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Mechanisms Conferring Risk versus Resilience for Autism Spectrum Disorder in Early Infancy

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Mechanisms Conferring Risk versus Resilience for Autism Spectrum Disorder in Early Infancy

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Psychology

by

Tawny Tsang

2018

ABSTRACT OF THE DISSERTATION

Mechanisms Conferring Risk versus Resilience for

Autism Spectrum Disorder in Early Infancy

by

Tawny Tsang

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2018

Professor Mirella Dapretto, Co-Chair

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Autism spectrum disorder (ASD) is a highly prevalent, lifelong condition characterized by impairments in social communication and the presence of restrictive, repetitive behaviors. Identifying the earliest signs of ASD is a critical factor in promoting optimal long-term outcomes for those affected. The developmental origins of ASD, including symptom onset and progression, remain poorly understood. Current known markers of ASD are centered on deviations in social and communicative skills that typically emerge around the first birthday; however, the presence of overt impairments reflects the *consequence* of an altered trajectory of social development, rather than the *causes and underlying mechanisms* from which these impairments arose. This has motivated the search for early risk markers more proximal to the

source of deficits, including aberrations in early emerging neural systems that scaffold social development.

Prior behavioral research has primarily focused the search within a single developmental domain, but this approach has fallen short of identifying reliable markers of ASD risk within the first 12 months. Instead, infants who develop ASD may exhibit impairments across several developmental domains, including diminished social attention in both visual and auditory domains as well as atypical brain organization in early infancy. This dissertation builds upon these bodies of research to provide a comprehensive profile of ASD risk in early infancy. The studies take a multimodal approach and employ eye-tracking, task-based and resting-state functional neuroimaging, and behavioral measures to investigate developmental antecedents of the social deficits and other core behavioral symptoms associated with ASD across early emerging domains critical for social development.

Study 1 used eye-tracking methods to examine visual social attention to faces from 3- to 12-months of age in infants at high- and low-risk for ASD, as well as factors that may moderate developmental trajectories. Greater parental affectedness of ASD-related behaviors predicted slower developmental increases in attention to faces, indicating that parents' social communicative skills influence their infant's social development. Moreover, developmental trajectories in face-looking in high-risk infants predicted social communicative functioning. Altogether the findings suggest that parent-mediated interventions targeting parent-child interactions may have positive effects on social communicative development in infants with familial risk for ASD.

Study 2 used a passive listening stimulus-evoked functional magnetic resonance imaging (fMRI) paradigm to evaluate native language processing (i.e., auditory social attention) in 1.5

and 9-month-old infants at familial risk for ASD. At 1.5 months, high-risk infants already showed evidence for suboptimal language processing. At 9-months, high-risk infants exhibited attenuated neural responses to language relative to their low-risk peers, and this effect was particularly pronounced in high-risk infants who later displayed delayed language development. Severity of social impairments was higher for high-risk infants with delayed language than those without. Deviations in language processing may constitute an early marker of social communicative difficulties associated with ASD risk.

Study 3 combined eye-tracking and resting-state fMRI methods to evaluate Salience Network connectivity at 6 weeks and its association with subsequent social communicative skills and sensory processing abilities. Six-week-old high-risk infants demonstrated hyperconnectivity with sensorimotor regions, whereas low-risk demonstrated hyperconnectivity with prefrontal regions involved in social attention. Infants with *higher* connectivity with sensorimotor regions had *lower* connectivity with prefrontal regions, suggesting a direct attentional tradeoff for sensory versus socially-relevant information. Alterations in network connectivity at six weeks predicted 12-month ASD symptomatology, providing the earliest mechanistic account for the unfolding of atypical trajectories associated with ASD risk. Initial disruptions in brain systems involved in sensory/attentional processes are a critical antecedent to the later-emerging social symptoms of ASD. Taken together, the findings from these studies indicate that a multimodal approach that includes early brain-based measures has the incredible potential to improve the early detection of ASD risk as well as uncover mechanisms associated with the emergence of ASD symptomatology within the first few weeks of postnatal life.

The dissertation of Tawny Tsang is approved.

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2018

DEDICATION

This dissertation is dedicated to my younger sister, Melody, for inspiring me to pursue autism research.

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Study 1 is a version that has been submitted for review at Autism Research (Tsang, T., Jeste, S., Dapretto, M., & Johnson, S.P. The Effects of Early Social Environment and Stimulus on Visual Social Attention for Infant at Risk for ASD*).* Study 2 is a version of a manuscript in preparation of publication (Tsang, T., Liu, J., Jackson, L., Bookheimer, S.Y., Dapretto, M. Neural Correlates of Early Language Processing during the First Postnatal Year in Infants at Risk for ASD). Study 3 is a version that has been submitted for review (Tsang, T., Green, S., Liu, J., Jeste, S., Bookheimer, S.Y., & Dapretto, M. Altered Salience Network Connectivity in 6-week-old Infants at Risk for Autism). Thank you to my co-authors for their contribution to the work.

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Oral Presentations

Tsang, T., Hutman, T., Jeste, S., Dapretto, M., & Johnson S.P. (2015, March). *Sensitivity and specificity of face perception for normative development*. Oral presentation at SRCD pre-conference on face processing, Philadelphia, Pennsylvania.

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Tsang, T., Atagi, N., Johnson, S.P. (2017, March). *The Roles of Early Language Environment and Language Skills on Infants' Visual Attention to Mouths*. Oral presentation at the Society for Research on Child Development (SRCD), Austin, TX.

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General Introduction

Autism spectrum disorder (ASD) is a highly prevalent, lifelong condition characterized by impairments in social communication and the presence of restrictive, repetitive behaviors (American Psychiatric Association, 2013). There is clear evidence that early detection and intervention for ASD lead to better prognosis, including improved adaptive functioning and interpersonal relationships, as well as reduced maladaptive behaviors (Eaves & Ho, 2004; Harris & Handelman, 2000). Improving the long-term outcomes of those affected can also ultimately reduce the substantial familial and societal costs associated with the disorder across the lifespan (Buescher, Cidav, Knapp et al., 2014). In addition to these clinical implications, there are profound theoretical implications for detecting markers of ASD risk in early infancy, including the prospect of unveiling antecedent neurobiological mechanisms that give rise to overt ASD symptoms. Altogether, this line of work presents an opportunity to take advantage of brain plasticity to potentially modify altered developmental trajectories—thus preventing the full onset and progression of ASD, rather than just ameliorating its effects (Dawson, 2008). Therefore, a major goal of ASD research is to identify reliable biomarkers that index developmental vulnerabilities during the prodromal period for the disorder (NIH, 2014).

Research endeavors focused on identifying early markers of ASD have leveraged the heritability of ASD by prospectively tracking the development of infant siblings of children with ASD (Zwaigenbaum et al., 2005). Younger, infant siblings of children with ASD are 20 times more likely than the general population to be diagnosed with the disorder (Ozonoff, Young, Carter et al., 2011) and are also at elevated risk for other suboptimal developmental outcomes such as social communicative impairments, language delays, and attentional problems (Gamliel, Yirmiya, Jaffe, Manor, & Sigman, 2009; Miller, Iosif, et al., 2015; Tsang, Gillespie-Lynch, &

Hutman, 2016)). Altogether, the prospective study of high-risk (HR) infant siblings versus low-risk (LR) infants without a family history of developmental disorders has critically informed how familial risk for ASD confers vulnerabilities across social and cognitive domains, including emergent behavioral profiles associated with atypical social communicative development.

Current symptom-based markers of ASD are centered on deviations in social and communicative skills that typically emerge around the first birthday, including shared attention with others and language skills (Zwaigenbaum et al., 2005). The emphasis on social behaviors is in line with theoretical frameworks positing that early disruptions in social orienting mechanisms cascade into core diagnostic features of ASD (Baron-Cohen et al., 2000; Charman, 2003; Klin, Jones, Schultz, Volkmar, & Cohen, 2002a). However, the reliance on observational measures to assess symptom-based, behavioral markers of ASD, in conjunction with the limited behavioral repertoire of young infants, has hindered the search for reliable prodromal signs of ASD prior to 12 months of age (Bryson, Zwaigenbaum, McDermott, Rombough, & Brian, 2008; Kleinman et al., 2008). Moreover, the presence of overt impairments reflects the *consequence* of an altered trajectory of social communicative development rather than the *causes and underlying mechanisms* from which these impairments arise. Using brain-based measures more proximal to the source of deficits may be more sensitive for detecting risk markers within the first 12 months (e.g., Elsabbagh et al., 2009; Bosl, Tierney, Tager-Flusberg et al., 2011; McCleery, Akshoomoff, Dobkins et a., 2009), and in turn further our understanding of the developmental origins of ASD, including symptom onset and progression.

Research on the neocortical architecture of children with ASD suggests that the disorder may stem from altered prenatal neural development (Stoner et al., 2014). Indeed, mounting evidence suggests that atypicalities in structural and functional brain organization are present at

least by 6 months of age in infants who develop ASD (Shen & Piven, 2014). This includes accelerated cortical surface area expansion, excess extra-axial cranial fluid, and altered functional and structural connectivity (Hazlett et al., 2017; Shen et al., 2013; Shen & Piven, 2014; Swanson et al., 2017; Wolff et al., 2012). These studies not only demonstrate that neuroimaging methods are sensitive at detecting early differences in neural development, but also lend support to the theory that ASD stems from altered connectivity across brain regions associated with higher-order cognitive processes, including social attention (Geschwind & Levitt, 2007). However, the body of work evaluating early brain development in HR infants has primarily focused on global indices and has thus provided limited data on the specific processes by which aberrant developmental trajectories relate to the onset of social and nonsocial dimensions of core ASD symptomatology. As such, the studies outlined here focus on bridging the gap between brain with behavior to examine the effects of familial risk for ASD on specific early attentional systems in infancy and their predictive validity with respect to subsequent symptom-based markers of ASD risk.

The search for early markers of ASD has primarily focused on atypicalities within a single developmental domain. Although prior work has found diminished social attention in visual and auditory domains and atypical brain organization in HR infants prior to 12 months, no single developmental process is affected in all HR infants (S. Ozonoff et al., 2010; Rogers, 2009; Zwaigenbaum et al., 2005). Instead, as for the diagnosis of ASD, identifying a collection of social attentional and brain abnormalities may be more informative about ASD risk (Dawson, 2008). Sampling risk markers across domains may thus enhance our ability to detect infants with developmental vulnerabilities and provide insight into a comprehensive profile of ASD in infancy. The studies that comprise this dissertation employed eye-tracking and functional

magnetic resonance imaging (fMRI) methods to investigate early developments in domains critical for social communication—1) attention to faces (Haan, 2002), 2) language processing (Kuhl, 2007), and 3) functional brain connectivity (M. H. Johnson, 2011). Due to the iterative nature of development, early derailments in these domains bear clinical significance as they may later cascade into social impairments and other core ASD deficits.

In sum, the overarching goal of the body of work presented here was to characterize the early expression of familial risk for ASD. The research approach leveraged individual variability to examine the extent to which deviations in mechanisms implicated in social attention –*across* neural and behavioral levels of analyses– are apparent in infants with familial risk for ASD. This multimodal approach may be more sensitive at detecting subtle deviations across developing neural systems in early infancy that map onto the emergence of symptoms that are consistent with the current ASD diagnostic framework.

Overview of Studies

Study 1: Visual Social Attention. 6-month-old HR infants later diagnosed with ASD show diminished attention to social scenes and, in particular, to people's faces (Chawarska, Macari, & Shic, 2012). This may indicate deviations in normative trends of increased attention to faces in complex scenes across the first year (Frank, Vul, & Johnson, 2009). However, HR infants do not appear to show a global impairment in attending to social information (Elsabbagh, Gliga, et al., 2013). Notably, subtle differences in visual social attention between HR and low-risk (LR) infants become more apparent when social cues become more obstensive (e.g., talking versus a smiling face; Shic, Macari & Chawarska, 2014). The hypothesis that subtle difficulties in visual social attention can be accentuated under more social demanding contexts has been previously raised (Elsabbagh, Bedford, Senju et al., 2013), but not evaluated longitudinally

within the first postnatal year.

A second point of focus was to examine how an infant's early social environment may influence visual social attention. In addition to increased genetic vulnerabilities associated with ASD (Devlin & Scherer, 2012), familial risk for ASD may also manifest as reduced social learning opportunities for the infant. First-degree relatives of individuals with ASD often exhibit features of the broad autism phenotype, which are a constellation of traits that include social aloofness, rigid personalities, and social communication difficulties (Bernier, Gerdts, Munson, Dawson, & Estes, 2012; Joseph Piven, 2001). Given that parent-child dyadic interactions largely characterize the social landscape of a developing infant, whether parental affectedness of ASD traits moderates trajectories of visual social attention was also examined.

Using eye-tracking methods, this study examined factors that influence developmental trajectories in visual attention to faces from 3- to 12-months in HR and low-risk (LR) infants, both by varying the social complexity of the stimuli used to gauge visual social attention and by examining the role of parental affectedness of ASD-related behaviors. Results showed that both HR and LR infants looked less to faces in complex, naturalistic social stimuli, relative to schematic, cartoon renditions of social interactions. While there was no effect by infant risk status, greater parental affectedness of ASD-related behaviors was associated with slower developmental increases in attention to faces from 3- to 12-months of age. For HR infants, greater attention to faces was associated with better response to joint attention cues and lower ASD symptom severity at 12- and 18-months, respectively. Data from this study provide preliminary evidence that developmental trajectories in visual social attention are modifiable, and highlight the clinical relevance of evaluating an infant's social environment when considering intervention strategies.

Study 2: Auditory Social Attention. Delays in speech and language are one of the first signs of ASD-related concerns (Zwaigenbaum, Bryson, & Garon, 2013), and are associated with atypical processing of social input in young children with ASD (Eyler, Pierce, & Courchesne, 2012). Early attunement to native language input is crucial for normative language (Kuhl et al., 2011) and social development (Kuhl, 2007); however, the neural correlates of native language processing have not been evaluated in infants at risk for ASD. Research examining social attention in the auditory domain has found that HR infants lack a normative preference for speech over non-speech sounds at 12 months (Curtin & Vouloumanos, 2013), which may stem from earlier deviations in language processing (Seery, Vogel-Farley, Tager-Flusberg, & Nelson, 2012) and affect subsequent language growth (Lombardo et al., 2015). Toddlers with ASD show reduced neural activity in response to speech (Redcay & Courchesne, 2008), especially among those with delayed language (Lombardo et al., 2015).

Prior work has primarily evaluated early neural attunement to language by comparing neural response to human speech relative to non-human primate sounds, non-speech sounds (e.g., coughing), or backwards speech (Redcay & Courchesne, 2008; Shultz, Vouloumanos, Bennett, & Pelphrey, 2014). The present study used another metric of auditory social attention by evaluating neural responses to native versus non-native speech. Based on prenatal exposure, neonates prefer the language they heard in utero (Moon, Cooper, & Fifer, 1993). Assessing infants' native language preference can thus provide a measure of their attention to and uptake of the most relevant linguistic input in their immediate environment (e.g., Kuhl, 2004).

