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Temperament in Infants At-Risk for Autism Spectrum Disorder

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**Abstract**

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Current research efforts are focused on identifying early signs of autism spectrum disorders (ASD) given improved outcomes for children with ASD through early behavioral intervention. Retrospective studies cite early temperamental abnormalities (e.g., passivity to marked irritability) as well as decreased expression of positive emotion and social engagement in the development of ASD. Given the limitations of retrospective report, prospective studies are needed to determine if these early risk signs are related to later development of ASD. Infant siblings of children with ASD are at increased risk for developing ASD. As such, this population provides a window into the early manifestation of ASD and early indicators of the disorder. The main objective of the current study was to determine if parent, observational, and EEG measures of temperament at 6 and 12 months of age were predictive of the development of early ASD symptoms. Infant siblings of children with ASD (high-risk infants,  $n = 43$ ) and infant siblings of children who do not have an older sibling with ASD or language impairment (low-risk infants,  $n = 45$ ) underwent cognitive and autism symptom assessments at 6, 12, 18, and 24 months of age.

At both 6 and 12 months of age, EEG was collected while infants watched a video of social stimuli (women telling nursery rhymes) and non-social stimuli (dynamic toys). Parents of these infants completed an early temperament questionnaire (the Infant Behavior Questionnaire – Revised) and independent behavioral coders conducted observational temperament coding at 6 and 12 months of age. Parent report of “cuddliness” at 6 and 12 months and behavioral observation of “social engagement” at 12 months significantly differentiated infants who later developed ASD. Other temperamental factors hypothesized to be related to ASD such as activity level, soothability, and distress to limitations were not found to be significant in this sample. EEG results reveal that infants who later developed ASD exhibited increased brain activation while watching dynamic toys compared to watching social stimuli at 12 months of age. These results support the theory of ASD as a failure to attend preferentially to social stimuli and a possible preference for non-social stimuli.

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## **DEDICATION**

To my parents...

who inspired me to pursue my dreams and continually provide me strength to achieve them.

## CHAPTER 1

### BACKGROUND AND RATIONALE

#### 1. Introduction

##### A. Overview of the study

Younger siblings of children with autism spectrum disorders (ASD) experience a 2 to 50-fold increase in risk for developing ASD. In the first year of life, these high-risk infants who later go on to develop ASD, may exhibit subtle disruptions in temperament that occur prior to the onset of clinical symptoms. Temperament has been defined as constitutional differences in reactivity and self-regulation that is influenced over time by heredity, maturation, and experience (Rothbart & Bates, 1998; Rothbart & Bates, 2006; Rothbart & Derryberry, 1981). It is biological in origin (Buss & Plomin, 1984) and individual differences in temperament are considered stable and enduring tendencies to react cognitively, behaviorally, and emotionally throughout the lifetime (Rothbart & Bates, 1998; Rothbart & Bates, 2006). Temperament is found to be predictive of social development, affective style, and developmental psychopathology. Evidence suggests that specific temperament characteristics and profiles influence psychopathology and perhaps confer risk factors. To date, little is known about how temperament influences the development of ASD symptoms. Broadly, the study of temperament in ASD can provide important information about early diagnosis, individual variability, intervention response, and aid in better understanding of the broader autism phenotype.

The current paper will first review what is known about the early development of ASD including the variability, stability, and early risk markers observed in the syndrome. Then, the literature regarding the development of temperament and the various methodologies of measuring temperament will be discussed including parent report, observational, and

electrophysiological (EEG) methods. Given that the earliest behavioral markers of ASD are not present until 12 months, the use of psychophysiological measures such as EEG may aid in the identification of earlier biomarkers of ASD. Next, the relationship between temperament and social development and psychopathology will be reviewed. Finally, the recent studies examining temperament in ASD and in infant siblings of children with ASD will be considered with respect to specific factors that distinguish individuals with ASD from other disorders, contribute to variability in the syndrome, and predict the development of ASD.

The main goal of the project was to examine temperament in infant siblings of children with ASD (high-risk infants) and infants with no known genetic risk for ASD (low-risk infants) at 6 and 12 months of age using parent, observational, and EEG measures of temperament to determine if temperament measures are related to early ASD risk symptoms at 18 and 24 months of age. This project is the first of its kind to examine multiple modal assessments of temperament, including parent report, observational measures, and EEG as early risk markers for autism.

## **B. Brief description of autism spectrum disorders**

Autism Spectrum Disorders (ASD) which encompass Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) are a group of neurodevelopmental disorders characterized by impairments in social and communication behaviors, and a restricted range of activities and interest (Diagnostic and Statistical Manual of Mental Disorders- 4<sup>th</sup> Edition- Text Revision; American Psychiatric Association, 2000). Although ASD is typically described as one syndrome, autism spectrum disorders represent complex developmental disabilities with considerable variability in clinical presentation. This variability may have important implications for understanding the etiologies

of ASD, stability of symptoms, individual differences in the age of onset, treatment response, and profile of social-emotional development that have been observed among children with ASD.

Various researchers have attempted to identify subtypes of ASD in order to better understand the heterogeneity in the behavioral phenotype. One strategy is to use a biological subtyping that focuses on biological etiologies or known medical conditions. Miles and colleagues (2005) identified individuals with genetic and medical syndromes that often co-occur with autism (e.g., fragile X syndrome) as the “complex” autism group. The complex group tended to have lower IQs, increased seizures/dysmorphic features, and poorer prognosis. The “essential” autism group tended to have higher IQs, higher rates of family history of ASD, and higher rates of regression than the complex group. Others researchers have focused on a cognitive, neurocognitive, and adaptive functioning subtypes. Studies have found evidence for distinct subtypes of ASD that differ in the severity of intellectual ability (Munson et al., 2008). In addition, verbal and nonverbal abilities are used for subtyping with one study finding that children with discrepantly high nonverbal skills relative to verbal skills had greater social impairment, beyond level of verbal ability or overall IQ (Tager-Flusberg & Joseph, 2003). Another classification system focuses on social functioning and personality characteristics in ASD. One such system developed by Wing and Gould (1979) is based on behavioral profiles and classifies individuals into one of three subtypes: aloof, passive, and active-but-odd. Children in the “aloof” subtype rarely initiate spontaneous social approaches to others and tend to reject approaches of others. This group is typically most impaired with the lowest IQ, lowest adaptive functioning, lowest communication skills, and highest rates of repetitive behaviors (Borden & Ollendick, 1994; Castelloe & Dawson, 1993). Children in the “passive” subtype rarely spontaneously approach others but typically can be engaged in activities in a passive manner.

Finally, children in the “active-but-odd” subtype may make spontaneous social approaches to others but in a one-sided and peculiar manner. Castelloe and Dawson (1993) suggested that the “aloof” and “active-but-odd” groups fall at two ends of a continuum. Overall, the social subtyping research has supported the Wing classification and the importance of social symptoms for subtyping and understanding the behavioral phenotype in ASD (Beglinger & Smith, 2001).

### **C. ASD development during infancy**

Parents and professional often begin to note concerns by the second year of life for the majority of children with ASD (Chawarska, Klin, Paul, & Volkmar, 2007; Ozonoff, Williams, & Landa, 2005). However, the average age of diagnosis is not until 4 years of age or older (De Giacomo & Fombonne, 1998; Flanagan & Nuallain, 2001). Therefore, it is vital to examine this early developmental period to identify early symptoms and to better understand the course of symptom expression. Understanding ASD in infancy will provide further information regarding the stability of ASD characteristics, the presence of potential subgroups, and symptom severity over time.

#### *Retrospective studies of ASD in infancy*

Since ASD is not typically diagnosed until at least two years of age, it is often difficult to determine its early symptoms and causes. Retrospective reports based on parental report, medical records, and videotapes indicate abnormalities are often present during the first year of life. When asked about their initial concerns regarding their child with ASD, at least 30-50% of parents recall abnormalities during the first year of life including extremes in temperament ranging from passivity to marked irritability, poor eye contact, lack of response to parents’ voices, and decreased play and social interaction (De Giacomo & Fombonne, 1998; Gillberg et al., 1990). In fact, the average age of parental concern is between 15 and 18 months of age

(Chawarska et al., 2007). Several studies of home videotape reveal that core symptoms of autism are apparent by 12 months of age such as reduced social interaction, lack of social smiling, lack of facial expression, failure to orient to name, lack of pointing/showing, and decreased orienting to faces (Baranek, 1999; Osterling & Dawson, 1994; Werner, Dawson, Osterling, & Dinno, 2000). Symptoms present in toddlers later diagnosed with ASD include similar symptoms such as limited response to name, decreased joint attention, poor eye contact, lack of pointing as well as delayed functional and symbolic play skills and verbal and nonverbal skills (Chawarska et al., 2007).

#### *Stability of ASD in infancy*

Efforts are focused on examining the stability of ASD during the first three years of life. Multiple studies reveal that the majority of children who have ASD symptoms in the second year of life continue to do so at 3 to 4 years of age (Chawarska, Klin, Paul, Macari, & Volkmar, 2009; Chawarska et al., 2007; Cox et al., 1999; Kleinman et al., 2008). However, other studies have found less stability for milder forms of ASD and increased stability of diagnosis if made at 3 years of age (Cox et al., 1999; Turner & Stone, 2007). Although there is overall high stability of the ASD diagnosis at 2 years of age, there is considerable variation and change in clinical presentation that could be attributed to natural variation in the course of ASD (Chawarska et al., 2009). In this study, most of the toddlers continued to have symptoms of autism during preschool but in a minority of cases there was marked improvement in social-affective skills. The majority of toddlers with milder symptoms continued to display less severe social symptoms and maintain a good rate of progress. One explanation is that there is rapid development in cognitive, social, and language skills during toddlerhood such that the most changes in clinical presentation are expected to occur during this period of development (Lord et al., 2006). As

such, the diagnostic measures and strategies utilized in older children may not be as effective for children under 30 months of age (Turner & Stone, 2007). The variation in symptoms across development suggests the need for the creation of developmentally appropriate identification measures during infancy.

*Prospective studies of infants at risk for ASD*

One way to identify early signs in ASD is to gather longitudinal information about infant siblings of children with ASD. Autism is a highly heritable neurodevelopmental disorder; as such, siblings of children with autism are at higher risk for developing autism spectrum disorders with recent estimates suggesting nearly 20 percent of infant siblings developing ASD, with higher rates for male infants and multiplex families (Ozonoff et al., 2011). Furthermore, ASD is one of the earliest emerging neurodevelopmental disorders with DSM-IV (1994) requiring abnormality to be apparent in the first three years of life. Thus, prospective studies of infant siblings allows for the examination of behavioral characteristics that are related to the development of ASD. Benefits of prospective studies are that infants can be identified at or prior to birth based on risk status (i.e., having a sibling with ASD), methods can be standardized, and assuming that infants are ascertained independent of any specific risk signs (i.e., based on risk status alone) they are likely representative of the general population of ASD. In addition, prospective studies allow for the examination of the timing and patterns of early symptom development and how the onset and progression of early signs might vary.

A number of prospective studies are examining the early development of ASD. These ongoing studies are longitudinally tracking younger siblings of children with ASD to determine if reliable early behavioral and neural indicators exist for ASD. Studies with follow-up outcomes inform the early indicators of ASD. However, studies examining infant siblings as a

group also provide vital data about the developmental trajectory of ASD by studying infants who do and those that do not go on to develop ASD to determine the genetic and environmental effects of the development of ASD and possible protective factors (Dawson, 2008; Elsabbagh & Johnson, 2007, 2010; Rogers, 2009).

*Early developmental characteristics in infants at risk for ASD*

These prospective studies of high-risk infant siblings show that behavioral group differences between high-risk siblings and low-risk infants (those without an older sibling with ASD) on standardized behavioral measures are often apparent by 12 months but not 6 months. However, a recent large-scale study of infant siblings at risk for ASD did find parent report behavioral differences at 6 months for children who developed ASD with respect to decreased social skills, communication, and fine motor skills (Bolton et al., 2012). Studies find that the performance gap between the high-risk and low-risk groups widens from 12 months to 24 months with the high-risk group performing more poorly on developmental assessments at later ages (Bolton et al., 2012; Brian et al., 2008; Stone, McMahon, Yoder, & Walden, 2007). Early signs often include precursors to later deficits in social and communicative behaviors (e.g., delayed language) but can also include other behaviors that are considered outside the core symptoms of ASD such as temperamental and motor characteristics.

In these prospective studies, infant siblings of children with ASD show decreased social interest and smiling, reduced expression of positive emotion, and reduced eye contact compared to low-risk infants by 12 months of age (Bryson et al., 2007; Landa & Garrett-Mayer, 2006; Yirmiya et al., 2006; Zwaigenbaum et al., 2005). Prospective studies of high-risk infants who developed ASD found delayed verbal and nonverbal communication and decreased social engagement (e.g., frequency of gazes at faces and shared smiles) beginning at 12 months of age

(Landa & Garrett-Mayer, 2006; Rozga et al., 2011; Ozonoff et al., 2010; Yirmiya et al., 2006; Yoder, Stone, Walden, & Malesa, 2009; Zwaigenbaum et al., 2005). In the communication domain, high-risk infants as a group demonstrate fewer responses to joint attention at 15 months than low-risk infants (Presmanes, Walden, Stone, & Yoder, 2007). A follow-up of this sample revealed that response to joint attention at 12 months predicted the degree of social impairment and ASD diagnosis at 33 months (Yoder et al., 2009). Another study found that high-risk infants who were showing social or language delays at 24 months had significantly more problems following joint attention probes than those high-risk infants without delays (Sullivan et al., 2007).

Prospective studies of high-risk infants have also found higher rates of repetitive behaviors and atypical behaviors beginning at 12 months. Ozonoff and colleagues (2008) found that 12 month olds who later developed ASD had higher rates of atypical behaviors (e.g., spinning and unusual visual regard). In this sample, there were no significant differences on typical motor milestones between the high-risk and low-risk group (without ASD outcome status). Similarly, Iverson and Wozniak (2007) found no differences on age of motor milestone but there were a higher proportion of late onset of milestones in the high-risk group. A study of postures and repetitive movements in high-risk infants found that arm waving and covering ears occurred more often in the high-risk group than the low-risk group and arm waving occurred significantly more in infants who developed ASD (Loh et al., 2007).

Zwaigenbaum and colleagues (2005) reported that 12 month old high-risk infants who were later diagnosed with ASD spent longer periods of time looking at objects by parent report, than low-risk infants and high-risk infants who do not go on to develop ASD. This increased attention to objects has also been found in other prospective high-risk studies (Bryson et al.,

2007; Wetherby et al., 2004). Zwaigenbaum and colleagues (2005) were the first to report that over- or under-responsivity to sensory stimuli differentiated infants who would later develop ASD at 12 months, but not 6 months. In addition, smoothness of visual tracking differentiated the high-risk group who developed ASD from both the non-ASD high-risk group and the low-risk group at 12 months of age. Although these differences were not apparent at 6 months of age, infants who later developed ASD showed increasing delays in the speed that they could disengage from one stimulus to another between the ages of 6 and 12 months.

Subtle disruptions in temperament may occur prior to the onset of clinical symptoms of ASD. Parent report of temperament found that high-risk infants who developed ASD were not temperamentally more difficult at 6 months of age compared to high-risk infants who did not develop ASD or low-risk infants (Zwaigenbaum et al., 2005). However, temperamental differences became more apparent over time with more intense distress and more time spent fixating on objects. At 24 months, factors on the temperament measure (behavioral approach and effortful emotional regulation) differentiated high-risk infants with ASD, high-risk infants without ASD, and low-risk infants. Specifically, behavioral approach differentiated high-risk siblings who did and did not later develop ASD and was related to symptom severity (Garon et al., 2009). Thus, temperament serves as a factor that may influence the trajectory of ASD and provide information regarding early diagnosis and behavioral variability in ASD.

Neurophysiological risk measures are also being investigated in prospective studies to search for early markers for ASD. Several studies have found atypicalities in visual processing of both social and nonsocial stimuli in high-risk infants as a group (Elsabbagh & Johnson, 2007; McCleery, Allman, Carver, & Dobkins, 2007). An electrophysiological study found that high-risk siblings processed objects faster than faces compared to low-risk siblings (McCleery,

Akshoomoff, Dobkins, & Carver, 2009). One prospective study found neural differences at 9 month of age with high-risk infants showing prolonged latency to direct gaze than low-risk infants as well as differences in EEG spontaneous resting states and gamma frequency (Elsabbagh et al., 2009). A measure of EEG complexity tended to differentiate high-risk from low-risk infants mostly clearly from 9-12 months (Bosl, Tierney, Tager-Flusberg, & Nelson, 2011). Furthermore, a recent multi-site prospective infant sibling project found that infants who later developed ASD displayed atypical white matter organization suggestive of abnormal brain connectivity in the first year of life, prior to the onset of behavioral symptoms (Wolff et al., 2012). Multiple research groups are currently examining similar neurophysiological measures with the hope of uncovering biomarkers that may be present even before behavioral differences emerge. However, researchers have cautioned about the interpretation and clinical utilization of these results suggesting the biomarkers should be evaluated in a population-based context in relationship to specific ASD symptoms to determine specificity and sensitivity to ASD (Griffin & Westbury, 2011; Walsh, Elsabbagh, Bolton, & Singh, 2011).

#### *Broader autism phenotype in infancy*

Infant siblings often share characteristics with individuals with ASD even though most infant siblings do not go on to receive a full diagnosis of ASD. The “broader autism phenotype” (BAP) refers to subclinical cognitive and neural characteristics of ASD that occur at a higher rate in first-degree relatives of individuals with ASD. These characteristics include overlapping clinical characteristics such as social relatedness, pragmatics of communication, and the presence of restricted interests as well as differences in face processing and executive functioning (Baron-Cohen & Hammer, 1997; Bolton et al., 1994; Dawson et al., 2002; Pickles et al., 2000). However, it remains unclear how exactly the broader autism phenotype manifests in infancy and

its long-term relation to later development. Many of the broader autism phenotype characteristics such as executive functioning deficits, peer relations, and verbal fluency impairments are not detected until older ages. It is estimated that up to 12-20% of high-risk infants who do not go on to develop ASD will exhibit a lesser variant of ASD and associated language and communication delays (Bolton et al., 1994; Landa & Garrett-Mayer, 2006; Zwaigenbaum et al., 2005). A study examining the broader phenotype in toddler siblings of children with ASD found that non-ASD high-risk infants demonstrated social communication, cognitive ability, adaptive ability, and language deficits in both observational and parent report measures (Toth, Dawson, Meltzoff, Greenson, & Fein, 2007; Toth, Munson, Meltzoff, & Dawson, 2006). However, another study reported that high-risk siblings who did not develop ASD had language delays at 14 and 24 months that were resolved by 36 and 54 months of age (Gamliel, Yirmiya, & Sigman, 2007). This finding suggests that there may be variation in the developmental trajectory of high-risk infants with some infants who show delays earlier on “recovering” from these delays by toddlerhood. It also raises important questions such as why some high-risk infants siblings present with early deficits that later resolve.

Studies examining the broader phenotype in high-risk infants have found that high-risk infants who do not develop ASD do not show the same temperamental problems, motor difficulties, and repetitive behaviors that the ASD high-risk infants demonstrate. In fact, one study found lower rates of sensory and repetitive behaviors and temperamental difficulties in the non-ASD high-risk group compared to the low-risk group at a mean age of 20 months (Toth et al., 2007). However, this may be an effect of parent report. Thus, it will be important to follow up these high-risk infants who do not develop ASD to determine long-term outcome and if they will show the same atypicalities associated with BAP in older children and adults.

#### **D. ASD development summary**

Overall, the results of these studies suggest that ASD represents a heterogeneous group with variability in the onset, developmental course, and behavioral phenotype. Research into subtyping ASD suggests strong evidence for the role of biological, cognitive, and social features in developing classification systems. Beglinger and Smith (2001) theorize that symptom heterogeneity can be represented by three continuous factors (developmental delay, social impairment, and repetitive behaviors) and that there are “continuum containing subgroups”. Existence of specific profiles raises the question if there are some subgroups of children showing unique patterns of onset of symptoms or if onset is more continuous with some children showing a rapid course and others showing a more gradual one. The research examining the course and timing of the onset of ASD symptoms in infancy suggests that patterns tend to be characterized by a slow onset of symptoms and a deceleration of cognitive and social development.

Examining ASD in infancy allows researchers to determine what factors are involved in the development of ASD and the broader phenotype in young children. Both retrospective and prospective studies have contributed vital information in this regard. The literature reveals that behavioral characteristics of ASD are not usually apparent until 12 months of age. The lack of behavioral markers at 6 months indicates a possible dis-continuity of social behavior across infancy and the expression of ASD (Rogers, 2009). In addition, most of these early differences are subtle and are over-arching across multiple domains (e.g., visual attention and temperamental difficulties).

Overall, there tends to be a high stability of ASD diagnosis by 2 years of age but still tremendous variation and change in clinical presentation. In addition, there seems to be a wide range of severity in symptom expression in infancy and toddlerhood. What factors may

contribute to this variation? In the infant sibling literature, many of these non-ASD high-risk infants have early social and communication impairments that resolve by later ages, while other infants continue to display these deficits. It is still unknown whether these symptoms will persist over time and if these siblings will go on to develop the broader autism phenotype.

Identifying other characteristics of ASD in infancy can also inform the course of symptom expression and severity over time. For instance, Rogers (2009) notes that many of the case studies of early ASD reveal that symptoms that were thought to be secondary such as irritability, sensory responsivity, activity level, and poor gross motor abilities appear concurrently or even before core symptoms of ASD. This suggests that ASD may not only affect social development but multiple domains of development. Thus, examining variables such as temperament that may moderate or overlap with ASD symptoms may prove critical for understanding the presentation and development course of ASD.

## **2. Temperament**

### **A. Temperament background**

Rothbart defines temperament as constitutional differences in reactivity and self-regulation, which influences the ways in which individuals adjust to and respond to environmental changes (Rothbart & Goldsmith, 1985). Increased attention has been devoted to the study of infant temperament and later social and personality development (Rothbart & Bates, 1998). Temperament can be reliably measured in infancy and is shown to be predictive of language development, internalizing and externalizing symptoms, and social competence in childhood (Rothbart, Posner, & Hershey, 1995; Sanson, Hemphill, & Smart, 2004).

Temperament traits are moderately stable over time while the bases for changes in temperament are currently poorly understood (Putnam, Ellis, & Rothbart, 2001). The following sections will

review the literature related to the definition, development, stability, heritability, and measurement of temperament as well as the relationship to psychopathology, specifically ASD.

## **B. Temperament definition and dimensions**

Although there has been disagreement over the exact definition of temperament, a consensus is that temperament is a relatively stable individual characteristic that is biologically based and is influenced by the environment and maturity (Bates, 1989; Rothbart, Derryberry, & Hershey, 2000a). Specifically, temperament has been defined as “constitutionally based individual differences in reactivity and self-regulation, as observed in the domains of emotionality, motor activity, and attention” (Rothbart, Posner, & Kieras, 2006). Other researchers may define temperament as a social construct that is generated from the interactions between the child and the environment. However, these definitions may not be mutually exclusive such that an infant’s initial tendencies may interact with the environment to influence the stability of these traits. Although there are various theories regarding the development of temperament and researchers use differing terms and measures, there is general agreement regarding the manifestation of temperament in infancy, its genetic influence, and its moderate stability over time.

Nine dimensions of temperament were originally identified by parent report in the seminal New York Longitudinal Study (Thomas & Chess, 1977). These included activity level, approach/withdrawal, intensity, threshold of responsiveness, adaptability, rhythmicity, mood, attention span persistence, and distractibility. However, concerns about the conceptual overlap among some of these scales led to theoretical refinement in which reactivity and self-regulation processes form the basis of temperament with three temperament dimensions: *self-regulation*, *extraversion/surgency*, and *reactivity/negative affectivity* (Rothbart et al., 2000). This popular

formulation has now gained wide acceptance and is often utilized in temperament research. *Self-regulation* has two subcomponents, effortful control of attention (e.g., persistence, non-distractibility) and of emotions (e.g., self-soothing). *Extraversion/surgency* can also be termed as approach-withdrawal, inhibition, or sociability and describes the tendency to approach or withdraw from novel situations/people. *Reactivity/negative affectivity* refers to irritability, negative mood, and high-intensity negative reactions and can be differentiated into distress to limitations (irritability, anger) and distress to novel situations/people (fearfulness). Factor analysis has also revealed narrower band factors such as *Rhythmicity* and *Activity level* (Sanson, Smart, Prior, & Oberklaid, 1993).

Rothbart and colleagues have further shown that rating scales could be consolidated through development (Putnam et al., 2001; Rothbart, Ahadi, & Evans, 2000; Rothbart & Bates, 1998; Rothbart & Derryberry, 1981). In infancy, five or six dimensions are prominent: positive affect, two kinds of negative affect (fear/anxiety and anger/irritability), activity level, and rhythmicity. At that age, a three-factor structure is defined by surgency/extraversion, negative affect, and affiliation (i.e., behaviors such as cuddliness and soothability, as well as orienting). However, by toddlerhood and early childhood, affiliation is moderated by attentional control and termed “effortful control”. In adolescence, affiliation is separated as a fourth factor, distinct from effortful control, such that the model of temperament begins to closely resemble the model proposed for personality.

### **C. Development and relationship to personality**

Temperament characteristics can be identified and reliably measured in infancy (Rothbart & Bates, 1998). Newborns show distress and avoidant movements and by 2 to 3 months, show approach reactions such as smiling, laughter, and body movements which maps onto the

extraversion factor. Physical approach begins when the infant becomes mobile, usually by 4 to 6 months, and activity level is found to increase throughout infancy (Buss & Plomin, 1975). Negative affectivity refers to an individual's proneness to negative affect such as fear, anxiety, sadness, and anger. Anger or frustration can be seen at 2 to 3 months of age and fear can be differentiated from anger by 4 to 8 months (Rothbart, Ahadi, Hershey, & Fisher, 2001). Fear can operate as a moderator of approach and aggression such that fear in infancy predicts later fearfulness and low aggression (Rothbart & Bates, 2006). Fear can also act as a reactive dimension that can regulate behavior (withdrawal from a threatening situation) and also operate to capture attention (Rothbart & Sheese, 2007). Duration of orienting has been found to decrease between 6 and 9 months followed by an increase between 9 and 12 months (Carranza, Perez-Lopez, Gonzalez, & Martinez-Fuentes, 2000). Effortful control (EC), which refers to the ability to regulate attention, emotions, and behavior, does not develop until the end of the first year and continues to develop during the preschool period (Rothbart & Putnam, 2002). By 30 months of age, children can consistently perform effortful control tasks (Kochanska, Murray, & Harlan, 2000).

There has been disagreement in the field about the distinction between temperament and personality (Rutter, 1987). Temperament has been conceptualized as a constitutionally based behavioral style in young children (Goldsmith et al., 1987) while personality is thought to be shaped throughout the lifetime through experiences (Mayer, 2005). However, both appear early in life, have similar heritability and stability, and are related to emotional and motivational aspects of behavior (Nigg, 2006). Several studies have examined associations between temperament and the five-factor model of personality (comprising Extraversion, Neuroticism or Emotional Stability, Agreeableness, Conscientiousness, and Intellect or Openness to

Experience). Despite difficulties comparing studies due to the differing temperament dimensions and scales used, there are some consistent trends. Extraversion and agreeableness have each been linked to activity and inhibition/approach, conscientiousness to persistence, neuroticism to emotionality/negative reactivity, and intellect/openness to inhibition/approach (Angleitner & Ostendorf, 1994; Hagekull & Bohlin, 1998). Although the literature is still unclear about specific links between temperament and personality, it is understood that temperament interacts with social development to influence personality.

#### **D. Heritability and stability**

Twin and adoption studies suggest that heritability is generally in the range of .4 to .6 (Braungart, Plomin, DeFries, & Fulker, 1992; Cypher, Phillips, Fulker, & Mrazek, 1990). Temperament has very low heritability in the neonatal period (Matheny, 1989; Riese, 1990) with higher heritability by toddlerhood for major traits (Matheny, 1989; Saudino & Cherny, 2001). Rothbart and Bates (1998) propose that the gradual emergence of the moderately stable traits may be consistent with the idea that traits are rooted in motivational response systems that have a greater influence on behavior through development. By early to middle childhood, most temperament traits have moderate heritability in the range of .3 to .6 across traits, significant effects of non-shared environment, and typically little or no contribution of shared environment (Nigg & Goldsmith, 1998; Saudino, 2005). However, some aspects of temperament (e.g., activity) appear to be more strongly influenced by heredity than others (e.g., attention span, soothability, and rhythmicity) (Schmitz, Saudino, Plomin, Fulker, & DeFries, 1996).

