Cornish, 2004; Kogan, Boutet, et al., 2004). The dorsal stream is one of two primary cortical pathways through which the lateral geniculate nucleus transmits information from the retina to primary visual cortex (Felleman & Van Essen, 1991). In healthy individuals, FMRP is highly expressed in magnocellular pathways involved in the dorsal stream, and the absence of FMRP has been posited to explain observed abnormalities in magnocellular pathway morphology in FXS (Kogan, Boutet, et al., 2004). Psychophysiological studies in FXS have supported this association by identifying a number of functional dorsal deficits, such as maintaining identity of dynamic but not static objects when occluded (Farzin & Rivera, 2010) detecting temporal change and motion (Farzin, Whitney, Hagerman, & Rivera, 2008; Kogan, Boutet, et al., 2004), and processing multimodal stimuli (Scerif, Longhi, Cole, Karmiloff-Smith, & Cornish, 2012). It is possible that abnormal dorsal functioning contributes to orienting deficits in FXS, particularly given recent evidence of abnormal associations between attention orienting and dorsal stream connectivity, measured via radial diffusivity of the splenium, among infants later

diagnosed with ASD (Elison et al., 2013). Notably, abnormal dorsal stream functioning has been similarly reported in other neurodevelopmental disorders such as Prader-Willi syndrome (Woodcock, Humphreys, & Oliver, 2009), Dravet syndrome (Ricci et al., 2015), and non-FXS associated ASD (Spencer et al., 2000), suggesting a potential shared biological pathway among neurodevelopmental disorders rather than syndrome-specific endophenotype to FXS.

The Present Study

Cross-syndrome comparisons may inform the early emergence of ASD by identifying both shared pathways of risk and sources of heterogeneity. Although ASD symptoms are common in FXS (Bailey, Raspa, et al., 2008; Farzin et al., 2006; Kaufmann et al., 2004; Sally J Rogers et al., 2001), few studies have examined early trajectories of ASD emergence in infants with FXS (Roberts et al., 2011; Tonnsen, Malone, et al., 2013), and no studies to-date have contrasted these early trajectories to other high-risk groups such as ASIBs. The present study examined cross-sectional profiles of visual attention orienting and concurrent heart-defined sustained attention in 9 and 12-month infants with FXS contrasted to a subset of our previously published ASIB data (Study 2). This work aimed to establish whether abnormalities we previously reported in ASIBs similarly manifest in infants with FXS, informing potential heterogeneity in early ASD predictors and mechanisms.

Three primary research questions were examined: (1) Do behavioral and heart-defined patterns of attention differ between FXS and both ASIB and LR groups? (2) Are biobehavioral associations between behavioral and heart-defined attention similar in FXS versus ASIB and LR groups, and (3) Within FXS, do behavioral and heart-defined

attention indicators predict clinical ASD risk? In Study 2, we examined these questions in ASIBs and LR controls using an expanded, longitudinal sample. In contrast, the present study focuses on attention in FXS compared to ASIB and LR groups.

We hypothesized that although both FXS and ASIB groups would display "sticky attention" (longer latencies to disengage attention, greater proportion of failed trials), associations between behavioral and heart-defined attention would differ across groups. In light of previous findings (Roberts et al., 2011), we hypothesized that infants with FXS would show longer latency to disengage attention but shorter and shallower SA, suggesting dysregulation from typical associations between slower disengagement indexed and greater sustained attention (Casey & Richards, 1988; Lansink & Richards, 1997; Richards, 1997). Increased dysregulation would be associated with higher clinical ASD risk features. This pattern would parallel our previous reports that greater clinical ASD risk in ASIBs is associated with reduced IBI change during SA and IBI variability during a passive viewing task (Study 1) as well as greater difficulty disengaging attention during a gap-overlap task (Study 2). Although we did not identify associations between behavioral and heart-defined attention in ASIBs during the gap-overlap task in Study 2, we anticipated these associations would be present in FXS due to the cumulative effects of self-regulatory and visual processing deficits in the disorder.

Methods

Measures, stimuli, and behavioral coding procedures were identical to Study 2. Data from 113 assessments were collected from 62 participants (17 FXS, 21 ASIB, 24 LR). The proportion of participants with both 9 and 12 assessments was similar across groups ($X^2(2) = 1.50$, $p = .47$; FXS=59%; ASIB=76%; LR=63%). All participants were

required to be born full term (37 weeks or later, >2000 grams) and live with their biological mother. Fragile X diagnoses were verified with genetic report (>200 CGG repeats on the *FMR*1 gene). The ASIB and LR groups were required to have no diagnosed genetic or medical conditions. Infant siblings were required to be full biological siblings of a child with an ASD diagnosis documented by a licensed psychologist. Due to the lower risk for ASD in females (Bertrand et al., 2001; Nassar et al., 2009) and high variability in developmental skills in females with FXS (Clifford et al., 2007; Hatton et al., 2009), efforts were made to recruit a predominately male sample. Several females were permitted in each group (FXS 7/17; ASIB 6/21, LR 3/24). The ASIB and LR sample for the present study overlapped considerably with Study 2, with minor differences due to matching and age (e.g. 6m ASIB data examined in Study 2, additional LR female included in Study 3 to parallel higher proportion females in FXS sample). In contrast to Study 2's focus on longitudinal patterns of individual differences in ASIBs contrasted to LR controls, the present study focuses on cross-sectional patterns of attention in 9 and 12 month infants with FXS. As such, differences between ASIB and LR groups are reported for reference but are presented in greater detail and using complete longitudinal data in Study 2.

To characterize both age and developmental processes related to attention, we examined FXS data in relation to both chronological and mental age groups. Chronological age comparisons were conducted across FXS, ASIB, and LR groups at 9 and 12 months. The FXS group exhibited mental ages approximately 3 months behind the LR group at each time point. Thus, to determine whether differences in the FXS group were accounted for by mental age, the FXS group was also compared to mental age (MA)

control groups by contrasting 12 month FXS and 9 month LR groups, as well as 9 month FXS and 6 month LR groups (6 month LR group not included in chronological age comparisons). Although mental age was not measured during the 6 month assessment, it was estimated for each participant by multiplying chronological age by the average ratio of mental to chronological age in the LR group at 9 and 12 months (1.07). Although coarse, these calculations suggested relatively similar mental ages across groups. Mental age contrasts were not conducted for physiological variables given expected maturation in autonomic functioning across age.

Missing data were similar to previous work in similarly sized samples (Elsabbagh, Volein, et al., 2009). In addition to 113 assessments included in analyses, 16 additional assessments were conducted but excluded due technology problems (n = 13; 4 ASIB, 9 LR) and noncompliance (n = 3; 1 FXS, 2 ASIBs) yielding 12% missing behavioral data. In addition, physiological data were excluded for 18 assessments (4 FXS, 8 ASIB, 6 LR) due to high artifacts, were not collected from 7 participants (2 FXS, 2 ASIB, 3 LR) during the initial battery implementation, and were not collected for 1 LR participant due to parental preference, yielding 19% missing physiological data. Five LR participants were excluded due to developmental concerns (1epilepsy, 3 suspected ASD, 1 suspected family history of ASD).

Analyses

Analyses were conducting using SAS 9.3 (Apex, NC) with α set to less than .05. Due to the relatively small sample and uneven number of assessments across participants, nonparametric techniques were used to test differences in levels of each dependent variable at two time points: 9 months (range 8 to 11 months) and 12 months (range 11 to

14 months). No participant was observed more than one time in each age range. Employing nonparametric analyses is appropriate for small, ordinally distributed samples but precludes conclusions about within-individual change over time, which we previously examined in our expanded longitudinal ASIB and LR samples (Studies 1 and 2). Group differences in dependent variables were analyzed using nonparametric Wilcoxon-Mann-Whitney tests, which compare dependent variable ranks across groups. Correspondence between behavioral and heart-defined attention variables were analyzed using Spearman correlations, collapsed across ages. Chronological and mental age were covaried for all correlations, and baseline latency was covaried for gap and overlap latencies.