This research examined the neural circuitry subserving language processing in HR and LR infants during the first postnatal year using functional magnetic resonance imaging (fMRI) to identify patterns of brain activity that may predict atypicalities in social communicative

development, and in particular language development. At 6 weeks of age, HR infants already exhibited atypical routes for processing native versus non-native language and at 9 months, they showed attenuated neural response to language, particularly in regions associated with social reward and learning. Similar to prior work, at 9 months HR infants with delayed language not only showed much less neural activity in response to language, but also were more impaired in social functioning than HR infants with normative verbal growth. These data indicate that language is a particularly informative domain for parsing heterogeneity in developmental trajectories in infants at high familial risk for ASD.

Study 3: Functional Brain Connectivity. Neural connectivity subserves both attentional and learning processes (Fox & Raichle, 2007; van den Heuvel & Hulshoff Pol, 2010), and disruptions in brain connectivity have been implicated in ASD (Geschwind & Levitt, 2007; Hernandez, Rudie, Green, Bookheimer, & Dapretto, 2015). Of particular interest here is the Salience Network (SN), an early emerging network (Alcauter, Lin, Keith Smith, Gilmore, & Gao, 2013; Gao, Alcauter, Smith, Gilmore, & Lin, 2014) believed to be involved in guiding attention to the most salient internal and environmental stimuli (L. Q. Uddin, 2014). Importantly, there is growing reason to believe that patterns of connectivity within the SN may be a biomarker of ASD as characteristics of the SN have been found to discriminate children with ASD from neurotypical controls with high sensitivity and specificity (L. Q. Uddin et al., 2013). Moreover, youth with ASD, and especially those with significant sensory over-responsivity, display heightened connectivity between the hub of the SN and somatosensory cortices (S. A. Green, Hernandez, Bookheimer, & Dapretto, 2016). These data suggest that the core social and sensory atypicalities of ASD may arise as a consequence of heightened attention to nonsocial stimuli at the expense of more developmentally relevant social stimuli.

Using resting-state fMRI, this study examined SN connectivity in 6-week-old infants at high and low risk for ASD. Six-week-old high-risk infants demonstrated hyperconnectivity with sensorimotor regions, whereas low-risk demonstrated hyperconnectivity with prefrontal regions involved in social attention. Notably, infants who showed *stronger* connectivity with sensorimotor regions had *weaker* connectivity with prefrontal regions, suggesting a direct tradeoff in attention to sensory versus socially-relevant information. Patterns of SN connectivity at 6-weeks also predicted level of later symptom-based markers of ASD, including social impairment and atypical sensory processing, lending further evidence that altered SN connectivity in early infancy imposes a developmental vulnerability for subsequent social cognitive development. This is the first study to demonstrate the relation between very early differences in brain network dynamics in infants at risk for ASD and later behavioral symptoms of the disorder, providing a mechanistic account for the emergence of ASD in late infancy.

The Effects of Early Social Environment and Social Complexity on Visual Social Attention to Faces in Infants at Risk for ASD

Diminished attention to socially relevant information appears to be an early emerging risk factor associated with autism spectrum disorders (ASD). However, inconsistencies across studies suggest that atypicalities in visual social attention in infants at high-risk for ASD during the first postnatal year may be subtle and only apparent under certain contexts. Here we explore factors that may moderate developmental trajectories in attention to faces, including the social complexity of the dynamic visual stimuli used to measure visual social attention and the early social environment of the infant as indexed by parental affectedness of ASD-related traits. Across infants at both high (HR) and low risk (LR) for ASD, attention to faces increased during the first postnatal year, with overall greater attention being allocated to schematic faces in the simpler video stimulus. Moreover, greater parental affectedness of ASD-related traits was associated with reduced developmental gains in attention to faces. For HR infants, greater attention to faces was positively associated with social communicative competence, including better joint attention skills and lower social impairments. Altogether, our findings highlight the importance of considering developmental level when selecting stimuli to longitudinally examine visual social attention, and the clinical relevance of including measures of infant's social environment in understanding early markers of ASD risk.

Faces are a rich source of social communicative cues and constitute a particularly salient class of stimulus in an infant's environment (Gliga, Elsabbagh, & Andravizou, 2009; Grossmann & Johnson, 2007). Infants preferentially orient to face-like stimuli shortly after birth (Morton & Johnson, 1991) and, in typical development, they increasingly look at faces during the first postnatal year (Frank, Vul, & Johnson, 2009; Frank, Vul, & Saxe, 2011). In contrast, infants who develop autism spectrum disorder (ASD) may deviate from normative patterns of attention to faces during the first postnatal year (Jones & Klin, 2013). Visual attention is an important mechanism for learning across social and cognitive developmental domains during infancy (Amso, Fitzgerald, Davidow, Gilhooly, & Tottenham, 2010; S. P. Johnson, Amso, & Slemmer, 2003), and it provides quantifiable indices of social and communicative functioning in both typical and atypical development (Campbell, Shic, Macari, & Chawarska, 2014; Tsang, Atagi, & Johnson, 2018; Young, Merin, Rogers, & Ozonoff, 2009). Early disruptions in attention to socially relevant stimuli likely impose cascading effects on social communicative development and is theorized to be a contributing factor to the emergence of the social impairments diagnostic to ASD (Elsabbagh & Johnson, 2016; Klin, Jones, Schultz, & Volkmar, 2003). A close examination of factors that moderate developmental trajectories of attention to faces during infancy provide greater specificity on how visual social attention is impacted in infants at risk for ASD.

Infants with an older sibling with ASD have approximately a 20% risk of developing ASD themselves (Sally Ozonoff et al., 2011). The prospective study of these high-risk (HR) infant siblings has begun to elucidate the extent to which attenuated attention to faces during the first postnatal year constitutes a prodromal symptom of the disorder. While there is evidence that infants who develop ASD show a reduced preference for social information (Chawarska, Macari,

& Shic, 2013; Jones & Klin, 2013; Shic, Macari, & Chawarska, 2014), there has not been unequivocal support for the finding that diminished visual social attention differentiates HR infants from low-risk (LR) controls (for review, see Jones, Gliga, Bedford, Charman, & Johnson, 2014). Inconsistent findings across studies may in part be due to the heterogeneity in genetic factors that contribute to ASD risk in HR samples. However, it may also in part be attributed to aspects of the stimuli and age of evaluation. For instance, some studies that have yielded null differences between HR and LR infants have used simplistic social stimuli, such as still photographs (Elsabbagh et al., 2009; Elsabbagh, Gliga, et al., 2013) or silent videos (Bedford et al., 2012). This suggests that deviations from normative patterns of visual social attention are subtle and are accentuated under more complex social contexts (Speer, Cook, McMahon, & Clark, 2007). When the social demands varied within a study, differences in social attention between 6-month-old HR infants later diagnosed with ASD and low-risk (LR) controls became apparent under more socially ostensive contexts (e.g., while viewing a talking rather than a smiling or neutral face) (Shic, Macari, & Chawarska, 2014). Varying the social complexity of the stimuli used to evaluate face looking may provide a more sensitive metric of the atypicalities in visual social attention in infants at high risk for ASD.

Other studies that have used more complex, naturalistic social stimuli but did not find group differences only sampled one time-point within the first 12 months (Bedford et al., 2012; Elsabbagh, Fernandes, et al., 2013; Young et al., 2009). Because differences in social attention are apparent in the second postnatal year, Elsabbagh and colleagues (2013) speculated that infants who develop ASD increasingly diverge from normative patterns of visual social attention as a cumulative effect of the atypical interactions with their social environment. Examining trajectories in visual social attention, rather than individual time points, likely more appropriately

captures differences in attention to faces and its association with social communicative development. Indeed, a longitudinal eye-tracking study that sampled multiple time points within the first postnatal year found that deviations in normative trajectories in face scanning are predictive of both individual diagnostic classification and level of social disability at 24 months in HR infants who developed ASD (Jones & Klin, 2013). In light of these findings, the current study examined attention to faces in HR and LR infants while viewing social scenes of varying social complexity (i.e., both schematic/cartoon-like and naturalistic, live-action social scenes) across the first postnatal year. This approach will inform the extent to which context affects developmental differences in visual social attention between infants at high versus low risk for ASD.

In addition to stimulus-specific qualities that may emphasize developmental differences in attention to faces, the social environment in which an infant develops may also impact visual social attention by affecting the availability of social learning opportunities afforded to them. There is some evidence that the landscape of HR infants' social environments may differ from those of LR infants. Subclinical characteristics associated with the autism phenotype, such as difficulties with social communication, aloofness, and rigidity, are continuously distributed in the general population and are particularly common among first degree relatives of individuals with ASD (Constantino & Todd, 2003; Lundström et al., 2012; Pickles, Starr, Kazak et al., 2000; Sucksmith, et al., 2011). Collectively, these traits are referred to as the "broader autism phenotype" (BAP). The Social Responsiveness Scale (SRS, Constantino, 2002) is an instrument that has been used to quantify affectedness of autism-related traits in first-degree relatives (Schwichtenberg, Young, Sigman, Hutman, & Ozonoff, 2010; Virkud, Todd, Abbacchi, Zhang, & Constantino, 2008). Importantly, parents of children with ASD and developmental concerns

exhibit elevated social and communicative features of the BAP (Schwichtenberg et al., 2010), which may have downstream effects on their child's social communicative development.

Prior work evaluating BAP features in parents of children with ASD have primarily focused on intergenerational transmission of autism-related traits (Bernier et al., 2012; Dawson et al., 2007; Gerdts, Bernier, Dawson, & Estes, 2013). The data indicate heritability of autism-related traits, but also suggest potential differences in the early social environment of HR families relative to families without a history of ASD (Gerdts et al., 2013). This, in conjunction with increased genetic susceptibility for ASD, may impose an additional risk for atypical social cognitive development. Clinical observation of parents with multiple children with autism noted reduced mutual eye-gaze, social smiling, and elevated rigid behaviors (Gerdts et al., 2013), which may inadvertently impact the quality of parent-child interactions in HR families (Wan et al., 2013). Moreover, the effectiveness of parent-mediated interventions for HR infants on improving infant attentiveness and parental responsiveness to infant's communicative bids, as well as reducing prodromal ASD symptoms (Green et al., 2015, 2017), further highlight the likelihood that parent-child interactions shape social functioning. Whether parental affectedness of ASD-related traits also influences developmental trajectories of visual social attention to faces in infancy is a point of investigation here.

The current study examined developmental trajectories in attention to faces in infants from 3- to 12-months of age. Specifically, we evaluated the extent to which complexity of dynamic social stimuli and parents' ASD-related traits moderate developmental trajectories in face looking in HR and LR infants. We hypothesized that differences between HR and LR infants may be more apparent while viewing complex realistic social scenes than while viewing

simpler, schematic/cartoon scenes. In addition, more ASD-related traits in parents may be associated with lower social attention in infants.

Furthermore, we examined the relation between developmental trajectories in attention to faces and behavioral measures of social communicative competence. We used two metrics of social communicative competence. The first was engagement in joint attention, or the coordination of attention between social partners to share interest in an object or event, as measured by the Early Social Communication Scales (Mundy, Delgado, & Block, 2003). Impairments in joint attention behaviors have been documented as an early risk marker of ASD (Zwaigenbaum et al., 2013); such impairments differentiate toddlers with ASD from individuals with more general developmental delays (Dawson et al., 2004) and are associated with symptom severity in toddlers with ASD (Charman, 2003). Our second measure of social communicative competence was the degree of social impairment specific to ASD, as operationalized by the Autism Diagnostic Observation Schedule-Toddler Module (ADOS-T) (Luyster et al., 2009). We predicted that greater developmental increases in attention to faces would be associated with more spontaneous joint attention behaviors (a sign of better social communicative skills) and lower levels of social ASD symptoms.

Methods

Participants. Infant participants were enrolled in this study as part of a larger UCLA Autism Center of Excellence (ACE; NICHD P50 HD055784) project. Infants were grouped in accordance to family risk status. Low-risk infants had no family history of ASD or related neurodevelopmental disorders. High-risk infants had at least one older sibling with a confirmed ASD diagnosis. Infants were eye-tracked at 3, 6, 9, and 12 months of age. The final sample comprised 83 infants (52 HR 31 LR), each contributing a total of 5.02 $\pm$ 2.06 eye-tracking trials

across 2.75 $\pm$ 1.03 time points. The quantity of data provided by each infant did not differ by risk status (trial: $t(78)$=1.61, p = .11; session $t(78)$=1.77, p = .08). An additional 26 (13 HR, 23 LR) 3-month-old, 28 (11 HR, 17 LR) 6-month-old, 20 (9 HR 11 LR) 9-month-old, and 8 (2 HR, 6 LR) 12 month-old infants were observed but excluded due to excessive fussiness which compromised data quality (i.e., point-of-gaze was recorded for less than 30% of viewing time) and/or inability to complete the eye tracking calibration procedure described below.

Behavioral Measures. The Social Responsiveness Scale (SRS, Constantino, 2002) was used to quantify the degree of autistic-like traits for each infant's parents. The SRS is a questionnaire that provides a quantitative measure of reciprocal and social communication behaviors. Parents completed the SRS for each other upon study enrollment. The average of the parents' scores was used both as a quantitative metric of parental social responsiveness (Lyall et al., 2014) and as proxy measure for infant's social environment (see Virkud et al., 2008; Schwichtenberg, Young, Sigman et al., 2010). The average parental SRS score is referred to as parental affectedness of ASD-related traits.

At 12-months, infants were administered the Early Social Communication Scales (ESCS; Mundy, Delgado, & Block, 2003) and the Mullen Scales of Early Learning (MSEL; Mullen, 1995). The ESCS is a play-based standardized assessment of nonverbal social communication skills, including frequency of initiating and responding to joint attention cues. The MSEL is a standardized, normed developmental assessment and provides an overall metric of cognitive functioning (i.e., the Early Learning Composite [ELC]).

At 18 months, infants were administered the Autism Diagnostic Observation Schedule-Toddler Module (ADOS-T) (Luyster et al., 2009) to measure core ASD symptomatology. Social

symptoms are reflected in the Social Affect (SA) Score. Higher values indicate greater level of impairment.

Eye-tracking Stimuli. Infants viewed two 2-minute audiovisual segments taken from a cartoon (*A Charlie Brown Christmas*) and a children's television program (*Sesame Street,* see Figure 1.1A). Videos were matched for duration, action sequences, motion, social interactions, as well as musical and linguistic content. Video frames were 8-bit color images and 720 by 480 pixels in resolution. Each frame was hand-traced for areas of interest (AOIs) encompassing each character's face as in prior research using identical stimuli (Frank, Amso, & Johnson, 2014; cf. Frank et al., 2009) using software written in MATLAB (MathWorks, Inc; Natick, MA). Faces on average comprised 38.21% of the visual display. Visual fixations (points of gaze within a 30 pixel radius for a minimum of 100 ms) were classified by the eye tracker, and accumulated fixations within these AOIs were recorded. The primary dependent eye-tracking measure was percent looking at faces, computed as fixations within face AOIs relative to any part of the display. On-screen viewing time was calculated as a percent of total dwell time relative to clip length. This was used as a measure of overall task engagement.

Eye-tracking Procedure. Eye-tracking protocols were completed at 3, 6, 9, and 12 months of age using a Tobii T60XL, a remote corneal-reflection binocular eye-tracker, which records eye movements at high spatial and temporal resolution (spatially < .5 degree of a visual angle; temporally samples at 60HZ). Infants sat on a caregiver's lap approximately 60 cm from the 65-cm video display monitor. Caregivers were explicitly instructed not to distract their infant's attention from the screen during stimuli presentation. Each infant's POG was calibrated using a 9-point calibration scheme prior to data collection. The calibration scheme was repeated

until infant's POG was within 1^{o} of the center of the target and repeated between the two trials. The video stimuli were presented only after the calibration criterion had been reached.