Temperament traits are typically moderately stable over time, with correlations in the range of .2 to .4 (Slabach, Morrow, & Wachs, 1991), although stability may be as high as .7 to .8 when measurement error is taken into account (Oberklaid, Sanson, Pedlow, & Prior, 1993).

Observational assessments of temperament tend to report lower stability correlations compared to questionnaire assessments (McDevitt, 1986; Rothbart, Derryberry, & Hershey, 2000b). This may be an artifact of parent report measures such that parents view temperament as fixed or are influenced by overall impressions throughout development rather than one time point. Some studies employing parent report measures report increasing stability of temperament over time (McDevitt, 1986; Plomin, Corley, Caspi, Fulker, & DeFries, 1998) whereas others find stability to be similar across ages (Saudino & Cherny, 2001). Initial temperamental variations are not necessarily stable at birth, although they show moderate continuity beginning at some point in infancy (Kagan & Snidman, 2004) and even more so by the toddler years (Lemery, Goldsmith, Klinnert, & Mrazek, 1999; Oberklaid et al., 1993). These moderately stable coefficients imply a considerable amount of change in children's temperament over time.

At present, the bases for changes in temperament are poorly understood (Putnam, Sanson, & Rothbart, 2002). One reason for the moderate stability may be that different aspects of temperament are salient at different points in development. For example, negative affect may be more apparent in infants than in older children, and self-regulation is more apparent in older children than toddlers. Activity level is a prominent characteristic of young children, but does not tend to emerge as a separate major trait in older children and adults (Putnam et al., 2001). Affiliation is more salient in adolescence than in the toddler years (Putnam et al., 2001). Variability in expression may be another consideration. For instance, inhibited behavior at age 3 years may be different from inhibited behavior at age 9. In addition, temperament may be affected by environmental change and adaptation (Kagan & Snidman, 2004). Finally, moderate stability correlations could be due to psychometric issues such as varying methodologies of measuring temperament. The types of methods used to capture temperament will be discussed

next.

## **E. Measurement**

### *Parent report measures*

The most widely used method of temperament assessment has been parent rated questionnaires. Advantages include (1) ability to rate a child's temperament across time, (2) ease of administration, and (3) good psychometric properties. However, it is well known that parent report of temperament may be biased with some researchers suggesting that parent reports should not be used (Kagan, 1998).

Rothbart and colleagues have developed parent measures to assess temperament across childhood. Rothbart designed questionnaires in attempt to avoid parent using global judgments or relying on past child behavior. For instance, the Infant Behavior Questionnaire (IBQ; Rothbart, 1981) was worded to ask caregivers to report on the frequency of certain behaviors in the past few weeks using a 7-point, Likert-like scale (never, very rarely, less than half the time, half the time, more than half the time, almost always, always). The IBQ was designed to enhance recall, to focus on specific events to limit global impressions, limit comparative judgments, and limit social desirability. The IBQ scales were developed to assess *activity level, smiling and laughter, fear, distress to limitations, duration of orienting, soothability, and vocal reactivity*. Reliability, convergent validity, and relative stability using this scale has been demonstrated with infants as young as 2 weeks of age (Worobey & Blajda, 1989). New scales were derived for the Infant Behavior Questionnaire – Revised (Gartstein & Rothbart, 2003). The Children's Behavior Questionnaire (Rothbart, Ahadi, Hershey, & Fisher, 2001b) was particularly influential in determining these factors on the IBQ-R that would have a downward extension into infancy including *positive anticipation (approach), falling reactivity, high and low intensity*

*pleasure, perceptual sensitivity, sadness, and cuddliness/affiliation.* Rather than capturing a broad infant trait, the IBQ-R measures specific dimensions of temperament with 14 subscales (see Table 1). *Approach* is defined as positive excitement and rapid approach toward pleasurable activities. *Falling reactivity* is defined as the rate of recovery from peak distress, excitement, or general arousal and reflects the infant's ability to regulate his/her own state. *High and low intensity pleasure* refer to enjoyment related to stimulus characteristics (e.g., high or low intensity, rate, complexity, novelty, and incongruity). *Perceptual sensitivity* is defined by the detection of slight, low intensity environmental stimuli. *Sadness* is defined as general low mood, or lowered mood and activity related to personal suffering, physical state, object loss, or inability to perform a desired action. *Cuddliness/affiliation* is defined as expression of enjoyment and molding of the body to being held by the caregiver. Rothbart and colleagues also have developed assessments for older children such as the Early Childhood Behavior Questionnaire with a three-factor structure of Surgency/extraversion, Affectivity, and Effortful control (Putnam, Gartstein, & Rothbart, 2006a). These instruments have shown to have good to excellent reliability and validity (Gartstein & Rothbart, 2003; Putnam, Gartstein, & Rothbart, 2006b; Putnam & Rothbart, 2006; Rothbart et al., 2000; Rothbart et al., 2001).

Table 1. Infant Behavior Questionnaire – Revised: Scales and definitions

<b>Scale</b>	<b>Definition</b>	<b>Example item</b>
Approach	Rapid approach, excitement, and positive anticipation of pleasurable activities.	When familiar relatives visited, how often did the baby get excited?
Vocal Reactivity	Amount of vocalization exhibited by the baby in daily activities.	When being dressed/undressed during the last week, how often did the baby coo or vocalize?
Smile and Laughter	Smiling or laughter during general caretaking and play.	How often during the last week did the baby smile or laugh when given a toy?
Activity Level	Gross motor activity, including movement of arms and legs, squirming and locomotor activity.	When put into the bath water, how often did the baby splash or kick?
Perceptual Sensitivity	Detection of slight, low intensity stimuli from the external environment.	How often did the baby notice fabrics with scratchy texture?
Sadness	Lowered mood and activity related to suffering, physical state, object loss, or inability to perform a desired action; general low mood.	Did the baby seem sad when the caregiver was gone for an unusually long period of time?
Distress to Limitations	Fussing, crying or showing distress while (a) in a confining place; (b) in caretaking activities; (c) unable to perform a desired action	When placed on his/her back, how often did the baby fuss or protest?
Fear	Startle or distress to sudden change in stimulation, novel physical objects or social stimuli; inhibited approach to novelty.	How often during the last week did the baby startle to a sudden or loud noise?
Falling Reactivity	Rate of recovery from peak distress, excitement, or general arousal; ease of falling asleep.	When frustrated, how often did the baby calm down within 5 min?
High Intensity Pleasure	Amount of pleasure or enjoyment related to high stimulus intensity, rate, complexity, novelty, and incongruity.	How often did the baby smile during a peekaboo game?
Low Intensity Pleasure	Amount of pleasure or enjoyment related to low stimulus intensity, rate, complexity, novel and incongruity.	When playing quietly with one of his/her favorite toys, how often did the baby show pleasure?
Cuddliness	Expression of enjoyment and molding of the body to being held by a caregiver.	When rocked or hugged, during the last week, how often did the baby seem to enjoy him/herself?
Duration of Orienting	Attention to and/or interaction with a single object for extended period of time.	How often during the last week did the baby stare at a mobile, crib bumper, or picture for 5 min or longer?
Soothability	Reduction of fussing, crying, or distress when soothing techniques are used by the caregiver.	When patting or gently rubbing some part of the baby's body, how often did s/he soothe immediately?

Source: Adapted from Gartstein and Rothbart, 2003, p. 72

Limitations of parent report include: (a) differences in frame of reference, (b) rater characteristics, (c) quality of parent-child relations, (d) contrast effects such as exaggerating sibling differences, and (e) or assimilation effects such as overestimating effects of sibling similarities (Rothbart & Bates, 1998; Rothbart & Goldsmith, 1985). It is known that rating

characteristics such as parental depression and stress as well as parents own childhood experiences can affect the validity of parent report (Forman et al., 2003; Leerkes & Crockenberg, 2003; Mednick, Hocesvar, Baker, & Schulsinger, 1996). Research has shown that depressed parents are more likely to rate their infants as more difficult and to misinterpret their infants' signals than parents who are not depressed (Mebert, 1991; Parade & Leerkes, 2008; Schuetze & Zeskind, 2001). Parental perceptions of temperament rather than actual temperamental characteristics have implications for their parent-child interactions. For instance, one study found that parents who rated their younger sibling as higher in negative emotionality than the older sibling tended to differentially treat the older sibling more favorably. However, when the older sibling was rated as higher or similar, parental treatment was equal (Brody, Stoneman, & McCoy, 1992). Contrast effects were originally reported in behavioral genetic twin studies examining genetic and environment contributions to temperament. Studies have found moderate correlations in monozygotic twins but very low correlations in dizygotic twins suggesting parental contrast effects since some convergence would be expected in dizygotic twins (Eaves et al., 2000; Plomin et al., 1993b). However, this tendency to evaluate children relative to each other exists in non-twin studies as well. Saudino et al. (2004) found that although parental report showed zero or negative correlations in siblings, observational measures of temperament in siblings found positive correlations suggesting contrast effects. Temperamental traits that have been most prone to parental bias include activity level, shyness, attention/persistence, sociability, and emotionality (Majdandzic, van den Boom, & Heesbeen, 2008; Plomin et al., 1993a, 1993b; Saudino & Cherny, 2001) whereas parent ratings of more affective behavioral dimensions such as approach, fear, pleasure, and smiling do not seem to show this contrast effect (Saudino, Wertz, Gagne, & Chawla, 2004). The methods of assessing temperament appear to affect the level of

contrast effects with questionnaires asking the parent to make more global judgments of temperament consistently yielding contrast effects compared to questionnaires that ask the parent to report on the frequency of behavior within a specified context (e.g., the Infant Behavioral Questionnaire (IBQ; Rothbart, 1981)) that do not tend to reveal this bias.

### *Observational measures*

Another system of assessment utilizes standardized observational or laboratory measures in which specific tasks are administered and behavior is coded by trained observers. Although observational measures have their own limitations such as the range of sampled behavior and may be affected by idiosyncratic factors (e.g., child's mood and alertness), observational measures may provide a less biased measurement of infant temperament. Observers have the added benefit of being trained to reliably identify and code certain behaviors and also review child behavior via videotape while parents are limited to recall and do not have training to identify behaviors reliably (Goldsmith & Hewitt, 2003; Rothbart & Bates, 1998).

Rothbart and colleagues have developed standardized laboratory assessments (e.g., the Laboratory Temperament Assessment Battery (Lab-TAB: Goldsmith, Reilly, Lemery, Longley & Prescott, 1995)) designed to elicit specific behavioral or emotion responses assumed to reflect temperamental traits. These observational batteries have been used in infancy and in early childhood. The Lab-TAB was developed to be used in conjunction with the two Rothbart early temperament parent questionnaires (IBQ-R and ECBQ). Another measure of temperament used in observational methods of temperament is the Infant Behavior Record (IBR; Bayley, 1969). The IBR was originally designed to assess infant behavior during a cognitive assessment and has been used to assess temperament dimensions such as social orientation, emotional regulation, task orientation, and activity level (Goldsmith & Gottesman, 1981; Matheny, 1983). It has been

utilized in laboratory settings (Carnicero, Perez- Lopez, Del Carmen, Salinas, & Martinez-Fuentes, 2000; Stifter & Corey, 2001) and home visits (Stifter et al., 2008).

*Parent and observation measurement convergence*

Overall, convergence between parental ratings and observational assessments of temperament has generally been found to be modest to moderate at best (Majdandzic et al., 2008; Mangelsdorf, Shoppe, & Burr, 2000; Rothbart & Bates, 1998). Concordance between parent reports and observed indices of temperament is strongest when well-established parent report measures are used, caretakers and observers rate infant behavior in similar situations, and comparisons are made between the same dimensions of temperament (Rothbart & Bates, 1998). The lack of high convergence in some studies may be due to the fact that methods of measuring temperament tap behavior in different context. For instance, dimensions such as inhibition, activity level, and attention show a situation specific effect (Majdandzic & van den Boom, 2007). Another possibility for modest correspondence is method specific bias noted previously such as rater characteristics, contrast or assimilation effects for parent report, and limited sampling of behavior and contextual factors for laboratory observations (Kagan, 1998; Rothbart & Bates, 1998).

A number of studies have cited parental characteristics such as depression, personality, psychopathology, and stress as reasons for greater discrepancies between parent reports and observational assessments (Forman et al., 2003; Mebert, 1991). Parental factors may alter parent perceptions of their child's behavior and impact their ability to correctly identify and report on their behavior. For instance, in high-risk samples, maternal characteristics have been found to be more predictive of maternal reports of temperament than observed infant behavior (Sameroff, Seifer, & Elias, 1982). Similarly, concordance between maternal reports and observed

temperament were found to be higher when mothers report less stress and hostility and exhibit low depressive symptoms (Forman et al., 2003).

Recent research suggests different factors may have different convergence. For example, Stifter et al. (2008) found a moderate degree of convergence between parents and observers in rating infant positivity but little or no convergence with respect to negativity. Supporting this finding, Majdankdzic et al. (2008) found significant correlations between observational and parental rating of children's shyness, activity level, interest, and fear and low correlations for anger and sadness. These differences may be due differences in context specificity across traits. In addition, parents may downplay the negativity of their own child. Seifer et al. (2004) found that parents observed their own child and an unfamiliar child during the same task and found that parents tended to underrate their own child's negativity. Another reason may be that parents take into account their child's potential for negativity while an observer would not have this comparison.

Together, these studies suggest that each method may tap into different aspects of temperament and that utilizing both parent and observational measures provides important information about the infant's abilities (Rothbart & Bates, 1998). Kraemer and colleagues (2003) suggested that no one informant of child behavior should be treated as more valid than another. Instead, they suggest that an index of temperament is ideally derived by isolating the shared variance from multiple sources of behavior gathered across multiple contexts. Although multiple temperament researchers have suggested the optimal solution is to use multiple methods of assessing temperament, relatively few studies currently adopt this procedure.

*Electrophysiology measurement of temperament*

Current research has started to further elucidate the biological basis of temperament. The majority of measures of temperament are behavioral in nature, although most temperament researchers agree that temperament has biological underpinnings (Buss & Plomin, 1975; Kagan, 1994; Rothbart, 1989a; Thomas & Chess, 1977). While it is agreed upon that there is an underlying biological basis towards specific temperament characteristics, it is unclear the association between physiological systems and temperamental traits (Quas, Hong, Alkon, & Boyce, 2000). There is a need to measure physiological correlates of temperament as the behavioral phenotype often develops and changes over time possibly masking the original temperamental biases. Thus, it is advantageous to identify both biological and behavioral measures to understand the physiological correlates of temperament characteristics.

One of the promising biological measures of temperament is electrophysiology (EEG). EEG frontal asymmetry refers to the degree of asymmetry in neural activity across the frontal cortex, observed at rest or during emotional tasks. Fox and colleagues (2001) have suggested that the pattern of resting frontal EEG reflects individual differences in affective styles in infants. The spontaneous rhythmic activity of the brain can be broken down into frequency bands that are related to various mental states. Synchronized neuronal activity with a frequency of 8 to 13 Hz in adults is called alpha and is greatest during attentive and awake states while not activity engaged in cognitive tasks (Schaul, 1998; Shagass, 1972). Power in the alpha frequency band is reduced when the person undergoes a cognitive task such that magnitude of the band is inversely proportional to cortical activation (i.e., reduced power reflects increased activation). There is debate over the precise boundaries of the alpha bands and how it changes over infancy and childhood. However, it is generally agreed upon that at 3 months of age, the occipital rhythm

occurs at a frequency of 3-5 Hz and increases to 6-7 Hz by the end of the first year of life (Marshall, Bar-Haim, & Fox, 2002).

One method for assessing hemispheric asymmetry is by recording EEG from homologous sites over the left and right hemispheres resulting in an index of asymmetry based on EEG power band. Researchers typically use a difference or laterality ratio score between the left and right sites. Since alpha power and cortical activation is inverse, a decrease in alpha power in the EEG recorded from the left scalp electrode relative to power in the right electrode has been taken as increased activation in the left frontal region or “left frontal asymmetry”. “Right frontal asymmetry” refers to a pattern where activation in the right frontal region is greater than activation in the left frontal region (lower alpha power in the right compared to the left). It is known that individuals vary in the lateralization of their alpha resting state where some individuals show greater lateralization on the left side while others are more active on the right. It is unclear why these asymmetries occur but it could be due to asymmetric neural projections from subcortical structures to each hemisphere or inherent differences in intrahemispheric patterns of arousal (Davidson, 1988). Limbic and cortical regions are known to affect alpha activity in the frontal region and can influence the hemispheric asymmetries in alpha activity. Thus, these asymmetries may reflect activation of specific areas of the prefrontal cortex as they modulate or inhibit the activity of these subcortical areas such as the amygdala (Davidson, 1998).

It is suggested that frontal EEG asymmetries reflect the activity of brain systems that moderate trait tendencies to approach (indexed by relatively greater left frontal activity) or withdrawal (indexed by relatively greater right frontal activity) from novel stimuli and mediate approach and withdrawal-motivational tendencies that underlie affective responses (Davidson, 1998). The approach and withdrawal model of emotion is based on Gray’s (1972) behavioral

activation and inhibition systems model (BAS/BIS) in which high trait behavioral activation is associated with a tendency to seek out reinforcement and greater positive affect while behavioral inactivation is associated with behavioral inhibition and stronger negative affect. Fox and Davidson (1984) proposed that areas of the cortex are lateralized to support motor and cognitive processes underlying approach or withdrawal behaviors and through experience and development, these prefrontal regions become specialized for approach/withdrawal responses.

EEG asymmetry stability has been assessed in different age groups from infancy through childhood and adulthood. Fox and colleagues (1992) found fair to moderate stability in frontal EEG asymmetry across a one month interval but lack of stability across five months. Bell and Fox (1994) found modest stability across a 6-month interval. Jones and colleagues (1997a) reported that frontal EEG asymmetry was moderately stable from the first six months of life to the second year of life. Overall, stability of EEG asymmetry in childhood is estimated to be low to moderate (Vuga, Fox, Cohn, Kovacs, & George, 2008). Research with adults shows moderate long-term stability in frontal asymmetry (Vuga et al., 2006). The lack of EEG asymmetry stability in infancy and early childhood may be due to possible changes in asymmetry as a result of early experience. Little is known about the developmental origins of frontal EEG asymmetry. It is unclear if it is present at birth or develops over the first years of life. Behavioral genetic findings suggest that heritability of frontal EEG asymmetry is relatively modest (<30% of the variance) suggesting that environment plays a substantial role in the development of frontal asymmetry (Anokhin, Heath, & Myers, 2006).

There is debate as to whether alpha asymmetry represents a stable temperamental trait or a transient state with studies finding support for both perspectives. In general, spontaneous or induced emotional states related to approach or positive affect are associated with greater left

activity whereas emotional states associated with withdrawal or negative affect are associated with greater right frontal activity. Davidson and Fox (1989) found that 10-12 month old infants showed evidence of increased left frontal activation in response to watching a video of an actress displaying a happy face and greater right activation while watching a video of an actress crying. In fact, infants as young as 2-3 days old exhibited an increase in left frontal asymmetry to drops of sugar water on their tongue and right frontal asymmetry to neutrally flavored water (Fox & Davidson, 1987). During the expression of positive affect, infants exhibit left frontal asymmetry while during the expression of negative affect, infants demonstrate right frontal EEG asymmetry supporting findings of EEG as a state marker (Fox and Davidson, 1988). Fox and colleagues found infants displayed greater right activation during the approach of a stranger (Fox et al., 1992; Fox & Davidson, 1987). In addition, adult studies have shown that the right frontal region is more specialized for negative emotions such as fear, anxiety, and sadness (Davidson, Ekman, Saron, Senulis, & Friesen, 1990). These research studies suggest that frontal EEG asymmetry can be utilized as a state marker of positive and negative emotion and approach-withdrawal tendencies.

Frontal EEG asymmetry has also been utilized as a trait marker for the disposition to express positive/negative affect and approach/withdrawal tendencies. Fox argues that the left frontal region mediates social approach behaviors while the right frontal region mediates social withdrawal behaviors (Fox, 1994). Davidson and Fox (1989) reported that infants' baseline EEG activity at 10 months of age predicted their response to maternal separation such that infants who displayed right frontal asymmetry were more likely to cry to separation than those demonstrating left frontal asymmetry. Fox and colleagues have built upon this early work and find that infants who display left frontal EEG asymmetry at rest are characterized as having

“easy” temperaments, whereas infants who display right frontal EEG at rest are characterized as having “negative” temperaments (Fox et al., 1995; Fox et al., 1996; Fox et al., 2001). In typically developing individuals, those with right frontal EEG symmetry tend to be more withdrawn and express negative affect whereas individuals with left frontal EEG asymmetry tend to be more approach oriented and express positive affect (Baving, Laucht, & Schmidt, 2002; Hane, Fox, Henderson, & Marshall, 2008). Fox and colleagues (1995) found that children with right frontal EEG asymmetry were generally more socially inhibited and scored lower on measures of social competence while children who were more socially competent had greater left frontal activity. These differences are hypothesized to be related to early emotional reactivity as well as the ability to regulate these emotional tendencies (Fox, 1994). A recent examination of a parent report measure of temperament, the Infant Behavior Questionnaire-Revised (IBQ-R) and concurrent measures of frontal asymmetry recorded while 7-9 month old infants viewed emotionally salient videos found domains of approach and engagement were generally associated with greater left frontal activation and falling reactivity (e.g., longer time for an infant to calm down) was associated with greater right frontal activation (LoBue, Coan, Thrasher, & DeLoache, 2011). These studies suggest that frontal EEG asymmetry is a reliable correlate of infant temperament dimensions.

Studies have also shown a relationship between EEG asymmetry and temperament dimensions at later ages. Infants who showed more extreme negative affect at 4 months of age tended to have right frontal EEG asymmetry at 9 months of age and were more behaviorally inhibited at 14 months of age compared with infants who showed high positive affect or low levels of positive and negative reactivity at 4 months of age (Calkins, Fox, & Marshall, 1996). In this sample, the combination of infant negative temperament and right frontal EEG asymmetry

was the best predictor of social inhibition at 4 years of age (Henderson, Fox, & Rubin, 2001). A study of 6 month old infants found that higher levels of cortisol was associated with right EEG asymmetry and increased expression of sadness during approach of a stranger (Buss et al., 2003).

Studies have found that continuity in temperament is strongest for children whose behavioral profile is associated with the corresponding profile of frontal asymmetry (i.e., approach-related tendencies with left frontal asymmetry and withdrawal-related behaviors with right frontal asymmetry) (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Henderson et al., 2001; Henderson, Marshall, Fox, & Rubin, 2004). Hane and colleagues (2008) found that negatively reactive infants showed significantly more avoidance than positively reactive infants and displayed a pattern of right frontal EEG while positively reactive infants exhibited more approach behavior and exhibited a pattern of left frontal asymmetry compared to controls. A study examining stability found that 9-month-old negative emotionality predicted social wariness at 4 years of age but only for infants who displayed greater right frontal EEG at 9 months of age (Henderson, Fox, & Rubin, 2001). Another longitudinal study of EEG and temperament found that children who were highly reactive in infancy and became highly fearful in toddlerhood were more likely to have demonstrated right frontal activation at 4 months of age (McManis, Kagan, Snidman, & Woodward, 2002). In a similar study, infants who were exhibiting extreme right frontal asymmetries at 3 month of age continued to do so at 3 years of age and were more inhibited and less empathetic (Jones et al., 1997a). Interestingly, some children who originally had a right frontal bias and were highly inhibited but became less inhibited with age revealed a corresponding change in their EEG pattern from right to left frontal asymmetry (Fox et al., 2001). The authors suggest that this may have been a result of early experience in childcare.

Thus, although EEG profiles appear to be moderately stable in the first few years of life, early experiential factors appear to influence the stability.

Overall, the data suggest that frontal asymmetry activation can reflect both stable moods as well as transient states and may be a result of the interaction between trait and state variables (Coan & Allen, 2003). A state-trait analysis conducted in adults suggests that 60% of the variance of the asymmetry measure was due to individual differences while 40% was due to state-specific changes. Future developmental research is necessary to determine if EEG asymmetry may be a mediator where the neural activation produces approach or withdrawal tendencies or may act as a moderator of behavior.

## **F. Temperament predicting developmental outcome**

### *Models of temperament and developmental psychopathology*

Several researchers have suggested that the study of temperament is a promising avenue for understanding vulnerability to psychopathology (Muris & Ollendick, 2005; Nigg, 2006). However, it is difficult to examine the relationship between psychopathology and temperament since many of the constructs overlap (Lahey & Waldman, 2003). Various models exist to describe the relationship between psychopathology and temperament. The spectrum model or common cause model suggests that temperament is basically a sub-clinical manifestation of psychopathology, with shared etiological determinants (Tackett, Krueger, Iacono, & McGue, 2005). An example of the spectrum model is the proposal that ADHD symptoms represent the extreme of temperament dimensions of effortful control, activity level, and negative affectivity. However, other studies suggest that there are qualitative differences between psychopathology and temperament dimensions with temperament explaining a substantial amount but not all of the variance in a diagnosis suggesting that a spectrum model cannot fully account for the

development of a disorder (Nigg, 2006). In the vulnerability or resilience model, certain temperament traits predispose or protect from certain kinds of psychopathology (Colder, Mott, & Berman, 2002). This would be consistent with a diathesis-stress model, in which the diathesis may be specific temperamental traits (Ingram & Luxton, 2005). Support has been found for the vulnerability model in which there are mediating and moderating influences of temperament on psychopathology. For instance, in children with anxiety disorders, an increased bias towards attention to potential threat cues mediates the relation between anxiety symptoms and temperament dimensions of negative affectivity and effortful control (Lonigan, Vasey, Phillips, & Hazen, 2004). Although various models are proposed and future research is needed to determine the relationship between temperament and psychopathology, studies demonstrate that temperamental characteristics are different from symptoms of psychological disorders. A factor analysis of temperament and psychopathology in children showed that temperament and psychopathology items loaded separately with expert ratings confirming these separate factors (Lemery, Essex, & Smider, 2002). Confirming this finding, Lengua, West, and Sandler (1998) found that there was still a relationship between temperament and psychological symptoms even after accounting for the overlap giving less support for the spectrum model.

#### *Temperament characteristics relation to psychopathology*

A large body of research has focused on the relationship between specific temperament characteristics and the development of psychopathology. A number of studies have demonstrated that early temperamental characteristics are associated with the development of psychological disorders. One of the first studies to demonstrate this association found that the temperament dimension of withdrawal at 3-5 years of age, predicted the presence of internalizing symptoms of those children in middle childhood and adolescence (Caspi, Henry, McGee,

Moffitt, & Silva, 1995). Further studies have investigated the relationship between temperamental inhibition in infancy and later inhibition and anxiety symptoms. Kagan and colleagues (2004) followed 4 month old infants to 7 years of age and found that infants who were highly reactive according to levels of distress and activity level at 4 months of age, showed more fear and inhibition as toddlers, were more likely to be withdrawn at 4 years of age, and exhibited more anxiety symptoms at 7 years of age. However, it may be difficult to determine distinctions since there is overlap between the temperament dimension (e.g., inhibition) and the outcome measure (e.g., social withdrawal).

Extraversion/surgency has been linked to greater externalizing problems and fewer internalizing problems. Anger and frustration predict both internalizing and externalizing problems, however, fear is more strongly related to internalizing and anger to externalizing difficulties. (Rothbart & Bates, 2006; Ormel et al., 2005). Low effortful control is a strong predictor of externalizing problems but a less strong predictor of internalizing problems. In addition, it acts as a moderator such that children who are highly negative but also have higher effortful control will be less likely to show problems (Rothbart & Bates, 2006; Rothbart et al., 2006). Children with high levels of effortful control are capable of regulating these negative emotions by employing more strategic, flexible, and effective coping strategies (Lengua & Long, 2002). A recent longitudinal study examined temperament predictors from 3 months to 15 years of age (Pitzer, Esser, Schmidt, & Laucht, 2009). Low regulative abilities at 3 months predicted adolescent behavioral and attentional problems over and above obstetric and psychosocial risks. Regulatory abilities had a larger contribution than other temperament factors describing aspects of mood and extraversion.