Results

Group Differences

Behavioral attention. Behavioral attention differed between FXS and comparison groups, with greatest abnormalities within FXS. Tables 4.3 and 4.4 detail chronological and mental age comparisons, respectively, for behavioral and physiological variables. Compared to same-aged LR controls, the FXS group exhibited longer overlap latencies (9 and 12 months), greater proportion of failed trials (9 months), and less inattention (greater attentiveness; 9 months). Compared to ASIBs, the 9-month FXS group exhibited longer overlap latencies and greater attentiveness. These group differences are depicted in Figures 4.1-4.3, which portray boxplots of individual values across groups and chronological age categories, with significant differences indicated by red brackets. Only 12-month overlap latencies remained significantly different compared to MA controls. Together, these behavioral results suggest longer latencies to disengage, greater proportion of stuck trials, and increased inattention in FXS, particularly in

younger participants. The FXS group exhibited similar latencies to MA-controls at 9 but not 12 months, suggesting delays that increasingly deviated from developmental expectations over time.

Physiological variables. Physiological profiles differed across groups. Compared to LR controls, the 9-month FXS group exhibited marginally longer IBI (p=.08) and higher RSA (p=.06). As depicted in Figure 4.3, the 9-month FXS group exhibited greater change in IBI during SA than both LR and ASIB groups, which did not differ from each other. The 12-month FXS group also spent a larger proportion of time in SA compared to LR controls. No additional group comparisons were significant at 9 or 12 months. Thus, physiological profiles in FXS were marked by increased depth of SA at 9 months and greater proportion of time in SA at 12 months, with a trend toward general hypoarousal at younger ages.

Correspondence between Behavioral and Heart-Defined Attention

Table 4.3 details correlations between behavioral and heart-defined variables for each group. Across groups, proportion of time in behavioral and heart-defined attention positively correlated. Sustained attention parameters also correlated with behavioral attention in the FXS and LR groups, although specific associations varied by group. Among infants with FXS, longer overlap latencies and greater proportion of stuck trials were associated with less change in IBI during SA, and longer overlap latencies were also associated with less variability in IBI during SA. However among LR controls, longer overlap latencies were associated with greater proportion of time in SA but were not significantly associated with change or variability of IBI during SA. Thus we observed paradoxical patterns of reduced SA parameters associated with increased attention in

FXS, versus typical associations between increased SA and increased attention in LR controls. Consistent with Study 2, no significant correlations between behavioral and heart-defined attention were observed among ASIBs.

Correlations with Clinical Autism Risk in FXS

Controlling for chronological age, longer baseline latencies and greater proportion of failed trials predicted higher clinical autism risk in 12-month infants with FXS. The effects between behavioral performance and clinical markers were no longer significant when mental age was included as a covariate, likely reflecting the strong negative correlation between ASD clinical markers and developmental abilities (-.50, $p = .01$). Thus, although associations between baseline latencies and clinical ASD risk are present in FXS, developmental ability accounts for a large proportion of variance in risk.

Discussion

Cross-syndrome comparisons may inform the etiology and developmental mechanisms of ASD by clarifying both shared pathways of risk and syndrome-specific mechanisms. The present study employed a cross-syndrome approach to investigate visual orienting in two samples of infants at "high risk" for ASD: infant siblings of children with ASD (ASIBs) and infants with fragile X syndrome (FXS). We observed persistent, early emerging orienting deficits in FXS increasingly deviated from developmental expectations and were more robust than differences observed in ASIBs. The magnitude of these deficits predicted clinical ASD risk in FXS, which was also highly associated with mental age. Importantly, we observed dissociations between patterns of physiology that related to global attention versus attention orienting in FXS, potentially indicating dysregulated intersection of attentional and physiological

processes. Furthermore, although behavioral abnormalities were similar across FXS and ASIB groups, physiological profiles differed. These distinct profiles suggest potentially heterogeneous pathways to abnormal attention across infant groups at high risk for ASD.

Visual Attention in FXS

As hypothesized, we observed early-emerging and persistent attentional deficits in FXS, consistent with a previous study of orienting in a small sample of infants (Roberts et al., 2011). These deficits worsened over time; although overlap latencies at 9 months were longer than CA controls but similar to MA controls, the FXS group exhibited longer overlap latencies than both CA and MA controls at 12 months, suggesting increasing deviation from developmental norms across age. The FXS group also exhibited greater proportion of failed trails and abnormally increased attentiveness at 9 months, although these differences were not significant compared to MA-matched controls. Thus, although a portion of attentional deficits in FXS may reflect developmental delay rather than deficit, the magnitude of saccade latency differences accelerated over time relative to MA-matched controls, suggesting increasingly greater deficits than expected based on developmental abilities alone. This pattern is distinct from abnormal orienting we previously described in ASIBs (Study 2), as abnormalities in FXS relate to mental age, whereas ASIB differences persisted when MA was covaried in models

Similar to our previous study of ASIBs (Study 2), behavioral attention also correlated with clinical ASD risk within FXS, suggesting attention abnormalities may index or contribute to ASD features. Specifically, longer baseline latencies and higher proportions of failed trials were associated with higher AOSI scores in FXS, paralleling previous reports of higher ASD symptoms in infants with FXS who exhibited

increasingly abnormal attention across early infancy (Roberts et al., 2011), as well as a number of studies linking abnormal orienting to ASD in ASIBs (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). Notably, these effects were no longer significant when mental age was covaried, likely due to the high proportion of variance in ASD risk explained by developmental ability. This differential association with and without controlling for MA does not necessarily suggest that orienting-ASD associations are insignificant in FXS, but instead may reflect the higher presence of ASD among individuals – with and without FXS – who exhibit lower intellectual abilities (de Bildt, Sytema, Kraijer, & Minderaa, 2005; Deb & Prasad, 1994; S. Hall et al., 2010). Examining these effects in larger FXS samples stratified by developmental ability may inform the impact of mental age on the attention-ASD relationship. Similarly, comparing attention in FXS to other groups with similar developmental delays would inform the specificity of impairments to FXS. Notably, sampling characteristics may have also tempered associations between behavioral attention and clinical ASD risk, as we included a high proportion of females who typically exhibit less ASD risk within FXS (Bailey, Raspa, et al., 2008). Thus is possible that effects may be salient in male-only samples or among infants with lower developmental abilities. Efforts are underway to expand the present FXS sample to include more male participants, increasing our capacity to examine whether effects differ in male-only samples.

Heart Activity in FXS

Physiological data also revealed unique patterns of autonomic dysfunction within FXS that may inform the mechanisms of abnormal attention in this population. Compared to ASIB and LR groups, the 9 month FXS group exhibited marginally longer IBI and

higher RSA, as well as greater IBI change during SA. Twelve-month infants with FXS also spent more time in heart-defined sustained attention than LR controls. Interestingly, these patterns of hypoarousal deviate somewhat from the general consensus that individuals with FXS are hyperaroused (Hall et al., 2009; Heilman et al., 2011; Roberts et al., 2001, 2012; Tonnsen, Shinkareva, et al., 2013) and display lower RSA at rest (Boccia & Roberts, 2000; S. S. Hall et al., 2009; Roberts et al., 2012). This divergence supports emerging theories that hyperarousal may emerge secondary to a period of hypoarousal in infants with FXS. For example, Roberts and colleagues (Roberts et al., 2012) reported higher autistic symptoms in FXS were associated with hypoarousal in young infants (<10 months) but hyperarousal in older infants (>37 months), and a number of additional studies in infants and toddlers failed to identify FXS-specific differences in arousal (Roberts et al., 2011; Tonnsen, Shinkareva, et al., 2013), perhaps due to shifting patterns that dilute group differences. Our finding of marginally higher RSA in FXS may similarly reflect reduced suppression of vagal tone in response to the cognitive load of the task, similar to findings of reduced vagal suppression in older children with FXS (Heilman et al., 2011). Thus, although somewhat inconsistent with findings in older samples, our results converge with emerging evidence of shifting arousal patterns in infants with FXS and warrant additional work in this area.

These potentially changing self-regulatory profiles across infancy have important implications for early detection and intervention efforts in FXS. First, our findings suggest that early self-regulatory profiles in FXS may not linearly predict later development, lending support to the increasingly popular conceptualization of neurodevelopmental disorders as developmental phenotypes that evolve over time

(Karmiloff-Smith, 2009). Second, although further work is needed to characterize self-regulation among infants with FXS, it is possible that future interventions may be designed to capitalize on these early periods of rapid developmental change, consistent with the increasing impetus on preventing rather than treating developmental disorders (Dawson, 2008). Identifying "hot spots" of developmental vulnerability, as well as the neurobiological systems contributing to those vulnerabilities, may permit interventions that alter early neurodevelopmental trajectories and improve developmental outcomes. However, additional prospective, longitudinal studies of infants with FXS are likely necessary to inform the specific developmental landscape of infant arousal within FXS, as well as the feasibility and consequences of potential arousal-related interventions.