Statistical Analyses. Percent looking at faces was longitudinally analyzed with a 3-level hierarchical linear model (individual time points nested within clip type within infant; risk status and parental affectedness as time-invariant subject level predictors) using the nlme package in R (Pinheiro, Bates, DebRoy, et al., 2018). On-screen viewing time was similarly longitudinally analyzed. Age at session, risk status (High vs. Low Risk), parental affectedness (average parental SRS score), and clip type (*Charlie Brown* vs. *Sesame Street*) were included as predictors in the model. The age variable was centered at 3 months such that the intercept represents mean attention to faces at 3 months of age. Similarly, parental affectedness was mean centered for ease of interpreting interaction terms.

As noted previously, we were interested in evaluating whether developmental trajectories are moderated by a) familial risk status, b) parental affectedness and c) clip type. This was statistically modeled as the interaction between age and each of the moderating variables. Additional interactions of interest concerned effects of risk status and family affectedness on developmental trends in attention to faces by clip type (i.e., three-way interaction between age, risk status, and clip type, as well as between age, parental affectedness, and clip type). We were also interested in evaluating a potential interaction effect between risk status and parental affectedness on the developmental trajectories in attention to faces (i.e., a three-way interaction between age, risk status, and parental affectedness). This would reveal whether there is a dosage effect of parental affectedness on developmental trajectories in attention to faces by risk status (e.g., greater parental affectedness in HR infants may have more of an impact on developmental trends in face-looking than greater affectedness in low-risk infants).

To examine how developmental trends in attention to faces may be associated with measures of social competence, we ran Pearson correlations between rate of change in attention to faces and ADOS-T SA scores and ESCS scores. We had a priori hypotheses that these measures would be inter-correlated with one another. Estimates of individual trajectories in attention to faces from 3- to 12-months were derived from the model using the coef function in R. This metric is a sum of the fixed effect and random effect for the age term for each infant and provides the estimated slope for face-looking from 3-12 months. Because prior work has found that cognitive level is associated with ASD symptom severity in young children with ASD (Ben-itzchak & Zachor, 2007) and cognitive profiles moderate trajectories of visual social attention to faces in youth with ASD (Rice, Moriuchi, Jones, & Klin, 2012), we also ran correlations partialling out the effect of cognitive skill level when evaluating associations between ADOS T scores and developmental trajectories in visual attention to faces (e.g., Bedford et al., 2012). This specifically examines the relation between face-looking and social impairments over and above the effects of cognitive functioning. Zero-order and partial correlations are reported.

Results

Behavioral Profiles. Parental affectedness did not differ between HR and LR infants ($t(81)=1.712$, $p = .09$). At 12 months, HR and LR infants had comparable cognitive scores ($t(77)=1.67$, $p=.099$) and similar rates of spontaneous initiation of, or response to, joint attention bids (ESCS IJA: $t(70)=1.499$, $p =.138$; ESCS RJA: $t(70)=1.208$, $p = .231$). At 18 months, HR infants showed greater social impairments than LR infants ($t(66)=2.007$, $p = .04$, see Table 1).

Across all infants, parental affectedness correlated with Mullen ELC ($r=-.30$, $p = .007$) and ADOS T SA ($r=.30$, $p =.012$), but not ESCS IJA and RJA scores. Mullen ELC was

positively correlated with ESCS RJA scores (r=.283, p = .016) and negatively correlated with ADOS T SA (r=-.370, p = .028).

Table 1.1: Behavioral Profiles of Infants at High vs. Low-Risk for ASD

Measure	High Risk	Low Risk	*t*	*p*
Parental Affectedness	28.25 (15.07)	22.33 (15.71)	1.712	.091
Mullen ELC	104.89 (14.37)	109.91 (14.37)	1.670	0.099
ESCS IJA	0.87 (0.53)	0.69 (0.44)	1.499	.138
ESCS RJA	0.26 (0.27)	0.33 (0.22)	1.208	.231
ADOS T SA	6.37 (4.71)	4.15 (3.60)	2.077	.042

Note. Standard deviations appear in parentheses below means. ELC, Early Learning Composite; ESCS, Early Social Communication Scales; IJA, Initiation of joint attention; RJA, Response to joint attention; ADOS T SA, social affect score

On-screen Viewing Time. An unconditional growth model was first run to evaluate the proportion of variance explained by each level. Intraclass correlation coefficients (ICC) were calculated to examine the correlation among observations within the same level. The ICC for clip type nested within subject was .07, suggesting that clip type was not an informative level to evaluate trends in on-screen viewing time; that is, the observations for *Charlie Brown* were no more similar than the observations for *Sesame Street* and vice versa. The ICC for between-subject variability was .29 and within-subject variability was .63. Thus, the hierarchical linear model reported below excludes clip type as a separate level and only includes infant age at evaluation, parental affectedness, risk status, and their interactions as predictors and percent on-screen viewing time as the criterion (see Table 1.2).

On-screen viewing time did not vary with age (β=0.43, t=0.89, p=.37) and developmental trajectories in on-screen viewing time did not vary by parental affectedness

(β=.05, *t*=1.58, *p*=.11) or infant risk status (β=-0.56, *t*=-0.89, *p*=.38). The dynamic stimuli were thus equally engaging for all infants across all time points.

Table 1.2: Results from Linear Mixed Effects Model Predicting On-Screen Viewing

Fixed Effects	Estimate	Standard Error	*df*	*t*	Pr (>|t|)
Intercept	67.501	3.445	326	19.592	<.001
Age	0.433	0.484	326	0.895	.371
Parental Affectedness	-0.396	0.223	78	-1.772	.080
Risk	.390	4.376	78	0.089	.929
Age X Parental Affectedness	0.049	0.031	326	1.588	.113
Age X Risk	-.561	.633	326	-0.888	.375
Parental Affectedness X Risk	.602	.259	78	2.322	.023
Age X Parental Affectedness X Risk	-0.058	0.036	326	-1.610	.108

Note. T-tests are calculated using Satterthwaite approximations of degrees of freedom

Developmental Trends in Attention to Faces. An unconditional growth model was used to calculate the ICC clip type, between-, and within-subject variance. ICC for clip type nested within subject was .55, justifying its inclusion as a level for the hierarchical linear model. ICC for between-subject variance was .14 and for within-subject variance was .31.

Attention to faces increased from 3- to 12-months of age across all infants (β=2.020, *t*=4.860, *p*<.001). There was also a main effect of clip type; infants looked less to faces in *Sesame Street* than in *Charlie Brown* (β=-26.589, *t*=-8.734, *p*<.001). There was no main effect of either parental affectedness (β=0.162, *t*=1.627, *p*= .11) or risk status (β=0-.214, *t*=-0.060, *p*=.952) on overall attention to faces (see Figure 1.1B).

There was a significant three-way interaction between age, parental affectedness, and clip type (β=0.447, *t*=2.687, *p* = .008). To decompose this interaction, separate hierarchical linear models were run for eye-tracking data corresponding to *Charlie Brown* and *Sesame Street* with age, parental affectedness, and their interaction as predictors. There was a significant interaction between age and parental affectedness for *Charlie Brown* (β=-0.39, *t*=-2.819, *p* = .006) but not

for *Sesame Street* (β=0.002, *t*=.147, *p* = .883); higher values in parental affectedness were associated with lower developmental change in face-looking in *Charlie Brown* from 3- to 12-months. This was also reflected in a significant interaction between parental affectedness and age (β=-0.038, *t*=-2.33, *p* = .020); higher values in parental affectedness were associated with lower developmental change in face-looking (see Table 1.3).

There was also a clip type by parental affectedness interaction (β=-0.217, *t*=-2.08, *p* = .041). Higher parental SRS scores were associated with overall lower attention to faces in *Sesame Street* relative to *Charlie Brown*. There were no significant interactions with risk status on developmental trends in attention to faces or parental affectedness.

Table 1.3: Results from Linear Mixed Effects Model Predicting Looking at Faces

Fixed Effects	Estimate	Standard Error	*df*	*t*	Pr (>|t|)
Intercept	54.026	2.732	246	19.778	<.001
Age	2.019	0.415	246	4.859	<.001
Parental Affectedness	0.162	0.099	79	1.629	.108
Clip Type	-26.588	3.044	74	-8.734	<.001
Risk	-.214	3.488	79	-0.059	.953
Age X Parental Affectedness	-0.038	0.016	246	-2.338	.020
Age X Clip Type	-0.348	0.489	246	-0.711	.477
Age X Risk	0.149	0.554	246	0.269	.788
Clip Type X Parental Affectedness	-0.217	0.104	74	-2.077	.042
Clip Type X Risk	4.748	4.050	74	1.172	.245
Age X Parental Affectedness X Clip Type	0.045	0.017	246	2.687	.008
Age X Risk X Clip Type	-0.899	0.658	246	-1.368	.173
Age X Parental Affectedness X Risk	-0.0004	0.011	246	-0.034	.973

Note. T-tests are calculated using Satterthwaite approximations of degrees of freedom

Associations between Developmental Trends in Attention to Faces and Social Communicative Competence. The null interaction between age and clip type suggests that developmental trends in attention to faces for *Charlie Brown* and *Sesame Street* were comparable. Indeed, estimates of individual slopes in attention to face while viewing *Charlie Brown* and *Sesame Street* were correlated with one another (*r*=.425, *p*<.001). Thus, estimates of

overall attention to faces across clip types were used to examine associations between developmental trends in face looking and social communicative competence. Across all participants, greater attention to faces was associated with lower social symptom severity (r=-.245, p=.044, see Figure 2); significance in zero-order correlations between ADOS-T SA and face looking did not hold when evaluating HR and LR groups separately. Partialling the effects of cognitive skill level, the negative correlation between attention to faces and ADOS-T SA scores across all participants still held (r=-.267, p=.034) and became significant for HR infants (r=-.319, p = .048). Greater developmental increases in attention to faces, therefore, were associated with lower levels of social symptom severity at 18 months.

Attention to faces from 3- to 12-months in HR infants was positively correlated with 12 month ESCS RJA scores (r=.316, p = .04) but not IJA scores (r = -.045, p = .780). The relation was not significant across all participants (ESCS IJA: r=-.016, p =.895; ESCS RJA: r=.165, p=.166, see Figure 2).

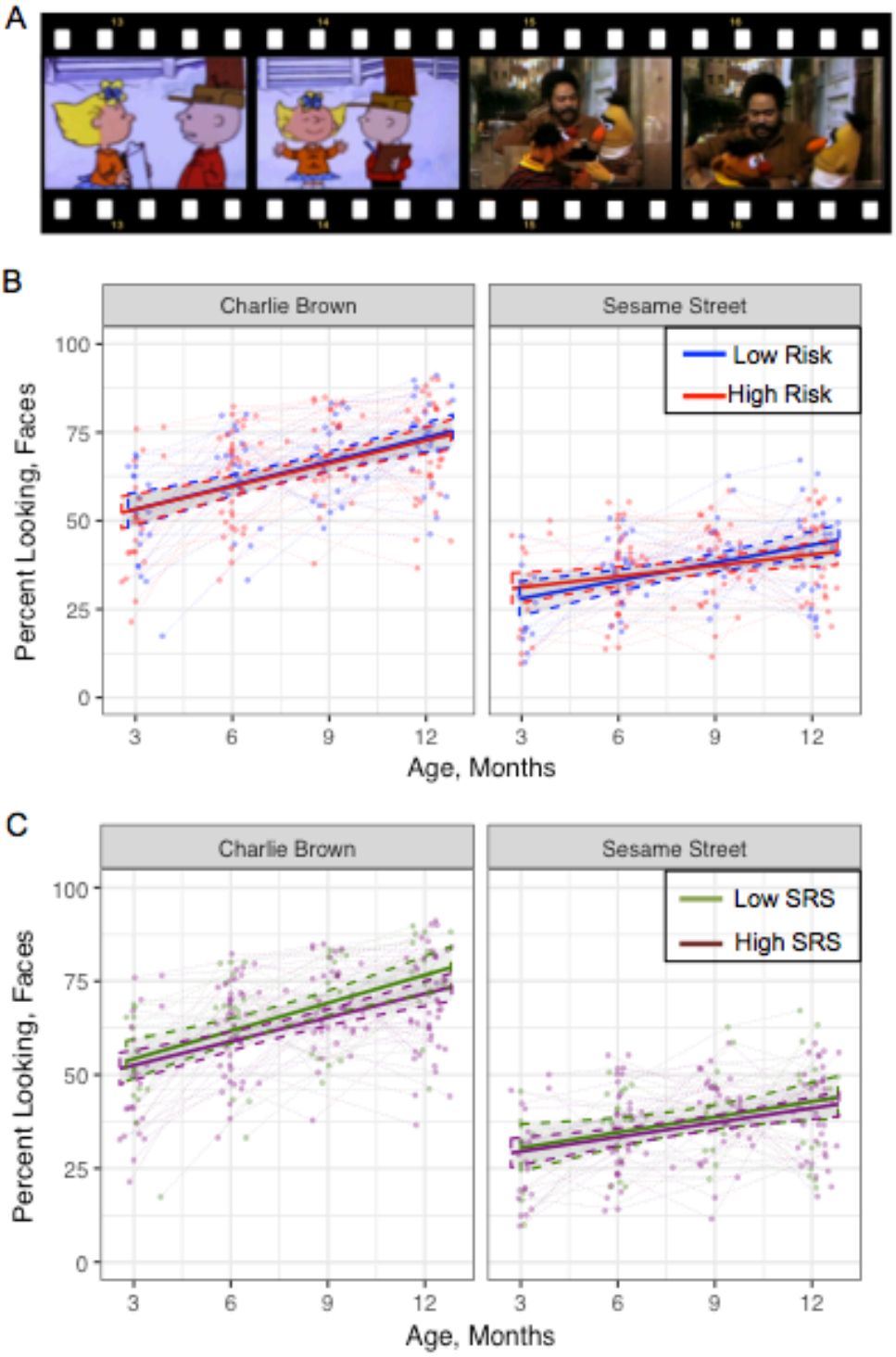
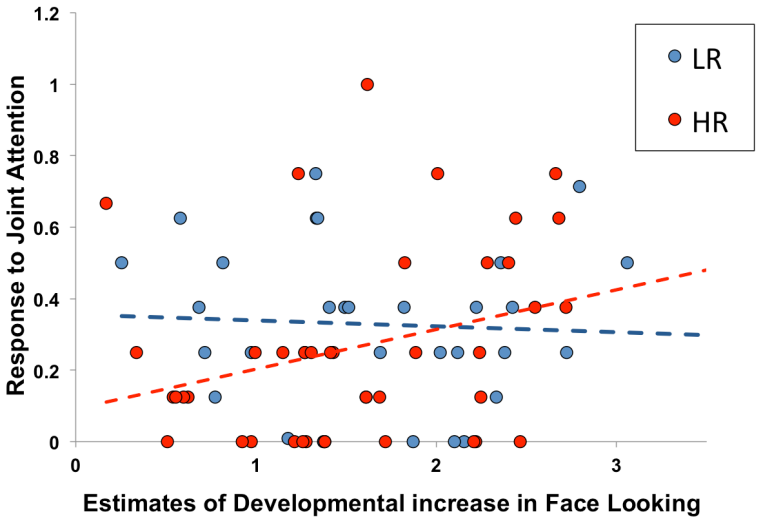


Figure 1.1 A) Sample stills from *Charlie Brown* and *Sesame Street.* B) Developmental trajectories in attention to faces by infant risk status and parental affectedness of ASD-related traits. C) For visualization purposes, infants were grouped based on their parent's average SRS scores. Low SRS includes infants whose parents' SRS scores were below the group average; High SRS includes infants whose parents' SRS scores were above the group average.