*EEG relationship to psychopathology*

The association between frontal EEG asymmetry and affective style suggests that EEG asymmetry may serve as a useful endophenotypic marker of vulnerability for certain forms of psychopathology or emotional dysregulation. Given the moderate stability in frontal asymmetry in early childhood (Bell & Fox, 1994; Fox, Calkins, & Bell, 1994), EEG asymmetry methodology may prove to be more useful in predicting developmental outcome than behavioral indicators. To date, the degree to which frontal EEG asymmetry represents an episode, liability, or genetic vulnerability marker for psychopathology is unclear. Current research suggests that EEG asymmetry does not characterize all individuals with psychopathology, but may index vulnerability for certain psychopathologies, including depression and anxiety.

Most research examining EEG asymmetry and psychopathology has focused on anxiety and depression. A multitude of studies has linked greater right frontal EEG resting activity to depression (Henriques & Davidson, 1991; Schaffer, Davidson, & Saron, 1983). Allen et al. (2004) found evidence of adequate stability in resting frontal EEG asymmetry in depressed patients. Further evidence of a relationship between maternal depression and right frontal activity in infants suggests a link between EEG and depression. Dawson and colleagues (1997) found that infants of depressed mothers exhibited a pattern of right frontal asymmetry compared with infants of non-depressed mothers. This pattern of frontal EEG activity distinguished infants of mothers with major depression and those who had sub-threshold symptoms. In addition, infants of depressed mothers were found to be less affectionate and had less left frontal activity at rest and during interactions with their mothers and familiar strangers (Dawson et al., 1999). These findings have been replicated with further studies finding greater relative right frontal EEG patterns in infants of depressed mothers (Field et al., 2001; Field, Fox, Pickens, &

Nawrocki, 1995; Jones et al., 1997a; Lundy et al., 1999). Overall, these data support behavioral findings that maternal depression is linked to increased likelihood of the development of negative affect, inhibition, and withdrawal in infants (Field, Pickens, Fox, Gonzalez, & Nawrocki, 1998). Importantly, the data suggest that EEG asymmetry may serve as a marker of genetic vulnerability.

Several studies have also found a relationship between EEG asymmetry and anxiety with greater right frontal activation being associated with inhibition (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Davidson & Rickman, 1999; Davidson et al., 2000). Smith and Bell (2010) found that children with stable left frontal EEG asymmetry during infancy were rated as higher in externalizing behaviors whereas children with stable right frontal EEG asymmetry were rated higher in internalizing behaviors. Since relatively greater right frontal activity has been associated with anxiety and depression, it has been suggested that right asymmetry may represent a general predisposition for internalizing disorders (Davidson et al., 2000). Studies examining the extent that EEG asymmetry serves as a liability marker reveal that EEG asymmetry is relatively independent of clinical status suggesting it can serve as a state-independent marker of risk for psychopathology, particularly depression (Gotlib, 1998). A recent study reported that relative right frontal EEG asymmetry was associated with elevated depression symptoms while watching either happy or sad film clips (Feng et al., 2012). However, some studies have found that massage therapy can attenuate the pattern of relatively greater right frontal activity seen in depressed adolescents (Jones & Field, 1999) and 1-month-old infants (Jones, Field, & Davalos, 1998). In general, EEG asymmetries demonstrate trait-like stability across time in depressed and non-depressed individuals and variations in frontal EEG typically do not vary with clinical state.

Frontal asymmetry may also be a bio-behavioral marker of individual differences in social motivation and emotional development in ASD. Dawson and colleagues (1995) identified children with ASD according to the Wing classification system (Wing & Gould, 1979) and measured resting EEG in these individuals. Researchers found that socially passive children demonstrated reduced delta and theta power while children classified by passive compared to children classified active but odd were distinguished by the power of the alpha band in the frontal region (Dawson, Klinger, Panagiotides, Lewy, & Castelloe, 1995). In general, comparison of children with autism and controls revealed reduced EEG power in the frontal and temporal regions, but not the parietal region in the autism group. One study of high functioning children with autism found that those children with left frontal asymmetry compared to those with right or intermediate frontal asymmetry, demonstrated fewer social impairments but higher levels of cognitive flexibility, emotional distress, and difficulty with interpersonal relationships (Sutton et al., 2005). A replication of this work in a high functioning ASD sample, found that children who displayed a pattern of left frontal EEG asymmetry tended to display milder levels of social symptoms (Burnette et al., 2011). This study also revealed that parents of children with left frontal asymmetry recalled that they initially became concerned about the development of their child at significantly later ages than did parents of children with right frontal asymmetry. Thus, frontal EEG asymmetry may be related to the course of early symptom presentation. These results suggest that EEG asymmetry may have the potential to be used as a biological marker of individual differences in ASD, aid in defining subgroups, and contribute to understanding the early course of ASD.

Collectively, the research examining the interaction of EEG and temperament suggests that EEG asymmetry is a useful psychophysiological measurement of temperament.

Specifically, given its relation to approach and withdrawal behavior it may prove fruitful in the search for early neurophysiological risk markers for ASD before behavioral indicators are apparent. These findings suggest that psychophysiological measures of temperament may provide important information about the early variability observed in high-risk infants and provide a direction for future investigation of biomarkers for ASD. The impact of such research could be critical to understanding the etiology and course of ASD, and can ultimately impact methods of early identification and intervention.

### **3. Temperament in ASD**

Temperament may provide a useful construct in understanding early developmental differences in children who go on to develop ASD. There is some overlap between the dimensions of temperament and behaviors that are seen in ASD including poor adaptation to novelty or change, early abnormalities in attention, behavioral reactivity, emotional regulation, and activity level (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000; Kasari & Sigman, 1997). These early abnormalities may influence early social interactions that can cause downstream consequences for social-emotional development. Thus, temperament can serve as a framework for examining the emergence of symptoms and trajectory of ASD.

#### **A. Group differences**

Recently researchers have begun to explore how temperament profiles can help in distinguishing individuals with ASD from typically developing and developmentally delayed populations. Most studies of temperament in ASD examine temperamental characteristics in middle childhood using parent report. Studies using the Child Behavior Questionnaire (Rothbart, 1996) have found that children with ASD are distinguished from matched comparison groups on the scales that make up the effortful control factor. Children with ASD are rated lower than

children with Down syndrome and typically developing children on effortful control, particularly with difficulties on focusing attention, shifting attention, and inhibiting prepotent responses (Konstantareas & Stewart, 2006; Landry, 2000). On the affectivity scales, children with ASD are rated by their parents as being less soothable, displaying more negative affect and withdrawal behaviors, and exhibiting less pleasure than children with Down syndrome and typically developing children (Landry, 2000). In addition, children with autism are rated as more active and more easily prone to anger and frustration than typically developing children (Landry, 2000).

Other studies of temperament in ASD have used the Behavior Style Questionnaire (BSQ; Carey & McDevitt, 1978). The BSQ has temperament domains that are conceptually similar to Rothbart's temperament dimensions. Kasari and Sigman (1997) found using the BSQ that parents rated their children with ASD as having more difficult temperaments than children with Down syndrome. Hepburn and Stone (2006) also found that children with ASD were lower on effortful control on the BSQ being rated by their parents as less adaptable, less persistent, and requiring more intense stimulation from the environment relative to parent report of temperament in typically developing children. Children with ASD and children with Fragile X are reported to be slower to adapt, less persistent, and more withdrawing than typically developing children (Bailey et al., 2000). When compared to a non-ASD group with Fragile X, children with ASD were rated as less distractible, less responsive to stimuli, and less emotionally intense (Bailey et al., 2000). A recent report found when compared to a developmentally delayed group, children with ASD were rated as showing less approach and less distractibility (Brock et al., 2012).

Bieberich and Morgan (1998) utilized an observation measure, the Minnesota Preschool Affect Rating, to observe temperament during a play session with the caregiver. Compared to children with Down syndrome, children with ASD scored lower on positive affect and higher on

negative affect. The largest group difference was on self-regulation which is strongly linked to effortful control. The children with ASD were less flexible and used fewer effective regulation strategies, especially in the control of attention. These children were followed up two years later and demonstrated consistent deficits in self-regulation suggesting high stability of this factor (Bieberich & Morgan, 2004).

Studies of adolescents and adults with ASD suggest that temperament characteristics are stable from childhood through adulthood. On a self-report measure, the Temperament and Character Inventory (TCI), adults with ASD scored lower on novelty seeking, harm avoidance, and reward dependence (Anckarsater et al., 2006; Soderstrom, Rastam, & Gillberg, 2002). Another study found that adolescents and adults scored higher on the social introversion scale and the depression scale of the MMPI-2 (Ozonoff, Garcia, Clark, & Lainhart, 2005). In addition, adults with autism may have lower effortful control/constraint exhibited by lower self-reported levels of self-directiveness on the TCI (Anckarsater et al., 2006; Soderstrom et al., 2002) and increased DSM symptoms of inattention (Bradley & Isaacs, 2006).

Overall, the examination of temperament in ASD strongly suggests deficits in effortful control such as difficulties focusing and shifting attention and inhibiting a prepotent response. There is some evidence that children with ASD tend to show lower positive affect and higher negative affect. Some of the variability in findings of the affect scales may be due to discrepancies between observation and parental report methods.

### **B. Individual variation**

Individual variation in temperament may be informative in understanding the behavioral variability commonly seen in children with ASD. Kasari and Sigman (1997) found that children with ASD who were rated by their parents as more difficult displayed less engagement and

responsiveness during social interaction with their parent and examiner. This reduced responsiveness suggests a relation between temperament and social skills in individuals with ASD. Aspects of temperament such as increased surgency (the ability to gain pleasure from high intensity activities) and effortful control (ability to shift attention and inhibit inappropriate behavior) were found to be predictive of adaptive outcomes for individuals with ASD (Schwartz et al., 2009).

Temperament may also be useful in examining the broader ASD phenotype. Relatives of children with ASD have also been found to score lower on measures associated with executive function and effortful control and higher on scales associated with negative affectivity (Murphy et al., 2000; Ozonoff, Rogers, Farnham, & Pennington, 1993). Collectively, these studies suggest temperament measures may be helpful in understanding individual differences in symptom presentation, social skills, and psychopathology in ASD and might inform how to individualize treatments leading to more adaptive outcomes for individuals with ASD.

### **C. Retrospective reports**

When asked about their initial concerns regarding their child with autism, at least 30-50% of parents recall abnormalities during the first year of life including extremes of temperament ranging from extreme passivity to intense irritability (Gillberg et al., 1990; Hoshino et al., 1987). Retrospective parent reports of children diagnosed with ASD suggest that, by the first year of life, these children had more difficulty with self-regulation, displayed higher negative emotional reactivity, and low motivation to interact with others compared to typically developing children and children with developmental delays (Gomez & Baird, 2005; Watson et al., 2007). Thus, attention to self-regulation difficulties may be important in the identification of ASD.

#### **D. Temperament in high-risk infant siblings of children with ASD**

Until recently, research exploring early temperament characteristics in ASD has been mostly limited to parent report that is subject to recall error and bias. Prospective studies allow for a fuller picture of the early behavioral profiles and developmental trajectories that may distinguish young children with ASD. A prospective study of infant siblings of children with ASD found that infants who were later diagnosed with ASD displayed higher levels of passivity and decreased activity at 6 months but were not found to be more temperamentally difficult than either high-risk infants who did not develop ASD or low-risk infants at 6 months of age (Zwaigenbaum et al., 2005). However, by 12 months of age, temperament differences became more apparent such that the high-risk infants who later developed ASD showed more frequent and intense distress reactions to a variety of stimuli, spent more time fixating on objects, and had a decreased expression of positive affect than the group who did not develop ASD. At 24 months, these siblings who developed ASD were reported to have less attention shifting, less inhibitory control, and less positive anticipation and affective responses relative to other high-risk siblings and low-risk siblings who did not develop ASD (Zwaigenbaum et al., 2005).

A follow-up analysis of a subgroup of these infants who developed ASD examined early behavioral and temperamental profiles in these children (Bryson et al., 2007). One subgroup of infants was distinguished by a marked change early in development that persisted and was more evident by 18 months. Between 6 and 12 months of age, these infants became more difficult to engage socially, expressed little pleasure in interacting with others, displayed a lack of interest and exploration of toys, and increased visual fixation. All of the children who developed ASD showed a distinct temperament profile that was characterized by marked irritability, intolerance of intrusions, proneness to distress/negative affect, and difficulties with self-regulation and with

being comforted or settled by others. However, it is unclear whether this temperamental profile is associated with or preceded by the emergence of ASD symptoms. Some infants were more passive and content at 6 months and with the onset of symptoms changed from being “easy” to being readily irritated and distressed and difficult to console. In other infants, there was some indication that they had more difficult temperaments at 6 months of age and that these difficulties became more striking with the emergence of ASD symptoms. These infants showed prolonged visual fixation and early irritability and proneness to distress that the authors suggest may be early indicators of behavioral inflexibility that may be consistent with executive dysfunction deficits seen in ASD.

This same sample of children were followed until 36 months of age to determine whether particular temperamental traits at 24 months predicted ASD at 36 months, examine the heterogeneity in ASD, explore the association between symptom severity and temperamental traits, and determine if subgroups could be derived from specific temperamental profiles (Garon et al., 2009). Researchers utilized a parent report measure to assess temperament, the Toddler Behavior Assessment Questionnaire-Revised (TBAQ-R; Rothbart, Ellis, Rueda, & Posner, 2003), to assess activity level, anger/frustration, positive anticipation, and social fear. The temperamental profile of low positive anticipation, high activity level, and low attention shifting (labeled “behavioral approach”) at 24 months was associated with a diagnosis of ASD at 36 months while a temperamental profile of low positive affect, poor regulation of negative emotions and difficulty with attention control (labeled “effortful emotion regulation”) at 24 months distinguished high-risk infants from low-risk infants at 36 months (Garon et al., 2009). The results also indicate that temperament provides unique information relating to a diagnosis of ASD beyond what is accounted for by IQ and ASD symptoms at 24 months. The behavioral

approach function best distinguished the ASD group from the other two groups. In general, individuals higher on behavioral approach tend to be rated higher on activity level; however, in this sample, the children diagnosed with ASD had high motor activity with low positive anticipation suggesting a dissociation between affect and motor activity in ASD. The effortful emotion regulation function differs in that children may show approach behavior but have difficulty regulating behavior, which supports previous studies of parent and observational measures in ASD. Interestingly, the non-ASD high-risk group actually had higher levels of behavioral approach than the control group. The authors propose that high approach tendencies may act as a protective factor for infants at risk of developing ASD. The authors also purport that these two functions may play different roles in differentiating groups. For instance, both behavioral approach and effortful emotion regulation are useful in distinguish ASD high-risk siblings from controls; while only the behavioral approach function was needed to distinguish ASD high-risk siblings from non-ASD high-risk siblings.

These research studies also suggest that there may be a change in temperament profiles in development. Bolton et al. (2012) found decreased activity at younger ages changing to increased activity later. The results suggest that specific temperamental factors and profiles may change between 6 and 24 months of age. This may occur due to the onset of ASD symptoms and related developmental changes.

All of the prospective infant sibling work has been conducted with parent report measures which as already discussed, is subject to report biases. Parents of children in the high-risk group may be more sensitive to any subtle difficulties and differences in their infants since they may be looking for any early indicator of ASD. Alternatively, the opposite argument could be made that parents may anchor their knowledge of development to their older child with ASD

and thus may under-report any potential difficulties seen in their younger infant. Garon and colleagues (2009) found higher behavioral approach in the non-ASD high-risk group control group compared to the low-risk infants. In addition to the proposal that behavioral approach serves as a protective factor for these infants, another potential explanation for this result is that parents may have inflated ratings by using their child with ASD as a point of comparison. Furthermore, parent report of temperament may change over time based on their knowledge of ASD symptoms. For instance, how a parent interprets their infant's temperament may change depending on their heightened awareness of ASD symptoms (e.g., possible coincidence of birth of a new child with the older sibling's diagnosis). Thus, it is necessary to verify if these temperamental profiles exist in high-risk infants by using direct observational measures of temperament. Examining temperament using multiple methods at multiple time points will help clarify the stability of temperament in this high-risk group.

#### **4. Summary**

ASD is a highly heterogeneous disorder with multiple factors influencing the development, stability, and behavioral phenotype of the disorder. One of the most promising factors in explaining the variability seen in ASD and its early symptoms is temperament. Studies of temperament in ASD have consistently found difficulties with self-regulation, low motivation for social interaction, and higher negative emotional reactivity and negative affect. Retrospective and prospective reports of infants who develop ASD suggest that temperamental characteristics such as increased irritability, intense distress reactions/negative affect, and difficulties with regulations are present in the first year of life and often precede the emergence of ASD symptoms. Specifically, behavioral approach and extraversion appear to be the best discriminators of ASD from other developmental disorders. In addition, temperament profiles

have been shown to provide unique variance to the prediction of ASD diagnosis not accounted for by IQ and social symptoms.

However, it remains unclear the relationship between the temperament profile seen in ASD and the emergence of symptoms. Thus far, most of the research has utilized parent report. Particularly for infant siblings of ASD, parent's rating may be influenced by their older child's behavior, their knowledge of ASD symptoms, and parental characteristics (e.g., depression). Thus, the inclusion of other types of measurement such as observational and electrophysiological measures, are necessary to replicate the early temperamental differences identified in ASD.

Identifying early temperamental characteristics in high-risk infants will provide further information regarding the interaction between these factors and early risk signs in the manifestation and development of ASD symptoms. It is still unknown whether these temperament factors are early signs that predict the onset of ASD or if they may represent endophenotypes that are present in the broader phenotype. The onset of ASD symptoms may lead to changes in temperament or potentially another unknown variable mediating the association (Garon et al., 2009). Another possibility is that similar biological systems are involved in temperament and the behavioral phenotype. The use of multiple methods of temperament across the time period when behavior risk signs are usually first apparent will aid in our understanding of this question. Additionally, since ASD is a highly genetic disorder, we can assume that early brain differences are present in the first year of life and are detectable before behavioral markers of ASD are apparent. Measures of EEG asymmetry may provide earlier identification methods even before these behavioral characteristics are identified. In addition, the relationship between temperament and underlying brain systems may provide clues into the development of ASD.

## 5. The Current Project

The goal of the current study was to examine early temperament characteristics in infants at risk for autism to determine if differences in early temperament are predictive of the development of autism symptoms. Infant siblings of children with ASD were the target population given their increased genetic risk for the development of ASD. High-risk infants and low-risk infants who had no relatives in the immediate family affected by ASD were assessed in the first year of life (at both 6 and 12 months of age). This project took a novel approach of examining parent report, observational, and electrophysiological measures of temperament as early measures of temperament.

Given the interest in early ASD symptoms, these infants were assessed at 18 and 24 months for ASD. Infants who developed ASD as well as high-risk infants and low-risk infants who did not develop ASD ( $HR_{noASD}$  and  $LR_{noASD}$ ) were examined in order to determine if there are any early temperamental differences between groups at 6 and 12 months of age on parent report, observation, and electrophysiological measures of temperament.

The assessment of temperamental traits via multiple formats allows for multi-informants (expert raters and parent report) across various settings/observations. This allows for the examination of the convergence of these measures. Given that parents of children with ASD may anchor their impressions of their infant to their older child, it is informative to compare their ratings with observational reports. Thus, a secondary goal was to examine the convergence of parent and observational report of temperament measures among high-risk and low-risk infants. Given that the goal was to determine if having an older child affected parent's perception, these convergence and stability analyses were conducted by sibling status (HR and LR) rather than diagnostic status (ASD,  $HR_{noASD}$ , and  $LR_{noASD}$ ).

The addition of an electrophysiological measure of EEG may serve as a bio-behavioral marker of temperament. It is advantageous to gather behavioral and biological signs of temperament to understand physiological correlates and behavioral manifestations of temperament. Elucidating both behavioral and physiological characteristics associated with ASD symptoms may facilitate early detection efforts. It may also contribute to our understanding of the variability of symptom presentation that can eventually inform our understanding of the development of ASD and aid in the development of treatments for ASD. The specific aims were as follows:

**A. Aim 1. To examine parent and observation measures of temperament at 6 and 12 months of age in infants who develop ASD, high-risk infants who do not develop ASD, and low-risk infants who do not develop ASD.**

High-risk in comparison to low-risk infants have been found to show more difficulty with regulation, assessed via parent report at 1 year of age (Gomez & Baird, 2005; Watson et al., 2007). Aspects of temperament, specifically decreased activity levels, decreased expression of positive affect, extreme distress reactions, and a tendency to fixate on particular objects in the environment have also been related to ASD risk symptoms by parent report (Zwaigenbaum et al., 2005; Bryson et al., 2007; Wai Wan et al., 2012). No study thus far has utilized standardized observational measures of temperament in infants at risk for ASD. Given parent and observational measures have only modest to moderate convergence (Majdankdzic et al., 2008); it is important to determine if there are observational reports of early temperamental differences in ASD. This project is the first known study to test if ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub> infants differ on *both* parent and observation measures of temperament at 6 and 12 months of age.

Given possible contrast effects for parent report of temperament, convergence of parent report and observation of temperament measures was assessed by infant sibling group status (HR and LR) at 6 and 12 months. To determine if there were differences in parent and observational report by infant sibling group status (HR and LR) over time, stability of temperament was assessed from 6 to 12 months.

**B. Aim 2. To examine electrophysiological (EEG) measures of temperament at 6 and 12 months of age in infants who develop ASD, high-risk infants who do not develop ASD, and low-risk infants who do not develop ASD.**

Fox and colleagues (2001) have suggested that the pattern of resting frontal EEG reflects individual differences in affective temperament styles in infants. For example, infants with right frontal EEG asymmetry tend to be more withdrawn and express negative affect whereas infants with left frontal EEG asymmetry tend to be more approach oriented and express positive affect (Fox et al., 2001). EEG measures can provide information about heterogeneity in ASD. Sutton et al. (2005) found that high functioning children with ASD with left asymmetry (higher approach) had fewer social problems, more cognitive flexibility, and increased social awareness. EEG power in the frontal region has also discriminated socially passive children with ASD (Dawson et al., 1998). Thus, this project assessed frontal EEG asymmetry and EEG power in ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub> infants at 6 to 12 months to determine if there were group differences.

Given relationships between temperament and EEG, a secondary goal was to examine the relationship between parent and observational report of temperament and EEG. We wanted to assess the effect of having an older child with ASD on parent report, so these comparisons were conducted on HR and LR without regard for ASD outcome status.

## CHAPTER 2

### METHODS

#### 1. Participants

Participants were recruited from the NIH-funded University of Washington Autism Center of Excellence Early Connections Project. The Early Connections project had two broad goals: (1) to identify neurophysiological risk indices for ASD; and (2) to assess whether it is possible to alter hypothesized risk processes via early intervention with high-risk infants, thereby reducing severity of autism symptoms. Specifically, the larger project investigated the efficacy of an early intervention that is designed to enhance early social responsiveness and communication in infants who are at risk for autism. In the Early Connections project, high-risk and low-risk infant participants were assessed at 6, 12, 18, and 24 months. The current study leveraged the structure of the Early Connections study, allowing for the use of the core diagnostic measures that were being collected as part of the large project to complete the specific aims of this project. The University of Washington Institutional Review Board approved the study.

#### A. Recruitment strategies

Recruitment took place through the University of Washington Autism Center. As part of the NIH funded projects, a network of recruitment sites including Birth-to-Three centers, local pediatric practices, speech therapy offices, occupational therapy offices, medical clinics, children's therapy units, and parent advocacy groups was successfully established. In addition to a local network of medical practices and parent advocacy groups that facilitate family contact with the University of Washington Autism Center on a regular basis, infants were recruited through the University of Washington Autism Center Registry, which contains a list of families

who have previously participated or who have contacted the University of Washington Autism Center and expressed interest in participating in a research study; individuals receiving clinical services at University of Washington Autism Center; and through flyers and newsletters in the local community.

### **B. Inclusion and exclusion criteria**

Inclusion criteria for high-risk infant siblings included age ( $\leq 6$  months), presence of autism in a non-adopted older sib, anticipated residence in the greater Seattle area (within 1.5 hours driving distance from the UW) for the next 2 years, and parents' willingness to participate in a 10-week early intervention program. If infants were recruited after 6 months of age, these infants were included at the 12-month time point. As part of the initial screening process, the Autism Diagnostic Interview-Revised (ADI-R) was administered by phone to ensure that the older sibling met criteria for ASD. In addition, medical records were collected to confirm that the older sibling had received a diagnosis of ASD based on DSM-IV criteria from a psychologist or physician. If medical records indicated that the older sibling had an identifiable genetic disorder or known genetic syndrome associated with ASD, that family was excluded.

Inclusion criteria for the low-risk infant siblings included age ( $\leq 6$  months), a non-adopted older sib without a diagnosis of ASD or language impairment, and anticipated residence in the greater Seattle area.

Given the aim of examining infants at genetic risk for ASD, adoption was an exclusionary criterion. Additional exclusion criteria for the all infant siblings included variables that impact may impact developmental functioning such as diagnosis or physical signs (e.g., dysmorphic features) of known genetic syndromes, serious medical or neurological conditions (e.g., encephalitis, concussion, seizure disorder, diabetes, congenital heart disease),

neurocutaneous markings, or sensory impairments such as vision or hearing loss; serious motor impairment; birth weight < 2000 grams and/or gestational age < 37 wks, history of intraventricular hemorrhage, exposure to neurotoxins (including alcohol, drugs), and gestational diabetes. In addition, variables that may impact family functioning (e.g., serious parental substance abuse, bipolar disorder, or psychosis) or affect participation in the study such as non-English speaking parents were exclusion criteria. Additional exclusionary criteria for non-risk infant siblings included a known family history of ASD in 1<sup>st</sup> or 2<sup>nd</sup> degree relatives.

### **C. Final sample and demographics**

Participants included 43 infants (males  $n = 28$ ) who were full-biological siblings of children with autism (high-risk infant siblings) and their parents and 45 infant siblings of children who do not have an older sibling with ASD or language impairment (low-risk infant siblings) (males  $n = 26$ ) and their parents. The high-risk group will be referred to as the “HR” group while the low-risk group will be referred to as the “LR” group. The sample consisted of 71 Caucasian participants, 5 Asian participants, and 12 participants of more than one race. Race was evenly divided between HR and LR infants.

In order to maximize power in the sample, analyses were conducted on all available infant data at each time point so there were different participant numbers for each group at each time point (see Tables 2 and 3). Nine participants (5 LR and 4 HR) withdrew prior to the 12 month timepoint and four HR infants and two LR infants were enrolled at 12 months. Two LR families declined to complete the 12 month measures. At 18 months, one LR infant’s family withdrew from the study, one HR infant’s family declined to participate, and one HR family moved and was unable to complete the visit.

### *Diagnostic groups*

Three diagnostic groups were formed based on sibling status and ASD diagnostic outcome at 18 and 24 months. These groups included 1) low-risk infants who did not develop ASD (LR<sub>noASD</sub>) (n = 44), 2) high-risk infants who did not develop ASD (HR<sub>noASD</sub>) (n = 33), and 3) an ASD group (n = 11). The “ASD” group was classified by infants who received a diagnosis of “Autism” or “Autism Spectrum” on the Autism Diagnostic Observation Schedule (ADOS) or DSM-IV clinical diagnosis of “Autism” or “PDD-NOS” at *both* 18 and 24. The ASD group consisted of 11 infants (10 HR and 1 LR) who developed ASD.

### *Demographics*

Demographic information such as gender, race, and age was analyzed using Analysis of Variance (ANOVA) for all three groups (LR<sub>noASD</sub>, HR<sub>noASD</sub>, and ASD). In addition, an infant measure of autism symptoms (the Autism Observation Scale for Infants (AOSI)) and an infant measure of cognition (Mullen) was analyzed using ANOVA to determine if there were group differences at 6, 12, and 18 months. The Mullen contains 5 domains (Visual Reception, Fine Motor, Expressive Language, Receptive Language, and Gross Motor). Verbal score is the sum of the Receptive Language and Expressive Language T-scores and Non-verbal score is the sum of the Visual Reception and Fine Motor T-scores. The Composite score is the standardized score of the sum of the Verbal and Non-verbal scores. Post-hoc analyses were conducted to determine significant difference between groups.