Given the elevated behavioral attention observed in our FXS group, it is also possible that hypoarousal reflected increased behavioral attention rather than "resting" differences in autonomic functioning. Several of our findings support this conclusion: proportion of time in behavioral and heart-defined attention correlated across groups, infants with FXS exhibited the highest behavioral attentiveness, and the 9-month FXS group exhibited deeper decelerations in IBI during SA compared to both ASIB and LR samples. However, conflicting patterns emerged related to gap-overlap task performance, potentially suggesting different mechanisms of abnormal attention at gross (overall looking) versus specific (visual orienting and disengagement) levels. Consistent with typical associations between longer saccade latencies and greater SA (Casey & Richards, 1988; Richards, 1997), we observed positive associations between longer overlap latencies and increased proportion of time in SA among our LR group. In contrast, longer overlap latencies in FXS were associated with less variability and change in IBI during

SA, despite deeper IBI decelerations in the FXS group overall. In other words, although overt looking correlated with proportion of time in SA within FXS, longer saccade latencies were associated with reduced quality of SA within FXS. These paradoxical patterns are consistent with previous reports of shallower SA-related IBI decelerations and less IBI variability during an attention toy play task in an independent sample of infants with FXS (Roberts et al., 2011) as well as our previous findings that decreasing IBI decelerations and variability during SA predicted clinical ASD risk among ASIBS (Study 2). These converging findings raise questions as to the biological processes maintaining abnormal attention in FXS.

A possible explanation for these paradoxical findings is that abnormal attention orienting and attention engagement are both present in FXS yet are subserved by different biological processes: abnormal overall attentional engagement may relate to general patterns of arousal in FXS, whereas specific abnormalities in attention orienting may emerge secondary to neurobiological processes such as dorsal stream dysfunction. From this framework, disengagement difficulties in FXS may index abnormal visual perception of competing, dynamic stimuli rather than general "over-interest" in a primary stimulus. Our data can be interpreted in a manner consistent with this hypothesis. The FXS sample exhibited greater looking time overall, as well as deeper IBI deceleration during SA and greater proportion of time in SA. These patterns suggest SA is a valid biomarker of overall attentional engagement in FXS, which was elevated in our sample. This biobehavioral association was reversed in relation to overlap saccade latencies: participants with FXS and more typical (shorter) overlap latencies exhibited deeper SA than participants with FXS and more atypical (longer) overlap latencies. It is possible that

the more extreme overlap latencies observed in FXS indexed abnormal visual processing of the dynamic stimuli that "interrupted" typical physiological engagement, reflected by less SA (shallower decelerations) despite abnormal latencies. Participants with FXS and less extreme overlap latencies, on the other hand, may exhibit less dorsal dysfunction than other participants with FXS and therefore maintain increased overall physiological engagement characteristic of the FXS group, resulting in deeper IBI decelerations relative to controls. Of course, this cursory theory is just one possible explanation of the present findings. Further testing of dorsal function in FXS as related to both neuroanatomical and physiological functioning is needed to test these relationships empirically.

Interestingly, we similarly observed dysynchrony among ASIBs, who did not exhibit any associations between qualities of SA and either overt looking or saccade latencies in the present cross-sectional study or our prior longitudinal analyses of gap-overlap data (Study 2), despite the presence of these associations in LR controls. Instead, elevated attention was associated with higher RSA across analyses. Again, at least two explanations could account for these patterns. First, it is possible that elevated behavioral attention among ASIBs is driven by poorer suppression of vagal activity during attention. Infant siblings with lower developmental abilities may be particularly vulnerable to RSA-related over-attention, as we previously observed higher RSA in ASIBs with lower developmental ability (Study 2). This RSA-attention association was not significant in our previous passive viewing task (Study 1; p=.11), suggesting more robust associations may have been driven by cognitive engagement in a more complex task. Interestingly, RSA was not associated with behavioral attention in FXS, which may suggest differences

in biological vulnerabilities across groups, with abnormal parasympathetic suppression in ASIBs compared to intersecting physiological and visual factors in FXS.

Alternately, it is also possible that abnormal visuo-perceptual processes interrupted physiological engagement among ASIBs – similar to FXS – particularly given recently reported associations between dorsal connectivity and orienting among ASIBs (Elison et al., 2013) and the reduced associations we observed between SA and overlap latencies among ASIBs versus LR controls (Study 2). During passive-viewing (Study 1), higher clinical ASD features in ASIBs were also associated with increasingly reduced IBI change and SD during SA in the context of a passive viewing task, suggesting reduced qualities of SA that, similar to FXS in the present study, did not distinguish ASIBs but did predict within-group variability. Given these effects emerged during passive viewing (Study 1) but not gap-overlap (Study 2) tasks, additional work is needed to clarify these potential dissociations using systematic comparisons of autonomic functioning across task types (e.g. high versus low cognitive load) and stimuli-specific demands (e.g. requiring dorsal versus ventral functions) in further cross-syndrome studies. Specifically designing studies to differentiate between autonomic and visual perceptual influences will be important to informing the mechanisms of abnormal attention, as well.

A long-term goal of this work will be to inform translational science surrounding abnormal orienting as a risk factor for ASD. Although we observed behavioral orienting as a common predictor of clinical ASD risk across both ASIB and FXS groups, our physiological data also raise questions as to whether these behaviors undergo distinct developmental trajectories and are subserved by different processes. These differences may inform the etiology and treatment of ASD. Etiologically, if orienting is related to

different physiological processes, why do orienting processes still predict symptoms across high risk groups? Does attention compound later downstream developmental processes, as has been previously proposed in the ASD literature (Keehn et al., 2013)? Alternately, are we capturing associations between orienting and specific features of ASD that are common in ASIB and FXS samples? Answering these questions will both inform etiology and pose direct implications for treatment. Although orienting has already been integrated into early interventions for ASD (Rogers et al., 2014), our data suggest that supplementing these efforts with mechanism-specific protocols may increase treatment effectiveness. For example, children whose abnormal orienting is sustained by abnormal arousal may respond to treatments targeting self-regulation, whereas children whose deficits emerge secondary to abnormal dorsal functioning may instead respond to visual training. In other words, although abnormal orienting is associated with clinical ASD risk across ASIBs and FXS, reducing the downstream effects of this common process may require mechanism-specific interventions targeting the processes, rather than symptoms, of impairment.

Limitations

It is important to temper these cross-group differences within the context of our study sample, as the more severe symptoms we observed in FXS may relate to a number of factors beyond the FXS phenotype. One interpretation of our findings is that the magnitude of effects in the FXS group, but not ASIB group, was great enough to be detected using coarse cross-sectional methods. Indeed, we hypothesized that abnormalities in FXS would be more severe given the higher presence of ASD symptoms in this population, greater intellectual impairment, and increased physiological and visual

processing vulnerabilities. However, it is also possible that group differences reflect diverging emergent ASD symptomatology across groups, as between 25% and 60% of children with FXS generally meet diagnostic criteria for ASD (Farzin et al., 2006; Kaufmann et al., 2004; Rogers et al., 2001) compared to approximately 20% of ASIBs (Ozonoff et al., 2011). Continuing to follow these samples over time will facilitate differentiating between endophenotypes of ASD – features that relate to genetic risk regardless of diagnostic outcome – versus specific predictors of ASD diagnoses. Given previous evidence that abnormal attention relates to abnormal developmental features in ASIBs aside from ASD (Elsabbagh et al., 2013), dissociating predictors of ASD versus other outcomes will be particularly important as we continue to follow these samples over time.

Our study is also limited by coarse cross-sectional methods, which are necessary due to the availability of FXS data but likely mask developmental trends that may differentiate groups. Indeed, the effects we identified among ASIBs in Study 2 were primarily longitudinal in nature and were therefore "missed" by static age comparisons in the present study. As such, it is likely that longitudinal characterization of our FXS data would reveal similarly complex age associations, particularly given previous evidence that developmental changes in attention and physiology relate to ASD features in infants with FXS (Roberts et al., 2011, 2012). Efforts to track attention in an expanded, longitudinal FXS cohort are underway and may further elucidate the developmental patterns that subserve abnormal attention in ASIB and FXS samples. Notably, this expanded dataset is projected to include additional male participants, permitting us to

also examine whether the high presence of females in the FXS group are tempering group differences.