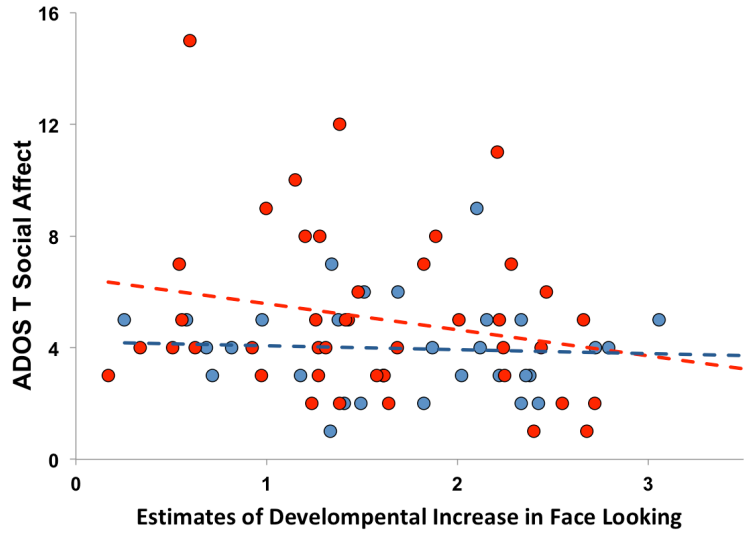


Figure 1.2. Zero-order scatterplots in estimates of developmental increases in face looking and ESCS RJA scores, and ADOS T SA scores. For HR infants, increased developmental rates in face looking were associated with better response to joint attention skills. Increased developmental rates in face looking were associated with lower ASD social symptom severity across all infants, and in HR infants when controlling for cognitive level.

Discussion

Attenuated engagement with the social world is a hallmark feature of ASD that also reflects the degree of social impairments in children and adults with ASD (Jones, Carr, & Klin, 2008; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Frazier et al., 2016). There is some evidence that diminished attention to socially relevant stimuli is an early emerging risk marker for ASD (Chawarska et al., 2013; Shic et al., 2014). Here, we explored factors that may influence observed developmental trajectories in attention to faces in infants at high and low risk for ASD, and the extent to which individual differences in attention to faces constitute a performance-based measure of social communicative competence. Both complexity of the stimulus and parental affectedness of ASD-related traits influenced attention to faces during the first postnatal year. These findings inform not only the type of experimental stimuli that may be sensitive at detecting differences in social attention in high vs. low-risk infants, but also potential modifiable aspects of early visual social attention.

Stimulus-specific Features Influence Visual Social Attention. We found that the complexity of the stimulus influenced infants' social attention, regardless of risk status. While developmental trends in attention to faces were comparable between *Charlie Brown* and *Sesame Street* (i.e., simple versus complex stimuli), infants overall looked more to faces in the simple schematic social stimulus (cf. Frank et al., 2014). This is consistent with prior eye-tracking studies demonstrating that visual behaviors are context-dependent and vary as a function of the social demands of the task in both typical development (Tenenbaum, Shah, Sobel, Malle, & Morgan, 2013) and ASD (Chawarska, Macari, & Shic, 2012; Elsabbagh, Bedford, et al., 2013). The ability to detect faces in complex, dynamic displays appears contingent upon an infant's attentional capacities (Frank et al., 2014), and the stimuli employed here contained dynamic

social action which required rapid deployment of attention. The exaggerated features of the schematic faces in *Charlie Brown* may represent a supernormal stimulus that facilitated the attentional capture of faces for both high- and low-risk infants.

Infants may also more readily attend to socially relevant features of a scene when such information is more accessible to them. Both schematic and live-action clips included segments of two to three characters engaged in conversation, which would require triadic attention. Although infants appear to be sensitive to shifts in gaze from dyadic to triadic interactions in live-settings (Striano & Stahl, 2005), the ability to coordinate attention and effectively monitor interactions involving at least two social agents does not become robust until the end of the first postnatal year (Carpenter & Nagell, 1998). The simpler presentation of the social scenes in *Charlie Brown* may have distilled the complexity of the social interactions such that irrelevant objects and events were not depicted and accentuated relevant social actions. For instance, the background in *Sesame Street* contained more details than in *Charlie Brown*, which may have detracted attention from faces. The departure from the busy complexity of naturalistic social situations, in conjunction with the exaggerated facial shapes and features in *Charlie Brown*, may have accounted for the elevated overall attention to faces in our schematic versus live-action stimuli.

Notably, attention to faces in the two kinds of social stimulus did not differ across the HR and LR infants we observed. Social stimuli used in some prior research examining visual social attention as an early risk marker of ASD featured a single actor engaging in ostensive social bids with the infant viewing the scene, emulating dyadic infant-caregiver social engagement (e.g., Jones et al., 2014). In contrast, the stimuli used here emulated complex social situations typical of playground settings, which may be more developmentally appropriate for older infants or

toddlers. Regardless, the stimuli used here appear to be valid in quantifying behavioral correlates of social competence in HR infants. Greater increases in attention to faces from 3- to 12-months of age was associated with greater responses to joint attention cues at 12 months, and lower levels of social symptom severity at 18 months, independent of cognitive levels. This corroborates prior work demonstrating that reduced attention to the eyes between 2-6 months in infants who develop ASD is associated with greater social impairment at 24 months (Jones & Klin, 2013). Performance-based measures of spontaneous attention to socially relevant information during infancy, therefore, can capture several aspects of social communicative development, highlighting the clinical significance for these early behaviors. More normative patterns of visual social attention in infancy may be a potential protective factor for HR infants with regards to their social functioning.

Social Environmental Features Influence Visual Social Attention. Our findings provide evidence that infants 'early social environments influence their cognitive and social development. Similar to prior work (Bernier et al., 2012), we did not observe differences between parents of HR and LR infants in BAP features. The comparable distribution of affectedness of ASD-related traits across risk cohorts suggests that parents of HR infants in our study are not be qualitatively different than parents of LR infants. Notably, however, elevated parental affectedness of autism-related traits predicted slower developmental increases in attention to faces across all infants, which suggests that parental behaviors play a role in modifying developmental trajectories of visual social attention in infancy. Given that increased rates of face-looking was associated with greater social competence in HR infants, improving social responsiveness in parents may have positive effects in promoting social communicative

development in infants at risk for ASD and other developmental disabilities that affect social function.

The association between parental SRS scores and developmental trajectories in attention to faces that we observed may not necessarily be attributable to the availability or overture of social communicative bids from the caregiver. If this were the case, we would have observed a significant association between degree of parent's SRS scores and infants' joint attention and language skills (e.g., Siller & Sigman, 2002). Shared genetic variation between parent and infant may account for the effect of social communication function in parents on infant's trajectories of visual social attention (e.g., Constantino et al., 2017). Alternatively, elevated BAP features in parents may impact the quality of parent-child interactions, such that they are more directive and less synchronous with the communicative needs of the infant (Parr, Gray, Wigham, & Mcconachie, 2015). Infants, including those at high risk for ASD, are sensitive to reciprocity in dyadic interactions and divert their gaze from their mother's face when there are breaks in contingency (Rozga et al., 2011). Reduced affect and synchrony in infant-initiated interactions in 4-month-old HR infants, however, suggest that HR infants may be less adept at engaging their caregivers in a reciprocal social interactions (Yirmiya et al., 2006). Following a transactional model of parent-infant interaction on social development, these two factors may iteratively compromise the social learning opportunities afforded by face-to-face interactions in HR infants (whose parents themselves may have elevated BAP features). This may manifest as reduced attention to faces, again presenting implications for developing interventions geared at ameliorating prodromal social symptoms in high-risk infants.

There is general support for the possibility that parent-mediated interventions can promote social communicative skills in children with ASD (Mcconachie & Diggle, 2007), and

earlier age of enrollment in parent-mediated intervention is associated with greater degree of improved social functioning (Rogers et al., 2012). Preliminary evidence from a randomized trial of parent-mediated intervention in 7- to 10-month-old HR infants suggests that optimizing parent-child interactive behaviors (e.g., parental responsiveness and emotional attunement to their infant's communication needs) can ameliorate behavioral and attentional prodromal symptoms of ASD (J. Green et al., 2017). For parents who may exhibit more social or language difficulties associated with BAP, such training may be particularly beneficial in promoting positive and sensitive parent-child interactions. Although prior work has found that parents with ASD are equally attuned to their child's communicative needs as parents of children with developmental delays and typical development, children with ASD may require higher levels of parent-child synchrony than their peers to attain benefits in joint attention and language skills (Siller & Sigman, 2002). Further research will be necessary to pinpoint how parent-mediated interventions affect autism-related traits in parents, and whether developmental changes in attention to faces can serve as an intermediary, and objective, outcome measure associated with social learning and social communicative competence in such interventions.

The findings from the present study contribute to our understanding of developmental differences in visual social attention among infants at high vs. low risk for ASD in three important ways. First, our results highlight the importance of considering developmental level when selecting dynamic social stimuli in longitudinal designs of visual social attention to faces. During the first postnatal year, infants transition from primarily engaging in dyadic social interactions to participating in triadic interactions. Stimuli that require monitoring triadic interactions may not be the most sensitive in identifying early deviations in visual social attention in high-risk infants, unlike the case for older individuals with ASD (Klin et al., 2002b;

Rice et al., 2012). Second, the comparable developmental trends in HR and LR infants' looking to faces in our study suggest that familial risk for ASD does not confer gross deficits in visual social attention. Given the genetic heterogeneity of the HR group, it is likely that overt impairments in social attention converge via different mechanistic routes. Rather, it may be possible that more subtle difficulties in modulating visual attention (Elsabbagh & Volein, 2009) initially impede the capacity to attend to the most socially relevant information in complex dynamic stimuli. Research to test whether lower level deficits in attention affects higher-order visual social attention in HR infants could clarify this issue. Finally, we found that ASD-related traits in parents moderated developmental trajectories in face looking during the first postnatal year, and that increased rates in HR infants' attention to faces were associated with social competency. This is a point of optimism because it identifies a modifiable aspect of an early symptom-based marker of ASD and presents clinical implications for developing interventions aimed at promoting social communicative skills in HR infants.

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Neural Correlates of Native Language Processing during the First Postnatal Year in Infants at Risk for ASD

Heterogeneity in language profiles is a distinct feature of the ASD phenotype, and a substantial proportion of infants at high familial risk for ASD exhibit language and communicative difficulties. Understanding the neural correlates of early language processing can not only inform the mechanisms underlying suboptimal language outcomes, but can also parse the heterogeneity in language functioning in ASD. Language learning is a highly social process and native language processing is crucial for language acquisition. Here we examined native versus nonnative language processing in infants with familial risk for ASD at 1.5 and 9 months of age, and its association with subsequent trajectories of language growth and symptom severity. We found that attenuated neural responses to language, a lack of left hemispheric specialization for language, and diminished trajectories of lexical growth were common across all infants at risk for ASD. Notably, stratifying HR infants based on language developmental profiles provided meaningful subgroups with distinct neural signatures for language processing. In line with prior work, HR infants with delayed language showed marked attenuated neural response for native and nonnative language; HR infants without delayed language showed non-normative routes for language processing. Altogether these findings suggest that developmental deviations in native versus nonnative language processing may constitute an early marker for the social communicative difficulties associated with ASD risk.

The symptom profile of autism spectrum disorder (ASD) is notably heterogeneous and particularly so within the language domain. Although language functioning is no longer considered within the current diagnostic framework for ASD, language substantially informs the ASD phenotype. Delays in language development are one of the earliest parent-reported concerns related to ASD (Tager-Flusberg, Paul, & Lord, 2005) and are regarded as an early behavioral marker of ASD risk (Zwaigenbaum et al., 2005). Moreover, verbal skills are predictive of long-term prognosis; early language profiles inform changes in ASD symptom severity during childhood, (Gotham, Pickles, & Lord, 2012), with some evidence indicating that better language skills in childhood are associated with better social functioning in adulthood (Howlin, Goode, Hutton, & Rutter, 2004). Given that language is a form of social communication, and ASD is characterized by social communicative deficits, examining language development early in infancy may provide insights into the emergence of ASD symptoms.

Capturing Language Development in ASD

Initial language acquisition lies at the intersection between social communicative and lexical development. Teasing apart these interlocking aspects of language learning by using converging measures of language development appears sensitive in determining how early language profiles may be affected in infants at familial risk for ASD (Hudry et al., 2014). Some measures of language development, such as the Macarthur Bates Communicative Development Inventory (MCDI), primarily gauge lexical development by characterizing both receptive and expressive vocabulary growth. Other measures, such as the Mullen Scales of Early Learning (MSEL), quantify communicative intent in young infants and evaluate language skills such as using jargon with gestures and responding to requests.

In typical development, the number of words an infant comprehends precedes and substantially exceeds the number of words produced (Bates, 1993). Whereas typically developing infants show a rapid growth in their receptive vocabularies from 6-24 months of age, infants at high-risk for ASD (HR) show minimal gains in a receptive advantage during this period (Hudry et al., 2014). Because children cannot meaningfully use words they do not themselves understand, a smaller difference in receptive versus expressive vocabulary may then translate into potential delays in *functional* language use. Indeed, declining trajectories in age-equivalent expressive and receptive verbal scores on the MSEL, a measure that can assess communicative intent and language use, from 6-24 months of age is predictive of an ASD diagnosis in HR infants (Longard et al., 2017). Moreover, difficulties in appropriate use of language (e.g., pragmatic language skills) are common among preschool-aged children with a family history of ASD, regardless of developmental outcome (Miller, Young, et al., 2015). Altogether this suggests that examining trajectories of both lexical and social communicative development of language throughout infancy can inform where impairments in language skills may arise in infants at risk for ASD. Furthermore, understanding the neural correlates of language learning may inform our understanding of the underlying mechanisms subserving language difficulties that are associated with familial risk for ASD.

Recent fMRI studies have shown that toddlers with ASD display reduced neural activity during speech processing (Eyler, Pierce, & Courchesne, 2012; Redcay & Courchesne, 2008), especially those with significant speech delays (Lombardo et al., 2015). Early atypicalities in the neural networks subserving language processing and the social and communicative difficulties in young children with ASD may reciprocally exacerbate one another with development. Moreover, given that language skills predict prognosis in ASD (Gotham et al., 2012; Tager-Flusberg, Paul,

& Lord, 2005), characterizing the neural profiles associated with early language development may be an informative dimension for parsing the heterogeneity in ASD. The first postnatal year marks incredible strides in initial language acquisition as infants go from being preverbal to making their first single-word utterances. Here, we examined the neural signatures of language processing at two time points to capture developmental changes in infants at low-risk (LR) and high familial risk for ASD (HR).