There were no differences between groups on gender, race, or age (see Tables 2 and 3). The results of the cognitive (Mullen) and early autism symptom measure (AOSI) by ASD outcome reveal some interesting findings (see Table 3). There was a significant difference

between groups for the total AOSI score at 6 months, ( $F(2, 75) = 4.04, p = .02$ ). Post-hoc analyses reveal that infants who later developed ASD scored higher on the AOSI (suggestive of increased early risk concerns) than LR<sub>noASD</sub> infants ( $p = .02$ ) and HR<sub>noASD</sub> infants ( $p = .01$ ). At 12 months, there was a trend for a difference between groups on total AOSI score, ( $F(2, 79) = 2.75, p = .07$ ). Post-hoc analyses reveal that ASD infants displayed higher AOSI scores at 12 months compared to LR<sub>noASD</sub> infants ( $p = .02$ ) and a trend for ASD infants to score higher than HR<sub>noASD</sub> infants ( $p = .06$ ).

At 12 months, there was a significant difference between groups on Verbal, ( $F(2, 79) = 4.28, p = .02$ ), Non-verbal, ( $F(2, 79) = 6.30, p < .01$ ), and Composite ( $F(2, 79) = 7.70, p < .01$ ) scores of an infant cognitive measure (Mullen). Post-hoc analyses reveal at 12 months, infants who later developed ASD displayed lower scores on the Verbal score than LR<sub>noASD</sub> infants ( $p = .02$ ) and HR<sub>noASD</sub> infants ( $p = .01$ ); displayed lower scores on the Non-verbal score than LR<sub>noASD</sub> infants ( $p = .001$ ) and HR<sub>noASD</sub> infants ( $p = .03$ ); and lower scores on the Composite score than LR<sub>noASD</sub> infants ( $p < .001$ ) and HR<sub>noASD</sub> infants ( $p = .01$ ).

At 18 months, there was a significant difference between groups for ADOS-T social-affect total score, ( $F(2, 76) = 48.74, p < .001$ ), Verbal score, ( $F(2, 75) = 7.95, p = .001$ ), Non-verbal score, ( $F(2, 75) = 8.89, p < .001$ ), and Composite score, ( $F(2, 75) = 12.48, p < .001$ ). Post-hoc analyses reveal that infants who later developed ASD exhibited higher ADOS-T scores compared to LR<sub>noASD</sub> infants ( $p < .001$ ) and HR<sub>noASD</sub> infants ( $p < .001$ ). Similar to the 12 month results, at 18 months, the ASD group also exhibited lower scores on the Verbal score than the LR<sub>noASD</sub> infants ( $p = .001$ ) and HR<sub>noASD</sub> infants ( $p < .001$ ); lower scores on the Non-verbal score

than the LR<sub>noASD</sub> infants ( $p < .001$ ) and HR<sub>noASD</sub> infants ( $p < .001$ ); and lower scores on the Composite score than LR<sub>noASD</sub> infants ( $p < .001$ ) and HR<sub>noASD</sub> infants ( $p < .001$ ).

Table 2. Gender and race of participants

	LR <sub>noASD</sub> (n=44)	HR <sub>noASD</sub> (n=33)	ASD (n=11)	p-value
Gender	Male=25 Female=19	Male=24 Female=9	Male=5 Female=6	NS
Race	White: n=36 Asian: n=3 More than one race n=5	White: n=28 Asian: n=1 More than one race n=4	White: n=7 Asian: n=1 More than one race n=3	NS

Table 3. Sample characteristics (cognitive and early symptom measures)

	LR <sub>noASD</sub>		HR <sub>noASD</sub>		ASD		p-value	Post-hoc significant comparisons
	M	Range (SD)	M	Range (SD)	M	Range (SD)		
<b>6 month</b>	n = 44		n = 33		n = 10			
Age	6.09	6-8 (.37)	6.17	6-9 (.60)	6.17	6-9 (.60)	.54	
Verbal	94.69	50-141 (14.88)	91.21	60-118 (12.31)	94.00	75-99 (6.38)	NS	
Non-verbal	98.76	53-126 (16.15)	99.28	73-136 (13.23)	96.90	85-109 (6.58)	NS	
Composite	96.76	51-122 (13.23)	95.24	75-118 (10.84)	94.00	83-104 (6.38)	NS	
AOSI score	8.55	2-22 (4.2)	8.96	2-17 (3.41)	12.70	6-24 (5.85)	.02*	ASD>LR <sub>noASD</sub> * ASD>HR <sub>noASD</sub> *
<b>12 month</b>	n = 39		n = 32		n = 11			
Age	12.08	11-14 (.62)	12.06	11-13 (.44)	12.27	12-15 (.91)	.59	
Verbal	94.62	73-127 (13.57)	92.97	69-127 (14.64)	80.91	59-96 (12.52)	.02*	ASD<LR <sub>noASD</sub> * ASD<HR <sub>noASD</sub> *
Non-verbal	120.95	91-146 (12.34)	115.75	90-136 (11.92)	105.82	81-132 (15.94)	.003**	ASD<LR <sub>noASD</sub> ** ASD<HR <sub>noASD</sub> *
Composite	107.92	87-136 (11.05)	104.38	84-132 (11.03)	93.18	70-107 (10.75)	.001**	ASD<LR <sub>noASD</sub> ** ASD<HR <sub>noASD</sub> *
AOSI score	5.23	0-21 (4.62)	4.59	0-16 (3.98)	8.09	1-15 (4.23)	.07 <sup>†</sup>	ASD>LR <sub>noASD</sub> * ASD>HR <sub>noASD</sub> <sup>†</sup>
<b>18 month</b>	n = 38		n = 30		n = 11			
Age	18.11	17-20 (.57)	18.06	17-20 (.44)	17.90	15-19 (1.10)	.64	
Verbal	97.44	61-130 (18.38)	101.77	75-134 (17.27)	74.6	54-125 (24.78)	.001**	ASD<LR <sub>noASD</sub> ** ASD<HR <sub>noASD</sub> **
Non-verbal	106.82	79-136 (11.99)	107.06	92-136 (10.34)	90.8	78-110 (11.31)	<.001**	ASD<LR <sub>noASD</sub> ** ASD<HR <sub>noASD</sub> **
Composite	102.07	77-122 (13.12)	104.36	88-134 (11.13)	81.9	65-111 (15.21)	<.001**	ASD<LR <sub>noASD</sub> ** ASD<HR <sub>noASD</sub> **
ADOS-T social-affect total	3.13	0-8 (2.57)	4.61	0-11 (3.00)	13.2	6-19 (4.37)	<.001**	ASD>LR <sub>noASD</sub> ** ASD>HR <sub>noASD</sub> **

Note: †  $p \leq .1$ , \* $p < .05$ , \*\* $p < .01$ .

Note: Verbal = Receptive Language + Expressive Language T-scores. The Non-verbal = Visual Reception + Fine Motor T-scores. Composite score = Average of the Non-verbal and Verbal scores.

## **2. Measures**

### **A. Measures of autism symptoms and cognition**

#### *6 and 12 month measures*

As part of the Early Connections project, at both the 6 and 12-month visits, measures of autism symptoms and cognition were undertaken. These measures included the Mullen Scales of Early Learning (Mullen, 1997), which is a standardized measure of cognitive functioning for children and assesses skills in five areas: Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language and the Autism Observation Scale for Infants (AOSI: Bryson et al., 2008), which is an 18-item direct observational measure designed to detect and monitor early signs of autism in infants aged 6-18 months. During the AOSI, infants are engaged in a semi-structured play, and systematic presses are designed to assess various target behaviors, including visual tracking and attention disengagement, coordination of eye gaze and action, imitation, affective responses, early social-communicative behaviors, behavioral reactivity, and sensory-motor development. Each behavior is rated on a scale from 0 to 2 or 3, where 0 suggests typical function, and higher values indicate increasing deviation. Total score of the 18-item measure can range from 0 to 50 where higher scores indicate more severity (Bryson et al., 2008). Total elevated scores of 7 or more at 12 months have been found to be predictive of an ASD diagnosis at 3 years of age (Zwaigenbaum et al., 2005).

#### *18 and 24 month measures*

At 18 months of age, infants were administered the Mullen and assessed for ASD symptoms via the Autism Diagnostic Observation Schedule – Toddler Module (ADOS-T; Luyster et al., 2009). This is a play-based observation scale administered by a trained research reliable examiner. The ADOS-T is designed to assess social-communicative behaviors

characteristic of autism in children 12-36 months. Infants are engaged in a semi-structured play with developmentally appropriate activities designed to elicit early social-communicative behaviors, language and communication, play and stereotyped/restricted behaviors or interests. The ADOS-T yields an algorithm with two classifications: ASD or non-spectrum.

At 24 months of age, infants were administered the Mullen and assessed for ASD symptoms via the ADOS (Lord et al., 2000). The ADOS is a play-based assessment intended to directly assess ASD symptoms through a series of age appropriate activities designed to elicit social interactions. The ADOS takes approximately 45 minutes to complete. There are four modules that differ in terms of required language ability and age of the individual. Updated algorithms contain a subtotal for codes relating to social communication skills (ADOS Social Affect) and restricted/repetitive behaviors and interests (ADOS Restricted and Repetitive Behavior) as well as a total score summing these domains (ADOS Total). The ADOS classifies children as “Autism”, “Autism Spectrum”, or “non-spectrum” depending on algorithm cut-off values.

At 18 and 24 months of age, a clinical diagnosis was given as defined in the DSM-IV (American Psychiatric Association, 1994) that was based on all available information obtained through the ADOS, cognitive testing, and any other clinical experiences with the infants. Based on this information, infants were classified as having “Autistic Disorder”, “Pervasive Developmental Disorder- Not Otherwise Specified” or “no diagnosis”.

## **B. Temperament measures**

### *Parent measure of temperament*

As part of the Early Connections Project, the Infant Behavior Questionnaire – Revised (IBQ-R; Gartstein & Rothbart, 2003) was administered to high-risk infants and low-risk infants

at 6 and 12 months. 78 participants (40 LR and 38 HR) completed the IBQ-R at 6 months; 69 participants (34 LR and 35 HR) completed the IBQ-R at 12 months. Three parents of LR infants declined to complete 6 month questionnaires and 7 LR and 7 HR participants declined to complete 12 month questionnaires. The IBQ-R is a 191 item parent-report questionnaire assessing 14 temperament domains: Activity Level, Distress to Limitations, Approach, Fear, Duration of Orienting, Smiling and Laughter, Vocal Reactivity, Sadness, Perceptual Sensitivity, High Intensity Pleasure, Low Intensity Pleasure, Cuddliness, Soothability, and Vocal Reactivity (see Table 1). It is designed to assess infant temperament between the ages of three and twelve months. Mothers rated the frequency of infant behaviors on a scale from 1 (never) to 7 (always). Each domain has a scale score (range from 1 to 7) which is the average of the items comprising the scale. A higher sub-scale score indicates higher levels of that domain (e.g., higher activity level reflects an infant who squirms and moves his/her limbs often and high distress to limitations indicates an infant who cries and fusses often). Internal reliability has been demonstrated to be good for each of the subscales (Gartstein & Rothbart, 2003; Parade & Leerkes, 2008).

#### *Observational measure of temperament*

Each infant's temperament was coded from videotaped interactions during two behavioral tasks in order to assess temperament across different types of behavioral tasks. These behavioral measures were administered to high-risk and low-risk infant siblings as part of the Early Connections project at 6 and 12 months. Videos of the Mullen Scales of Early Learning and the AOSI were coded for observational measures of temperament. These two behavioral tasks were chosen since it would allow for coding of temperament across a cognitive demand task (Mullen) and a social engagement task (AOSI). Both of these standardized measures were

completed at 6 and 12 months with the same examiner administering the Mullen and AOSI. Domains of interest such as social engagement, positive and negative affect, activity level, frustration (e.g., toys taken away), and soothability were observable during these two behavioral tasks. However, it is important to note that given temperament was coded from these cognitive and early symptoms measures and from the same session, observational coding of temperament was not truly independent from cognitive and symptom measures.

The Behavior Rating Scale (BRS) was adapted to assess infant behavior during the Mullen and AOSI. The Behavior Rating Scale is the second revision based on the Infant Behavioral Rating Scale (IBR) (Bayley, 1969) which was originally designed to assess infant behavior after completion of the Bayley, an infant cognitive assessment. It has since been used in lab and home visits as a temperament assessment reflecting such dimensions as social approach, affect, task orientation, and activity level (Stifter & Corey, 2001; Stifter et al., 2008; Goldsmith & Gottesman, 1981; Matheny, 1983). An advantage of the BRS is that it requires minimal training and takes into account the infant across a variety of contexts thus mimicking parent ratings of temperament.

For the purpose of this study, the BRS was modified to include objectively defined behaviors and a coding manual was developed. This coding manual is included in Appendix A. Behavioral coding was conducted separately for each the Mullen and AOSI at each time point. At 6 months, 40 LR and 36 HR infants had validly coded data. Three infants LR and two HR with valid Mullen and AOSI data at 6 months did not have a valid video for coders to complete coding (e.g., not videotaped). At 12 months, 40 LR and 42 HR had validly coded data.

Coding was conducted on selected items from the Mullen and AOSI to assess domains of interest including: Energy Level, Social Engagement, Positive Affect, Soothability, and

Frustration. Rather than using the BRS, which is a gestalt coding system, the current coding system was anchored to specific tasks to facilitate operationally defined coding and increase the inter-rating reliability process (e.g., coding for pre-identified tasks allowed for coding and communication about specific opportunities for observable behaviors). Mullen tasks selected were non-verbal (e.g., visual reception and fine motor tasks) and tasks that all infants would engage in despite their developmental level. Mullen items were selected to examine specific areas of interest such as frustration, soothability, and energy level. Example of Mullen items included the infant finding a ring under a washcloth and picking up a cheerio. AOSI items coded included “peek-a-boo”, “imitation”, and “ball play” in order to assess social engagement and positive affect. Each item was coded on a 5-point Likert-like scale. Higher scores reflect increased examples of that behavior. For example, positive affect was defined as “the degree to which the infant displays positive affect in response to either the test materials or to the examiner and caregiver”. Positive affect includes smiling, laughing, or making sounds that are perceived to be expressions of excitement, happiness or pleasure. The ratings refer to both duration (amount of time) and intensity of affect (e.g., mild, moderate, high). Positive affect was coded as 1: Very low = no positive affect, 2: Low = one or two brief or mild displays (< 2 seconds), 3: Moderate = three or more brief or mild displays, 4: High = one or two prolonged (>2 seconds) or intense display of positive affect (e.g. laughing), and 5: Very High = three or more prolonged (>2 seconds) or intense display of positive affect (e.g., laughing). Thus, higher scores on positive affect indicated more instances and more intense displays of positive affect. Similarly, higher scores on negative affect reflected more instances and more intense displays of negative affect.

All coding was conducted by coders who were naïve with respect to group status (high-risk versus low-risk). Behavioral coders consisted of five undergraduate students who

participated for research course credit. Behavioral coders underwent extensive training including observing example tapes with “gold standard” codes completed by the study investigator and were required to establish 80% reliability with example tapes before coding experimental videos. At the start of training, all coders coded the same 5 subjects and met weekly to determine consensus. Weekly “coding meetings” were held to discuss behavioral coding and in which all coders coded the same tape and determined consensus. Nearly fifty percent of all 6 and 12 month coding (70 of 158) was double-coded between coders for reliability. Overall Kappa was .67 for the 6 month time point and .72 for the 12 month time point. Ongoing reliability was monitored by the study investigator. If Kappa was below .70 between two coders, those coders re-coded through consensus coding (e.g., discussed the items in disagreement and reached a resolution). This often included discussion with the study investigator. If the two coders were unable to meet to determine consensus, a third coder coded the same participant and the final data used were the agreement codes between two of the three coders.

#### *Electrophysiological measure*

As part of the Early Connections project, EEG was recorded from high-risk and low-risk infants at 6 and 12 months. 42 LR and 36 HR were tested at 6 months; 38 LR and 39 HR were tested at 12 months. At 6 months, 3 subjects (2 HR, 1 LR) were excluded due to excessive artifact and 1 LR subject’s EEG file was corrupt. At 12 months, 2 HR infants’ data were excluded due to excessive artifact, 3 LR infants refused to wear the net, and 1 LR subject’s EEG file was corrupt.

EEG was recorded continuously throughout the session, with a concurrent video record of the infant’s behavior time-locked to the EEG record. EEG was collected while infants watch a video of social stimuli (women telling nursery rhymes) and non-social stimuli (dynamic toys). A

similar paradigm has been used in other infant studies of EEG temperament (Hane & Fox, 2006; Marshall, Bar-Haim, & Fox, 2002). EEG was recorded from 128 electrodes using the Geodesic Sensor Net (Electrical Geodesics, OR), on Net Station 4.3 data acquisition software. A 128 lead Geodesic sensor net was dipped into KCl electrolyte solution and placed on the infant's head and fitted. Electrodes were arranged to symmetrically cover the scalp from nasion to inion and left to right ears. Impedances were typically  $< 50$  kOhm. EEG was recorded with reference to the vertex (Cz) electrode, amplified, and analog filtered (elliptical) between .1 and 100 Hz. Signals were digitized at 250 samples/second. Recordings were carried out in an electrically shielded, sound attenuated booth. During testing, the infants were monitored for eye and head movements.

To assess EEG asymmetry, EEG data were analyzed for spectral power using Matlab. Our lab has used this method successfully to examine EEG asymmetry in this age group (Jones et al., 2010). EEG data were filtered to remove high frequency noise. EEG data were manually edited to remove segments with artifact due to eye movements or motion, and to reject electrodes with a preponderance of noise resulting from poor electrode contact with the scalp. An amplitude threshold criterion was applied to reject electrodes that exceeded  $\pm 200$  microvolts during a trial, and if any given electrode was rejected in more than 25% of trials, it was eliminated entirely for that participant. Averaged power spectra for each subject were visually inspected at all electrodes for artifact contamination. The mean number of trials, standard deviation, and range for each condition by time-point are presented in Table 4.

Table 4. Mean, SD, and range of EEG trials extracted per condition by time-point

	6 month EEG trials		12 month EEG trials	
	Social	Non-Social	Social	Non-Social
Mean (SD)	81.30 (37.12)	92.77 (37.12)	132.75 (53.85)	127.25 (48.47)
Range	16 - 207	16 - 223	37 - 247	37 - 247

At each time point, the potentials at each electrode were re-referenced to the instantaneous average of all electrodes, yielding average referenced potentials. In order to calculate the frontal power indices, natural log (ln) 6- to 9-Hz alpha power data from the midfrontal (approximate electrode locations F3/F4) were used. Frontal asymmetry was computed as power in the right hemisphere minus power in the left hemisphere following current procedures in the literature (Coan & Allen, 2003; Jones et al., 1997). Negative asymmetry index scores represent right EEG frontal alpha asymmetry (increased activity in the right frontal region), while positive index scores represent left EEG frontal alpha asymmetry (increased activity in the left frontal region). Social and nonsocial conditions were analyzed separately. For the purposes of group EEG comparisons, participants were divided into two groups for each condition: infants who displayed a left asymmetry bias and infants who displayed a right frontal asymmetry bias. This was calculated by conducting a t-test to determine the difference value that was significantly different from 0. The value of +/- .05 was found to be significantly different than 0 ( $t(72) = (-2.36)$ ,  $p < .05$ ). Thus, scores that were below -.049 were categorized as “right asymmetry” while those that were .051 or above were classified as “left asymmetry”. Values between +/- .05 were categorized as “no difference”.

### C. Temperament comparisons

Specific dimensions of the parent report measure (IBQ-R) were mapped with conceptually similar items on the observational scale (BRS) and EEG asymmetry. For the purpose of the current study, only Cuddliness, Smiling and Laughter, Soothability, Activity Level, and Distress to Limitations on the IBQ-R were examined since these are the scales that have conceptual overlap with dimensions on the observational measure (BRS) and have been found to be associated in ASD (see Table 5). IBQ-R domains and related items BRS such as Social Engagement, Positive Affect, Negative Affect, and Soothability were examined in relation to EEG asymmetry scores based on empirical association between frontal EEG asymmetry and affective style in infants (see Table 5).

Table 5. Parent Report, Observational, and EEG Measure Comparisons

<b>Parent Measure (IBQ-R domain)</b>	<b>Observation Measure (BRS items)</b>	<b>EEG asymmetry</b>
Cuddliness	Social engagement	Left asymmetry
Smiling and Laughter	Positive affect	Left asymmetry
Soothability	Soothability when upset	Left asymmetry
Sadness	Negative affect	Right asymmetry
Distress to Limitations	Frustration	Right asymmetry
Activity Level	Energy	Not Applicable

### 3. Statistical Analyses

Given the interest in detecting early temperamental differences associated with a later diagnosis of ASD, a third grouping variable, “ASD” was included in group analyses for temperamental domains (e.g., parent report, observation, and EEG). The purpose was to determine if early temperamental characteristics are different in infants who go on to develop ASD.

Since the purpose of convergence and stability analyses was to determine the psychometrics of these temperament measures concurrently and across time, rather than examine ASD outcome, these analyses only included the two grouping variables “HR” and “LR” infants which included the infants who developed ASD. In addition, the current literature has examined parent report of temperament for infant siblings across time without regard to ASD outcome and we hoped to compare our results to these studies. Furthermore, for analyses examining parent report, our goal was to determine if having an older child with ASD might influence a parent’s perception of the temperament of their younger infant.

**A. Aim 1. To examine parent and observation measures of temperament at 6 and 12 months of age in infants who develop ASD, high-risk infants who do not develop ASD, and low-risk infants who do not develop ASD.**

#### *Parent report at 6 and 12 months*

Specific temperament domains of Cuddliness, Smiling and Laughter, Soothability, Activity Level, and Distress to Limitations were chosen based on previous studies finding a relationship of these domains to ASD. Based on previous parent report findings suggestive of no differences in temperament at 6 months of age, it was hypothesized that parent report (IBQ-R) of temperament will not differ between ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub> infants at 6 months of age.

Given previously reported parent temperament differences at 12 months of age, there were a few alternative hypotheses about group differences at this age. At 12 months, there could be differences between HR<sub>noASD</sub> and LR<sub>noASD</sub> groups, which might suggest certain temperamental traits are part of the broader autism phenotype in the HR<sub>noASD</sub> group. Alternatively, it may be that temperamental differences only arise for infants who later develop ASD and that early temperament differences are only detected in this group rather than the HR<sub>noASD</sub> group. It was hypothesized that at 12 months, group differences would arise reflective of *decreased* Cuddliness, Smiling and Laughter, Activity Level, and Soothability and *increased* Distress to Limitations on the IBQ-R. It was uncertain whether this pattern would appear only for the ASD group or in both the ASD and HR<sub>noASD</sub> groups. Group performance between ASD, LR<sub>noASD</sub>, and HR<sub>noASD</sub> groups was compared using a multivariate analysis of variance with select IBQ-R domains (Cuddliness, Smiling and Laughter, Soothability, Activity Level, and Distress to Limitations) entered as dependent variables. Post-hoc analyses included Bonferroni corrections.

#### *Observational measure at 6 and 12 months*

It was hypothesized that observations of temperament using the BRS coding of the Mullen and AOSI, would reveal *decreased* Social Engagement, Positive Affect, Soothability, Energy, and *increased* Frustration at *both* 6 and 12 months of age for HR<sub>noASD</sub> compared to LR<sub>noASD</sub> infants. This prediction was based on preliminary data suggesting that high-risk compared to low-risk infants show more variability in behavior across specific constrained tasks (Venema, Jones, Webb et al., 2009). In addition, it was hypothesized that infants who later developed ASD would show this same pattern. Group performance was compared using multivariate analysis of variance with dependent variables of the BRS (Social Approach, Positive Affect, Soothability, Energy, Frustration) entered as dependent variables and group (ASD,

LR<sub>noASD</sub>, HR<sub>noASD</sub>) as the independent variables. Post-hoc analyses included Bonferroni corrections.

*Convergence of parent report and observational measures at 6 and 12 months*

In order to determine if having an older child diagnosed with ASD influenced parent report, convergence analyses were run for high-risk and low-risks groups (which included infants who developed ASD). It was expected that there would be lower convergence between parent report and observation in the high-risk group than the low-risk group at both 6 and 12 months, possibly due to parent bias such as a contrast effect or child temperament contextual instability (e.g., familiar context vs. novel lab context). Multiple linear regressions were conducted in which group, parent report, and parent report by group interaction were entered as predictors of observational measure of temperament for each temperament domain (see Table 5 for corresponding parent and observational domains). These analyses were conducted separately at each time point (6 and 12 months). A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

*Stability of parent report measure*

It was predicted that stability over time would be lower for parent report for the high-risk group compared to the low-risk group possibly due to parents being influenced by increasing concerns about possible ASD symptoms. Multiple regression analyses were conducted in which group, parent report at 6 months, and parent report at 6 months by group interaction were entered as predictors of parent report at 12 months for each temperament domain (see Table 5). A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

*Stability of observational measure*

It was expected that observational stability would be similar across both groups. Multiple regression analyses were conducted in which group, observation at 6 months, and observation at 6 months by group interaction were entered as predictors of observation at 12 months for each temperament domain (see Table 5). A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

**B. Aim 2. To examine electrophysiological (EEG) measures of temperament at 6 and 12 months of age in infants who develop ASD, high-risk infants who do not develop ASD, and low-risk infants who do not develop ASD.**

*EEG at 6 and 12 months of age*

It was predicted that ASD and HR<sub>noASD</sub> infants would show greater right frontal asymmetry than low-risk infants in observed EEG alpha power suggestive of less social approach and more negative affective at 6 and 12 months. Repeated-measures ANOVA was conducted on EEG log alpha power with condition (social, non-social) and hemisphere (left, right) as the repeated measures with group (ASD, LR<sub>noASD</sub>, HR<sub>noASD</sub>) as the independent variable at both 6 and 12 months of age.

*Stability of EEG frontal asymmetry*

Following the statistical approach for the other stability aims, stability of EEG was examined for the high-risk infants and low-risk infants without regard to ASD outcome for comparability with other analyses. Based on the typically developing literature showing moderate stability for EEG frontal asymmetry during the first year of life, it was predicted that the low-risk infants would have stability in EEG frontal asymmetry from 6 to 12 months of age. It was predicted that stability over time would be lower for the high-risk group compared to the

low-risk group possibly due to the onset of early ASD symptoms. Multiple regression analyses were conducted in which group, EEG frontal asymmetry value at 6 months, and EEG frontal asymmetry value at 6 months by group interaction were entered as predictors of EEG frontal asymmetry value at 12 months. A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

*Parent report measure and EEG asymmetry*

Previous reports suggest a significant relation between parent report and EEG frontal asymmetry. It was hypothesized that the current study would replicate this relationship in the low-risk group. The high-risk group was hypothesized to show lower correlations between parent report and EEG due to parent bias in reporting or possibly due to more variability in child behavior. Multiple regression analyses were conducted in which group, parent report, and parent report by group interaction were entered as predictors of EEG asymmetry value. These were conducted for each IBQ-R domain of Cuddliness, Smiling and Laughter, Soothability, Sadness, and Distress to Limitations (see Table 5 for parent report and EEG comparisons). A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

*Observational measure and EEG asymmetry*

The relation between observational and EEG measures for both groups was hypothesized to be significant as both were obtained in the same context and time. A regression analysis was conducted in which group, observation, and observation by group interaction were entered as predictors of EEG asymmetry value. These were conducted for each observation domain of Social Engagement, Positive Affect, Soothability, Negative Affect, and Frustration (see Table 5

for parent report and EEG comparisons). A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

## CHAPTER 3

### RESULTS

#### 1. Aim 1

##### A. Parent report of temperament (IBQ-R)

###### *6-month results*

The overall MANOVA revealed a significant difference between ASD, LR<sub>noASD</sub>, and HR<sub>noASD</sub> groups at 6 months for parent report of Cuddliness,  $F(2, 381.08) = 3.27, p = .04$ . Post-hoc pair-wise comparisons including Bonferroni corrections suggest that the ASD group ( $M = 93.3, SD = 18.6$ ) were rated lower on Cuddliness than the HR<sub>noASD</sub> group ( $M = 103.31, SD = 7.39$ ) ( $p = .04$ ) and a trend for the ASD group to be rated lower than the LR<sub>noASD</sub> group ( $M = 101.68, SD = 10.36$ ) ( $p = .09$ ). There was no significant difference between the LR<sub>noASD</sub> and HR<sub>noASD</sub> groups (see Table 6 for means, standard deviations, and range for each group). There were no significant results for Smiling and Laughter, Activity Level, Soothability, or Distress to Limitations.