A related limitation that is reflected both in the present study and in the broader literature of "high risk" infants is the potential for spontaneous findings that emerge due to examining different variables in the same longitudinal cohort over time. Of note, the utility and need for controlling for multiple comparisons has been debated in the broader quantitative literature due, in part, to the likelihood of corrections actually increasing the likelihood of missing "true" effects (Rothman, 1990). Although the philosophy of multiple comparisons is certainly relevant to the present study, there are a number of practical issues in early detection research that further complicate the discussion of whether – and how – to control for multiple comparisons. First is the issue of non-independence. It is common for a single infant with FXS to be enrolled in multiple research studies, and ASD data are often submitted to national data repositories for use by future researchers (e.g. National Dataset for Autism Research; Hall, Huerta, McAuliffe, & Farber, 2012), thus even studies published by separate labs are often not fully independent. Although family-wise error could hypothetically be controlled within certain measures or constructs, truly controlling for multiple comparisons would require tracing single participants' data across all ongoing studies and evaluating significant findings in the context of cross-lab findings. These comparisons would create such stringent criteria for significance that virtually no effects – even true effects – would exceed the adjusted threshold. A second challenges is that high-risk samples are often expensive and difficult to recruit, thus it is not feasible for scientists to obtain research-naïve samples for each study and research question. Given these challenges, we have

addressed issues of independence and multiple comparisons using a five-pronged approach: (1) use theory to guide study design and hypothesis testing rather than "fishing" for significant findings, (2) specify non-independence of ASIB and LR samples across papers, as well as cross-study differences in sampling and exclusions, (3) explicitly state that our FXS sample is independent from previously published studies by our group examining similar constructs (Roberts et al., 2011), (4) focus on effect sizes in addition to traditional *p* values, and (5) articulate the limitations of non-independent samples explicitly. Although these strategies are an initial step toward increased sensitivity toward issues of non-independence and multiple comparisons, we would advocate for a broader discussion of these issues across the early detection field.

Conclusion

Prospective infant studies have transformed early detection of ASD by emphasizing prospective surveillance of prodromal ASD features that are being translated to earlier detection and treatment. The present study is among the first to compare risk factors in ASIBs to other high risk groups, and our results provide initial evidence that despite similar behavior profiles (e.g. "sticky attention"), the biological mechanisms subserving these behaviors may vary across genetic samples. Consistent with our dynamic developmental framework, these biobehavioral group differences should not be interpreted as counterevidence for exploring ASD in FXS due to potential FXS-specific mechanisms. Instead, we propose these data contribute to a growing discussion of how genetic disorders may inform meaningful heterogeneity of ASD symptom expression, complementing growing emphasis on conceptualizing psychopathology across multiple layers of analysis (Cuthbert & Insel, 2013). Our data also suggest that similar behavioral

features, such as abnormal visual attention, may emerge through multiple developmental processes, consistent with the notion that broader ASD profiles may reflect a number of heterogeneous pathways of risk (Yuen et al., 2015). Together, these findings support continued prospective cross-group comparisons of ASD emergence that may inform mechanistically-sensitive early detection and treatment efforts.

Table 4.1 Behavioral Descriptive Statistics by Group and Categorical Age

	Chronological Age Comparison									Mental Age Comparison		
	FXS			ASIB			LR (CA)			LR (MA)		
9 Month	*n*	$\bar{X}$	SD	*n*	$\bar{X}$	SD	*n*	$\bar{X}$	SD	*n*	$\bar{X}$	SD
Age in months	13	9.36	0.63	19	9.45	0.55	18	9.43	0.37	12	6.38	0.63
MSEL Std. Score	12	86.08	22.96	17	96.71	13.59	18	103.06	13.10			
MSEL Mental Age	12	7.48	2.92	17	9.09	1.16	18	10.15	2.41	12	6.83	0.67
Base. Latency	11	344.77	67.69	19	367.07	86.56	18	336.23	77.60	12	349.28	79.30
Overlap Latency	11	563.59	155.14	17	467.12	124.80	18	468.46	122.66	11	561.13	149.82
Gap Latency	12	316.17	110.01	19	311.73	87.21	18	290.03	88.86	12	282.59	85.32
% Failed Trials	13	0.16	0.16	19	0.08	0.07	18	0.07	0.05	12	0.07	0.07
Prop. Inattentive	11	0.29	0.18	16	0.49	0.18	16	0.52	0.19	6	0.41	0.23
12 Month												
Age in months	14	12.62	0.67	18	12.49	0.58	21	12.27	0.48	18	9.43	0.37
MSEL Std. Score	14	83.07	21.05	17	98.65	11.65	21	101.81	11.60	18	103.06	13.10
MSEL Mental Age	14	10.59	2.70	17	12.16	1.49	21	13.06	2.40	18	10.15	2.41
Base. Latency	13	401.54	193.87	18	350.29	81.40	20	321.30	67.74	18	336.23	77.60
Overlap Latency	11	601.46	214.08	12	489.36	83.98	21	464.64	130.18	18	468.46	122.66
Gap Latency	14	333.04	156.66	18	275.50	53.68	20	278.47	72.06	18	290.03	88.86
% Failed Trials	14	0.14	0.13	18	0.10	0.08	21	0.08	0.06	18	0.07	0.05
Prop. Inattentive	10	0.42	0.24	17	0.46	0.19	17	0.51	0.20	16	0.52	0.19

Table 4.2: Heart Activity Descriptive Statistics by Group and Categorical Age

	Chronological Age Comparison								
	FXS			ASIB			LR (CA)		
9 Month	*n*	$\bar{X}$	SD	*n*	$\bar{X}$	SD	*n*	$\bar{X}$	SD
Heart Activity									
Overall IBI	11	300.98	105.94	13	281.62	103.48	16	241.45	44.79
Overall RSA	11	2.74	1.02	13	2.34	1.05	16	2.10	0.39
Overall IBI SD	11	17.67	7.50	13	19.04	11.46	16	14.77	4.32
Sustained Attention									
Proportion SA	11	0.27	0.15	13	0.19	0.11	15	0.20	0.12
SA IBI SD	11	24.69	7.38	13	25.59	7.04	15	29.09	12.61
SA Mean IBI Δ	11	33.16	11.49	13	24.16	8.15	15	25.23	9.46
12 Month									
Heart Activity									
Overall IBI	10	281.41	97.01	13	283.16	85.30	17	279.21	104.32
Overall RSA	10	2.53	0.99	13	2.53	0.91	17	2.57	1.26
Overall IBI SD	10	16.40	4.41	13	19.01	8.28	17	18.12	9.97
Sustained Attention									
Proportion SA	9	0.27	0.13	13	0.24	0.09	16	0.18	0.06
SA IBI SD	9	32.06	8.70	13	32.06	15.13	16	29.20	11.42
SA Mean IBI Δ	9	32.25	12.65	13	26.58	10.57	16	26.66	8.26

Table 4.3. Partial Spearman Correlations between Behavioral and Heart-Defined Attention

	Baseline Latency		Overlap Latency		Gap Latency		% Stuck Trials		% Inattentive	
	n	*partial ρ*	*N*	*partial ρ*	*n*	*partial ρ*	*n*	*partial ρ*	*n*	*partial ρ*
FXS										
Overall IBI	19	-.21	18	-.28	19	.22	19	-.20	20	-.15
Overall RSA	19	-.22	18	-.35	19	.27	19	-.19	20	.11
Overall IBI SD	19	-.26	18	-.45	19	.21	19	-.34	20	.22
Proportion SA	18	.003	17	.01	18	-.05	18	.33	20	-.78*
SA IBI SD	18	.13	17	-.65 *	18	.21	18	-.27	20	.23
SA Mean IBI Δ	18	-.27	17	-.69 *	18	.22	18	-.50 *	20	.31
ASIB										
Overall IBI	26	-.02	19	.05	26	-.02	26	-.02	26	-.29
Overall RSA	26	.08	19	.06	26	-.02	26	.07	26	-.47*
Overall IBI SD	26	.17	19	-.12	26	.02	26	.04	26	.01
Proportion SA	26	-.16	19	.36	26	-.29	26	.08	26	-.69*
SA IBI SD	26	.01	19	-.14	26	.04	26	.02	26	.17
SA Mean IBI Δ	26	-.02	19	-.27	26	.02	26	-.16	26	.05
LR										
Overall IBI	32	.22	32	.03	31	.22	32	.30	31	-.12
Overall RSA	32	.09	32	-.09	31	.06	32	.15	31	-.06
Overall IBI SD	32	.02	32	-.04	31	.16	32	.17	31	-.03
Proportion SA	30	.16	30	.48 *	29	-.07	30	.15	31	-.82*
SA IBI SD	30	.12	30	-.03	29	.28	30	.30	31	-.18
SA Mean IBI Δ	30	.16	30	.03	29	.01	30	.23	31	-.19