Native Language Processing in Infancy

One very early index of language learning is a preference for the language an infant hears prenatally. Exposure to language begins early in life; human fetuses show evidence of distinguishing their mother's voice versus a female stranger's, and their native versus nonnative language, by 33 weeks of gestational age (Kisilevsky et al., 2009). Thus, relevant information about the prosodic contours of one's native language (i.e., a language's rhythm, stress, and intonation) is available and perceived by the infant prenatally. Shortly after birth, this is reflected in a native language preference (Moon, Cooper, & Fifer, 1993). Assessing infants' native language preference can thus provide a measure of their attention to and uptake of the most relevant linguistic input in their immediate environment (e.g., Kuhl, 2004).

Neural attunement to the phonemes of one's native language at 7 months, as measured by electroencephalography (EEG), is associated with better language skills in the second postnatal year (Kuhl et al., 2011), which indicates that native language processing can index language learning in preverbal infants. The better spatial resolution of functional magnetic resonance (fMRI), relative to EEG, can precisely identify the brain regions involved in language processing and passive fMRI during natural sleep has been used to successfully map neural responses to speech in infancy (Dehaene-Lambertz, Dehaene, & Hertz-Pannier, 2002; Dehaene-Lambertz,

Hertz-Pannier, & Dubois, 2006; Dehaene-Lambertz, Montavont, Jobert et al., 2010; Shultz, Vouloumanos, Bennett, & Pelphrey, 2014). Indeed, reliable activation in canonical fronto-temporal language areas has been observed in infants as early as 2 days after birth (Perani et al., 2011). Neural specialization for language shortly after birth indicates attunement and learning of to the linguistic properties of speech, such as prosodic contours and rhythm. Moreover, within the first few months of postnatal life, the neural circuitry subserving language processing undergoes developmental change such that temporal regions associating with language processing become increasingly specialized for speech over human non-speech sounds (Shultz & Vouloumanos, 2014). It is possible that subsequent difficulties in language and attenuated neural responses to language in ASD may stem from earlier differences in neural specialization for language between infants at high versus low-risk for ASD. Here, we examine one particular aspect of specialization—that is neural responses to native versus nonnative language.

The Current Study

The current study aimed to examine neural responses to native versus nonnative at 1.5- and 9-months of age in HR and LR infants. We used English and Japanese speech streams as instances of the infant's native and non-native languages, respectively. Sampling at these ages was meant to provide insight into the neural circuitry subserving language processing at different stages of language acquisition. At the earlier age, we expected LR infants to show evidence of neural differentiation of native versus nonnative language due to prenatal exposure to their native language. Moreover, if language in general is perceived as a relevant class of stimulus, then the unfamiliar language may recruit additional neural resources. At the later age, we expected LR infants to have acquired some native-language expertise (e.g., perceptual narrowing for one's native language would have occurred), which should also be reflected at the neural level as

greater activity for their native versus non-native language. Given prior research, we hypothesized that HR infants would exhibit attenuated neural responses for language and possibly fail to show differential activity between English and Japanese at both ages. In addition to neural differentiation between native and nonnative language, we also explored whether infants differed in lateralization of neural responses to language by risk status. Language processing becomes increasingly left lateralized with development during language acquisition (Ghislaine Dehaene-Lambertz et al., 2006); HR infants may not demonstrate normative patterns in hemispheric lateralization for language from 1.5- to 9-months.

In addition to examining neural responses to native versus nonnative language, we also characterized language skills in LR and HR infants from 6- to 18-months of age using two converging measures of communicative development. This served to provide a rich developmental description of language profiles in typical and atypical development. HR infants were deemed more likely to exhibit signs of language delays, and to show minimal, or slower growth in their receptive advantage relative to LR infants; individual differences in language development were expected to be associated with level of ASD social symptom severity. Moreover, the early neural signatures for native versus nonnative language processing were hypothesized to predict subsequent behavioral measures of language development in both HR and LR infants. Given that language profiles are vastly heterogeneous in ASD, the final, exploratory aim examined whether neural responses to language in HR infants could be stratified by their language developmental profiles such that HR infants who exhibit language delays may process both native and nonnative language differently than HR infants who exhibit more normative language profiles.

Methods

Participants. Participants in this study were enrolled as part of a longitudinal project examining early brain-based markers of ASD during the first year. Infants were assigned to risk cohorts based on family history. HR infants had at least one older sibling with a clinical ASD diagnosis whereas LR infants had no family history of ASD or any other developmental disorder. Racial background for HR and LR families were comparable ($X^2(3)=2.19$, $p=.53$). HR and LR infants were matched by gender and age at each scan session ($T1_{gender}$: $X^2(1)=0.41$, $p=.52$, $T2_{gender}$: $X^2(1)=1.95$, $p=.16$; $T1_{age}$: $t(51)=0.03$, $p=.94$, $T2_{age}$: $t(31)=0.85$, $p=.39$).

The final sample included 53 infants (HR=25; LR=28) at the 1.5-month scan and 32 infants (HR=21, LR=12) at 9-month scan (Table 1). Twenty-five infants (HR=14, LR=11) provided usable fMRI data at both time points. Infants were excluded from the final sample if the MRI scan could not be completed due to the infant waking up during the scan session (T1: N=8; T2: N=11), or equipment malfunction (e.g., headphones did not emit sounds; T1: N=0; T2: N=3).

Infants included in the study were from households where English was reported as the primary language. A caregiver completed a language exposure form at each scan, which included information about the number of languages spoken in the household and number of waking hours the infant interacted with each household member for each language reported. English exposure, and other relevant participant information are reported in Table 2.1. One LR infant was studied but excluded from the final sample due to greater than 50% non-English language exposure.

Table 2.1: Participant Characteristics

		High Risk	Low Risk	*T*	*p*
N (T1/T2)		26/21	25/12		
Age at scan, months	T1	1.43 (0.26)	1.52 (0.27)	0.78	.43
	T2	9.27 (0.28)	9.13 (0.33)	0.85	.39

Relative motion, mm	T1	0.20 (0.17)	0.35 (0.32)	1.17	.25
	T2	0.10 (0.09)	0.11 (0.10)	0.19	.85
English Exposure, %	T1	94.60 (7.83)	91.30 (14.98)	0.93	.36
	T2	95.55 (6.21)	96.72 (5.07)	0.12	.90

Notes. Standard deviations are listed in parentheses below group means.

fMRI Stimuli and Paradigm. The speech stimuli were recorded from different female native speakers of English and Japanese (8 segments in each language). Both English and Japanese stimuli were previously used in behavioral studies in infants to assess native language preference (Sundara, Polka, & Molnar, 2008) and were matched for duration, intensity, peak amplitude, pitch, and pitch range. Speech stimuli were presented in a traditional fMRI block design whereby alternating English and Japanese speech segments (18 s each) were interleaved with periods of silence (12 s each). Speech streams were presented via MRI-compatible headphones.

MRI Data Acquisition. MRI scans were acquired at approximately 1.5 and 9 months of age on a Siemens 3T Tim Trio scanner using a 12-channel head coil. A scout localizing scan was used for graphic prescription. The language paradigm was acquired with a T2*-weighted spin-echo functional sequence: TR=3000ms, TE=28ms, matrix size 64x64, FOV=192mm, 34 slices, 3mm in-plane resolution, with 4mm-thick axial slices. Structural matched bandwidth T2-weighted high-resolution echo planar images were acquired co-planar to the functional scan to ensure identical distortion characteristics to the fMRI scans: TR=5000ms, TE=34ms, matrix size 128x128, FOV=192mm, 34 slices, 1.5mm in-plane resolution, with 4mm-thick axial slices.

MRI data acquisition occurred during natural sleep. After the infant was asleep and swaddled, silicon earplugs were placed over the infant's ear canal and mini earmuffs were fitted

over the entire outer ears. Infants were then placed onto a custom-made bed that fit inside the head coil. To minimize movement, infants were secured on the scanner bed with a Velcro strap, given a weighted blanket, and cushioned with foam pads around their heads. MRI safety-certified personnel remained in the scan room to monitor the infant's behavior throughout the scan. Scanning was stopped if the infant awoke.

fMRI Preprocessing Analysis. Functional imaging data were preprocessed and analyzed using FSL version 5.0.8 (FMRIB's Software Library; Jenkinson et al., 2012; Smith et al., 2004; Woolrich et al., 2009). Preprocessing included co-registration of the fMRI data to the subject's corresponding high-resolution anatomical scan, registration to an infant brain template (Shi et al., 2011; neonatal template for 1.5 months scans and 1-year-old template for 9-month scans), image realignment to correct for head motion, and spatial smoothing (Gaussian kernel of 5mm FWHM) to increase signal-to-noise ratio. Registrations were performed with a 12-parameter affine-general transformation. Individual volumes were censored to attenuate the effects of motion-related artifacts on the data (Power et al., 2014; Spiegel et al., 2013).

Behavioral Measures. Language skills were prospectively evaluated by both observational and parent-report measures. Separate measures were used to characterize social communicative and lexical development. A trained clinician administered the Mullen Scales of Early Learning (MSEL, Mullen, 1995) at 6-, 12-, and 18-months of age. The MSEL is a standardized developmental assessment that measures gross and fine motor, visual reception, as well as receptive and expressive language skills. Language items on this measure assess skills such as following verbal requests, recognize own name and objects, plays gesture games, and jabbers with inflection. A verbal developmental quotient (VDQ) was calculated for each time point as a quotient between the average expressive and receptive age-equivalent scores and the

infant's chronological age. This provided a metric of the infant's verbal functioning normed based on chronological age.

The MacArthur-Bates Communication Development Inventory (MCDI, Fenson et al., 2007) is a parent-completed standardized questionnaire that measures an infant's receptive and expressive vocabulary. A parent completed the MCDI when the infant was 9-, 12-, and 18-months of age. A receptive advantage score was calculated for each time point as the difference between the number of words the infant comprehended and the number of words the infant could produce (Hudry et al., 2010).

Lastly, infants were administered the Autism Diagnostic Observation Schedule, Toddler Module (ADOS-T, Luyster et al., 2009) at 18 months to measure ASD symptomatology. Severity of ASD social symptoms was indexed by the Social Affect (SA) score.

fMRI Data Analysis. Single subject analyses were conducted with FSL FEAT. Multiple linear regressions were used to estimate the BOLD response to each condition of interest (i.e., activity during English and Japanese speech segments). This was obtained by convolving a square wave for each stimulation block of that condition with a double-gamma function approximating the hemodynamic response. Each subject's motion parameters were entered as covariates in the model. The regression model produced estimates of a percent signal change in BOLD response to English>Silence, Japanese>Silence, English>Japanese, and Japanese>English for each subject (cluster corrected to $Z>1.7$, $p< 0.05$).

Group-level analyses were also conducted with FSL FEAT. A mixed effects linear model (FLAME 1 + 2) was used to generate group activation maps within a common template space. Subjects were entered as random variables and language condition as a fixed variable. Planned contrasts were used to identify areas of significantly different activation between conditions and

HR and LR groups. Reported group maps are pre-threshold masked by the composite English and Japanese vs Silence activation maps that survived a $Z > 1.7$ threshold for both HR and LR infants. While these maps were more liberally thresholded, we report only clusters that exceeded a $Z > 2.3$ ($p < 0.01$) threshold (see Table 2.2).

Exploratory longitudinal analyses were conducted with the imaging data. Anatomically defined masks were created with temporal regions that have previously shown to undergo developmental changes in neural response to speech in early infancy (cf. Shultz et al., 2014). This included the middle temporal gyrus, superior temporal gyrus, and Heschl's gyrus. Separate masks were created for left and right hemispheres, as well as a conjoined bilateral mask. Parameter estimates of magnitude of activity in response to English and Japanese vs Silence were extracted from these temporal regions. To examine the extent that language processing becomes left lateralized with age, a laterality index was calculated as the difference in activity (i.e., magnitude of signal intensity) between left and right hemispheres divided by the sum of left and right hemisphere activity in temporal language regions (Swanson et al., 2017). Laterality indices in response to English and Japanese longitudinally analyzed using the nmle package in R. The model included age, risk, and language (English and Japanese) as predictors.

Behavioral Data Analysis. MSEL VDQ and MCDI Receptive Advantage scores were longitudinally analyzed in two separate linear mixed models using the nlme package in R. Each model included age and infant risk status as predictors, and verbal scores as the criterion. Estimates of developmental trends in language growth were obtained with the coef function. These values were included as bottom-up covariates of interest in fMRI data analysis to examine how native language processing at 1.5- and 9-months of age is associated with individual trends

in verbal skills and lexical development (i.e., MSEL VDQ and MCDI Receptive Advantage, respectively).

Longitudinal VDQ and MCDI scores were also analyzed to identify clusters of language developmental trajectories. This would allow us to stratify infants by language development. Longitudinal cluster analysis was done using the kml package in R, which uses a k-means clustering to determine groupings. It is able to deal with missing data and also provides metrics for determining the optimal number of clusters give the data (e.g., Calinksi and Harabatz, Ray and Turie, BIC).

Results

Neural Response to Native vs. Nonnative Language.

One and a half months. Data collected in 1.5 month-old infants showed robust activity in bilateral temporal language areas for both English and Japanese speech segments (Figure 2.1A). Between-group difference in neural response to either English or Japanese did not survive cluster-wide correction for multiple comparisons.

There were also significant between-language differences, indicative that infants differentiated between English and Japanese at the neural level (Figure 2.2). LR infants exhibited greater activity in regions associated with language processing (i.e., left STG and supramarginal gyrus) for English than for Japanese (Figure 2.2A). In contrast, HR infants showed greater response in bilateral amygdalae for English relative to Japanese. LR infants exhibited greater activity in regions associated with attention and prosodic processing (i.e., right inferior parietal lobule [IPL]) and speech processing (i.e., left MTG) for Japanese over English (Figure 2.2B). HR infants did not show any greater activity for Japanese relative to English.

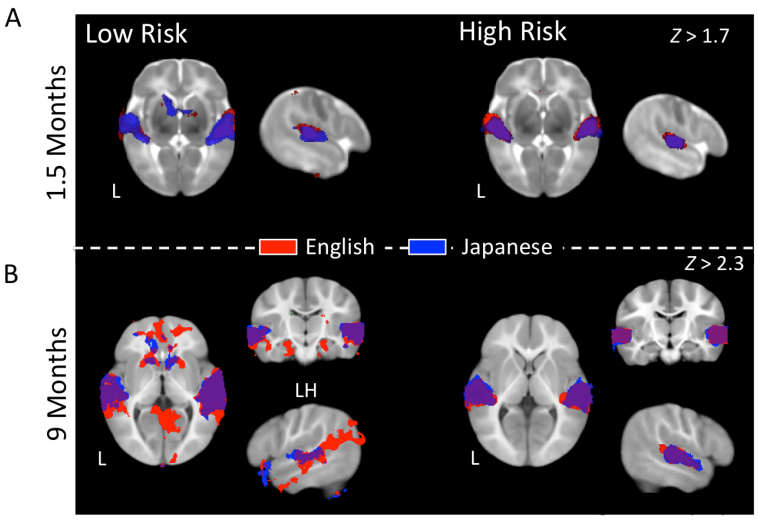

Figure 2.1: Activity maps in response to English and Japanese in infants at high- and low-risk for ASD at 1.5 and 9 months.