Table 6. Mean, SD, and range for parent report 6-month domains

Domain	ASD	HR <sub>noASD</sub>	LR <sub>noASD</sub>
	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>
Cuddliness	93.3 (18.60) (57-116)	103.31 (7.39) (89-116)	101.68 (10.36) (64-113)
Smiling and Laughter	46.48 (9.76) (22-64)	46.48 (9.79) (31-60)	43.79 (10.15) (21-60)
Activity Level	65.40 (16.00) (33-83)	64.21 (11.26) (42-87)	58.53 (13.58) (37-91)
Soothability	85.10 (13.57) (59-105)	88.45 (8.76) (71-106)	90.37 (10.64) (66-110)
Distress to Limitations	60.30 (17.81) (41-91)	61.86 (14.25) (38-89)	57.92 (12.30) (32-81)

### 12-month results

The overall MANOVA suggests a significant difference between ASD, LR<sub>noASD</sub>, and HR<sub>noASD</sub> groups at 12 months for parent report of Cuddliness,  $F(2, 980.61) = 5.26, p < .01$ . Post-hoc pair-wise comparisons including Bonferroni corrections suggest that the ASD group ( $M = 78.6, SD = 19.29$ ) were rated lower on Cuddliness than the HR<sub>noASD</sub> ( $M = 90.33, SD = 14.36$ ) ( $p < .01$ ) and a trend for the ASD group to be rated lower than the LR<sub>noASD</sub> ( $M = 95.08, SD = 9.69$ ) ( $p = .06$ ). There was no significant difference between the LR<sub>noASD</sub> and HR<sub>noASD</sub> groups (see Table 7 for means, standard deviations, and range for each group). There were no significant results for Smiling and Laughter, Activity Level, Soothability, or Distress to Limitations.

Table 7. Mean, SD, and range for parent report 12-month domains

Domain	ASD	HR <sub>noASD</sub>	LR <sub>noASD</sub>
	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>
Cuddliness	78.60 (19.29) (52-102)	95.08 (9.69) (80-116)	90.33 (14.36) (38-108)
Smiling and Laughter	45.70 (14.86) (24-69)	48.96 (10.49) (29-67)	47.42 (8.58) (28-63)
Activity Level	65.70 (8.48) (53-82)	66.04 (10.22) (51-85)	64.85 (12.61) (42-95)
Soothability	89.20 (14.90) (57-108)	89.58 (19.08) (45-119)	90.30 (14.70) (48-119)
Distress to Limitations	75.50 (11.19) (61-93)	71.65 (13.41) (46-98)	68.70 (11.83) (45-98)

In summary, parent report of Cuddliness was the only significant difference between groups at 6 and 12 months, with the ASD group rated lower on Cuddliness than the HR<sub>noASD</sub> group and a trend for the ASD group to be rated lower than the LR<sub>noASD</sub> group at both time points. There were no significant results for Smiling and Laughter, Activity Level, Soothability, or Distress to Limitations.

## B. Observational measure of temperament (BRS)

### *6-month results*

The overall MANOVA suggests a significant difference between ASD, LR<sub>noASD</sub>, and HR<sub>noASD</sub> groups at 6 months for observational coding of Social Engagement,  $F(2, 1.56) = 4.01, p = .02$ . Post-hoc pair-wise comparisons including Bonferroni corrections suggest a trend for the HR<sub>noASD</sub> group ( $M = 2.03, SD = 0.61$ ) to be rated lower on Social Engagement than the LR<sub>noASD</sub> group ( $M = 2.40, SD = 0.70$ ) ( $p = .06$ ). There was not a significant difference between the ASD and non-ASD groups (see Table 8 for means, standard deviations, and range for each group). There were no significant differences between groups on Positive Affect, Energy Level, or Frustration. There were not enough cases for the domain Soothability to be analyzed.

Table 8. Mean, SD, and range for observation 6-month domains

Domain	ASD	HR <sub>noASD</sub>	LR <sub>noASD</sub>
	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>
Social Engagement	1.93 (.42) (1.29-2.29)	2.03 (.61) (1.14-3.00)	2.40 (.67) (1.00-3.86)
Positive Affect	1.70 (.36) (1.0-2.29)	1.91 (.65) (1.00-3.43)	2.17 (.66) (1.14-3.71)
Energy Level	2.81 (.49) (1.86-3.57)	3.06 (.48) (2.29-4.71)	3.05 (.42) (1.57-3.86)
Frustration	1.36 (.50) (1.00-2.57)	1.20 (.35) (1.00-2.71)	1.16 (.25) (1.00-2.14)
Soothability	--	--	--

### *12-month results*

The overall MANOVA suggests a significant difference between ASD, LR<sub>noASD</sub>, and HR<sub>noASD</sub> groups at 12 months for observational coding of Social Engagement,  $F(2, 1.45) = 3.33, p = .04$  and Frustration,  $F(2, 0.14) = 3.61, p = .03$  at 12 months. Post-hoc pair-wise comparisons including Bonferroni corrections suggest that the ASD group ( $M = 2.27, SD = 0.61$ ) was rated

lower on Social Engagement than the HR<sub>noASD</sub> ( $M = 2.86$ ,  $SD = 0.68$ ),  $p = .04$  (see Table 9). For the Frustration domain, pair-wise comparisons reveal that the ASD group were rated as displaying increased Frustration ( $M = 1.22$ ,  $SD = .44$ ), compared to the LR<sub>noASD</sub> group ( $M = 1.05$ ,  $SD = 0.14$ ), ( $p = .04$ ) and marginal significance for the ASD group to display increased Frustration compared to the HR<sub>noASD</sub> group ( $M = 1.05$ ,  $SD = 0.11$ ), ( $p = .05$ ). There were no significant differences between groups on Positive Affect or Energy Level. There were not enough cases for the domain Soothability to be analyzed.

Table 9. Mean, SD, and range for observation 12-month domains

Domain	ASD	HR <sub>noASD</sub>	LR <sub>noASD</sub>
	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>
Social Engagement	2.27 (.60) (1.43-3.29)	2.87 (.68) (1.57-4.29)	2.71 (.66) (1.14-4.29)
Positive Affect	2.11 (.57) (1.14-2.71)	2.31 (.58) (1.14-3.43)	2.25 (.61) (1.29-3.67)
Energy Level	2.77 (.31) (2.14-3.14)	2.98 (.31) (2.14-3.57)	2.92 (.36) (2.14-3.57)
Frustration	1.22 (.44) (1.00-2.43)	1.05 (.11) (1.00-1.43)	1.05 (.14) (3.00-5.00)
Soothability	--	--	--

### C. Concurrent convergence of parent report and observation of temperament

Multiple linear regressions were conducted in which parent report, group (HR, LR), and parent report by group interactions were entered as predictors of observational measure of temperament for related temperament domains at each time point. A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

### 6-month results

Linear regression results suggest that parent report of Smiling and Laughter and group interaction by Smiling and Laughter predicted observational coding of Positive Affect at 6 months for Model 2 ( $R^2 = .13$ ,  $F(3, 69) = 3.37$ ,  $p = .02$ ) (see Table 10). However, these results were not significant after applying Bonferroni correction of alpha level of .01. Follow up analyses for each group found a trend for a correlation between Smiling and Laughter and positive affect for the low-risk group only ( $r = .32$   $p = .05$ ) but not for the high-risk group ( $r = -.13$   $p = .44$ ) (see Figure 1).

Table 10. Regression table for Smiling and Laughter to predict Positive Affect at 6 months

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	1.75	.33		5.24	< .001
	Smiling and Laughter	.006	.007	.095	.80	.426
2	(Constant)	1.28	.44		2.89	.005
	Smiling and Laughter	.02	.01	.33	2.07	.042
	Group	.90	.64	.70	1.41	.163
	Group* Smiling and Laughter	-.03	.01	-1.02	-1.99	.05

Note  $R^2 = .01$  for Model 1; change in  $R^2 = .12$  for Model 2 ( $p = .01$ ).

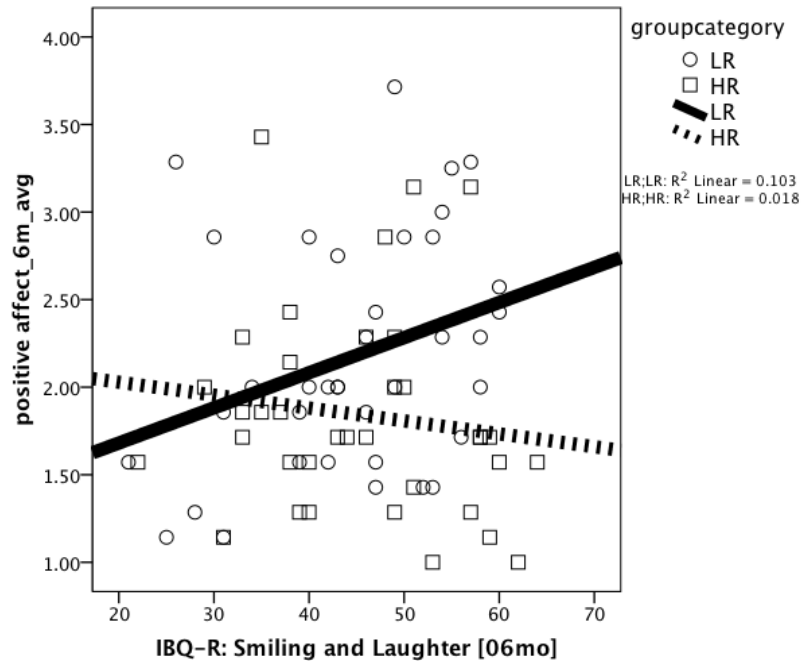


Figure 1. Relationship between parent and observational rating of Positive Affect at 6 months

There were no results for the other domains including Activity Level, Social Engagement, Soothability, and Distress to Limitation.

#### *12 months*

There were no significant relationships between parent report domains of temperament and observational report of temperament at 12 months.

#### **D. Stability of parent report of temperament**

To examine stability of parent report, multiple regression analyses were conducted in which parent report at 6 months, group, and parent report at 6 months by group interaction were entered as predictors of parent report at 12 months. Cuddliness at 6 months significantly predicted Cuddliness at 12 months ( $R^2 = .37$ ,  $F(1, 63) = 37.91$ ,  $p < .001$ ) (see Figure 2). There was a trend for group ( $p = .06$ ) and group by time point interaction ( $p = .06$ ) to explain a significant proportion of variance of 12 month Cuddliness. However, group was not significant

after applying Bonferroni corrections. Model 2 did not significantly predict Cuddliness above Model 1 ( $p = .17$ ) (see Table 11).

Table 11. Regression table for Cuddliness (6m) to predict Cuddliness (12m)

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	12.79	12.67		1.01	.31
	Cuddliness	.78	.13	.61	6.16	< .001
2	(Constant)	40.26	19.19		2.10	.04
	Cuddliness	.50	.19	.40	2.63	.01
	Group	-47.78	25.31	-1.89	-1.89	.06
	Group* Cuddliness	.48	.25	1.90	1.90	.06

Note  $R^2 = .38$  for Model 1; change in  $R^2 = .04$  for Model 2 ( $p = .17$ ).

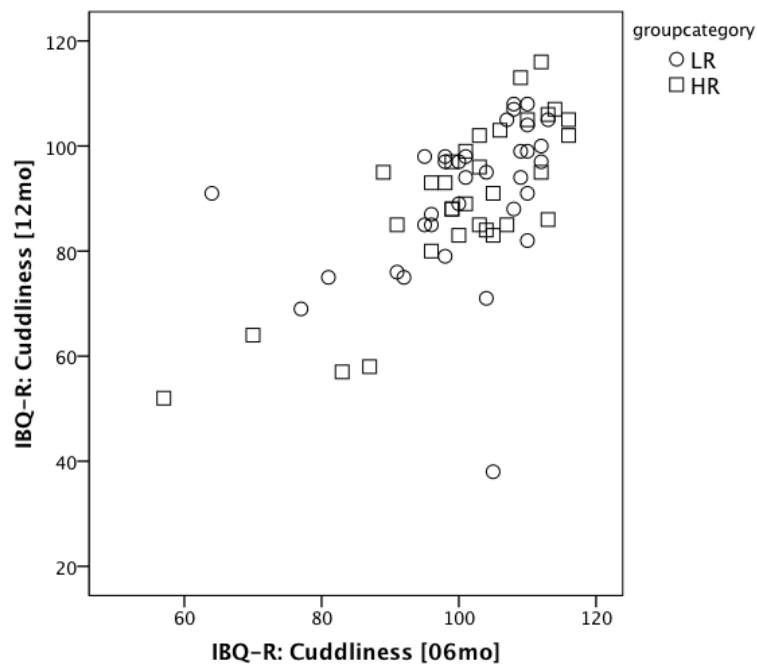


Figure 2. Stability of parent report of Cuddliness from 6 to 12 months

Smiling and Laughter at 6 months significantly predicted Smiling and Laughter at 12 months ( $R^2 = .46$ ,  $F(1, 63) = 53.24$ ,  $p < .001$ ) (see Figure 3). The addition of group and the interaction of group by Smiling and Laughter at 6 months accounted for additional variance of Smiling and Laughter at 12 months ( $R^2 = .51$ ,  $F(1, 63) = 20.93$ ,  $p < .001$ ). However, group was not significant after applying Bonferroni corrections. Correlation analyses reveal a positive relationship between Smiling and Laughter at 6 months and Smiling and Laughter at 12 months for the LR group ( $r = .50$ ,  $p = .003$ ) and the HR group ( $r = .79$ ,  $p < .001$ ) There was a trend for Model 2 to be a better fit than Model 1 ( $R^2 = .51$ ,  $p = .06$ ) (see Table 12).

Table 12. Regression table for Smiling and Laughter (6m) to predict Smiling and Laughter (12m)

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	18.48	4.14		4.47	< .001
	Smiling and Laughter	.65	.09	.67	7.30	< .001
2	(Constant)	28.06	5.98		4.69	< .001
	Smiling and Laughter	.42	.13	.44	3.24	.002
	Group	-16.96	8.07	-.85	-2.10	.04
	Group* Smiling and Laughter	.41	.17	.98	2.34	.02

Note  $R^2 = .46$  for Model 1; change in  $R^2 = .05$  for Model 2 ( $p = .06$ ).

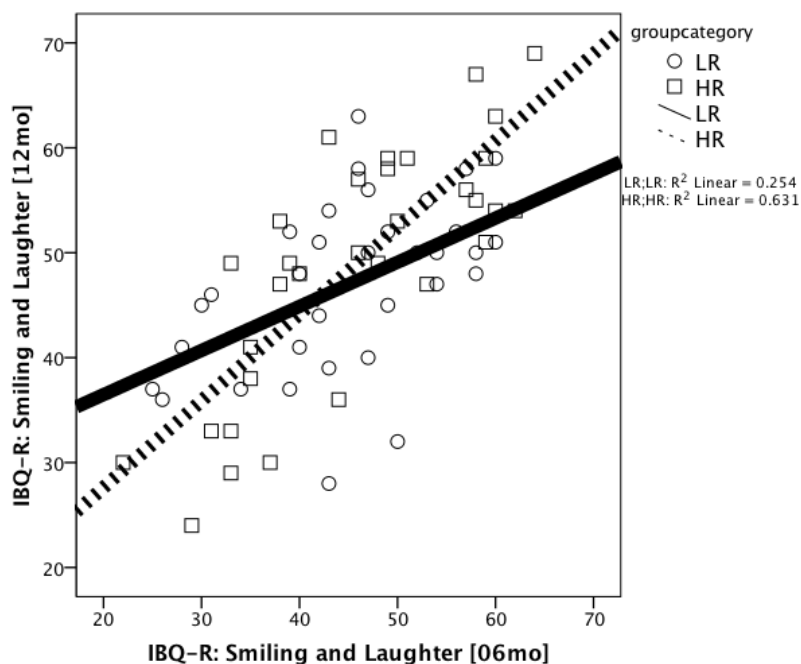


Figure 3. Stability of parent report of Smiling and Laughter from 6 to 12 months

Soothability, Activity Level, and Distress to Limitations 6 month values all significantly predicted to 12 months values ( $p < .01$ ). Group and group by temperament domain interaction did not contribute significantly to the models (see Tables 13-15).

Table 13. Regression table for Soothability (6m) to predict Soothability (12m)

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	23.61	14.82		1.59	.12
	Soothability	.74	.17	.50	4.49	< .001
2	(Constant)	23.16	22.51		1.03	< .001
	Soothability	.74	.25	.50	3.02	.002
	Group	.08	30.59	.003	.003	.99
	Group* Soothability	.01	.34	.02	.02	.99

Note  $R^2 = .25$  for Model 1; change in  $R^2 < .001$  for Model 2 ( $p = .99$ ).

Table 14. Regression table for Activity Level (6m) to predict Activity Level (12m)

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	36.50	5.52		6.61	< .001
	Activity Level	.47	.09	.56	5.34	< .001
2	(Constant)	34.12	7.34		4.65	< .001
	Activity Level	.51	.12	.61	4.20	< .001
	Group	5.17	11.69	.24	.44	.66
	Group* Activity Level	-.09	.18	-.28	-.49	.62

Note  $R^2 = .31$  for Model 1; change in  $R^2 = .003$  for Model 2 ( $p = .87$ ).

Table 15. Regression table for Distress to Limitations (6m) to predict Distress to Limitations (12m)

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	36.69	5.69		6.45	< .001
	Distress to Limitations	.58	.09	.61	6.16	< .001
2	(Constant)	42.47	8.53		4.98	< .001
	Distress to Limitations	.46	.15	.48	3.10	<.003
	Group	-8.45	11.66	-.34	-.73	.47
	Group* Distress to Limitations	.18	.19	.47	.95	.35

Note  $R^2 = .37$  for Model 1; change in  $R^2 = .02$  for Model 2 ( $p = .43$ ).

In summary, all of the parent report temperament domains were significantly related from 6 to 12 months of age. There was a trend for Cuddliness and Smiling and Laughter to differ by group across time.

### E. Stability of observation of temperament

Multiple regression analyses were conducted in which observation at 6 months, group, and observation at 6 months by group interaction were entered as predictors of observation at 12 months.

Social Engagement at 6 months significantly predicted Social Engagement at 12 months ( $R^2 = .11$ ,  $F(1, 73) = 9.09$ ,  $p < .01$ ) (see Figure 4). Adding group status and group by Social Engagement interaction term did not significantly improve model fit ( $R^2 = .130$  for Model 2 ( $p = .47$ )) (see Table 16). Correlation analyses found a significant positive relationship between Social Engagement at 6 months and Social Engagement at 12 months for the LR group ( $r = .40$ ,  $p = .01$ ), and the HR group ( $r = .32$ ,  $p = .05$ ).

Table 16. Regression table for Social Engagement (6m) to predict Social Engagement (12m)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	1.94	.27		7.11	< .001
	Social Engagement	.36	.12	.33	3.02	.004
2	(Constant)	1.76	.40		4.40	< .001
	Social Engagement	.39	.16	.37	2.24	.02
	Group	.13	.57	.10	.23	.82
	Group*Social Engagement	.03	.26	.05	.13	.90

Note  $R^2 = .33$  for Model 1; change in  $R^2 = .02$  for Model 2 ( $p = .47$ ).

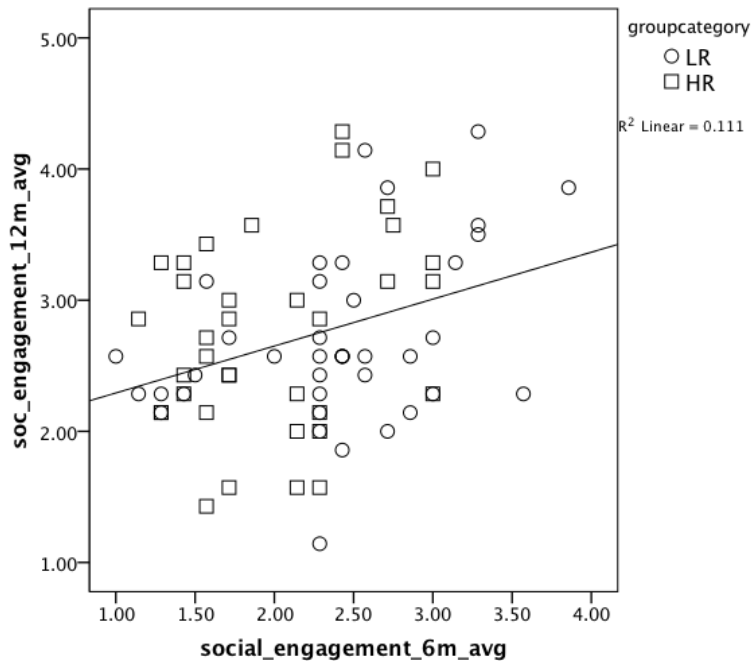


Figure 4. Stability of observational rating of Social Engagement from 6 to 12 months

There were no significant results for Positive Affect, Soothability, Energy Level, or Frustration.

## 2. Aim 2

### A. EEG group analyses

Differences between EEG log alpha power (left and right) and EEG log alpha asymmetry were analyzed by the 3 diagnostic groups (ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub>).

#### *6-month results*

A repeated-measures ANOVA was conducted on EEG log alpha power with condition (social, non-social) and hemisphere (left, right) as the repeated measures and group (ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub>) as the independent variable. There was a trend for EEG alpha power to differ by condition,  $F(1, 68) = 2.98, p = .09$ , and a significant difference between hemispheres,

$F(1, 68) = 6.35, p = .01$  (see Table 17 for means). No differences were found between groups,  $F(2, 68) = .652, p = .54$ .

Table 17. Mean, SD, and range for 6 month EEG log power

EEG Condition by Hemisphere	ASD	HR <sub>noASD</sub>	LR <sub>noASD</sub>
	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>	<i>M (SD)</i> <i>(Range)</i>
Social Left Alpha	.03 (.63) (-.63-1.42)	-.26 (.49) (-1.20-1.02)	-.10 (.50) (-1.13-1.36)
Social Right Alpha	-.13 (.64) (-1.10-1.23)	-.29 (.57) (-1.13-1.66)	-.19 (.48) (-1.39-1.08)
Non-Social Left Alpha	.07 (.61) (-.64-1.19)	-.19 (.52) (-1.04-.96)	-.12 (.50) (-1.15-1.23)
Non-Social Right Alpha	.04 (.60) (-1.12-.87)	-.18 (.60) (-1.04-1.69)	-.15 (.55) (-1.51-1.04)

EEG alpha was also examined in terms of an asymmetry value (negative asymmetry, no clear asymmetry, and positive asymmetry) to determine if there were any differences between the ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub> groups. A chi-square test revealed no significant differences by group,  $\chi^2(4, N = 73) = .94, p = .91$  (see Table 18). Social and non-social conditions were analyzed separately and similarly, no relationship was found. There were no significant group differences for theta power and asymmetry.

Table 18. EEG alpha asymmetry grouping at 6 months (N=73). Chi-square test suggests no significant differences between groups.

6 month EEG alpha asymmetry grouping	LR <sub>noASD</sub> (n=39)	HR <sub>noASD</sub> (n=24)	ASD (n=10)
Negative asymmetry	19	10	5
No clear asymmetry	9	8	3
Positive asymmetry	11	6	2

### 12-month results

A repeated-measures ANOVA was conducted on EEG log alpha power with condition (social, non-social) and hemisphere (left, right) as the repeated measures and group (ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub>) as the independent variable. Within subject results suggest a significant difference by hemisphere  $F(1, 67) = 4.72, p = .03$ , by condition  $F(1, 67) = 8.07, p = .006$ , and condition by group interaction,  $F(2, 67) = 3.66, p = .03$ . Post-hoc pair-wise analyses reveal a condition within group effect such that the ASD group displayed lower EEG power (higher activation) during the non-social condition than the social ( $p = .004$ ) (see Table 19). Post-hoc pair-wise analyses with Bonferroni corrections did not reveal a group within conditions effect (e.g., no difference between groups during social and non-social conditions).

Table 19. Mean, SD, and range for 12 month EEG log power

EEG Condition	ASD	HR <sub>noASD</sub>	LR <sub>noASD</sub>
	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>
	<i>(Range)</i>	<i>(Range)</i>	<i>(Range)</i>
Social Alpha	.51 (.66) (-.62-1.85)	.46 (.40) (-.19-1.53)	.60 (.45) (-.20 -1.74)
Non-Social Alpha	.33 (.72) (-.89-1.82)	.42 (.38) (-.35-1.18)	.60 (.45) (-.11-1.66)

EEG was also grouped by asymmetry value (negative asymmetry, no clear asymmetry, and positive asymmetry) to determine if there were any differences between the ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub> groups. A chi-square test revealed no significant differences by group collapsed across condition,  $\chi^2(4, N = 73) = 1.94, p = .75$  (see Table 20). Social and non-social conditions were analyzed separately and similarly, no relationship was found. There were no significant group differences for theta power and asymmetry.

Table 20. EEG asymmetry grouping at 12 months (N=73). Chi-square test suggests no significant relationship between groups.

12 month EEG alpha asymmetry grouping	LR <sub>noASD</sub> (n=39)	HR <sub>noASD</sub> (n=24)	ASD (n=10)
Negative asymmetry	17	13	6
No clear asymmetry	6	11	3
Positive asymmetry	11	28	2

In summary, results of EEG alpha power and alpha asymmetry and EEG theta power and theta asymmetry do not reveal group differences between ASD, LR<sub>noASD</sub>, and HR<sub>noASD</sub> at 6 months of age. However, at 12 months of age, there was a group by condition interaction for EEG power such that ASD infants displayed lower EEG power for the non-social condition than the social condition.

### **B. Stability of EEG log alpha power and asymmetry**

To examine the stability of EEG for social and non-social conditions from 6 to 12 months of age, multiple regression analyses were conducted in which EEG values at 6 months, group (HR and LR), and EEG values at 6 months by group were entered as predictors of 12 month corresponding values. These were conducted separately for log left alpha power, log right alpha power, and log asymmetry values.

For the social condition, log left alpha power, log right alpha power, and asymmetry values at 6 months significantly predicted corresponding 12 month values ( $p < .001$ ) (see Tables 21-23). Group and group by EEG interaction did not contribute significantly to any of the models.

Table 21. Regression table for Left Alpha EEG (6m) to predict Left Alpha EEG (12m) (social condition)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	.62	.05		13.89	< .001
	Left Alpha EEG	.55	.08	.65	6.72	< .001
2	(Constant)	.65	.06		10.50	< .001
	Left Alpha EEG	.65	.06	.61	4.23	< .001
	Group	.13	.57	-.08	-.80	.43
	Group*Left Alpha EEG	.03	.26	.05	.30	.76

Note  $R^2 = .43$  for Model 1; change in  $R^2 = .008$  for Model 2 ( $p = .66$ ).

Table 22. Regression table for Right Alpha EEG (6m) to predict Right Alpha EEG (12m) (social condition)

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	.65	.05		12.74	< .001
	Right alpha EEG	.53	.09	.61	6.00	< .001
2	(Constant)	.68	.07		9.39	< .001
	Right alpha EEG	.48	.14	.56	3.55	.001
	Group	-.06	.10	-.06	-.53	.60
	Group*Right Alpha EEG	.08	.18	.07	.44	.66

Note  $R^2 = .37$  for Model 1; change in  $R^2 = .007$  for Model 2 ( $p = .71$ ).

Table 23. Regression table for EEG asymmetry (6m) to predict EEG asymmetry (12m)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	.01	.03		.31	.76
	EEG asymmetry	.24	.11	.27	2.15	.04
2	(Constant)	-.03	.04		-.73	.47
	EEG asymmetry	-.02	.19	-.02	-.11	.91
	Group	.06	.06	.14	1.05	.30
	Group*EEG asymmetry	.40	.23	.36	1.72	.09

Note  $R^2 = .27$  for Model 1; change in  $R^2 = .05$  for Model 2 ( $p = .21$ ).