* Significant at $p \leq .05$

Table 4.4: Wilcoxon Cross-Group Chronological Age Comparisons at 9 and 12 Months

	FXS v LR						FXS v ASIB						ASIB v LR					
	9 months			12 months			9 months			12 months			9 months			12 months		
Variable	*Z*	*p*	*d*	*Z*	*p*	*d*	*Z*	*p*	*d*	*Z*	*p*	*d*	*Z*	*p*	*d*	*Z*	*p*	*d*
Behavior																		
Baseline Latency	0.25	0.4	0.12	1.53	0.06	0.40	-0.65	0.26	-0.30	0.74	0.23	0.25	-0.91	0.18	0.40	0.89	0.19	0.43
Overlap Latency	**1.89**	**0.03**	0.58	**1.75**	**0.04**	0.61	**2.07**	**0.02**	0.71	1.32	0.09	0.50	0.02	0.49	-0.01	0.88	0.19	0.19
Gap Latency	1.14	0.13	0.23	0.86	0.2	0.33	0.06	0.48	0.05	1.27	0.1	0.35	-0.88	0.19	0.24	0.23	0.41	-0.04
% Failed Trials	**1.8**	**0.04**	0.54	1.08	0.14	0.44	1.47	0.07	0.73	0.84	0.2	0.30	-0.11	0.46	0.20	0.71	0.24	0.33
Prop. Inattentive	**-2.89**	**0.002**	-1.21	-0.93	0.18	-0.35	**-2.59**	**0.005**	-1.07	-0.33	0.37	-0.16	0.25	0.4	-0.16	0.76	0.22	-0.25
Heart Activity																		
Overall IBI	1.41	0.08	0.53	0.33	0.37	0.02	0.93	0.18	0.26	-0.22	0.41	-0.02	0.15	0.44	0.90	0.59	0.28	0.04
Overall RSA	1.55	0.06	0.60	0.43	0.33	-0.04	1.22	0.11	0.56	-0.09	0.46	0.00	0.1	0.46	0.62	0.5	0.31	-0.03
Overall IBI SD	0.91	0.18	0.37	0.43	0.33	-0.37	0.12	0.45	-0.24	-0.53	0.3	-0.31	0.99	0.16	0.99	0.96	0.17	0.09
Sustained Attention																		
Proportion SA	1.25	0.11	0.44	**2.12**	**0.02**	0.65	1.39	0.08	0.60	0.67	0.25	0.33	-0.23	0.41	-0.08	1.34	0.09	1.00
SA IBI SD	-0.73	0.23	-0.57	0.93	0.18	0.31	-0.23	0.41	-0.08	0.2	0.42	0.00	-0.37	0.36	-0.28	0.33	0.37	0.25
SA Mean IBI Δ	**1.66**	**0.05**	0.65	0.99	0.16	0.41	**2.14**	**0.02**	0.87	0.8	0.21	0.57	0	0.5	-0.11	0.2	0.42	-0.01

Table 4.5: Wilcoxon Cross-Group Mental Age Comparisons at 9 and 12 Months

	9 Month FXS vs. LR						12 Month FXS vs. LR					
	Chronological Age (9 mo LR)			Mental Age* (6 mo LR)			Chronological Age (12 mo LR)			Mental Age (9 mo LR)		
Variable	*Z*	*p*	*d*	*Z*	*p*	*d*	*Z*	*p*	*d*	*Z*	*p*	*d*
Mental Age	--	--	--	1.13	0.13	0.21	--	--	--	0.55	0.29	0.16
Baseline Latency	0.25	0.4	0.12	-0.09	0.46	-0.06	1.53	0.06	0.82	0.98	0.16	0.32
Overlap Latency	**1.89**	**0.03**	0.58	0.13	0.45	0.02	**1.75**	**0.04**	0.40	**1.69**	**0.05**	0.59
Gap Latency	1.14	0.13	0.23	1.01	0.16	0.29	0.86	0.2	0.61	0.7	0.24	0.26
% Failed Trials	**1.8**	**0.04**	0.54	1.55	0.06	0.54	1.08	0.14	0.33	1.44	0.07	0.52
Prop. Inattentive	**-2.89**	**0.002**	-1.21	-1.16	0.12	-0.63	-0.93	0.18	0.44	-0.92	0.18	-0.39

Table 4.6: Partial Spearman Correlations among Chronological and Mental Age, Clinical Autism Risk and Attention in FXS

					Correlations with Clinical Autism Risk			
	CA		**MA**		**Controlling CA**		**Controlling CA+MA**	
Behavior	*ρ*	*p*	*ρ*	*p*	*partial ρ*	*p*	*partial ρ*	*p*
Baseline Latency	.19	.36	-.27	.20	**.43**	**.05**	.26	.26
Overlap Latency	.09	.69	-.14	.54	-.18	.48	-.22	.39
Gap Latency	.08	.71	-.27	.19	.14	.53	.11	.66
% Failed Trials	-.003	.99	-.22	.28	**.43**	**.04**	.27	.23
Prop. Inattentive	.31	.17	.10	.68	.16	.52	.01	.97
Overall Heart Activity								
Overall IBI	.008	.97	-.42	.07	.30	.22	.13	.61
Overall RSA	-.16	.48	-.33	.16	.29	.23	.10	.68
Overall IBI SD	-.05	.81	-.13	.58	.33	.17	.18	.46
Sustained Attention								
Proportion SA	.07	.76	-.07	.79	.03	.90	.18	.50
SA IBI SD	.37	.11	.41	.08	.14	.58	.20	.44
SA Mean IBI Δ	-.08	.75	.22	.36	.20	.43	.33	.19