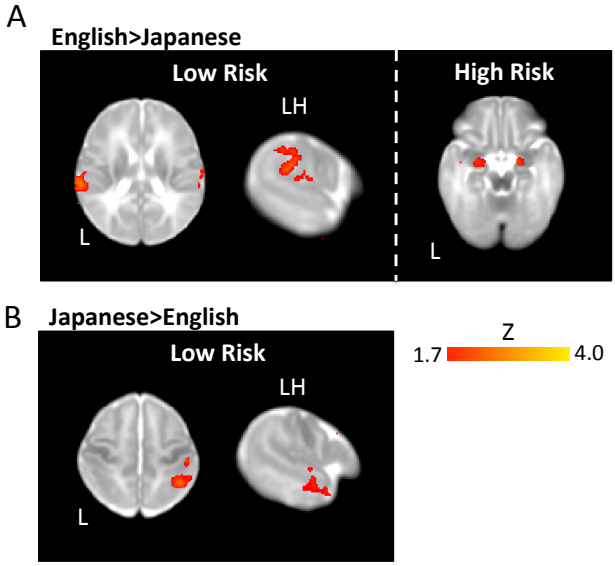

Figure 2.2: Activity maps demonstrating neural differentiation for familial vs. unfamiliar language (A) and unfamiliar vs. familiar language (B) in high- and low-risk infants at 1.5 months.

Nine months. The extent of activation in response to speech stimuli was expansive at 9 months (Figure 2.1B). While neural responses to English and Japanese were localized to bilateral temporal regions in HR infants, in LR infants activation extended beyond temporal regions to also include prefrontal regions, posterior cingulate, precuneus, and bilateral caudate, putamen, hippocampi. Relative to HR infants, LR infants showed greater activity in the precuneus, left

MTG, left STG, and right superior medial frontal gyrus for English, and right medial orbitofrontal cortex, cerebellum, and left MTG for Japanese.

LR infants showed greater activity for English relative to Japanese in posterior cingulate, cuneus, and bilateral hippocampi. No between-language differences were observed in HR infants.

Language Profiles. VDQ increased from 6- to 18-months of age (β=1.02, $t(141)=3.27$, $p=0.001$) and trajectories of verbal growth did not differ by risk status (age X risk: β=-0.74, $t(141)=1.82$, $p=0.07$). In contrast, there was a significant risk by age effect for receptive advantage scores (β=-6.21, $t(108)=3.31$, $p=0.001$). LR infants exhibited larger increases in receptive advantage from 9- to 18-months than HR infants. A diminished developmental gain in receptive advantage has been observed in other samples of infants with high familial risk for ASD (Hudry et al., 2010, 2014).

Longitudinal cluster analyses revealed two distinct trajectories in both receptive advantage scores and VDQ growth (see Figure 2.3). The receptive advantage clusters revealed two distinct trajectories in receptive advantage development (Cluster A: 63.8% of infants; Cluster B: 36.2%). Although both clusters included infants with gains in receptive advantage, the extent of the receptive advantage was greater for one cluster than the other. A binomial test revealed that HR infants were more likely to be categorized as the infants who showed smaller developmental gains in receptive advantage scores ($z=-2.36$, $p = .02$), recapitulating the group differences observed in the linear mixed model for receptive advantage scores. For VDQ, one cluster (66.7% of infants) was characterized by growth in verbal development and one cluster (33.3% of infants) was characterized by delays in verbal development. A binomial test revealed that cluster membership marginally reflected risk status ($z=1.91$, $p = 0.06$)—14 out of 32 HR

infants showed delayed language trajectories, whereas only 4 out of 26 LR infants showed delayed language trajectories. This suggests greater heterogeneity in language development in the HR group.

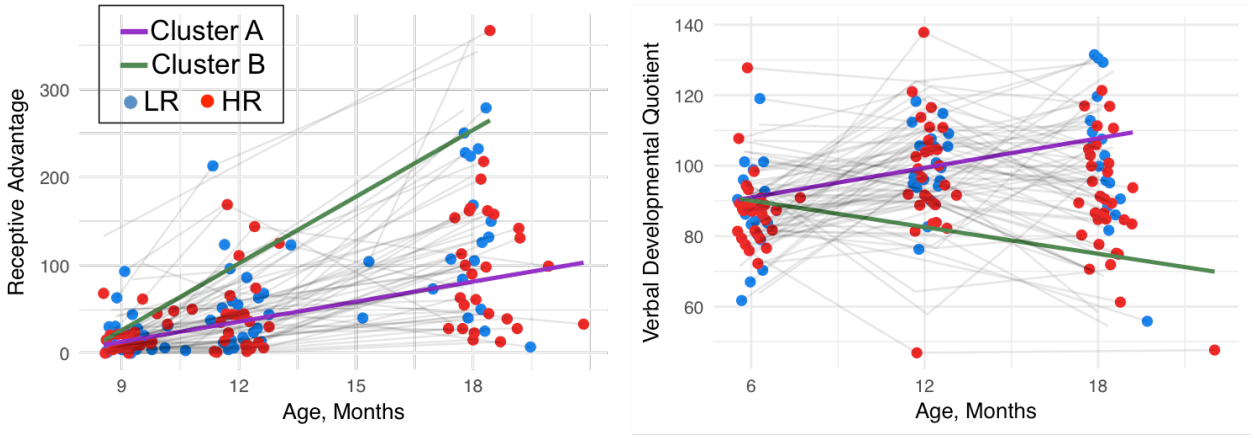

Figure 2.3: Results of longitudinal cluster analysis for Receptive Advantage and Verbal Developmental Quotient trajectories. The cluster analysis revealed two distinct trajectories for both Receptive Language and Verbal Developmental Quotient growth.

To examine how distinct language profiles may be associated with severity of ASD social symptoms, we stratified HR infants into two groups based on VDQ cluster membership (i.e., delay vs no delay) and compared ADOS T SA scores between the HR-delay vs HR-no delay groups. This measure was chosen over the MCDI because the clustering analysis for receptive advantage scores were indicative of infant's risk status; accordingly, stratifying by receptive advantage would not provide further information about variability within the HR group. HR infants with language delays had higher ADOS T SA scores than HR infants without language delays (HR-delay: M=9.55, SD=4.72; HR-no delay: M=3.29, SD=2.20; $t(26)=4.11$, $p=.001$).

Neural Responses to Native Language and Subsequent Language Development. To investigate how early native language processing at the neural level relates to trajectories in language development, we examined neural responses to English as a function of growth rates in

VDQ and receptive advantage (Figure 4). Across all infants, greater activity in the right STG, MTG and supramarginal gyrus, and left IFG at 1.5 months predicted greater growth in VDQ from 6 to 18 months. Greater activity in right MTG in response to English at 1.5 months predicted larger increases in receptive advantage scores from 9 to 18 months of age. At 9 months, greater activity in right MTG, STG, and IFG predicted greater changes in VDQ, and greater activity in bilateral IPL predicted larger increases in receptive advantage scores. Altogether these results suggest that different, but overlapping, neural regions are associated with functional language development versus lexical growth.

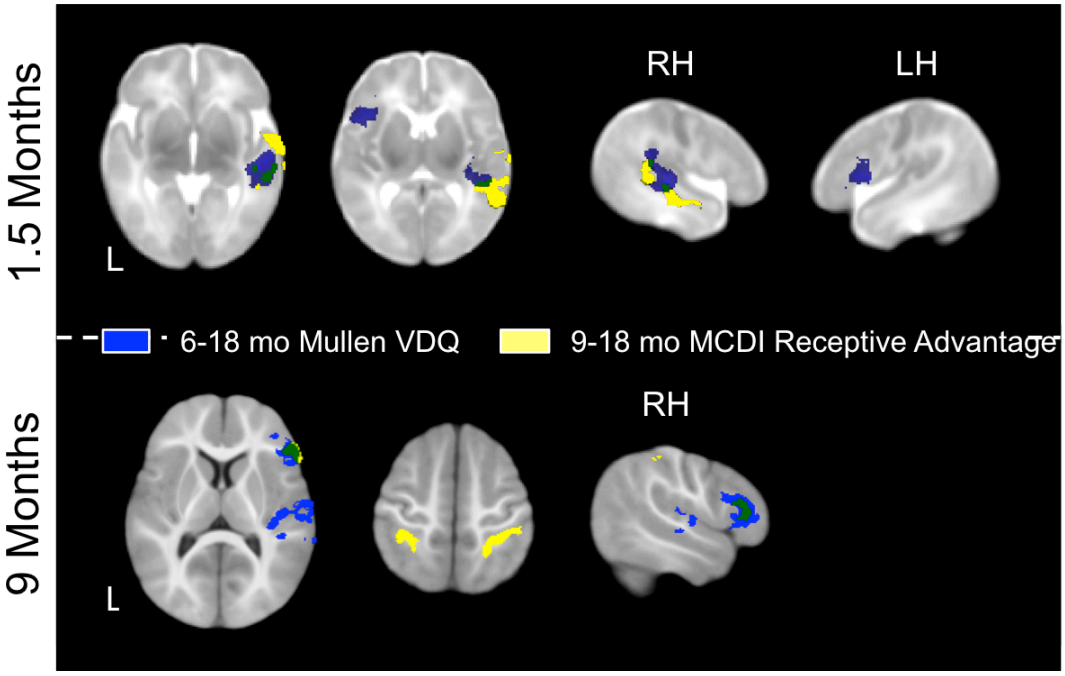

Figure 2.4: Neural regions in response to English that predicted developmental growth in Verbal and Receptive Advantage scores at 1.5 and 9 Months across high- and low-risk infants.

Laterality Index. We also longitudinally examined neural response to language by extracting parameter estimates from anatomically-defined temporal regions associated with processing speech (see Figure 2.5). There was a significant interaction between risk and age for a laterality index (β = -1.323, $t(109)$=-2.08, p=0.04). Language processing became increasingly left

lateralized for LR infants from 1.5 months to 9 months of age, whereas HR infants did not show evidence of hemispheric specificity for language processing.

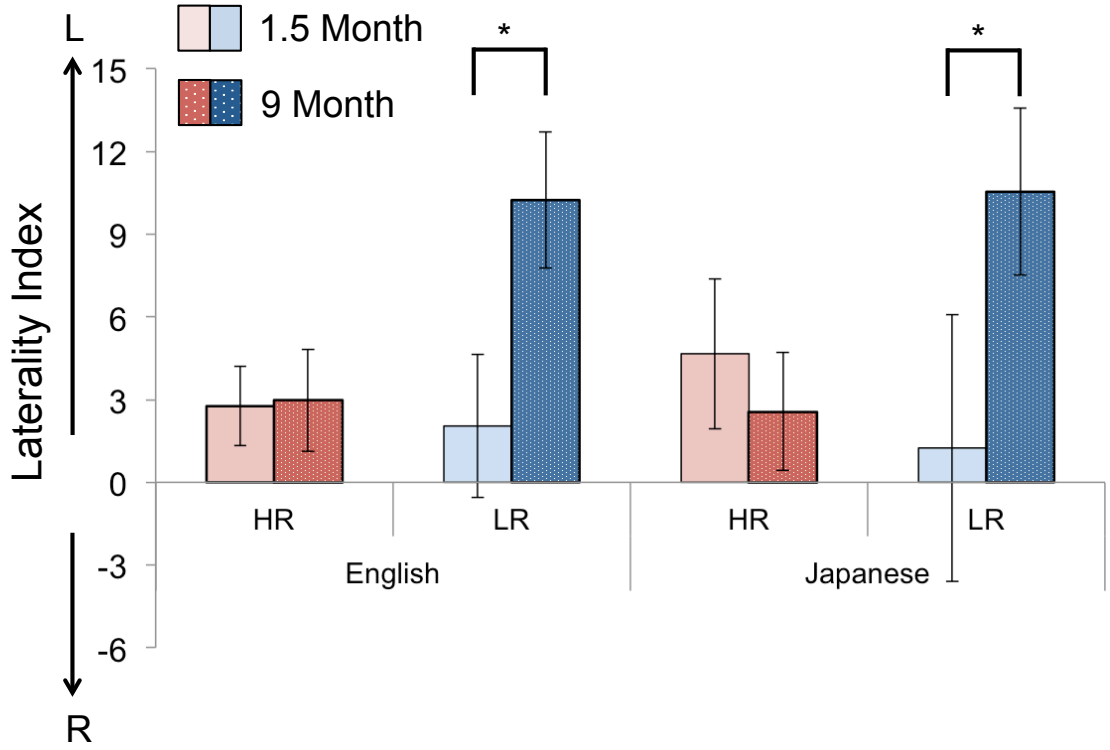

Figure 2.5: Laterality of activity in temporal language regions for English and Japanese at 1.5 months to 9 months.

Neural Responses to Language in HR-Delay versus HR-No Delay. To explore whether distinct language profiles in HR infants were associated with differences in language processing, we reanalyzed neural responses to English and Japanese (as compared to Silence) stratifying the HR infants into HR-Delay vs. HR-No delay subgroups, as determined by the cluster analysis on VDQ. No significant differences were observed at 1.5 months between these two groups. However, at 9 months, neural responses to both English and Japanese were significantly attenuated in HR-Delay (N=10) relative to HR-No Delay (N=11); see Figure 2.6, Table 2.4 and 2.5 for significant between-group differences. Notably, HR infants with normative language growth exhibited robust activity in frontal areas for both English and Japanese (and

activity in visual cortex for Japanese). Neither HR-No Delay nor HR-Delayed showed greater activity than LR infants in response to either Japanese or English; However, LR infants showed greater activity than both HR subgroups.

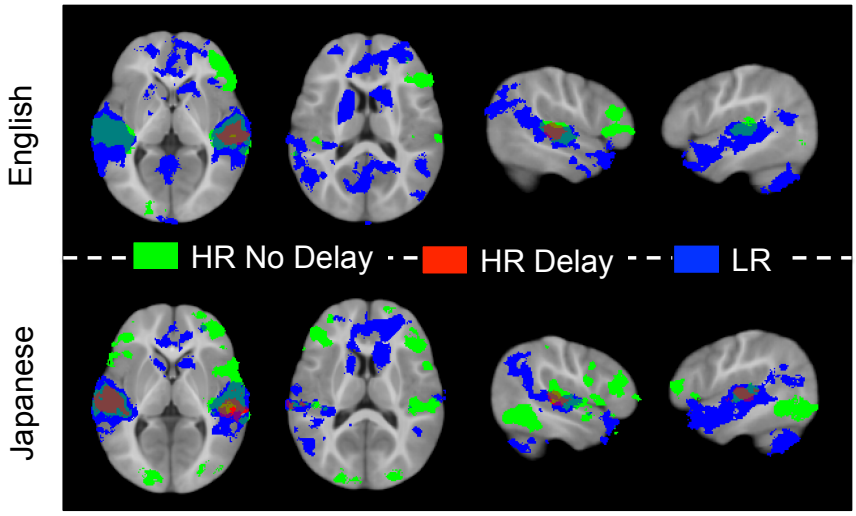

Figure 2.6: Activation maps in response to English and Japanese between HR infants with and without delayed language. LR maps are overlaid for visual comparison.