This suggests that (collapsed across groups) EEG values for the social condition are stable from 6 to 12 months. There was a trend for the interaction of group by social asymmetry at 6 months to predict social asymmetry scores at 12 months of age ( $p = .09$ ). Follow up correlations found that the HR group's 6 month social asymmetry score was positively correlated with the 12 month social asymmetry score,  $r = .42$ ,  $p = .02$  but the LR group's scores were not correlated,  $r = -.23$ ,  $p = .90$  (see Figure 5).

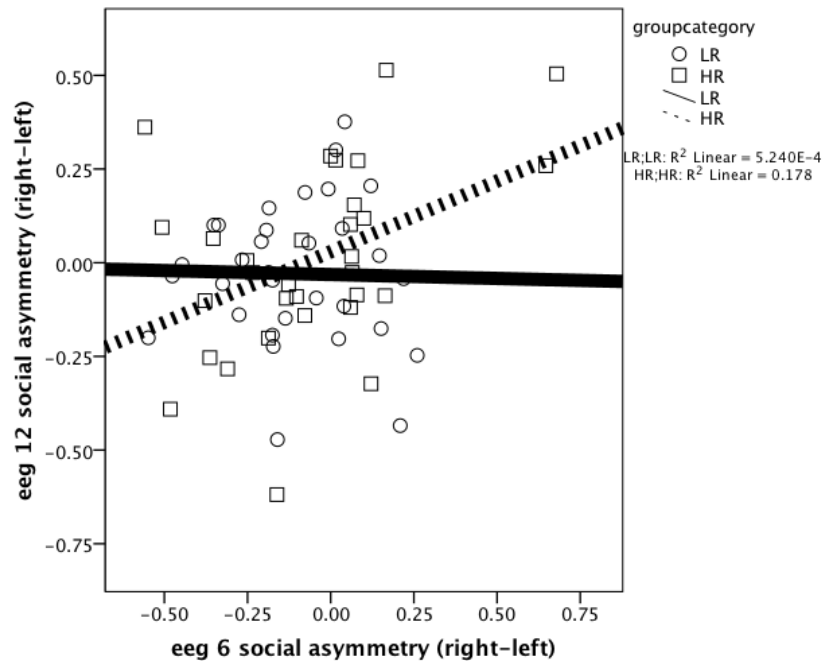


Figure 5. Stability of social asymmetry from 6 to 12 months

For the non-social condition, log left alpha and log right alpha at 6 months significantly predicted 12 month corresponding values ( $p < .001$ ). Group also explained a significant proportion of variance in 12 month left alpha values ( $p = .02$ ) (see Figure 6) and a trend for group to explain a significant proportion of variance in 12 month right alpha values ( $p = .06$ ). However, log asymmetry value at 6 month did not significantly predict 12 month values ( $p = .51$ ) (see Tables 24 – 26).

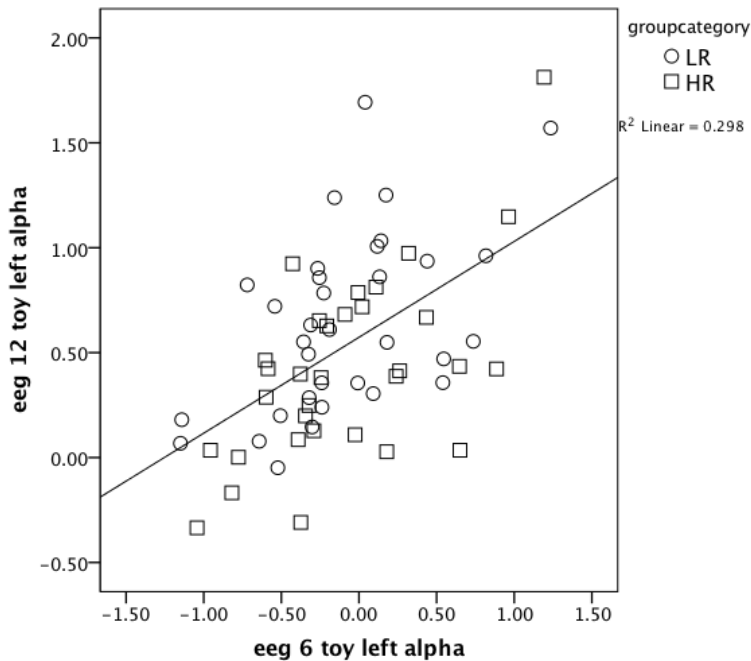


Figure 6. Relationship of EEG log left alpha from 6 to 12 months

Table 24. Regression table for Left Alpha EEG (6m) to predict Left Alpha EEG (12m) (social condition)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	.57	.05		12.08	< .001
	Left Alpha EEG	.46	.09	.55	5.18	< .001
2	(Constant)	.68	.06		10.62	< .001
	Left Alpha EEG	.46	.09	.55	5.33	< .001
	Group	-.22	.09	-.25	-2.43	.02
	Group*Left Alpha EEG	-.05	.29	.02	-.17	.86

Note  $R^2 = .55$  for Model 1; change in  $R^2 = .06$  for Model 2 ( $p = .06$ ).

Table 25. Regression table for Right Alpha EEG (6m) to predict Right Alpha EEG (12m) (non-social condition)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	.53	.06		9.58	< .001
	Right Alpha EEG	.39	.10	.46	4.12	< .001
2	(Constant)	.64	.08		8.18	< .001
	Right Alpha EEG	.41	.13	.48	3.11	.003
	Group	-.21	.11	-.22	-1.90	.06
	Group*Right Alpha EEG	-.01	.19	-.01	-.08	.94

Note  $R^2 = .21$  for Model 1; change in  $R^2 = .05$  for Model 2 ( $p = .16$ ).

Table 26. Regression table for EEG asymmetry (6m) to predict EEG asymmetry (12m) (non-social condition)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	-.04	.03		-1.24	.22
	EEG Asymmetry	.13	.14	.11	.88	.38
2	(Constant)	-.05	.05		-1.20	.24
	EEG Asymmetry	.08	.20	.07	.40	.69
	Group	.03	.06	.06	.45	.66
	Group*EEG Asymmetry	.08	.25	.05	.05	.75

Note  $R^2 = .01$  for Model 1; change in  $R^2 = .004$  for Model 2 ( $p = .88$ ).

In summary, stability results suggest that EEG power is stable for both left and right hemispheres for both groups and in both social and non-social conditions. EEG asymmetry for the social condition was stable for the HR group but not LR group. However, EEG asymmetry for the non-social response was not stable from 6 to 12 months for either group.

### **C. Convergence between parent report measure and EEG asymmetry**

To determine if parent report of temperament is related to EEG asymmetry, multiple regression analyses were conducted in which parent report, group (HR and LR), and parent report by group interaction were entered as predictors of EEG asymmetry value separately at 6 and 12 month time points. A Bonferroni correction was applied such that an alpha level of 0.05 was divided by the number of regression analyses to control for Type I error.

In general, left frontal asymmetry is typically related approach and positive affect while right frontal asymmetry is typically associated with withdrawal and negative affect.

#### *6-month social condition*

Cuddliness significantly predicted left frontal asymmetry at 6 months ( $R^2 = .21$ ,  $F(1, 67) = 17.36$ ,  $p < .001$ ) (see Figure 7). Adding group and group by Cuddliness interaction term did not significantly improve model fit ( $R^2 = .02$  for Model 2 ( $p = .54$ )) (see Table 27).

Table 27. Regression table for Cuddliness (6m) to predict EEG asymmetry (6m) (social condition)

Model		Unstandardized		Std.	T	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	-1.05	.24		-4.46	<.001
	Cuddliness	.01	.002	.45	4.17	<.001
2	(Constant)	-1.10	.36		-3.01	.004
	Cuddliness	.01	.004	.46	2.76	.008
	Group	.06	.48	.12	.13	.90
	Group* Cuddliness	1.21	.005	-.003	-.003	1.0

Note  $R^2 = .21$  for Model 1; change in  $R^2 = .02$  for Model 2 ( $p = .54$ ).

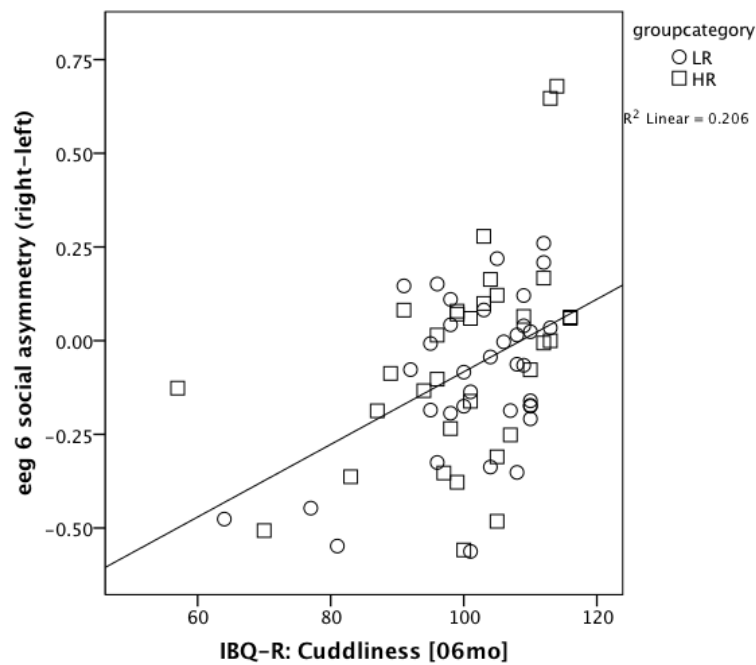


Figure 7. Relationship of Cuddliness and EEG social asymmetry at 6 months

There were no results for Smiling and Laughter, Soothability, Sadness, and Distress to

Limitations.

*6-month non-social condition*

Similar to the social results, parent report of Cuddliness was positively related to left frontal asymmetry scores ( $R^2 = .29$ ,  $F(1, 67) = 5.97$ ,  $p = .02$ ). However, these results were not significant when applying Bonferroni corrections and comparing to an alpha value of .01. Group and group by Cuddliness did not significantly contribute to the model above Cuddliness (see Table 28).

Table 28. Regression table for Cuddliness (6m) to predict EEG asymmetry (6m) (non-social condition)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	$\beta$		
1	(Constant)	-.57	.23		-2.52	.01
	Cuddliness	.01	.002	.29	2.44	.02
2	(Constant)	-.32	.35		-.92	.36
	Cuddliness	.003	.003	.15	.84	.40
	Group	-.435	.46	-.99	-.94	.35
	Group* Cuddliness	.01	.005	1.05	1.00	.32

Note  $R^2 = .29$  for Model 1; change in  $R^2 = .06$  for Model 2 ( $p = .56$ ).

There were no results for Smiling and Laughter, Distress to Limitations, Soothability, or Sadness.

*12-month social condition*

Unlike the 6-month results, there was no relationship between parent report of Cuddliness and EEG frontal asymmetry. Similarly, there were no significant findings for Smiling and Laughter, Soothability, Sadness, or Distress to Limitations.

### *12-month non-social condition*

There were no significant findings for Cuddliness, Smiling and Laughter, Soothability, Sadness, or Distress to Limitations.

### *Summary of parent report and EEG asymmetry findings*

As anticipated, 6-month-old HR and LR infants who were rated higher on parent report of Cuddliness revealed the related pattern of left frontal asymmetry thought to be related to approach and positive affect during the social videos (women saying nursery rhymes). There was a trend for the relationship between parent report of Cuddliness and left frontal asymmetry during the non-social video (dynamic toys). Unlike the 6-month-old findings, there was no relationship between parent report of Cuddliness and asymmetry scores for either group during the social EEG condition at 12 months. There were no other relationships between temperament domains and EEG asymmetry at 6 or 12 months.

## **D. Convergence between observational measure and EEG frontal asymmetry**

To examine the relation between observational measures of temperament and EEG frontal asymmetry, multiple regression analyses were conducted in which observation, group, and observation by group interaction were entered as predictors of EEG asymmetry value. These were conducted for each observation domain of Social Engagement, Positive Affect, Soothability, Negative Affect, and Frustration.

### *6-month social and non-social results*

There were no significant relationships between observational domains and EEG frontal alpha asymmetry for the social condition. For the non-social condition, Social Engagement and Positive Affect predicted left asymmetry. Infants who were rated as higher in Social Engagement ( $r = -.25, p = .04$ ) (see Figure 8) and higher in Positive Affect ( $r = -.26, p = .03$ ) tended to display

left frontal asymmetry. However, these results are not significant when applying Bonferroni corrections and comparing to an alpha value of .01. Group and group by Social Engagement and Positive Affect did not significantly contribute to the models (see Tables 29-30).

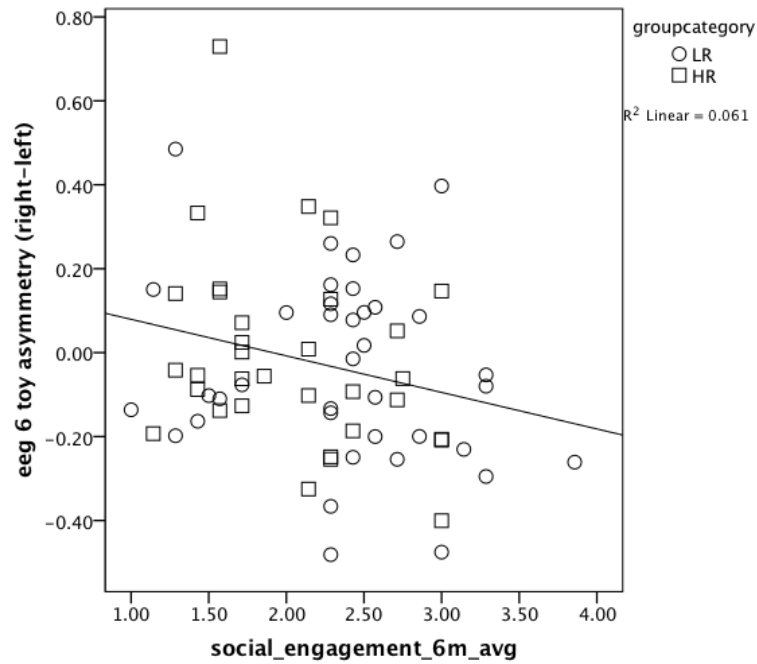


Figure 8. Relationship between observation of Social Engagement at 6 months and left EEG non-social asymmetry at 6 months

Table 29. Regression table for Social Engagement (6m) to predict EEG asymmetry (6m) (non-social condition)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	$\beta$		
1	(Constant)	.17	.10		1.75	.09
	Social Engagement	-.09	.04	-.25	-2.11	.04
2	(Constant)	.11	.14		.81	.42
	Social Engagement	-.06	.06	-.18	-1.14	.26
	Group	.13	.20	.29	.65	.52
	Group* Social Engagement	-.06	.09	-.29	-.67	.51

Note  $R^2 = .06$  for Model 1; change in  $R^2 = .006$  for Model 2 ( $p = .80$ ).

Table 30. Regression table for Positive Affect (6m) to predict EEG asymmetry (6m) (non-social condition)

Model		Unstandardized		Std.	t	Sig.
		$\beta$	Std. Error	B		
1	(Constant)	.16	.09		1.85	.07
	Positive Affect	-.09	.04	-.26	-2.27	.03
2	(Constant)	.14	.12		1.11	.27
	Positive Affect	-.08	.06	-.24	-1.50	.14
	Group	.05	.18	.11	.27	.79
	Group* Positive Affect	-.02	.09	-.10	-.24	.81

Note  $R^2 = .07$  for Model 1; change in  $R^2 = .001$  for Model 2 ( $p = .97$ ).

### *12-month social and non-social results*

There were no significant results for any of the other domains assessed: Social Engagement, Positive Affect, Soothability, Negative Affect, and Frustration.

### *Summary of observation of temperament and EEG asymmetry findings*

For the 6 month non-social condition, higher Social Engagement and Positive Affect was related to EEG right asymmetry for both HR and LR groups. However, these results are not significant when applying Bonferroni corrections. For the 6 month social condition, there were no significant relationships between observational domains and EEG asymmetry for the social condition. There were no significant relationships for observation of temperament and EEG asymmetry at 12 months for either condition.

### **3. Follow-up Analyses**

To examine the relationship among temperament domains in the ASD group, correlations were conducted among parent reported temperament domains of the IBQ-R. Cuddliness was significantly related to Smiling and Laughter at 6 months ( $r = .67, p = .04$ ) and at 12 months ( $r = .81, p = .005$ ). Cuddliness was also positively related to Approach at 12 months ( $r = .89, p = .001$ ).

Additional follow-up correlation analyses were conducted to examine possible relationship of Cuddliness to parent report of activity level at 6 and 12 months of age. Results found no significant relationships at 6 months ( $r = -.18, p = .11$ ) or 12 months ( $r = .03, p = .84$ ). Cuddliness was also examined in relationship to perceptual sensitivity but no significant results were found at 6 months ( $r = .20, p = .58$ ) or 12 months ( $r = -.07, p = .83$ ).

## CHAPTER 4

### DISCUSSION

The main objective of this study was to prospectively examine temperament in infant siblings of children with ASD (high-risk infants) and infants with no known genetic risk for ASD (low-risk infants) at 6 and 12 months of age using parent, observational, and EEG measures of temperament to determine if temperament measures are predictive of the development of early ASD risk symptoms. Another goal was to examine the stability across time for each temperament measure and concurrent convergence between measures of temperament. This project was the first of its kind to examine multiple modal assessments of temperament, including parent report, observational measures, and EEG as early risk markers for autism.

#### 1. Aim 1

The first aim was to determine if group differences existed between LR<sub>noASD</sub>, HR<sub>noASD</sub>, and ASD groups for parent and observational measures of temperament at both 6 and 12 month time points. HR and LR groups were examined across time points to determine stability for each measure as well as convergence within each time point.

Parent report of Cuddliness was the only domain to differentiate groups at 6 and 12 months with the infants who later developed ASD rated lower on Cuddliness than the LR<sub>noASD</sub> and HR<sub>noASD</sub> groups. Observational measure of temperament did not find any domains differing between ASD and non-ASD groups at 6 months but did find a trend for the HR<sub>noASD</sub> to be rated lower in Positive Affect than the LR<sub>noASD</sub> group. At 12 months, coders rated the ASD group lower in Social Engagement than the HR<sub>noASD</sub> group. Coders also rated the ASD group as

displaying increased Frustration compared to the HR<sub>noASD</sub> and the LR<sub>noASD</sub> group at 12 months of age.

These results suggest that parent report of Cuddliness and observation of Social Engagement were the main temperament factors to differentiate infants who later developed ASD. This is in line with other prospective infant studies finding social engagement at 12 and 24 months of age to differentiate HR siblings who developed ASD from those that did not (Garon et al., 2009; Ozonoff et al., 2010). Given the hypothesis that ASD is primarily a disorder of decreased social motivation (Dawson et al., 1998; Dawson, Webb, & McPartland, 2005), it would follow that diminished social engagement is one of the earliest deficits to differentiate infants who later develop ASD.

Consistently, parent report of Cuddliness at 6 and 12 months, differentiated infants who later developed ASD. Cuddliness was defined by the IBQ-R as “the baby’s expression of enjoyment and molding of the body to being held by a caregiver”. Questions within the domain included how often in the last week “did your infant seem to enjoy the closeness while being fed or held”, “snuggle after done feeding”, “mold to your body”, “enjoy being rocked or hugged”, and “seem eager to get away with hugged”. Cuddliness could reflect several dimensions such as infant social engagement with the caregiver, interest in physical proximity, motor abilities, and self-regulation strategies. Correlation analyses for temperament domains found significant positive relationships between Cuddliness and Smiling and Laughter and Approach at 6 and 12 months for the ASD group suggesting that parent report of cuddliness may group with factors of positive affect and approach.

Additional follow-up correlation analyses were run to examine additional hypotheses about the relationship of Cuddliness to other skills within the ASD group. To examine the

hypothesis that Cuddliness may be related to activity level or motor abilities, analyses were conducted for Cuddliness and parent report of Activity Level at 6 and 12 months (see follow-up analyses in results). However, no significant relationships were found. As well, given that children with ASD often display tactile sensitivity, decreased reports of Cuddliness may also reflect emerging sensory sensitivities. However, no relationship was found between Cuddliness and parent rated perceptual sensitivity at 6 or 12 months for the ASD group. It would be informative to conduct factor analysis to determine what other domains Cuddliness may be related to and what information caregivers are using to rate the cuddliness of their infant.

At 12 months, coders rated ASD infants as displaying increased frustration than the  $HR_{noASD}$  and the  $LR_{noASD}$  group. This is consistent with both retrospective and prospective studies detecting negative emotional reactivity and dysregulation in the first year of life for infants who later develop ASD (Bryson et al., 2007; Gomez and Baird, 2005; Watson et al., 2007; Zwaigenbaum et al., 2005).

Contrary to our hypotheses, parent report of temperament, but not observational measures of temperament, differentiated groups at 6 months of age. It may be that parents of infants with ASD were highly attuned to the early development of their younger child in order to detect possible ASD concerns (e.g., diminished cuddliness) and utilized a wider sampling of behavior across contexts compared to the observational coding. Furthermore, cuddliness may be easier for parents to operationally define and may be consistent across contextual factors. Another explanation could be that diminished social engagement may be more easily detectable by primary caregivers than independent observers given that the caregiver is the infant's primary social partner.

Observational raters detected diminished social engagement in the ASD group compared to the HR<sub>noASD</sub> group at 12 months of age. This supports other prospective infant findings that decreased social engagement by an observer in a lab context is not detectable until 12 months of age (Zwaigenbaum et al., 2005). This is also consistent with a profile of infants who develop ASD as decreasing in social engagement from 6 to 12 months of age (Bryson et al., 2007). It was interesting that there was no difference in observed social engagement between the LR<sub>noASD</sub> and HR<sub>noASD</sub> groups at 12 months as hypothesized. This suggests higher levels of social engagement may serve as a protective factor for infants at risk of developing ASD. Another hypothesis is that the clinicians administering the assessment may have been scaffolding social engagement and providing more support to HR infants as clinicians were not naïve to group status.

There was no significant relationship between parent and observational reports at 6 and 12 months after adjusting alpha level for multiple comparisons. Studies have found the strongest concordance rates between parent report and observed indices of temperament when well-established parent report measures are used, caretakers and observers rate infant behavior in similar situations, and comparisons are made between the same dimensions of temperament (Rothbart & Bates, 1998). Although this study utilized standardized measures of temperament and compared across the same dimensions of temperament, it may be that increased variability in infant behavior in the laboratory context contributed to lack of concordance between measures. In addition, the behavioral ratings were on a 5-point Likert-like scale and there may not have been enough variability in the coding system to determine a relationship with the parent report measure, which had a larger scale.

It was hypothesized that stability of temperament would be similar for both groups on the observational measure but parent report may differ due to increasing concerns for ASD and

parents' expectations of their child's behavior and development, particularly if the temperament questions were perceived as reporting on early autism risk. Parent report of Cuddliness was found to be stable across time. As shown in Figure 9, it is noteworthy that 4 out of the 6 lowest scores for Cuddliness were infants who later developed ASD and demonstrated a mean drop in approximately 20 points on Cuddliness from 6 to 12 months. Given that significant positive relationships were found between Cuddliness and Smiling and Laughter and Approach at 6 and 12 months for the ASD group, these parents may be reporting decreased Cuddliness reflecting related areas of concern such as decreased social approach and low positive affect. This would be consistent with another prospective infants sibling study finding a loss of loss of social-emotional connectedness between 6 and 12 months of age (see Bryson et al., 2007).

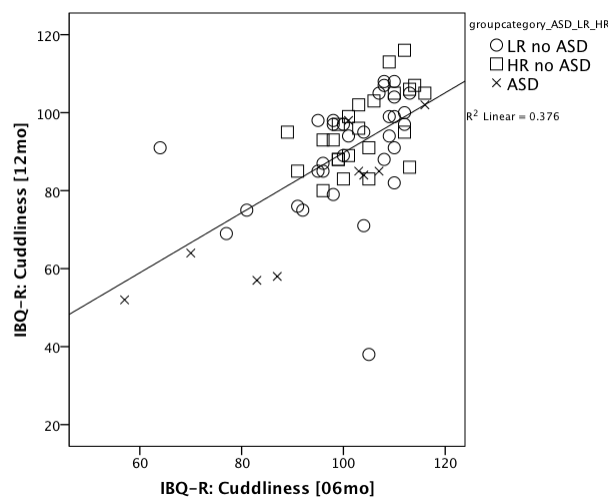


Figure 9. Stability of parent report of cuddliness by ASD outcome. Infants who developed ASD are marked by an X.

Social Engagement at 6 months was found to be predictive of Social Engagement at 12 months for observational coding of temperament but did not differ by groups across time. No other observational domains of temperament were found to be stable between 6 and 12 months. Social engagement may be one of the most stable and observable traits with the typically developing infant literature revealing social engagement tendencies are relatively stable from 6

to 18 months of age (see Marshall and Fox, 2006). An alternative hypothesis is that there was limited variability in the coding scheme given the range of 1 to 5 which may have constrained potential change in social engagement between groups between 6 and 12 months.

It was hypothesized that other temperamental domains such as Activity Level, Soothability, and Distress to Limitations would differ by groups at 6 and 12 months. Recent studies have found decreased activity level at 6 months by parent and observational measures in HR infants compared to LR infants (Wai Wan et al., 2012; Zwaigenbaum et al., 2005). It is unclear why this finding was not replicated in the current sample. It may be that these dimensions are variable across contexts; for instance, dimensions such as inhibition, activity level, and attention have been shown to have a situation specific effect (Majdandzic & van den Boom, 2007). In addition, activity level in the observational setting was restricted since infants were typically seated on their caregiver's lap at 6 months and in a high-chair at 12 months during the behavioral tasks.

Parent report of Soothability and Distress to Limitations were hypothesized to differ between groups based on parents of children with ASD retrospective report of self-regulation difficulties and higher negative emotional reactivity. In a large scale prospective study, parents of infants who developed ASD rated their infant's temperament as characterized by marked irritability, intolerance of intrusions, proneness to distress/negative affect, and difficulties with self-regulation and with being comforted or settled by others (Bryson et al., 2007). However, these results were not found in the current study. The study also found that HR infants who developed ASD were more passive and content at 6 months, and with the onset of symptoms changed from being "easy" to being readily irritated, distressed, and difficult to console at 12 months. It may be that our sample does consist of infants with these similar temperamental

profiles; however, our analyses did not allow for examination of these developmental trajectories. It would be informative to conduct functional discriminate analyses similar to Bryson and colleagues (2007) to determine if these developmental profiles exist. Soothability and frustration are typically low frequency behaviors and were not frequently observed in our laboratory assessments, limiting the ability to fully investigate this domain in the observational coding.

## **2. Aim 2**

The purpose of aim 2 was to determine if there were group differences in EEG alpha and asymmetry for LR<sub>noASD</sub>, HR<sub>noASD</sub>, and ASD groups. EEG frontal alpha and asymmetry stability from 6 to 12 months of age was examined as well as convergence with parent and observational measures of temperament for LR and HR infants.

Differences in EEG patterns among infants who developed ASD compared to the infants who did not develop ASD emerged at 12 months of age. Specifically, whereas the children in the HR<sub>noASD</sub> and the LR<sub>noASD</sub> groups showed very similar levels of brain activation (alpha EEG power) during the social versus non-social condition, the infants who developed ASD exhibited higher level of brain activation (lower EEG power) during the non-social condition as compared to the social condition. This suggests that infants who later develop ASD exhibited increased brain activation watching dynamic toys compared to watching social stimuli. This supports the theory of ASD as a failure to attend preferentially to social stimuli (Dawson et al., 1998) and possible preference for non-social stimuli. Multiple findings support the idea for increased preference for non-social stimuli than social stimuli. An electrophysiological study of 10-month-old high-risk infants found that HR infants as a group displayed an abnormal response of faster responses to objects compared to faces than low-risk infants (McCleery et al., 2009). A recent

study found that at 12 months of age, high-risk infants who later developed ASD tended to continue to observe a toy during a socially salient condition (Hutman, Chea, Gillespie-Lynch, & Sigman, 2012). Another prospective infant study found improved working memory for non-social stimuli for high-risk infants during the first year of life (Noland et al., 2010). Finally, young children with ASD have also been found to prefer non-social to social auditory stimuli and this pattern of response was related to abnormal electrophysiological response to sounds (Kuhl, Coffey-Corina, Padden, & Dawson, 2004). Thus, the current finding adds to the behavioral and electrophysiological literature of early processing differences between social and non-social stimuli in ASD.