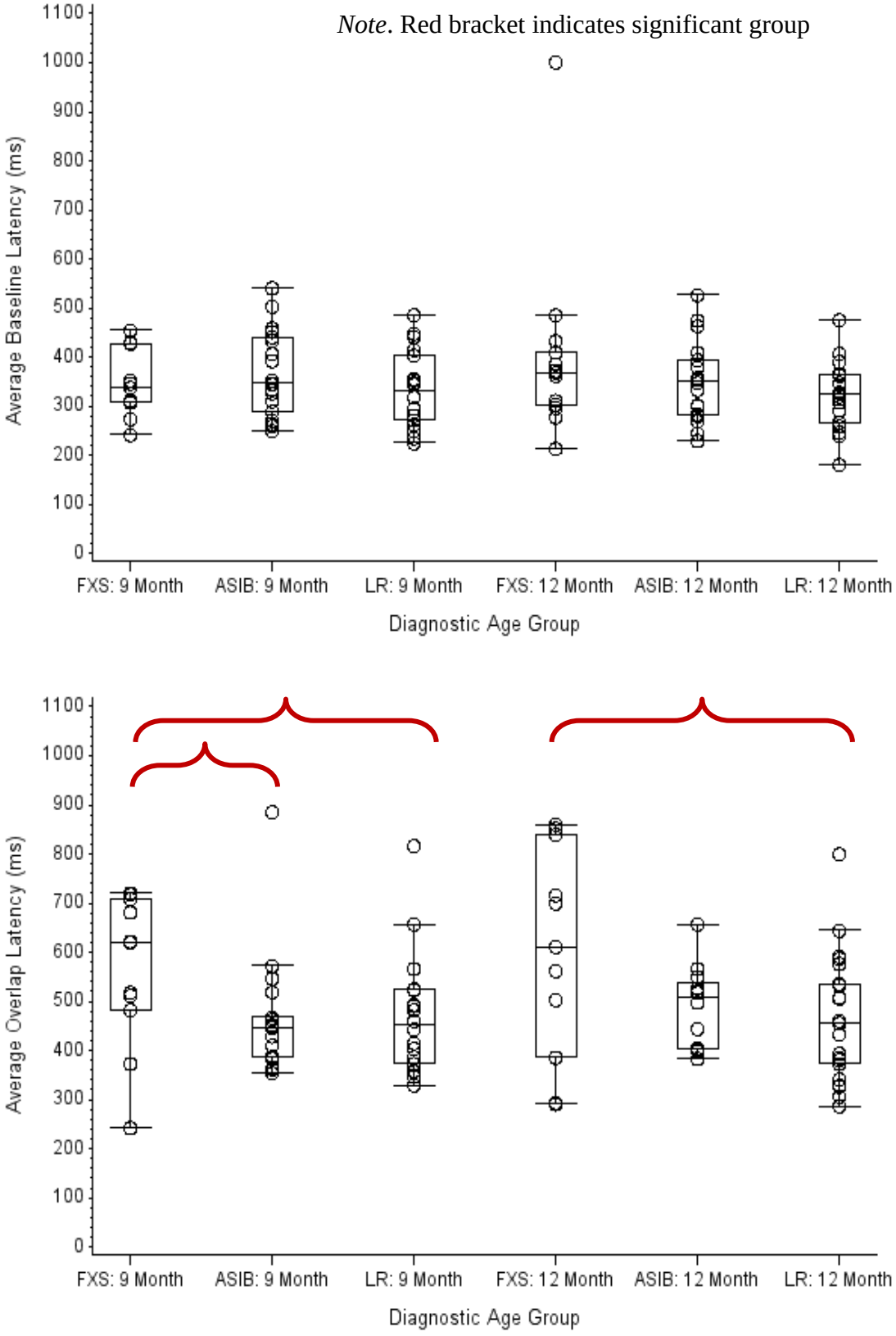

Figure 4.1: Group Differences in Saccade Latencies

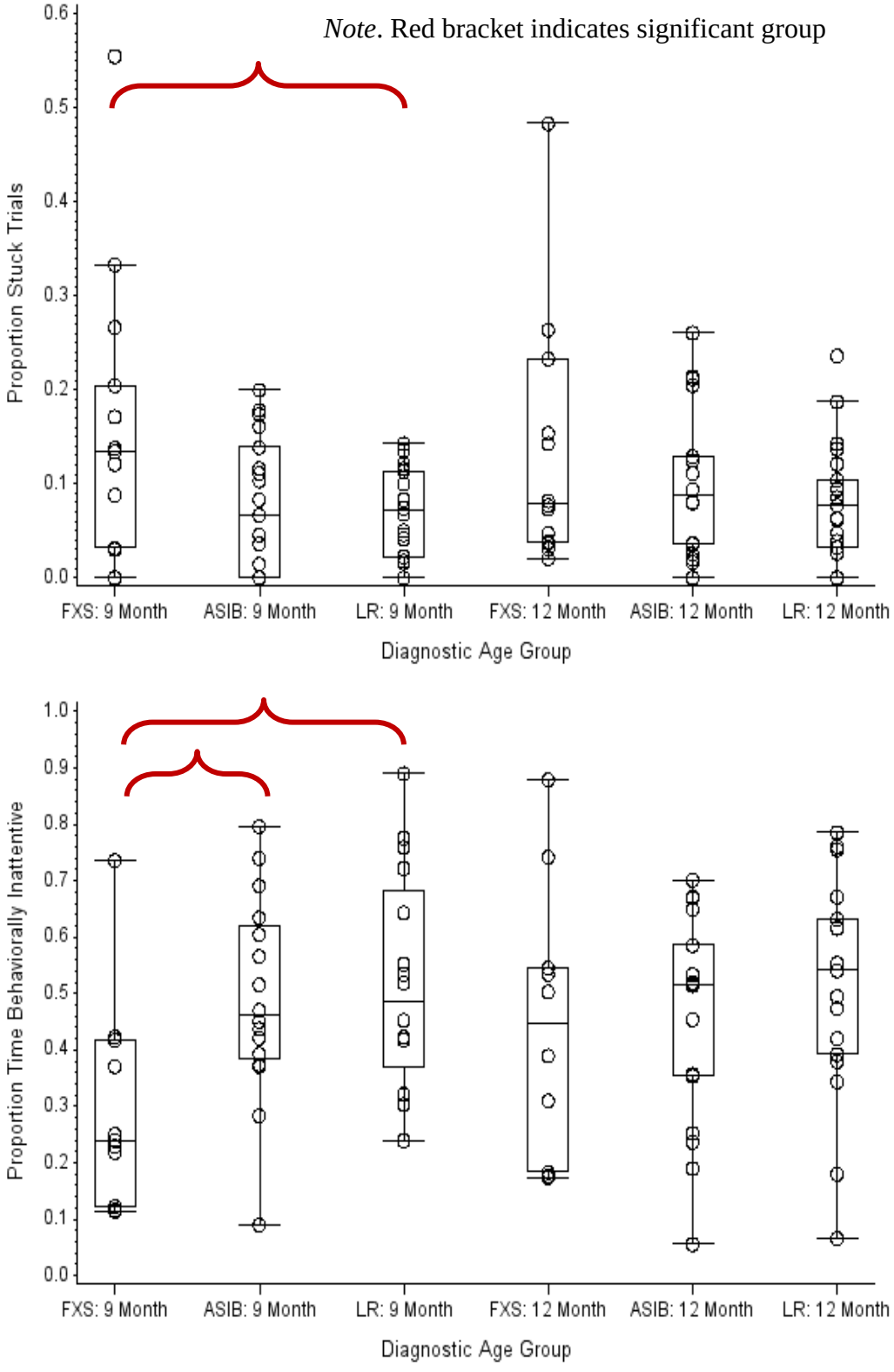

Figure 4.2: Behavioral Attention: Proportion Stuck Trials and Overall Looking

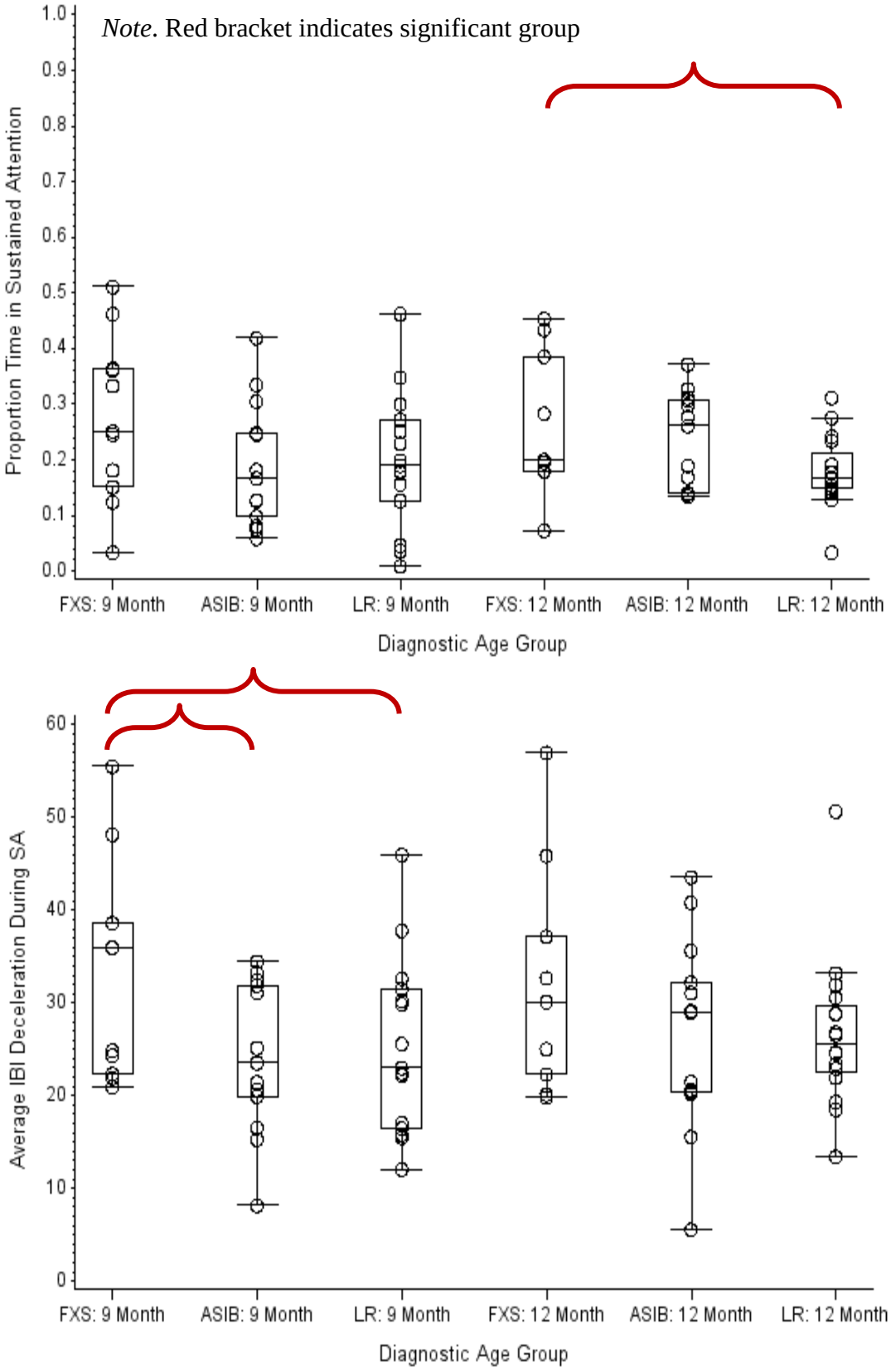

Figure 4.3: Heart-Defined Sustained Attention

CHAPTER 5

CONCLUDING REMARKS

In 2008, Geraldine Dawson made the provocative claim that "[f]or the first time, prevention of autism is plausible. Prevention will entail detecting infants at risk before the full syndrome is present and implementing treatments designed to alter the course of early behavioral and brain development" (Dawson, 2008, p. 775). Indeed, converging evidence from both surveillance and treatment studies suggest that prodromal features of autism spectrum disorder (ASD) are emerging as early as two months of age (Jones & Klin, 2013), and ASD outcomes can be altered – in some cases by intervening in symptomatic infants within the first year of life (Rogers et al., 2014). These advances suggest that characterizing and treating ASD in infancy – prior to full symptom expression – is likely key to reducing ASD-associated impairments. However, the early detection field remains limited in a number of domains. It is unclear which children are most likely to benefit from prevention efforts, as even within "high risk" groups, many children exhibit non-ASD outcomes (Ozonoff et al., 2011). The mechanisms of ASD emergence are also still unknown. Although abnormal visual attention orienting has predicted ASD across a number of studies (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005) and has been integrated into prevention efforts(Sally J Rogers et al., 2014), few studies have examined the biological mechanisms of abnormal attention in high-risk infants (Elison et al., 2013; Roberts et al., 2011). As a result, both prevention

and treatments often target ASD symptoms, rather than mechanisms, arguably reducing effectiveness in altering maladaptive developmental trajectories.

The present series of studies addressed these areas of need by unpacking the developmental and biological features of a commonly-reported predictor of ASD in infants: visual orienting. This work aimed to inform the onset, mechanisms, and generalizability of attention as an endophenotype of ASD. Across studies, we demonstrated abnormal patterns of both behavioral and heart-defined attention in infant siblings of children with ASD (ASIBs) and infants with fragile X syndrome (FXS), which emerged within the first year of life. We also provided novel evidence that although behavioral patterns of abnormal attention in ASIBs grossly generalize to high-risk infants with fragile X syndrome (FXS), the nuanced associations between attention and arousal systems differ across groups, potentially indicating diverging pathways to common behavioral features. Here, we synthesize these findings by (1) integrating results across studies and (2) discussing cross-study themes and implications.

Integrating Results

Across studies, we examined behavioral and heart-defined attention in high risk ASIBs (Studies 1-3) and infants with FXS (Study 3) during both passive viewing (Study 1) and visual orienting (Studies 2-3) tasks. The following sections integrate key behavioral (overall attention, saccade latencies) and physiological (global heart activity, heart-defined attention) findings, highlighting trends across studies. Additional discussion of these findings is provided within Chapters 2-4, thus the following sections provide a brief synopsis rather than exhaustive review. Specifically, findings are summarized in relation to (1) primary dependent variables (behavioral attention, saccade latencies, heart

activity), (2) association between behavioral and physiological measures, and (3) predictors of clinical ASD risk.

Behavioral attention. One of the most consistent findings across studies was abnormal behavioral attention (e.g. looking at the monitor) across high risk groups. In ASIBs, this effect was present during passive viewing (Study 1), during which ASIBs exhibited increased behavioral attention with age compared to decreased attention among low risk (LR) controls. Similarly, infants with FXS exhibited greater attention than LR controls and ASIBs at 9 months of age, with differences similar to younger LR children matched on MA. Two informative inconsistencies emerged in cross-study data. First, ASIBs' abnormal looking patterns were elicited by passive viewing (Study1) but not a gaze-contingent task (Study 2), suggesting typical gaze patterns in the presence of examiner and task-related prompts, but not in the absence of these supports. Second, the abnormalities in FXS appear to relate to mental age, whereas ASIB differences persisted when MA was covaried in models. Thus, although both groups exhibited similar behavioral symptoms, the developmental nature of abnormal overall attention varies across groups, potentially suggesting mechanisms related to developmental delay in FXS compared to potential ASD endophenotype-related differences among ASIBs.