Discussion

The aims of this study were threefold. First, we aimed to characterize neural responses to native versus non-native language in HR and LR infants at 1.5 and 9-months of age. Second, we sought to examine trajectories of communicative and lexical development, and its association with ASD risk and social symptomatology. Third, we aimed to explore whether early neural responses to language predicted subsequent language profiles. We found that both HR and LR infants demonstrated significant neural activity in response to English and Japanese in canonical temporal regions associated with language processing as early as 1.5 months of age, suggesting early functional attunement of temporal and voice-selective regions for language input. Interestingly, HR infants already differed from LR controls in native versus nonnative language processing at 1.5 months of age and failed to demonstrate normative development of left lateralization for language processing during the first postnatal year. Corroborating prior work,

we found that HR infants were not only more likely to exhibit minimal gains in receptive language advantage (Hudry et al., 2014), but HR infants with language delays also exhibited attenuated neural responses to language (Lombardo et al., 2015). Altogether, these findings illustrate language profiles that are a) common among infants with familial risk for ASD and b) specific to HR infants with language delays.

Language Processing in HR Infants. Our data indicate that infants with familial risk for ASD process language differently than LR infants quite early in development. For instance, HR infants exhibited greater amygdala reactivity for English than Japanese at 1.5 months of age. Initial amygdala responses for familiar speech streams may reflect an alternative, and perhaps maladaptive, route for language learning. In typical developing infants, greater amygdala volume is associated with lower expressive and receptive skills at 3 and 4 years of age (Ortiz-mantilla, Choe, Flax, Grant, & Benasich, 2010). Although structural and functional brain measures may not always converge, amygdala hyper-reactivity early in life is associated with increased volume (Tottenham & Sheridan, 2009). Work examining amygdala growth in ASD has found mixed associations with language and communication skills. Amygdala enlargement in toddlers with ASD was found to be associated with concurrent social and communicative deficits but predictive of better subsequent joint attention skills (Mosconi et al., 2009; Wolff, Jacob, & Elison, 2017). A longitudinal study in high-risk infant siblings found that larger amygdala volume at 12 months was associated with a smaller receptive language advantage (i.e., poorer language profile) at 24 months (Swanson et al., 2017). Moreover, given greater amygdala involvement in English versus Japanese processing at 1.5 months in HR infants, but not at 9 months, suggests nonlinear amygdala functional development. It is theorized that early exposure to highly stimulating inputs may initially lead to increased reactivity and a compensatory

response in the amygdala, resulting in overgrowth and overproduction of corticotrophins and glucocorticoids, which, in excess, would lead to eventual cell death (Schumann & Amaral, 2006). Given the amygdala's involvement in stress reactivity/arousal, social processing, and learning/memory, early reactivity may suggest that HR infants may perceive speech as a particularly arousing sensory input. However, in line with this "allostatic load" hypothesis, the amygdala and related regions may become less sensitive to speech over time. This effect may account for the attenuated neural responsivity to language we – and others – have observed, in HR infants towards the end of the first postnatal year (Lombardo et al., 2015; Redcay & Courchesne, 2008).

Language Processing in HR Infants with and without Delayed Language. Our exploratory analyses revealed that, relative to LR infants, HR infants with and without delayed language had attenuated neural responses to both English and Japanese at 9 months of age. What was particularly striking was that neural activity was limited to bilateral temporal regions in HR infants with delayed language, whereas HR infants with more normative language trajectories showed significant activity in frontal and temporal-parietal regions as well. Notably, HR infants with delayed language were also most likely to be socially impaired than HR infants without delays, which corroborates prior work demonstrating that language function and social function are, to some extent, mutually informative of one another (Gotham et al., 2012; Tager-Flusberg et al., 2005). Our findings indicate that language development is a useful domain to parse the heterogeneity in ASD phenotypes and that early neural responsivity to language may be a sensitive measure to identify potential subgroups among infants with familial risk for ASD.

Although behaviorally HR infants without delayed language were similar to LR infants in terms of their language growth, their neural signatures for native and nonnative language

processing indicate that they are nevertheless processing language differently than LR infants. For instance, we observed activity in visual cortex for Japanese in 9-month old HR infants without delayed language. Upregulation of visual cortex during simple auditory processing has been observed in children and adolescents with ASD, and atypical crossmodal modulation of visual-auditory inputs has been associated with symptom severity (Keehn et al., 2018). While we do not yet know the developmental outcomes for these HR infants, as a group they are more likely to exhibit social cognitive and attentional difficulties (Miller, Iosif, et al., 2015; Miller, Young, et al., 2015; Tsang, Gillespie-Lynch, & Hutman, 2016) which may stem from early, suboptimal routes for language processing.

Widening Gap in Language Abilities between HR and LR Infants. Our data suggest a widening gap in language processing abilities between infants with and without familial risk for ASD over the course of the first year; differences in neural responses to English and Japanese were more extensive at 9 months of age than at 1.5 months between LR and HR infants, both with and without language delays. Similarly, we observed that the receptive-advantage profiles of LR and HR infants increasingly diverged with age. This may suggest that early differences in language processing may be subtle at first and cumulatively cascade into overt delays in language development and difficulties in social communication.

At the behavioral level, HR infants displayed minimal gains in receptive language advantage from 9- to 18-months relative to LR infants. Prior work that similarly found this effect also noted graded differences in receptive versus expressive vocabulary by HR outcome, such that HR infants with typical developmental showed the largest receptive advantage followed by HR infants with developmental delays, and HR infants with ASD (Hudry et al., 2014). Given that word comprehension is necessary for functional language production, minimal gains in

receptive vocabulary relative to concurrent gains in expressive vocabulary is a limiting factor for subsequent language development. Nearly half of children with familial risk for ASD exhibit language difficulties during school-aged years, and language skills at that age are closely associated with nonverbal cognitive scores, adaptive functioning, and theory-of-mind abilities, illustrating the relevance of language for functioning across social and cognitive domains (Tsang et al., 2016). Thus, early deviations in lexical profiles falter the foundational skills necessary to use language for social communicative functions.

At the neural level, HR infants displayed attenuated neural response to both native and non-native language in subcortical regions associated with learning and reward processing. Moreover, our exploratory analyses revealed a lack of left temporal specialization for language during the first postnatal year in HR infants. These findings are in line with previous studies using EEG and fMRI to examine speech processing in HR infants (e.g., Seery et al., 2012; Eyler et al., 2012; Lombardo et al., 2015). Prior work in toddlers with ASD has suggested that hypoactivation in left language processing regions and compensatory recruitment of homologous right temporal regions not only reflects an inefficient route for language processing but may also impede normative functional development of right temporal regions for social processes (Eyler, Pierce, & Courchesne, 2012). Although we did not observe compensatory right hemispheric activity in response to English or Japanese, our data suggest that language may not necessarily elicit the same level of social reward in HR infants as it does in LR infants, given the attenuated response to language in learning and reward processing regions (e.g., the caudate, medial prefrontal regions, and orbitofrontal cortex).

It has been suggested that difficulties in social communication may constitute an endophenotype of ASD because it is commonly observed in infants and toddlers with genetic

liability for ASD, and in first degree relatives of individuals with ASD (Hudry et al., 2014; Miller, Young, et al., 2015; Sasson et al., 2013). Common variants in contactin-associated protein 2 (*CNTNAP2),* one of the most replicated genes associated with ASD, has been linked to language delays in children with ASD as well as neurotypical individuals (Alarcón et al., 2008; Peñagarikano & Geschwind, 2012; Whitehouse, Bishop, Ang, Pennell, & Fisher, 2011). *CNTNAP2* is a gene involved in early brain development and synaptic transmission; it is primarily expressed in brain regions integral for higher-order cognition and language learning (i.e., frontal, perisylvian, and temporal areas; Rodenas-Cuadrado, Ho, & Vernes, 2014). Imaging-genetic studies have found that carriers of *CNTNAP2* risk alleles have reduced gray and white matter volume in frontal cortices (Tan, Doke, Ashburner, Wood, & Frackowiak, 2010), as well as alterations in both structural (Dennis et al., 2011) and functional connectivity in networks involved in social cognition and language (Scott-Van Zeeland et al., 2010), independent of an ASD diagnosis. Although the mechanisms by which familial risk for ASD confers vulnerabilities for language development are heterogeneous, variation in *CNTNAP2* may be one possible source that accounts for some of common features (e.g., slow receptive versus expressive language growth and attenuated neural response to language) observed in HR samples.

Normative Language Development. Our findings also inform our understanding of the neural underpinnings of normative language development. Whereas prior research on early language processing has used either native *or* nonnative speech in contrast to non-speech sounds (e.g., Shultz et al., 2014; Perani et al., 2011; Dehaene-Lambertz et al., 2010), to our knowledge this is the first study that directly examined the neural correlates of native versus nonnative speech processing throughout the first postnatal year. We find evidence that neural

differentiation for native versus nonnative language is already present by 1.5 months. In LR infants, greater responses in left superior temporal gyrus and supramarginal gyrus for English relative to Japanese may reflect learning that has already occurred for their native language due to prenatal exposure. Relative to English, LR infants also had greater responses to Japanese in regions associated with attention and processing prosodic cues (e.g., left middle temporal gyrus and right IPL).

Greater neural responses for Japanese versus English is of note because it indicates that shortly after birth LR infants not only perceive different languages as distinct from one another but also process them as a relevant speech input. While prior work has interpreted attention to nonnative speech sounds as evidence for universal speech perception (Kuhl, 2004), neural discrimination between novel (i.e., nonnative) versus familiar (i.e., native) language may also reflect early auditory social attention and a general aptitude for language learning. Related research on speech perception has found that the developmental window for differentiating between familiar vs. novel speech sounds is protracted in bilingual infants relative to monolingual infants, which, presumably, indicates prolonged sensitivity to speech to learn relevant features about multiple languages (Weikum et al., 2007). Taken together, the neural differentiation we observed for native and nonnative languages may be a marker of plasticity for language learning.

By 9 months of age, we found that language processing had not only become increasingly left-lateralized, but that it also elicited a much more widespread neural activity than at 1.5 months. In addition to temporal language areas, both native and nonnative language activated neural regions associated with reward processing, social orienting, as well as learning and memory. This pattern of activity in response to language supports the hypothesis that language

learning is "gated" by the social brain (Kuhl, 2007). LR infants only exhibited greater neural response for English relative to Japanese, but not the converse, suggestive that some perceptual narrowing may have occurred by 9 months of age for the language infants are primarily exposed to.

Notably, neural responses to the infant's native language (i.e., English) were associated with individual differences in verbal and lexical development in regions functionally associated with language processing. Overall verbal communicative development – as indexed by change in VDQ from 6- to 18-months of age – was associated with greater activity in right temporal and inferior frontal regions at both 1.5 and 9 months of age, demonstrating developmental continuity in the neural underpinnings of language acquisition. Lexical vocabulary growth – as indexed by change in receptive advantage scores from 9- to 18-months of age – was associated with greater activity in voice-selective regions at 1.5 months (e.g., superior portions of the right MTG and STG) and with regions associated with word comprehension (e.g., IPL) at 9 months. Moreover, the functions of these regions align with language processing functions observed in adults, which indicates early tuning of these regions to language processing.

Conclusions. Despite our relatively small sample size, this study provides preliminary evidence that attunement to native versus nonnative language occurs early in postnatal life and that language processing increasingly diverges between HR and LR infants. Our data begin to address the role of language exposure and language acquisition on developmental changes in native versus nonnative language processing during the first postnatal year. These findings corroborate related work in language processing in ASD; however, replication with a larger sample is necessary to verify the validity of our observed effects.

What accounts for the greater attunement to native language observed in LR infants relative to HR infants by 1.5 months? One possibility is that the capacity to parse relevant from irrelevant social inputs is affected in infants at high risk for ASD. Taken together, our findings suggest that early differences in language processing among HR infants become increasingly divergent from normative patterns during the first postnatal year. Accordingly, language processing is a critical domain that can stratify developmental profiles in HR infants, thus affording an early means to parse the considerable heterogeneity observed in HR outcomes.

Table 2.2: Coordinates of Peak Activation in Response to Language Stimuli at 1.5 and 9 Months

	High Risk				Low Risk			
	MNI Peak mm				MNI Peak mm			
Region	x	y	z	Max Z	x	y	z	Max Z
	1.5 month response to English > Silence							
Left Superior Temporal Gyrus	-32	-15	5	7.94	-26	-22	8	5.14
Right Superior Temporal Gyrus	36	-13	5	5.1	33	-15	7	4.46
Right Heschl's Gyrus	25	-20	8	4.85				
	1.5 month response to Japanese > Silence							
Left Superior Temporal Gyrus	-32	-15	5	7.89	-37	-11	6	4.2
Right Superior Temporal Gyrus	34	-14	4	4.89	31	-17	5	4.39
	1.5 month response to English > Japanese							
Left Amygdala	-32	10	15	2.55				
Right Amygdala	19	-7	-6	3.32				
Left Superior Temporal Gyrus					-45	-23	13	3.15
Left Inferior Parietal Lobule					-41	-27	29	2.85
	1.5 month response to Japanese > English							
Left Middle Temporal Gyrus					-40	-5	-8	2.62
Right Inferior Temporal Lobule					26	-37	30	3.26
	9 month response to English > Silence							
Left Superior Temporal Gyrus	-45	-8	-2	4.48	-55	-5	1	9.87
Right Superior Temporal Gyrus	51	-16	3	8.14	54	2	-7	6.43
Left Middle Temporal Gyrus	-59	-9	-2	5.75	55	-12	-7	4.7
Anterior Cingulate Gyrus					-1	35	7	4.05
Right Orbitogrontal cortex (middle)					22	46	-9	4.22
Left Putamen					-17	0	14	4.19
Left Caudate					-14	5	12	3.36
	9 month response to Japanese > Silence							
Left Superior Temporal Gyrus	-47	-5	-2	6.44	-53	-17	3	9.48
Left Middle Temporal Gyrus	43	-16	0	6.55	54	3	-5	6.41
Right Superior Temporal Gyrus	52	-17	3	8.24	52	-10	3	6.45
Right Middle Temporal Gyrus	-52	-3	1	5.94				
Cerebellum					27	-59	-38	5.38
Right Caudate					11	10	13	4.61
Left Medial Superior Frontal Gyrus					-1	30	47	4.24
Left Supplementary Motor Area					-2	23	52	3.92
Right Medial Superior Frontal Gyrus					9	49	18	4.17
Right Middle Frontal Gyrus					22	41	8	4.05
Right Caudate					8	10	15	4.21
	9 month response English > Japanese							
Cuneus					6	-54	14	3.9
Left Orbitofrontal Cortex					-10	-47	1	3.01
Right Hippocampus					24	-12	-12	2.91
Left Hippocampus					-18	-10	-14	2.3

Table 2.3: Coordinates of Peak Activation of Between-Group Differences at 9 Months

	English: Low Risk> High Risk				Japanese: Low Risk> High Risk			
	MNI Peak mm				MNI Peak mm			
Region	x	y	z	Max Z	x	y	z	Max Z
Left Precuneus	-2	-51	12	4.94				
Left Middle Temporal Gyrus	-53	-3	-1	3.98				
Right Superior Frontal Gyrus (medial)	13	39	5	3.99				
Right Orbitofrontal Gyrus (medial)	2	45	-1	3.73	-53	4	-7	4.23
Right Cerebellum	-39	-45	-40	3.94	-25	-61	-39	4.57
Anterior Cingulate					8	27	-1	3.74
Right Rectal Gyrus					4	44	-12	3.7

Table 2.4: Coordinates of Peak Activation in Response to Language Stimuli at 9 Months in High-Risk Infants with and without Delayed Language