It was hypothesized that ASD and HR<sub>noASD</sub> infants would show greater right frontal asymmetry than LR infants suggestive of less social approach and more negative affective at 6 and 12 months. However, there were no asymmetry differences between the ASD, HR<sub>noASD</sub>, and LR<sub>noASD</sub> groups at 6 or 12 months of age. This was an exploratory aim as no studies thus far have examined EEG asymmetry for HR and LR infants. Studies have found that older children with ASD who displayed a pattern of left frontal EEG asymmetry tend to display milder forms of social symptoms and retrospective reports of parent noted concerns at later ages (Burnette et al., 2011; Sutton et al., 2005). However, in these studies, there were no diagnostic group differences in frontal activity suggesting that differences appeared to be related to general rather than syndrome specific factors (Burnette et al., 2011). Similarly, the current study did not find asymmetry differences between groups. It may be that EEG asymmetry does not distinguish between groups but operates as modifier in the expression of ASD as proposed by Mundy and colleagues (2007). Previous findings support this theory as frontal brain activity differentiates approach versus withdrawal subtype classification in ASD (Dawson et al., 1995).

Although EEG alpha power was found to be stable for both the HR and LR group, follow up analyses suggest that EEG asymmetry stability was only found for the HR group for the social condition. Asymmetry for the non-social condition was not stable from 6 to 12 months for either group. This seemingly contradictory result of stable EEG power but unstable asymmetry value is consistent with a study that found EEG asymmetry stability coefficients were consistently lower for EEG asymmetry than for EEG power in typically developing children (Vuga et al., 2008). The authors explain that the common component containing stable EEG power at each assessment becomes eliminated in the calculation of EEG asymmetry resulting in lower asymmetry stability. Overall, studies have found inconsistent EEG asymmetry stability results with some groups finding lack of stability across a five month period, while other groups reporting moderate stability across a 6 month interval and across the first few years of life (Bell & Fox, 1994; Vuga et al., 2008). A number of potential factors may influence the stability of frontal EEG power including child sex, parental history of depression, and brain development. While Vuga and colleagues (2008) did not find sex or parental depression to affect stability findings, this sample was too small to test for sex differences. Examining parental history of depression would be an interesting direction given the known increase of parent depression and stress in parents of children with ASD (Bitsika, & Sharpley, 2004; Dunn et al., 2001).

The second part of this aim was to determine if EEG frontal asymmetry is related to parent and observational report of temperamental characteristics given previous findings that infants with right frontal EEG asymmetry tend to be more withdrawn and express negative affect whereas infants with left frontal EEG asymmetry tend to be more approach oriented and express positive affect (Fox et al., 2001). The goal was to further expand upon the literature showing a

relationship between approach and withdrawal behaviors and EEG asymmetry in older children with ASD (Burnette et al., 2011; Dawson et al., 1995; Sutton et al., 2005).

Infants who displayed left asymmetry while watching a video of women saying nursery rhymes, displayed higher levels of Cuddliness according to parent report at 6 months, but not 12 months of age. These results replicate a recent EEG finding where left frontal activation related to parent report of approach and engagement in typically developing infants (LoBue, Coan, Thrasher, & DeLoache, 2011). Although not statistically analyzed, some of the infants with the lowest scores on Cuddliness and corresponding right frontal asymmetry at 12 months later developed ASD suggesting that this pattern may represent an early risk marker for ASD (see Figure 9). Overall, the findings suggest that there is a relationship between cuddliness and left EEG asymmetry, which supports previous findings between social approach and left asymmetry in typically developing infant studies. This is the first study to find such a relationship with a sample of infants at risk for ASD.

### **3. Summary**

The current study expands on the findings of temperament in prospective infant siblings studies. Similar to a previous large-scale longitudinal study which found that behavioral approach at 24 months differentiated high-risk siblings who did and did not later develop ASD (Garon et al., 2009), the present study found observation of Social Engagement to differentiate between infants who developed ASD and those that did not starting at 12 months. A novel finding is that parents of the ASD infants detected diminished Cuddliness at *both* 6 and 12 month on parent measures of temperament. The current study expands upon the literature of temperament in ASD by finding both parent report and independent observers reports of temperament differences during the first year of life. In addition, this study found that starting at

6 months of age, the ASD group scored higher on an early symptom measure (the Autism Observation Scale for Infants) compared to the HR<sub>noASD</sub> and the LR<sub>noASD</sub> groups. At both 12 and 18 months of age, the ASD group scored higher on early symptom measures and lower on cognitive measures than the HR<sub>noASD</sub> and the LR<sub>noASD</sub> groups. Overall, these results suggest that early signs are able to be detectable by 6 months of age and that differences between ASD and non-ASD groups grows larger during the next 12 months of life.

However, it is important to note that these temperament and early symptom differences were only apparent after being classified by risk status. It is unknown if these results would be generalizable and detect differences at a population based level. The current results suggest that aspects of temperament, specifically positive affect, cuddliness, social engagement, and increased frustration may be informative of the trajectory of ASD for infants with a familial risk for ASD. Given the finding of sub-groups of ASD infants displaying a temperamental profile of decreased social engagement and regulatory difficulties (Bryson et al., 2007; Garon et al., 2009), exploring differences in temperament patterns rather than separate temperament scales may be more useful for identification of ASD in infancy. In addition, combining temperament measures with early symptom and cognitive measures may contribute to a more complete picture of the manifestation of early risk symptoms in ASD.

This is the first study to our knowledge to examine EEG frontal alpha and asymmetry in a prospective high-risk infant sample for ASD. One of the main EEG findings is that at 12 months of age, infants who later developed ASD exhibited a higher level of brain activation (lower EEG power) during the non-social condition compared to the social condition. This suggests that infants who later develop ASD may display increased attention and a preference for non-social stimuli detectable at a neurophysiological level as early as 12 months of age.

Although there were no differences in EEG asymmetry between ASD and non-ASD infants, there were associations between temperament domains and EEG asymmetry such as a positive relationship between Cuddliness and left EEG asymmetry. This is the first study to find such a relationship with a sample of infants at risk for ASD and supports previous findings between social approach and left asymmetry in typically developing infants.

In summary, infants who later developed ASD tended to have higher brain activation (lower EEG alpha power) while watching non-social stimuli compared to social stimuli at 12 months of age. Similar to studies of typically developing infants and older children with ASD, there were associations between EEG profiles and approach behaviors suggesting that EEG power and asymmetry may inform the presentation and trajectory of symptoms and potentially response to intervention. Examination of EEG power and asymmetry along with other measures of early social-communication risk signs may inform the trajectory and identification of ASD in infancy.

#### **4. Limitations**

There are a number of important limitations to the current study. Consistent across many prospective infant siblings studies, one primary limitation is small sample size. Generally, infant siblings of children with ASD are difficult to recruit, especially within one geographic location. Despite this difficulty, the study was able to recruit 43 high-risk infants. ASD rates in the sample were similar to other prospective studies (Ozonoff et al., 2011) with 11 infants (10 HR and 1 LR) meeting criteria for ASD. However, this small group decreased overall power to detect group differences and correlations. Thus, the results of the current study should be interpreted within this constraint. In addition, given that not all of the infants have yet reached their 36-month-old time point, an age at which diagnosis may be more stable, it is unknown whether these infants

will consistently meet criteria for ASD or whether their symptoms at 18 months represent transient delays or difficulties that may resolve over time.

There are also a number of methodological limitations to the current study. One is that the coding system utilized in the current study has not been previously empirically validated. Although previous research has utilized the BRS to examine temperament, the purpose of the measure is for gestalt coding of domains of temperament. The current coding system was developed and definitions were modified in order to better operationally define coding items. This included high standards to obtain initial reliability and closely monitored ongoing reliability with high inter-rater agreement. Although it is the hope that this system improved observational coding of temperament, this is the first study to use the coding system in this manner. Given that the coding was conducted on specific items and not gestalt coding, specific behaviors may not have been observed during the available coding periods. One way to examine this would be to train coders on the gestalt coding system and to re-code the full Mullen and AOSI, allowing for more behaviors. This could then inform the research and clinical utility of utilizing a gestalt versus item-level observational coding method.

A second limitation is that the observational coding may have captured idiosyncratic factors (e.g., infant's mood and alertness). In addition, the observation of temperament was coded from the same measures that early symptoms and cognition was derived from. Therefore, the behaviors observed may reflect early ASD symptoms and are not a truly independent measure of temperament. Future studies should include assessment across multiple time points as well as naturalistic observations (e.g., during parent-child interactions).

In previous studies of HR samples, one concern is that those infants who go on to develop ASD may demonstrate behaviors that are suggestive of group status leading to a bias in

the coding of behaviors. Coders may have been able to detect early signs of ASD, which could bias their coding of behavior. In addition, some parents mentioned during the assessment that they have an older child with ASD. Thus, knowledge of infant risk status may have influenced coder's ratings of temperament. In addition, clinicians assessing infants at 6, 12, 18, and 24 months of age were not native to infant siblings status. Although early symptom and cognitive measures are standardized, this knowledge may have impacted clinician's scoring of early symptom measures.

A final limitation of the observational coding is that some of the behaviors of interest have a lower frequency of occurrence and may have not been observed within the context of the laboratory setting (e.g., soothability). One way to elicit these behaviors would be to provide opportunities in the laboratory setting for these behaviors to occur. For example, the Lab-TAB is a standardized laboratory measure developed by Rothbart and colleagues and designed to elicit specific behavioral or emotion responses assumed to reflect temperamental traits (e.g., toy retraction and caregiver separation). A future direction would be to utilize this measure with prospective HR infants to obtain a larger variety of behavior within a limited sampling opportunity.

In terms of parent report, the IBQ-R has been utilized in numerous infant studies and has recently started to be applied in HR populations. However, it is unclear how well suited the IBQ-R and other temperament measures are for ASD populations given the conceptual overlap between temperament and IBQ-R. For instance, the domain "Approach" has both social and non-social items comprising the total score (e.g., approach to toys and approach to people). Although it would be intuitive to think that an infant scoring high on "Approach" would engage in social approach behaviors, it could be that the parent is primarily relying on these non-social items.

Recent evidence suggests that differences in social and non-social attention may be one of the hallmarks of ASD, with early increased non-social attention as an early risk sign for ASD (Bryson et al., 2007; Wetherby et al., 2004; Zwaigenbaum et al., 2005). Infants at risk for ASD may be scoring high on “Approach” based on higher attention and approach oriented behaviors to non-social items. Thus, future research into temperament in ASD must clearly delineate social and non-social items and develop different factors for each. Within the current sample, a future direction would be to separate the social and non-social items in the IBQ-R and run the same analyses within this project to determine if any differences become apparent between temperamental domains. It may be that in ASD, specific temperament factors are expressed differently between social and non-social contexts.

Most research with EEG frontal asymmetry has been conducted during “resting states” rather than dynamic stimuli. The current study utilized socially engaging (e.g., women saying a nursery rhyme) and non-social videos (e.g., toys moving) in order to determine if EEG differed using these dynamic stimuli. This may explain why the current study did not fully replicate previous findings of temperament and EEG frontal asymmetry as prior studies often used a low-demand social engagement for baseline (e.g., an experimenter blowing bubbles or spinning a pinwheel). However, a recent study of typically developing infants utilized an EEG paradigm with emotionally salient stimuli (films of snakes and elephants) did find consistent patterns between left activity and parent rated temperament (LoBue et al., 2012) suggesting that dynamic stimuli can produce similar and possibly stronger results as it attempts to elicit affective and approach systems. Lastly, the infant’s affective state prior and during viewing the videos (e.g., happy versus distressed) may have influenced EEG frontal asymmetry. Given that the infants were filmed during EEG data acquisition, this question could be addressed in a follow-up analysis.

## 5. Future Directions for the Current Project

A few immediate next steps for the current project include longitudinal follow-up at 24 and 36 months to examine stability of ASD diagnosis. Furthermore, the longitudinal study obtained parent report of temperament and EEG data at 18 months as well. Thus, the trajectory of temperament and interaction with ASD symptomology can be examined through a development framework. Given the power limitation, it may also be fruitful to combine samples with other prospective infant sibling research groups to examine potential temperamental characteristics between HR infants who do and those who do not go on to develop ASD. This would also lend the data to more sophisticated modeling analyses that could allow for identification of developmental profiles for ASD as well as characterize protective factors.

Given the focus of the study, related questions were not investigated such as the relation between parental depression and stress to parental report of temperament and infant EEG. A number of studies have cited parental characteristics such as depression, personality, psychopathology, and stress as influencing parental report of temperament (Forman et al., 2003; Mebert, 1991). A potential factor in a prospective infant sibling sample is the impact of broader autism phenotype characteristics in parents that may influence their impressions of their infant's temperament. Maternal depression has been shown to be associated with infant EEG activation (e.g., greater relative right frontal EEG) (Field & Diego, 2008). Fortunately, measures of parental depression, stress, and self-reported broader autism phenotypic characteristics (e.g., interest in social relationships) were collected as part of the larger study and can be examined in the context of temperament factors and emerging ASD symptoms as well as the association with infant EEG activation patterns.

One of the major aims of the larger study was to examine the effects of an early parent-child intervention for HR infants. Thus, half of the HR infants were randomized into a 10-week intervention aimed at increasing parental sensitivity to their infant's cues with the goal of promoting the parent-child relationship. The results of the current study should be examined within the context of this intervention to determine the effects of intervention for parent report of temperament, observational report, and potentially EEG asymmetry. The treatment could moderate findings in a number of ways such as improving parents' perceptions of their infant's temperament (e.g., helping parents observe their child's behavior objectively) as well as potentially decrease parental stress/depression, which may influence parental ratings of temperament. In addition, a parent may become more in-tuned with their infant and accommodate temperamental challenges more easily (e.g., noticing warning signs for frustration before the behavior escalates). Alternatively, there may be a direct effect of intervention for the infant that may modify temperamental characteristics and early ASD risk signs. These are interesting questions to explore further in this sample.

## **6. Future Directions in the Field**

Identifying temperamental factors of ASD in infancy can inform the course of symptom expression and severity over time. For instance, Rogers (2009) notes that many of the case studies of early ASD reveal that symptoms that were thought to be secondary such as irritability, sensory responsivity, activity level, and poor gross motor abilities appear concurrently or even before core symptoms of ASD. This suggests that ASD may not only affect social development but multiple domains of development. Studies also suggest that there may be a change in temperament profiles in development. For example, Bolton et al. (2012) found decreased activity at younger ages changing to increased activity later in ASD. This result is consistent

with higher activity levels reported in older children with ASD and reports of passivity and decreased activity during infancy. Longitudinal high-risk studies suggest that specific temperamental factors and profiles may change between 6 and 24 months of age due to the onset of ASD symptoms and related developmental changes. Thus, examining variables such as temperament that may moderate or overlap with ASD symptoms may prove critical for understanding the presentation and development course of ASD.

It will be important for future research in the field of prospective studies of ASD to be able to develop a consistent and developmentally appropriate strategy to classify the broader autism phenotype (BAP) in HR infants (e.g., identifying subgroups based on language, social, and restricted interests and repetitive behaviors). Temperament may prove to be a useful construct for developing this BAP classification by examining what factors are consistent among HR infants who go on to develop ASD and those that do not. Protective factors against ASD can also be identified-- these may include temperamental traits such as increased positive affect and cuddliness since there were no differences between LR<sub>noASD</sub>, and HR<sub>noASD</sub> groups on these domains in the current sample.

It will be important to continue to expand the EEG literature in HR infants to determine if there is indeed a reliable biomarker that can identify infants who later develop ASD. A recent study found that a measure of resting state EEG complexity was able to reliably differentiate infants between HR and LR infants (Bosl, Tierney, Tager-Flusberg, & Nelson, 2011). Although this is an interesting finding, longitudinal analyses are necessary to determine if this EEG marker can reliably differentiate infants who later develop ASD.

Future research should focus on the specificity of temperamental and EEG factors/profiles in distinguishing infants who later develop ASD in a population-based sample. It

would be beneficial to include infants at-risk for other developmental disorders in order to determine if specific temperament profiles distinguish infants who later develop ASD from other developmentally delayed or other developmental psychopathology outcomes. The hope is to advance early detection efforts from a selective strategy (i.e., identifying “at-risk” infants for ASD based on familial risk) to a universal strategy. Inclusion of both “typically developing” and “at-risk” populations would have important early screening implications with the focus on improving identification of ASD by increasing not only sensitivity, but also specificity.

Ultimately, the study of temperament can be useful in not only the early identification of ASD, but also in explaining variability within the behavioral phenotype, which can help individualize interventions for children with ASD. Temperament may aid in developing treatment goals as well as examining response to intervention. Studying temperament in other disorders has led to a number of temperament-based interventions (McClowry, 1998) that could be applied to ASD interventions. For instance, children who are lower on approach and higher avoidance behavior are less responsive to behavioral treatment (Sherer & Schreibman, 2005). In addition, a child’s temperament and parent perception of their child’s temperament may impact the parent-child relationship indirectly affecting response to treatment. Therefore it is important to assess both temperament and parent-child relationship in order to provide effective teaching strategies that encourage social engagement. Brock and colleagues (2012) suggest that helping parents focus on positive characteristics of their child may be helpful for a child who has a difficult temperament or social withdraw. Thus, the study of temperament in ASD can provide relevant information for the development and modification of interventions for ASD by targeting specific temperamental characteristics that are known to be related to clinical outcomes for individuals with ASD.

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APPENDIX A.  
TEMPERAMENT CODING MANUAL

**Behavior Rating Scale  
-Early Connections Study-**

**THE EARLY CONNECTIONS STUDY:**

The Early Connections Study is a longitudinal study looking at infant siblings of children diagnosed with autism. Research has shown a greater likelihood of siblings of children with autism developing the disorder as well. We want to learn more about how temperamental factors may help in identifying early symptoms in infant at-risk for autism. Infants will be evaluated using behavioral and brain assessments at 6, 12 and 18 months of age. This project examines multiple assessments of temperament, including parent report, observational measures, and EEG as early risk markers for autism.

**AOSI AND MULLEN PROCEDURE:**

This sample consists ~45 minute videotaped standardized behavioral session with the experimenter and child. The play sample involves a set of toys, an unfamiliar environment, and a parent/primary caregiver.

The first assessment administered is the Autism Observation Schedule for Infants. The AOSI is an 18-item direct observational measure designed to detect and monitor early signs of autism in infants aged 6-18 months. Infants are engaged in a semi-structured play, and systematic presses are designed to assess various target behaviors, including visual tracking and attention disengagement, coordination of eye gaze and action, imitation, affective responses, early social-communicative behaviors, behavioral reactivity, and sensory-motor development.

The second assessment is the Mullen Scales of Early Learning which is a standardized measure of cognitive functioning for children and assesses skills in five areas: gross motor, visual reception, fine motor, receptive language, and expressive language. The infant completes a variety of tasks based on their developmental level.

Training Checklist for 499 Students

- Complete the Required Readings
  - Early Connections Study Coding Manual
  - Autism Spectrum Disorders: A Reference Handbook
  - Behavioral Manifestations of Autism in the First Year of Life (Zwaigenbaum et al., 2005).
  - Temperament and its Relationship to Autistic Symptoms in a High-Risk Infant Sib Cohort
- Watch the example DVDs to gain understanding of assessment and calibrate understanding of codes.
- Complete practice coding videos.

### General Tips for Coding

1. Always code what you see not how you feel about what you see. We are coding behaviors, not intentions. Be sure you are not trying to interpret the behavior but only coding exactly what you see the person doing.
2. Make sure you pay close attention to each item and the description. Re-read the codes each time you come in for a coding session. When you are coding a tape think carefully about which code best represents the behavior that you saw.
3. If for some reason you know the parent or child pictured immediately stop coding and inform a supervisor.
4. For the most part all five points on the scale should be used. It is easy to fall into the habit of always scoring in the middle, but try to avoid this.
5. The first time you watch a video clip, try to watch overall for impressions and write down preliminary codes. The second time watching, pay close attention to any codes you were unsure of and confirm codes.

### Coding Instructions

1. Code AOSI items
  - a. Code all items for AOSI tasks (Peekaboo, Imitation, and Ball)
  - b. Code as a “social task”. For instance, “persistence” = persistence in keeping social interaction going
2. Code Mullen items:
  - a. Code all items for Mullen tasks:
    - 6month: ring with string, ring under washcloth, peg, and cheerio
    - 12 month: ring under washcloth, teddy bear with cup, blocks, and pennies in bank
3. When coding each item, consider coding to start from time the examiner presents item to infant through transition time to next item (e.g., if infant gets fussy during the time of the transition, should be coded under the first item).
4. Complete categorical questions on last page once coding is finished. These questions should reflect an overall impression of the infant from watching both the AOSI and Mullen

## Behavior Rating Scale

### 1. Predominant State

This scale assesses the infant's state of arousal that is most prevalent during the test session. The states range from drowsy or asleep to awake and alert.

**Rating of [1]: Very Low** – Drowsy or asleep

**Rating of [2]: Low** – Typically drowsy, a few moment of wakefulness

**Rating of [3]: Moderate**- Drowsy half the time, awake and alert half the time

**Rating of [4]: High** – Typically awake and alert, a few moments of drowsiness

**Rating of [5]: Very High** – Awake and alert

### 2. Lability of State of Arousal

This scale assesses whether the infant's state of arousal changes during the session or whether one arousal state predominates. For example, the child may alternate between drowsy/sleepy and awake/alert state, or the infant may be sleepy during the beginning but become alert later in the session. Alternatively, the infant may remain awake and alert, or asleep, for the entire session.

**Rating of [1]: Constant changes** – From state of drowsiness or sleeping to alert

**Rating of [2]: Frequent changes** – From state of drowsiness or sleeping to alert

**Rating of [3]: Several changes** – From state of drowsiness or sleeping to alert

**Rating of [4]: One or two changes** – From state of drowsiness or sleeping to alert

**Rating of [5]: Constant** – State of drowsiness or sleeping to alert

### 3. Energy level

Behavior that is vigorous, robust, animated or expressive. Infant's gross motor activity, including movement of arms and legs, squirming, and locomotor activity.

**Rating of [1]: Very low** – Consistently lacks animation or energy; tired and lackluster, not engaging in task

**Rating of [2]: Low** – Typically tired and lackluster; one or two period of animation or energy; rarely engages in task

**Rating of [3]: Moderate** – Animated/energetic half the time; lackluster half

**Rating of [4]: High** – Typically animated/energetic; one or two periods of being lackluster, usually engaged in task

**Rating of [5]: Very High** – Consistently animated or energetic, consistently engaged

#### 4. Positive Affect

The degree to which the infant displays positive affect in response to either the test materials or to the examiner and caregiver. Positive affect includes smiling, laughing, or making sounds that are perceived to be expressions of excitement, happiness or pleasure. The ratings refer to both duration and intensity.

**Rating of [1]: Very low** – No positive affect displayed

**Rating of [2]: Low** – One or two brief displays of positive affect

**Rating of [3]: Moderate** – Three or more brief displays of positive affect

**Rating of [4]: High** – One or two intense, heightened, or prolonged displays of positive affect

**Rating of [5]: Very High** – Three or more intense, heightened, or prolonged displays of positive affect

#### 5. Negative Affect

The degree to which the infant displays negative affect in response to either the test materials or to the examiner and caregiver. Negative affect includes fussing, pouting, whining, crying, and vocal or physical expressions of anger. The ratings refer to both duration and intensity. (Note: this is reverse coded from the manual)

**Rating of [1]: Very low** – No negative affect displayed

**Rating of [2]: Low** – One or two brief displays of negative affect

**Rating of [3]: Moderate** – Three or more brief displays of negative affect

**Rating of [4]: High** – One or two intense, heightened, or prolonged displays of negative affect

**Rating of [5]: Very High** – Three or more intense, heightened, or prolonged displays of negative affect

#### 6. Soothability when upset

The amount of external support required to calm the infant once upset. Infant's reduction of fussing, crying, or distress when soothing techniques are used by the caretaker. Note: Record how many and what strategies utilized.

**Rating of [1]: Cannot be soothed** – Infant not able to calm down, task not completed

**Rating of [2]: High effort to soothe** – One or more strategies utilized, infant still upset

**Rating of [3]: Moderate effort to soothe** – One or more strategies, eventually calmed

**Rating of [4]: Low effort to soothe** – One strategy used, infant easily calmed (e.g., distracted easily)

**Rating of [5]: Does not need external assistance (e.g., self-soothed)**

**Rating of [9]: Not Applicable (NA)** – No instances of negative affect

## 7. Hypersensitivity to Test Material and Stimuli

The infant's excitability or sensitivity to the stimulation provided by the test material, the environment (e.g., lighting, background noise), or by the experimenter or caregiver as they interact with the infant. A hypersensitive child's disposition or organization might be so disrupted by the sights and sounds of the testing situation that she or he cannot attend to the tasks. A less sensitive infant may have an adverse reaction to only one or two types of stimuli, have a mild reaction to some stimuli, or not be disrupted at all by the stimuli.

**Rating of [1]: No hypersensitivity** – Consistently responds appropriately to task

**Rating of [2]: Low hypersensitive** – Possible odd reaction to item

**Rating of [3]: Moderate hypersensitive** – Hypersensitive in a few instances but does not disrupt testing

**Rating of [4]: High hypersensitive** – Returns to activity in one or two instances

**Rating of [5]: Constantly hypersensitive** – Hypersensitivity disrupts testing

## 8. Fearfulness

The extent to which the infant shows fear toward the examiner when the examiner makes a social or physical approach toward the infant or when the examiner presents material to the infant. For example, a fearful infant might try to hide behind his or her caregiver, bury her or his head in the caregiver's lap, or be hesitant to approach the examiner or the toys. A less fearful infant may exhibit fear only when the examiner gets too close.

**Rating of [1]: No fear** – Never fearful

**Rating of [2]: Possibly fearful** – Unclear if fearful

**Rating of [3]: Moderately** – Mildly fearful but does not disrupt testing

**Rating of [4]: Fearful** – One or two instances of fear and continues task

**Rating of [5]: Very Fearful** – Fearfulness disrupts testing

## 9. Orientation to the examiner

The degree to which the infant accepts the examiner during the session. A responsive infant accepts the examiner's presence and the examiner's approaches toward him or her. An avoidant child may turn his or her face or close his or her eyes when the examiner approaches. A resistant child may push examiner away when they touch or hold them.

**Rating of [1]: Constantly avoids** – Never responsive, doesn't look at examiner

**Rating of [2]: Typically avoids or resists** – One or two instances of looking

**Rating of [3]: Occasionally avoids or resists** – Half time looking at examiner

**Rating of [4]: Moderately responds** – Mostly looking at examiner

**Rating of [5]: High responsiveness** – Always looking and responsive

## 10. Social Engagement

The degree to which the infant willingly attempts to engage the examiner or the caregiver in social interaction (e.g., vocalizations, smiles, reaches). The infant may or may not use the materials to initiate interaction. Take into account how often infant initiates plus quality of social interaction.

**Rating of [1]: Very low** – No attempts to interact socially

**Rating of [2]: Low** – Possible attempt

**Rating of [3]: Moderate** – A few attempts, not highly engaged

**Rating of [4]: High** – Many attempts to interact socially

**Rating of [5]: Very High** – Constant attempts to interact socially

## 11. Cooperation

The degree to which the infant willingly responds to examiner's or parent's requests to perform the task. A child scoring high in compliance will make an effort to do what the adult asks or will respond quickly to the adult's subtle or overt suggestions. A child scoring low in compliance may refuse to cooperate with the adult. This child may actively avoid the activity by throwing materials or simply by ignoring the adults suggestions and engaging in other activities.

**Rating of [1]: Very Low** – Consistently resists; does not respond to suggestions or requests. The child may overtly demonstrate refusal to cooperate by throwing or pushing away materials, or may simply ignore the adult's suggestions.

**Rating of [2]: Low** – Typically resists; one or two instance of cooperation. While the child may occasionally attempt to cooperate with the adult's suggestions, the child is not cooperative for the majority the interaction.