Saccade latencies. Patterns of saccade latencies were similarly abnormal across both high-risk groups, although nuanced differences again emerged across samples. In Study 2, ASIBs exhibited distinct age-related patterns of initial abnormalities in orienting across trial types, which normalized (e.g. approached LR trajectories) at different rates over time. The increased differences between baseline and overlap latencies over time produced emerging "disengagement effects," in which ASIBs displayed worsened

abilities to disengage from competing stimuli, compared to their ability to shift attention in the absence of competing stimuli. Interestingly, infants with FXS also exhibited orienting deficits compared to both ASIB (9 month only) and LR groups (9 and 12 months), which persisted in relation to developmental controls at 12 months. However, these effects were specific to overlap trials only, in comparison to general orienting deficits across trial types among ASIBs. Thus, in contrast to ASIBs who exhibited poor orienting across trial types that was present in the youngest participants (6 months), deficits in FXS were initially similar to MA controls but worsened in comparison to both MA and CA controls with time. Thus, groups differed in both (1) the specificity of orienting deficits (disengagement in FXS, global orienting with particular disengagement deficits in ASIBs) and (2) the developmental sequaele of deficits over time relative to developmental abilities (present at 6 months in ASIBSs relative controlling MA, emerging between 9 and 12 months in FXS relative to MA controls). Again, these findings suggest behaviorally similar profiles with distinct developmental trajectories.

Heart activity. Patterns of heart activity across development varied across high risk groups. Young ASIBs exhibited hypoarousal during the gap-overlap (Study 2) but not passive viewing tasks (Study 1), whereas the FXS group exhibited marginally longer IBI and lower RSA at 9 months only. As discussed in Study 3, it is possible that these differences relate to patterns of shifting arousal across infancy, a theory that has been supported by preliminary data (Roberts et al., 2012) but requires increased longitudinal surveillance in larger samples to test empirically. However, our data lend preliminary support to theories of shifting arousal, as hypoarousal is present in the youngest high-risk samples only.

Both high risk groups also exhibited abnormal patterns of heart-defined attention. In ASIBs, these patterns were apparent during passive viewing, during which proportion of time in SA remained relatively stable over time, compared to age-related decreases in attention among LR controls. Notably, this effect was not present during the gap-overlap task in ASIBs, consistent with behavioral attention patterns. However, proportion of time in SA (12 months) and depth of deceleration (9 month) were abnormal in FXS during the gap-overlap task, paralleling previous reports of reduced IBI decelerations during attention tasks in FXS compared to LR controls (Roberts et al., 2011). Thus, both groups exhibited differences in proportion of time in SA, paralleling behavioral effects. Depth of IBI decelerations were also abnormal in FXS, suggesting qualities – in addition to quantity – of SA is distinct in this sample.

Biobehavioral Correlates

Biobehavioral correlations indicated distinct patterns of biological processes potentially underpinning behavioral features. Among ASIBs, greater behavioral attention corresponded with higher proportion of time in heart-defined SA during the gap-overlap task, with less robust, non-significant correlations emerging during passive viewing. Similarly, higher overall looking corresponded with higher RSA in the ASIB but not FXS group during the gap-overlap task. In contrast, longer overlap latencies and increased stuck trials in FXS were associated with less IBI SD and IBI change during SA. Thus, although overall looking during the gap-overlap task related to heart-defined SA across ASIB and FXS groups, gross behavioral differences (overall looking) relate to RSA among ASIBs, and saccade latencies relate to qualities of SA in FXS. These patterns may suggest distinct underlying processes contributing to attentional deficits across groups.