**Rating of [3]: Moderate** – Resists half the time, cooperates half time. The child attempts to cooperate with the adult's requests or suggestions about as often as he or she does not cooperate.

**Rating of [4]: High** – Typically cooperates; one or two instances of avoidance or resistance. He or she may occasionally refuse to cooperate but for the majority of the time attempts to follow the adults suggestions or requests.

**Rating of [5]: Very High** – Consistently cooperates; never avoidant or resistant. He or she responds quickly to both overt and subtle requests or suggestions.

## 12. Adaptation to change in test materials

The infant's ability to repeatedly relinquish material used for one item and accept the material for the next item. An infant who has difficulty making the transition may become upset to varying degrees when the examiner tries to induce him or her to relinquish the material. A child who easily makes the transition from one material to another will show interest in the new material, even though she or he was interested in what she or he was playing with, and readily relinquish the old material for the new material presented.

**Rating of [1]: Very Low** – Does not relinquish and/or is highly distressed, does not calm down with presentation of new item

**Rating of [2]: Low** – Distressed but will calm with presentation of new item or when distracted/comforted

**Rating of [3]: Moderate** – Mild display of distress but able to calm down quickly

**Rating of [4]: High** – Typically relinquishes and accepts new materials; may show some mild discomfort/distress

**Rating of [5]: Very High** – Easily relinquishes materials and accepts new materials.

## 13. Initial Interest in Test Materials and Stimuli

The amount of interest the infant displays in the materials or stimuli. This does not mean the amount of enthusiasm, persistence, or overall attention the child displays but rather, the degree to which the child initially attends to the task during each administration.

**Rating of [1]: Very Low** – Does not attend, off task, does not look at all

**Rating of [2]: Low** – Typically un-interested, attends in one or two instances.

**Rating of [3]: Moderate** – Interested half the time, uninterested in other half

**Rating of [4]: High** – Typically interested

**Rating of [5]: Very High** – Constantly attends

## 14. Initiative with task

The extent of the child's initiative in exploring the materials (e.g., touching, mouthing, looking). (Note: for social tasks, code infant's initiative in keeping interaction going.)

**Rating of [1]: Very Low** – Does not initiate task. May be extremely passive and inactive.

**Rating of [2]: Low** – Occasionally attempts to initiate activities. Mostly, infant is passive

**Rating of [3]: Moderate** – On several occasions the infant attempts to keep interaction going.

**Rating of [4]: High** – Infant consistently attempts to keep interaction, attention wanders briefly

**Rating of [5]: Very High** – Constantly initiates activities.

## 15. Exploration of Object/Surroundings

The degree to which the infant actively seeks out new aspects of the task, including visual, auditory, and tactile exploration. (Note: code entire interval for overall exploration (even if not specific to task).

**Rating of [1]: Very Low** – No exploration

**Rating of [2]: Low** – One or two instances of exploration

**Rating of [3]: Moderate** – Moderate exploration

**Rating of [4]: High** – Much exploration

**Rating of [5]: Very High** – Constant exploration

## 16. Attention to Task

The degree to which the infant remains focused on the task presented by the examiner, in other words, the degree to which the infant sustains interest in the task. Code duration of attention.

**Rating of [1]: Very Low** – The child never attends to the activity for more than a few seconds at a time. He/she may be completely inactive, avoidance of the activities, or may constantly change activities

**Rating of [2]: Low** – The child can be described as generally inattentive for the activity. Although the child sometimes participates in the activity, more often inactive or avoidant

**Rating of [3]: Moderate** – The child attends to the activities about as often as she does not. She has extended periods of time in which she participates in the activity as well as periods in which she is engaged in avoiding or changing activities.

**Rating of [4]: High** – The child “stays with” the activities for the majority of the session. She may have periods in which she is inattentive but these are short-lived and limited in number

**Rating of [5]: Very High** – Constantly attends; child “stays with” the activities throughout the session. The child participates in the activities without periods of inattention.

## 17. Persistence in Attempting to Complete Task

The degree to which the infant persists at tasks in attempting to complete them. Persistence should be distinguished from preservation, in which the infant repeats a part of the task without the aim of completing the entire task. (Note: for social items, code persistence in keeping social interaction going)

**Rating of [1]: Very Low** – The child never demonstrates repetition of a behavior. The infant who is low in persistence may never attempt a second try when having difficulty.

**Rating of [2]: Low** – The child infrequently demonstrates repetition of a behavior. She may occasionally make a second attempt when having difficulty but quickly gives up.

**Rating of [3]: Moderate** – The child has extended periods in which he or she seems to be practicing behaviors, but just as often has periods in which he does not practice. Similarly, there are periods in which the child continues to try when having difficulty about as often as there are periods in which she quickly gives up.

**Rating of [4]: High** – Although the child has some periods in which she quickly gives up or during which repetition of behavior is rarely seen. In general, the infant is high in persistence. She is often observed to practice behavior or makes second or third attempts when having difficulty.

**Rating of [5]: Very High** – The child frequently practices activities. He also may make repeated attempts at tasks when having difficulty.

## 18. Enthusiasm towards task

The degree to which the infant exhibits deep concentration, coupled with excitement or delight, in the material or task. (Note: must show excitement by either a positive vocalization, facial expression, or motor activity).

**Rating of [1]: Very Low** – Consistently unenthusiastic

**Rating of [2]: Low** – Typically unenthusiastic, one or two instances of enthusiasm

**Rating of [3]: Moderate** – Unenthusiastic half, enthusiastic half the time

**Rating of [4]: High** – Typically enthusiastic, unenthusiastic in one or two instances

**Rating of [5]: Very High** – The child frequently demonstrates enthusiasm.

## 19. Frustration with inability to complete task

The degree to which the infant becomes frustrated when she or he is unable to understand or complete a task. When coding consider, emotional reaction and if the response affected completion of the activity.

**Rating of [1]: No frustration**

**Rating of [2]: Low** – Possible mild frustration, such as unclear facial expression or vocalization

**Rating of [3]: Moderate** – Frustration in a few instances but does not disrupt testing (e.g., distress vocalization)

**Rating of [4]: High** – Often frustrated (e.g., whine/cry) but returns to activity in one or two instances

**Rating of [5]: Very High**- The child frequently becomes frustrated and it disrupts testing.

## 20. Gross-motor movement required by tasks.

The appropriateness of the child's movement and coordination in her or his arms, legs, and/or trunk, in response to the demands of the task.

**Rating of [1]: Inappropriate** – Definite inappropriate movement the entire time

**Rating of [2]: Low** – Definite most of the time

**Rating of [3]: Moderate** – Definite inappropriate movement but brief and mild

**Rating of [4]: High** – Typically appropriate, one or two instances of possible inappropriate gross-motor movement

**Rating of [5]: Consistently appropriate** – No inappropriate movement observed

## 21. Fine-motor movement required by tasks.

The appropriateness of the child's movement and coordination in her or his fingers and hands, in response to the demands of the task.

**Rating of [1]: Inappropriate** – Definite inappropriate movement the entire time

**Rating of [2]: Low** – Definite most of the time

**Rating of [3]: Moderate** – Definite inappropriate movement but brief and mild

**Rating of [4]: High** – Typically appropriate, one or two instances of possible inappropriate gross-motor movement

**Rating of [5]: Consistently appropriate** – No inappropriate movement observed

## 22. Tremulousness.

Tremors in the child's motor movements. Tremors may appear as intermittent spastic muscle movements or constant trembling of the limbs or other parts of the body.

**Rating of [1]: Constant** – Definite tremulousness movement the entire time

**Rating of [2]: Frequent** – Definite most of the time

**Rating of [3]: Moderate** – Definite movement but brief and mild

**Rating of [4]: Infrequent** – Possible instance

**Rating of [5]: None**

## 23. Hyperactivity

Behavior that is superfluous and impulsive. A hyperactive infant may squirm, kick, or flail arms/body.

**Rating of [1]: Constant** – Consistently hyperactive, fidgety and agitated in movement

**Rating of [2]: Frequent** – Typically hyperactive, one or two instances of appropriate activity level

**Rating of [3]: Moderate** – Hyperactive half the time, appropriate activity level half the time

**Rating of [4]: Infrequent** – Typically not hyperactive, one or two instances of hyperactivity

**Rating of [5]: None** – Consistently not hyperactive, never fidgety or agitated in movement

**Karen Burner, Ph.D.**  
**Curriculum Vitae**

**Education**

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- |           |   |
|-----------|---|
| 2006–2012 | Doctorate in Child Clinical Psychology<br>University of Washington, Seattle<br>Advisor: Geraldine Dawson, Ph.D.; Co-advisor: Wendy Stone, Ph.D.;<br>Autism Center Mentor: Sara Jane Webb, Ph.D.<br>Dissertation: Observational and Electrophysiological Assessment of<br>Temperament in Infants at Risk for ASD |
| 2006–2010 | M.S. in Clinical Psychology<br>University of Washington, Seattle<br>Thesis: Neural Correlates of Emotional Face Processing in Children with<br>Autism.  |
| 1999–2003 | B.A. in Human Development, Cum Laude, Departmental Honors with<br>Highest Distinction; Minor: Biology<br>University of California, San Diego  |
| 2001      | Study Abroad: University College London   |

**Research Support and Fellowships**

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|------|---|
| 2010 | Autism Science Foundation Pre-Doctoral Fellowship. \$25,000<br>“Observational and Electrophysiological Assessment of Temperament in<br>Infants at Risk for ASD” |
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**Awards and Honors**

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|------|--|
| 2009 | Travel Award, Nathaniel Wagner Memorial Endowment Fund<br>Clinical Psychology Training Program, University of Washington   |
| 2008 | Student Travel Award, International Meeting for Autism Research  |
| 2001 | Peterson Scholarship Award for students pursuing research careers in<br>developmental disabilities.<br><br>Foundation for Developmental Disabilities. San Diego, California. |

## Research Experience

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- 2007–2011 *Title:* Early Detection/Intervention in Infants at Risk for Autism, NIMH Autism Center of Excellence  
University of Washington Autism Center/Center on Human Development and Disability  
*Principal Investigators:* Sara Webb, Ph.D.; Bryan King, Ph.D.  
*Goal:* To identify neurophysiological risk indices for autism in high-risk infants and to assess whether it is possible to alter these risk processes via early intervention.  
*Role:* Graduate research assistant responsible for conducting diagnostic, cognitive, and neuropsychological assessments with infants longitudinally at 6, 12, 18, and 24 months of age. Wrote evaluation summaries and conducted feedback sessions with parents. Prepared human subjects applications and assisted in study flow and data tracking.
- 2007–2011 *Title:* Longitudinal MRI Study of Infants at Risk for Autism, NIMH Autism Center of Excellence, multi-site collaborative study  
University of Washington Autism Center/Center on Human Development and Disability  
*Principal Investigators:* Annette Estes, Ph.D.; Steve Dager, M.D.  
*Goal:* Investigate early symptoms of autism in sample of infant siblings of children with ASD.  
*Role:* Graduate research assistant responsible for conducting diagnostic, cognitive, and neuropsychological assessments of infants longitudinally at 6, 12, and 24 months of age. Wrote evaluation summaries and conducted feedback sessions with parents.
- 2006–2007 *Title:* Early Development Study, NICHD Collaborative Programs for Excellence in Autism (CPEA)  
University of Washington Autism Center/Center on Human Development and Disability  
*Goal:* To deepen understanding of neurobiological basis of autism, subtypes and outcome predictors, neurocognitive profiles, and individual differences in symptom expression.  
*Principal Investigator:* Geraldine Dawson, Ph.D.  
*Role:* Graduate research assistant responsible for conducting semiannual parent interviews to monitor child progress and interventions. Administered diagnostic reassessments of 9-year-old children with autism, including cognitive assessment, standardized measures of symptomatology, and social and neuropsychological measures.
- 2005–2006 *Title:* Computer-based receptive language training for young children with autism  
University of California, San Diego; Autism Research Laboratory  
*Goal:* Teach word-comprehension skills to typically developing infants and young children with autism.

*Principal Investigator:* Laura Schreibman, Ph.D.

*Role:* Project Coordinator. Established experimental design, set up research project, and prepared human subjects application. Recruited participants, administered standardized developmental assessments, and analyzed behavioral and electrophysiological data. Trained and supervised undergraduate research assistants.

2005–2006

*Title:* Visual processing in teenagers with autism spectrum disorders

University of California, San Diego; Vision Laboratory

*Goal:* To assess if adolescents with ASD show abnormal visual dorsal pathway processing.

*Principal Investigator:* Karen Dobkins, Ph.D.

*Role:* Conducted visual psychophysical assessments and behavioral standardized assessments with adolescents with ASD.

2004–2006

*Title:* Neural correlates of the processing of featural and configural face information

University of California, San Diego; Face Processing and Perception Laboratory

*Goal:* Employ event-related potentials to directly examine the neural correlates of face featural and configural information processing in adults.

*Principal Investigator:* Kang Lee, Ph.D.

*Role:* Collected and analyzed adult electrophysiological data. Provided input into the experimental design and contributed to the writing of a journal article.

2003–2005

*Title:* Early precursors to autism in infancy

University of California, San Diego; Developmental Neuroscience Laboratory

*Goal:* Investigate risk factors associated with the development of ASD in infant siblings of children with ASD longitudinally over the time course of 6-36 months.

*Principal Investigators:* Karen Dobkins, Ph.D.; Leslie Carver, Ph.D.

*Role:* Collected and analyzed infant psychophysical and electrophysiological data. Administered standardized developmental assessments. Trained and supervised undergraduate research assistants.

2002–2003

*Title:* Neural mechanisms underlying 12-month-olds' social referencing behavior

University of California, San Diego; Developmental Neuroscience Laboratory

*Goal:* Examine the relationship between infant social referencing behavior and corresponding brain development using event-related potentials.

*Principal Investigator:* Leslie Carver, Ph.D.

*Role:* Honors undergraduate student. Completed an honors thesis, aided in preparation of a poster presentation, and presented findings to Human Development Honors Colloquium.

2002 Center for Teratology Information Service; UCSD Medical Center, Department of Pediatrics  
*Role:* Student Intern. Led a prospective study examining the risks for developmental anomalies in infants prenatally exposed to anticonvulsant drugs. Collected and analyzed data from medical records to determine which anticonvulsant drugs were the most detrimental to development.  
*Supervisors:* Kenneth Jones, Ph.D.; Christina Chambers, Ph.D.

### **Clinical Experience**

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2011–2012 Psychology Resident  
 University of Washington School of Medicine  
 Seattle Children’s Hospital  
*Role:* Conduct psycho-social and neuropsychological evaluations as well as psychotherapy for children and adolescents with psychiatric conditions. Clinical rotations included inpatient psychiatry unit, pediatric consultation liaison, neuropsychology, and outpatient psychiatry clinic.  
*Supervisors:* Gretchen Gudmundsen, Ph.D.; David Breiger, Ph.D.; Elizabeth McCauley, Ph.D.; Cynthia Flynn, Ph.D.; Brent Collett, Ph.D., Molly Adrian, Ph.D., Heather Carmichael Olson, Ph.D., and Jim McKeever, Ph.D.

2007–2011 Psychological Services and Training Clinic (PSTC):  
 Department of Psychology, University of Washington, Seattle  
*Role:* Staff therapist providing outpatient psychotherapy to individual child and adult clients experiencing a variety of presenting problems and meeting criteria for Axis I disorders. Intervention modalities include CBT, ACT, FAP, behavioral, motivational interviewing, parent training, family therapy, and supportive therapy.  
*Supervisors:* Corey Fagan, Ph.D., Chris McCurry, Ph.D., Seth Noonan, Ph.D., Russell Hanford, Ph.D., Heidi Wasch, Ph.D., Steve Katz, Ph.D.

2010–2011 Therapist, Autism Center, Seattle Children’s Hospital  
*Supervisor:* Felice Orlich, Ph.D., Clinic Director  
*Role:* Staff therapist in charge of co-leading social skills group for children with ASD in a school setting. Attend weekly team meetings.

2010–2011 Trainee, Interdisciplinary Training Committee  
 Leadership Education in Neurodevelopmental and Related Disabilities (LEND)  
 University of Washington/Center on Human Development and Disability  
*Supervisors:* Sally Stuart, MSW; Sara Jane Webb, Ph.D.

- Role:* Participate in core seminars and leadership seminar curricula with goal of developing clinical and applied research expertise in prevention, early detection, assessment, treatment strategies, care coordination, evidence-based practice, and long-term management of individuals with neurodevelopmental and related disabilities within the context of an interdisciplinary model of training, service, and research.
- 2009–2011  
Anxiety Disorders Consult Group  
Department of Psychology, University of Washington, Seattle  
*Supervisor:* Lori Zoellner, Ph.D.
- 2010–2011  
Functional Analytic Psychotherapy Practicum  
Department of Psychology, University of Washington, Seattle  
*Supervisor:* Mavis Tsai, Ph.D.
- 2009–2010  
Family Therapy Consult Group  
Department of Psychology, University of Washington, Seattle  
*Supervisor:* Corey Fagan, Ph.D.
- 2009–2010  
Evaluator and Therapist, Treatment Practicum, Autism Center, Seattle Children's Hospital  
*Supervisor:* Felice Orlich, Ph.D., Clinic Director  
*Role:* Conducted assessment of symptom status; socio-emotional, cognitive, and neuropsychological functioning; and differential diagnosis in conjunction with licensed psychologist. Co-conducted intake interviews and parent feedback sessions. Wrote evaluation summaries, including suggestions for intervention and treatment planning. Provided long-term individual therapy to three children with developmental disabilities and associated medical and psychiatric conditions. Co-led social skills group for children with ASD and their typically developing siblings. Attended team meetings and rounds.
- 2008–2009  
Neuropsychological Testing Consultant, Assessment Practicum, Seattle Children's Hospital Inpatient Psychiatric Unit, Seattle, WA  
*Role:* Practicum student. Conducted cognitive, diagnostic, neuropsychological, and personality assessments for children and adolescents hospitalized in the Inpatient Psychiatric Unit. Provided additional testing support for outpatient services, including assessment of cognitive function for children with brain injury and cancer.  
*Supervisor:* David Breiger, Ph.D., Neuropsychology Clinic Director
- 2003  
Children's Care Connection Program, San Diego Children's Hospital  
Program to identify and provide free services for children ages 0-5 who are at risk for developmental and behavioral problems.  
*Role:* Summer intern. Assisted in data collection from parent phone interviews as well as data entry and organization. Aided in grant preparation and writing and searched for possible funding agencies.

Attended classes and seminars at developmental sites and observed developmental assessments.

*Supervisor:* Peter Doyle, M.A.

2001

San Diego Center for Children, San Diego, CA

*Role:* Summer volunteer. Provided in-patient behavioral support for children with severe psychopathology and/or behavioral problems.

### **Specialized Clinical Training**

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- July 2011 Dialectical Behavioral Therapy (DBT). Weekend workshop conducted by Kate Comtois, Ph.D. at Harborview Medical Center.
- August 2010 Fetal Alcohol Spectrum Disorders Training. One-day training including overview of current diagnostic and treatment strategies for FASD and observation of two comprehensive diagnostic evaluations. Training conducted by clinic director, Susan Astley, Ph.D., at the University of Washington.
- August 2010 Functional Analytic Psychotherapy (FAP): New Frontiers in Awareness, Courage, Love, and Behaviorism. Training conducted by Bob Kohlenberg, Ph.D., and Mavis Tsai, Ph.D., at the University of Washington.
- March 2010 A Two-Stage Model for Identifying Youth Who Are at Risk for Suicide. Training conducted by James Mazza, Ph.D., at the University of Washington.
- October 2009 CBT Workshop for Depression. Training conducted by Steve Hollon, Ph.D., at the University of Washington.
- May 2008 Autism Diagnostic Observation Schedule-Toddler module training. Videoconference training conducted by Cathy Lord, Ph.D., at the Autism & Communication Disorders Center at the University of Michigan.
- October 2007 Infant Behavioral Imaging Studies network training. Training workshop for administration and reliability on Mullen Scales of Early Learning, Autism Observation Scale for Infants, and Communication and Symbolic Behavior Scale. Training conducted by Annette Estes, Ph.D., and Lonnie Zwaigenbaum, M.D., at the Autism Center, University of Washington.
- July 2007 Clinical Personality Assessment: MMPI-2 and MCMI-III. Training conducted by Ronald Smith, Ph.D., at the University of Washington.

- March 2007 Autism Diagnostic Interview-Revised research reliability training workshop.  
Training conducted by Susan Risi, Ph.D., at the Autism & Communication Disorders Center at the University of Michigan.
- May 2005 Autism Diagnostic Observation Schedule research reliability training workshop.  
Training conducted by Natacha Akshoomoff, Ph.D., at the University of California, San Diego.

### **Teaching/Supervision Experience**

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- 2010, Fall Professional Development Seminar, Lecturer  
University of Washington Autism Center/Center for Human Development and Disability.  
*Role:* Deliver a series of weekly lectures on science career development to undergraduate research assistants. Assign readings and preparatory exercises and facilitate discussions related to topic.
- 2009, Fall Teaching Assistant for Graduate Course on Psychological Assessment of Children  
University of Washington, Department of Psychology  
*Role:* Aided in training of child clinical graduate students assessment abilities by providing feedback on their assessment skills and written reports. Taught a class on assessment of autism and current diagnostic measures.
- 2010 Pediatric Resident Lecture Series, Lecturer  
University of Washington School of Medicine and Children's Hospital  
*Role:* Deliver monthly lectures on autism spectrum disorders to pediatric residents and fourth year medical students on rotation at the Center for Human Development and Disability, University of Washington.
- 2008–2011 Advanced Graduate "Clinical Buddy"  
University of Washington, Department of Psychology  
*Role:* Serve as a mentor and provide constructive feedback to second year clinical graduate students starting to see their first clients.
- 2006–2011 Undergraduate Research Assistant Training, Supervisor  
Department of Psychology, University of Washington.  
*Role:* Interview, train, and supervise undergraduate psychology students to assist with study operations. Provide didactic and practical training.
- 2002 Teaching Assistant for Clinical Neuropsychology course  
University of California, San Diego  
*Role:* Aided in the preparation and grading of exams, held office hours for students.

### Invited Lectures and Presentations

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“Developmental Psychopathology”—an invited lecture to undergraduate Developmental Psychology class at University of Washington. July, 2010.

“Temperament In High-Risk Infants for ASD” Science and Sandwiches presentation at the International Meeting for Autism Research, Philadelphia, PA. May, 2010.

“Group Therapy for Children with Anxiety Symptoms” Science Informed Case Presentation. Presented to students and faculty of the University of Washington Clinical Psychology program, Seattle, WA. April, 2010.

“Autism 101”—a presentation for parents and caregivers of children with autism. Imagine Museum, Everett, WA. May, 2009.

“Autism 101”—a presentation for parents and caregivers of children recently diagnosed with autism. Seattle Children’s Hospital. January, 2009.

“Neural Correlates of Emotional Face Processing in Children with Autism”—a presentation at the University of Washington Psychology Department Research Festival. May, 2008.

“Current Brain Research and Clinical Services at the University of Washington”—a presentation to the Arc of Snohomish County Parent Group, Everett, WA. February, 2008.

“Current Research and Intervention in Autism at the UW Autism Center”—a presentation to the Arc of Snohomish County Parent Group, Everett, WA. August, 2008.

### Publications

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Dawson, G., & **Burner, K.** (2011). Behavioral interventions in children and adolescents with autism spectrum disorder: A review of recent findings. *Current Opinion in Pediatrics, 23* (6), 616-620.

Dawson, G., **Burner, K.**, & Klinger, L. (In preparation). Autism Spectrum Disorders. To appear in E. Mash & R. Barkley (Eds.), *Child Psychopathology, 3<sup>rd</sup> edition*. New York: Guilford Press.

**Burner, K.** & Bernier, R. (2011). Neurobiology and Genetics in ASD. Volkmar, F. (Ed). *The Encyclopedia of Autism Spectrum Disorders*. Springer Major Reference Work.

Webb, S.J., Bernier, R., **Burner, K.** & Murias, M. (2011). Electrophysiology & Evoked Potentials in Autism. In D. Amaral, G. Dawson, & D. Geschwind (Eds.), *Autism Spectrum Disorders*. Oxford University Press.

McCleery, J., Ceponiene, R., **Burner, K.**, Townsend, J., Williams, C., Kinnear, M., & Schreibman, L. (2009). Neural correlates of verbal and non-verbal semantic integration in children with autism. *Journal of Child Psychology and Psychiatry*.

## Conference Presentations

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**Burner, K.,** Faja, S., Murias, M. (2011, May). Frontal Asymmetry and Temperament in Young Children with ASD. Poster presented at the International Meeting for Autism Research, San Diego, CA.

Webb, S.J., Jones, E., **Burner, K.,** Robertson, C., R. Edwards, R., Tattersall, A., & Dawson, G. (2011, May). Developmental Trajectory of the N170 Marker of Face Processing In Children with ASD. Talk presented at the International Meeting for Autism Research, San Diego, CA.

**Burner, K.,** Venema, K., Jones, E., Estes, A., King, B., & Webb, S.J. (2010, May). Examining Temperament in a Longitudinal Study of High-Risk Infants for ASD. Poster presented at the International Meeting for Autism Research, Philadelphia, PA.

Estes, A.M., Alvarez, S., Dupont, E., **Burner, K.,** Kelly, J., Dawson, G., Munson, J., King, B., & Webb, S.J. (2010, May). Stress in Parents of 6-Month-Old Infants with Older Siblings with Autism Spectrum Disorders. Poster presented at the International Meeting for Autism Research, Philadelphia, PA.

**Burner, K.,** Greenson, J., Elder, L., Lindsey, J., & Dawson, G. (2009, May). Joint Attention, Play Behavior, and Language in Toddlers with ASD, Developmental Disorders, and Typical Development. Poster presented at the International Meeting for Autism Research, Chicago, IL.

Rivera, V., Estes, A., Munson, J., Elder, L., **Burner, K.,** & Cali, P. (2009, May). Problem Behavior, Social Functioning, and Academic Achievement in School-Aged Children with An Autism Spectrum Disorder. Poster presented at the International Meeting for Autism Research, Chicago, IL.

**Burner, K.,** Webb, S., Merkle, K., Murias, M., & Dawson, G. (2008, May). Neural Correlates of Emotional Face Processing in Children with Autism. Poster presented at the International Meeting for Autism Research, London, England.

McCleery, J. Ceponiene, R., **Burner, K.,** William, C., Townsend, J., Schreibman, L. (2008, May). Neural Correlates of Verbal and Non-Verbal Semantic Integration in Young Children with Autism. Poster presented at the International Meeting for Autism Research, London, England.

McCleery, J., Kinnear, M., **Burner, K.,** Stahmer, A., Carver, L., Ceponiene, R., & Schreibman, L. (2008, March) Computer-Based Receptive Language Training for Infants. Poster presented by Karen Burner at the International Meeting for Infant Studies, Vancouver, British Columbia.

McCleery, J.P., **Burner, K.M.,** & Dobkins, K.R. (2006, June) Autism associated with abnormal magnocellular visual pathway during development. Poster presented at the Annual Symposium by the Kavli Institute for Brain and Mind, Salk Institute, La Jolla, CA.

McCleery, J.P., **Burner, K.M.,** Dobkins, K.R., & Carver, L.J. (2006, May) Neural correlates of face processing in infant siblings of children with autism. Poster presented at the International Meeting for Autism Research, Vancouver, British Columbia.

Carver, L.J., McCleery, J.P., Reed, S.R., **Burner, K.M.**, Tully, L.M., & Dobkins, K.R. (2006, May) The broader phenotype in 10-month-old infant siblings of children with autism. Poster presented at the International Meeting for Autism Research, Vancouver, British Columbia.

McCleery, J.P., Allman, E.A., **Burner, K.M.**, Carver, L.J., & Dobkins, K.R. (2005, May) Psychophysical evidence for abnormal magnocellular processing in 6-month-old infants with autism in their family. Paper presented at the International Meeting for Autism Research, Boston, MA.

McCleery, J.P., **Burner, K.M.**, Carver, L.J., & Lee, K. (2004, October) Neural correlates of the processing of featural and configural information in faces. Paper presented in symposium Visual Cortex: Processing of Faces at the Annual Meeting of the Society for Neuroscience, San Diego, CA.