In Study 3, we introduced a theoretical conceptualization of the association between longer overlap latencies and abnormal SA (shorter IBI decelerations, less IBI variability) in FXS to potentially account for this observed paradoxical association. Specifically, we proposed that longer overlap latencies observed in FXS may index abnormal visual processing that "interrupts" physiological patterns of engagement, reflected by less SA (shallower decelerations) despite abnormal latencies. We proposed that in contrast, less extreme overlap latencies may reflect relatively intact dorsal function, thus patterns of physiological engagement would be more typical of FXS as a group (e.g. deeper IBI decelerations). Although this pattern may similarly explain the lack of biobehavioral associations among ASIBs, orienting deficits in ASIBs appear to be more closely related to parasympatethic functioning. Of course, these theories are speculative in nature and should be empirically tested by contrasting dorsally-mediated and non-dorsally mediated task performance with concurrent physiological monitoring.

Predictors of Clinical ASD Risk

Behavioral correlates of clinical ASD risk were similar across groups, although distinct physiological predictors emerged. Across both ASIBs and FXS, higher proportion of stuck trials and longer baseline latencies were shared predictors of increased clinical ASD risk. Within FXS, longer baseline latencies were also associated with higher clinical risk scores. The association between global heart activity and clinical autism risk factors varied by group and task. Among ASIBs, higher clinical risk was associated with abnormal patterns of global and heart-defined attention during passive viewing, with decreasing age-related patterns of both global (IBI, IBI SD) and heart-defined (IBI SD during SA, mean IBI change during SA) heart activity in ASIBs with

greater clinical risk. These associations only emerged during passive viewing. In contrast, physiological features were not associated with clinical autism risk in FXS. We proposed that these patterns speak to the utility of attention as a feature of the ASD endophenotype, with behavioral attention presenting a more global pattern of risk, versus physiologically-indexed attention more closely relating to risk in the ASIB group. However, it is possible that heart activity during passive viewing would similarly reveal unique phenotypic information in FXS. We did not examine passive viewing in FXS as part of these studies, although data collection is underway to examine these associations in the future.

Key Implications

The three presented studies generated a number of common themes that speak to the importance of this work. Two of the most compelling findings are improved characterization of aberrant attention in ASIBs, as well as novel information regarding the generalization of ASD endophenotype in ASIBs to infants with FXS. These themes are discussed at length throughout the previous studies thus are summarized briefly within the present discussion.

Characterizing Aberrant Attention in ASIBs

Across studies, we identified abnormal behavioral and heart-defined attention in ASIBs. Our identification of atypical orienting in very young (5-8 month) ASIBs is consistent with a subset of previous studies, although the developmental trends we reported differed from previous groups, likely reflecting sampling and methodological differences. Despite these differences across studies, our data generally converged with previous reports that atypical orienting may be a marker of ASD risk among ASIBs. In addition to potentially serving as an endophenotype of ASD, abnormal orienting also

predicted clinical ASD risk in our sample, consistent with previous findings of "sticky attention" in ASIBs who later meet ASD criteria (Elison et al., 2013; Elsabbagh et al., 2013; Zwaigenbaum et al., 2005). Interestingly, effects were stable across ages, suggesting differences may be detectable as early as 6 months of age. Future studies are needed to examine the long-term outcomes associated with abnormal orienting, as well as whether orienting differentiates ASD from other developmental concerns (Elsabbagh et al., 2013). However, in the interim, our findings support that (1) abnormal orienting is a salient feature of the ASD endophenotype, and (2) detectable effects may emerge earlier than previously reported.

Cross-Group Differences in Orienting and ASD risk

Our findings also provide novel evidence of distinct cross-group differences, as well as within-group predictors of clinical ASD risk, across ASIB and FXS groups. Chapter 4 discusses the implication of these cross-group findings in detail. In brief, developmental differences in symptom trajectories differed across groups, suggesting different etiological pathways to aberrant attention: ASIBs exhibited initial impairments that changed at differential rates over time, whereas abnormalities in FXS were specific to attention disengagement and increased in intensity with age, relevant to developmental controls. Given these differences, we proposed that abnormal attention orienting and attention engagement are both present in FXS and ASIB groups yet may be subserved by different biological processes. In FXS, disengagement difficulties in FXS may index abnormal visual perception of competing, dynamic stimuli rather than general "over-interest" in a primary stimulus. In contrast, elevated attention was associated with higher RSA in ASIBs, possibly indicating that abnormal elevations in attentional engagement

are driven by poorer suppression of vagal activity during attention. These differences highlight the heterogeneity of developmental processes related to ASD risk and the potential presence of similar behavioral profiles with distinct biological signatures.

Future Directions

Chapters 2-4 propose a number of compelling future directions for this work, particularly related to cross-syndrome investigations of ASD, employing developmentally-sensitive research designs, and integrating biomarkers to inform symptom profiles. In brief, our findings support further investigation of ASD emergence in non-ASIB samples, contributing to a growing discussion of how genetic disorders may inform meaningful heterogeneity of ASD symptom expression (Abrahams & Geschwind, 2008). Specifically, our data suggest that "risk factors" for ASD may be subserved by different biological mechanisms and developmental processes across infants with varied genetic risk factors, despite shared behavioral features that are similarly predictive of ASD risk. These findings are consistent with the increasingly popular notion that broader ASD profiles may reflect a number of heterogeneous pathways of risk (Yuen et al., 2015), supporting continued prospective cross-group comparisons of ASD emergence that may inform mechanistically-sensitive early detection and treatment efforts.

Our data also suggest that developmental surveillance – rather than age-related contrasts – are essential for teasing apart nuanced risk factors for ASD. Indeed, repeated assessments across short time periods are increasingly employed in ASIB studies (Jones & Klin, 2013; Ozonoff et al., 2010, 2014; Sacrey et al., 2013) but have not previously been applied to examining visual orienting and physiology. Indeed, Studies 1 and 2 unveiled patterns of abnormal attention previously unreported in the literature, which

likely reflect, in part, our use of longitudinal modeling rather than categorical age comparisons. In particular support of developmental surveillance, a number of our longitudinal findings were not duplicated in cross-sectional analyses of the same sample (Study 3), highlighting the importance of quantifying individual change rather than simply group-level data.

Finally, our integration of biomarkers clarified group differences and supported theoretical developments that would not have been possible with behavioral data alone. Indeed, although FXS and ASIB groups exhibited grossly similar behavioral orienting deficits, cross-group differences were most apparent when physiological data were integrated. Although biomarkers are arguably more difficult to translate into common practice, their integration into clinical research may inform heterogeneity of clinical features that are undetectable using standard observational methods. Establishing subtypes of mechanism-driven symptoms may, in turn, promote more sensitive and specific clinical tools that reflect the heterogeneity of ASD. For example, our results suggest that although visual attention in FXS is subserved by different biological processes in ASIBs and FXS, behavioral factors were similarly related to clinical ASD risk in both samples, warranting future study of whether behaviorally modifying orienting responses may have beneficial downstream effects across samples. However, it is likely that given distinct physiological underpinnings of shared behavioral features across groups, response to attention-related treatments may be more efficient if tailored to mechanisms of change. For example, efforts to improve dorsal function may improve orienting in FXS, whereas self-regulatory interventions may be more effective in ASIBs, given hyperarousal in FXS is a chronic state that does not predict clinical ASD risk and

physiological variables related to clinical ASD risk in ASIBs only. Although these theories must be tested empirically, our data support further study of the use of biomarkers in informing mechanism-informed early detection treatment protocols.

Conclusion

As rates of ASD diagnoses continue to increase, research methods for predicting and treating ASD must similarly be refined with time. Data from the present three studies suggest that cross-syndrome comparisons, developmentally-sensitive surveillance, and biomarker integration may offer novel glimpses into the emergence and etiology of ASD in infancy, potentially informing the complex heterogeneity of ASD that continues to challenge current science in this field. In addition to promoting earlier identification and treatment of ASD, this work supports an individualized, multifaceted approach to unpacking developmental differences, framed in the assumption that developmental disorders are emerging phenotypes rather than static outcomes of linear development (Karmiloff-Smith, 2009). Thus, our work provides novel contribution to both the emergent features of ASD and improved methods for studying this complex developmental process.

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