	High Risk-Delay				High Risk-No Delay			
	MNI Peak mm				MNI Peak mm			
Region	x	y	z	Max Z	x	y	z	Max Z
	9 month response to English > Silence							
Left Middle Temporal Gyrus	-57	-23	3	3.48				
Left Superior Temporal Gyrus	-46	-13	8	4.46	-38	-25	10	5.13
Right Middle Temporal Gyrus	52	-23	1	3.84	45	-16	4	7.34
Right Superior Temporal Gyrus	45	-19	4	5.34	52	-10	3	5.76
	9 month response to Japanese > Silence							
Left Superior Temporal Gyrus	-43	-14	7	4.87	-51	-20	4	5.49
Right Superior Temporal Gyrus	46	-22	-2	5	46	-12	3	5.88

Table 2.5: Coordinates of Peak Activation of High-Risk Between-Group Differences at 9 Months

	English				Japanese			
	MNI Peak mm				MNI Peak mm			
Region	x	y	z	Max Z	x	y	z	Max Z
	HR-No Delay > Delay				HR-No Delay > Delay			
Left Calcarine Cortex	-1	-68	-17	3.39				
Right Cuneus	13	-82	-20	3.35				
Right Inferior Frontal Gyrus (Triangularus)	40	24	9	3.88				
Right Inferior Temporal Gyrus	38	-37	-22	3.78	44	-46	-19	3.92
Right Precuneus					10	-36	-1	3.58
Right Heschl's Gyrus					34	-16	5	3.96
Right Middle Occipital Gyrus					30	-68	20	4
Left Middle Occipital Gyrus					-41	-63	-4	4.29
	LR > HR-No Delay				LR>HR-No Delay			
Left Temporal Pole (Middle)	-37	11	-24	4.13				
Left Superior Temporal Gyrus	-61	-20	8	3.61				
Left Middle Temporal Gyrus					-51	2	-6	5.26
Right Rectus Gyrus					3	24	-19	4.58
Cerebellum					-11	-70	-35	3.5
	LR > HR-Delay				LR>HR-Delay			
Right Lingual Gyrus	4	-32	0	4.49				
Right Superior Frontal Gyrus	11	41	8	4.25				
Precuneus	17	-44	20	4.13				
Cerebellum	-9	-86	-23	3.7	-43	-52	-30	4.23
Posterior Cingulate Cortex					-1	-30	8	4.08
Left Rectus Gyrus					1	45	-11	4.76
Anterior Cingulate Gyrus					12	38	5	4.04
Left Middle Temporal Gyrus					-57	-5	-5	4.26

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Autism Risk is Associated with Altered Salience Network Connectivity in 6-Week-Old Infants

Disrupted brain connectivity has been implicated in autism spectrum disorder (ASD). Here we examined the Salience Network (SN)--an early-emerging neural network involved in orienting attention to the most salient information in one's environment—as an underlying brain-based mechanism associated with behavioral manifestations of ASD risk. Six-week-old infants at high-risk for ASD exhibited stronger SN connectivity with sensorimotor regions; low-risk infants exhibited stronger SN connectivity with prefrontal regions involved in social attention. Infants with *higher* connectivity with sensorimotor regions had *lower* connectivity with prefrontal regions, suggesting a tradeoff for attention to basic sensory versus socially-relevant information. Higher SN connectivity with prefrontal regions predicted better social attention in low-risk infants. Early alterations in SN connectivity predicted subsequent ASD symptomatology. Contextualizing Salience Network connectivity with longitudinal eye-tracking and behavioral measures of social and sensory development provide a plausible mechanistic account for the unfolding of atypical developmental trajectories associated with ASD risk.

Shortly after birth, newborns display systematic preferences for faces (Morton & Johnson, 1991)(Morton & Johnson, 1991), voices (DeCasper & Fifer, 1980), and biological motion (Simion, Regolin, & Bulf, 2008). These early social orienting mechanisms are foundational for normative social communicative development (Grossmann & Johnson, 2007). However, the perceived salience of socially relevant information appears disrupted in autism spectrum disorders (ASD). Infants who develop ASD show altered developmental trajectories (Jones & Klin 2013) characterized by reduced attention to social information (Chawarska et al., 2013; Shic et al., 2014) and heightened awareness of non-social sensory inputs (Thye, Bednarz, Herringshaw, Sartin, & Kana, 2017). Additional behavioral markers of ASD become apparent after the first postnatal year, including reduced social communicative skills (e.g., gesturing and initiation of joint attention) and atypical visual exploration of objects (E. J. H. Jones et al., 2014). Atypicalities in social versus nonsocial attention are broadly recognized as a marker of ASD risk (Tager-Flusberg, 2010) and likely contribute to the emergence of social impairments and the restrictive and repetitive behaviors diagnostic of ASD. However, the neurobiological mechanisms underlying the early-emerging attentional abnormalities associated subsequent autism symptomatology remains unknown.

Examining early brain connectivity offers a promising lens for investigation (Hernandez et al., 2015). Indeed, most ASD risk genes impact synapse formation and function, presenting a biological pathway for ASD that converges on brain connectivity. Neuroimaging studies have consistently implicated atypical brain network dynamics in ASD (Hernandez et al., 2015). Moreover, recent work has found global differences in the structural and functional brain organization of 6-month-old infants who later develop ASD (Emerson et al., 2017; Hazlett et al., 2017; Shen & Piven, 2014; Wolff, Swanson, et al., 2017), lending support for the hypothesis that

ASD stems from early disruptions in the development of brain connectivity (Geschwind & Levitt, 2007). Extending this framework, these early deviations in brain network connectivity may provide a biomarker of ASD risk prior to the emergence of behavioral symptoms associated with the disorder such as attenuated attention to faces, subtle difficulties in social referencing, and atypicalities in sensory and motor behaviors (Zwaigenbaum et al., 2005).

One early emerging functional brain network of particular interest is the Salience Network (SN). The SN is an intrinsic resting state network believed to be integral in guiding attention to the most salient interoceptive and exteroceptive stimuli (Uddin 2014). Altered SN connectivity can discriminate children with ASD from neurotypical controls with high classification accuracy (L. Uddin & Supekar, 2013), and is associated with restrictive/repetitive behaviors (L. Uddin & Supekar, 2013) as well as atypical sensory processing (S. A. Green et al., 2016). Moreover, early symptom-based markers of ASD, such as deviations in processes that support social-communicative development (e.g., attention to faces)(Jones & Klin 2013), implicate potential disruptions in SN connectivity. As faces represent a highly salient class of stimuli for infants (Valenza, E., Simion, F., Cassia, V. M., & Umiltà, 1996), early SN connectivity may underlie the normative development of attention to faces (M. C. Frank et al., 2009) and the ostensive social communicative cues they afford (Mundy & Newell, 2007). Conversely, early disruptions in SN connectivity may iteratively derail processes that typically reinforce the perceived salience of faces by conferring heightened salience to non-social stimuli, ultimately contributing to the emergence of the social communicative impairments and altered sensitivity to sensory stimuli characteristic of ASD (J Piven, Elison, & Zylka, 2017).

To test this model, here we evaluate SN connectivity in 6-week-old infants at high (HR) and low (LR) familial risk for ASD and its association with early ASD symptom-based markers,

including atypicalities in visual social attention, social communicative development, and sensory processing. The present study combined resting-state fMRI with longitudinal eye-tracking and behavioral assessments of social communicative development and sensory processing atypicalities. This multimodal approach provides a direct and comprehensive assessment of the relationships between early functional brain connectivity and its downstream behavioral consequences on development. Between 4-8 weeks, early social-orienting behaviors transition from being under reflexive subcortical control to experience-dependent cortical control (M. Johnson, 1990). Thus, examining SN connectivity during this purported transitional period may reveal altered development of social attention and begin to provide a mechanistic account for the ontogeny of ASD symptomatology.

To examine visual social attention, infants' eye-movements were recorded as they were presented video excerpts from *Charlie Brown* and *Sesame Street* from 3- to 12-months of age (Frank et al. 2014; Frank et al. 2009). Social communicative development was evaluated at 12 months with the Early Social Communicative Scales (Mundy et al., 2003), which quantifies joint attention skills, an early social cognitive skill that is believed to be foundational for social cognitive and competence (Mundy & Jarrold, 2010). Moreover, a proposed model of the development of joint attention integrates neural systems involved in attention processing, including regions within the SN (e.g., anterior cingulate cortex and prefrontal association areas) (Mundy & Jarrold 2010). Social communicative impairments, as well as early markers of sensory atypicalities, were indexed by the Autism Observation Scale for Infants at 12 months(Bryson et al., 2008). Profiles of sensory sensitivity during the first postnatal year were informed by the Infant/Toddler Sensory Profile (Dunn & Daniels, 2000). Altogether these measures provide a rich sampling of behaviors that are affected in infants who later develop

ASD. We hypothesized that HR infants would show SN hyperconnectivity with sensorimotor regions (S. A. Green et al., 2016; L. Q. Uddin et al., 2013) and that distinct patterns of SN connectivity would predict individual trajectories of social attention, communicative development, and sensory sensitivities—early symptom-based markers of ASD risk.

Methods

Participants. Participants in this study were enrolled as part of a longitudinal project examining early brain-based markers of ASD during the first year. The Institutional Review Board (IRB) at the University of California, Los Angeles, approved all protocols associated with the project, and all enrolled participants had informed consent provided by their parent/legal guardian. Infants were assigned to risk-based cohorts based on family history: high-risk infants (HR) had at least one older sibling with a clinical ASD diagnosis whereas low-risk infants (LR) had no family history of ASD or any other developmental disorder. Prior research showed that the recurrence risk for developing ASD is approximately 20% in HR infants (Sally Ozonoff et al., 2011). Exclusionary criteria for both groups included: 1) indication of genetic or neurological conditions associated with ASD risk (e.g., fragile X syndrome, epilepsy, tuberous sclerosis), 2) significant perinatal insult or chronic medical conditions impacting development, 3) severe visual, hearing, or motor impairment, 4) non-English speaking parents, and 5) contraindication for MRI (e.g., metal implants). All participants were enrolled in the study prior to 6 weeks of age. HR and LR infants were matched by gender (Mann-Whitney U = 306, p=0.38), and birth weight (t(51)=0.43, p=0.67), as well as ethnicity and family socio-economic status (race: Mann-Whitney U=341.5, p=0.88; household income: Mann-Whitney U=282.5, p = 0.23). HR and LR infants did not differ on cognitive development at 12 months according to the Mullen Scales of Early Learning—Early Learning Composite (HR_{ELC} Mean=106.67, SD=17.24;

LR_{ELC} Mean=110.22, SD=11.83; $t(49)=0.87$, $p = 0.39$ (Table 3.1).

Table 3.1: Participant Demographics

	Low-Risk N=29		High-Risk N=24		
	N	%	N	%	P
Sex					0.38
Female	11	37.93	17	58.62	
Male	18	62.07	12	41.38	
Race					0.88
White	20	68.97	17	58.62	
Non-white	9	31.03	12	41.38	
Family Income					0.23
Not Answered	1	3.45	0	0.00	
<$50K	3	10.34	6	25.00	
$50-$75K	4	13.79	4	16.67	
$75-$100K	5	17.24	5	20.83	
$100-$125K	4	13.79	2	8.33	
>$125K	12	41.38	7	29.17	
	Mean	(SD)	Mean	(SD)	P
Birth Weight					
Pounds	7.65	1.67	7.41	2.43	0.67
Age at Scan					
Weeks	6.63	1.37	6.65	1.09	0.95
Relative Motion					
mm	0.23	0.82	0.10	0.07	0.44
Behavioral Scores at 12 months					
Mullen ELC	110.22	11.83	106.67	17.24	0.39
ESCS IJA	0.71	0.45	0.96	0.48	0.06
ESCS RJA	0.31	0.23	0.28	0.24	0.56
AOSI Total Score	4.12	1.83	4.71	3.07	0.97
Sensory Sensitivity	19.52	4.39	20.7	7.55	0.52

Infants at high- and low-risk for ASD are matched on demographic characteristics and developmental level at 12 months did not differ by risk status. ELC, Early Learning Composite; ESCS IJA, Early Social Communicative Scales Initiation Joint Attention; ESCS RJA, Early Social Communicative Scales Response to Joint Attention; AOSI, Autism Observation Scale for Infants

A total of 53 infants (N=24 HR, N=29 LR) underwent fMRI during natural sleep at approximately 6 weeks of age (HR_{age} Mean=6.63 weeks, SD=1.37 weeks; LR_{age} Mean=6.65

weeks, SD=1.09 weeks, $t(51)=0.062$, $p=0.95$). A subset of 51 infants (N=24 HR, N=27 LR) provided data from behavioral measures of social and cognitive development at 12 months (2 LR infants dropped out of the study). Of the 53 infants, 50 infants (N=24 HR; 26 LR) provided longitudinal eye-tracking data at 3-, 6-, 9-, and 12-months of age. An additional 3 infants participated in the study but were not included in the analyses due to excessive head motion during scanning and/or scanner artifacts.

Behavioral Measures. Infants were administered a battery of behavioral assessments during the first postnatal year to measure socio-communicative, cognitive, and sensory development (see Table 1.1). At 12 months, infants' developmental level was assessed on with the Mullen Scales of Early Learning (Mullen, 1995), nonverbal social communicative behaviors (i.e., rates of initiating of and responding to joint attention cues—IJA and RJA respectively) with the Early Social Communication Scales (ESCS; Mundy et al. 2003), and early signs of ASD symptomatology with the Autism Observation Scale for Infants (AOSI; Bryson et al. 2008). Approximately 20% of the total ESCS sample was double coded for reliability. Coders were trained undergraduate research assistants who were blind to ASD family risk status and other study variables. Intraclass correlations (ICC; absolute agreement, single measures) indicated good reliability for IJA (ICC = .96) and RJA (ICC = .89). Parents also completed the Infant/Toddler Sensory Profile (Dunn & Daniels, 2000), a standardized questionnaire tracking their child's sensitivity to sensory inputs and sensory-related difficulties, at 6, 9, and 12 months; the average raw score on the Sensory Sensitivity quadrant across time points was used as a general metric of sensitivity to visual, auditory, and tactile stimuli.

Eye-tracking Protocol. Infants were eye-tracked at the 3-, 6-, 9- and 12-month visits while they were presented with two, 2-mintue full audiovisual video segments taken from a

cartoon and live-action video; these video stimuli have been previously used in studies on visual social attention to faces in typically-developing infants.(M. C. Frank et al., 2014a, 2009) Infants sat on their caregiver's lap during the eye-tracking procedure at approximately 60 cm from the 65-cm video display monitor. Caregivers were explicitly instructed not to distract their infant's attention from the screen during stimuli presentation.

Point-of-gaze (POG) data were collected using a Tobii T60XL eye-tracker at 60 Hz with a spatial accuracy of approximately 0.5^{o} accuracy. Eye-movements (e.g., fixations, blinks, and saccades) were detected using the accompanying Tobii software. Infants' POG were calibrated using a 5-point calibration scheme prior to data collection. The calibration scheme was repeated until an infant's POG was within 1^{o} of the center of the target and repeated between the two trials. The video stimuli were presented only after the calibration criterion had been reached. Individual trials were removed from analyses in case of failure to initially calibrate the infant's eyes to the eye-tracking system (N_{trials}=9) or failure to track an infant's eyes from excessive movement or fussiness (N_{trials} =31).

Video frames were 8-bit color images and 720 by 480 pixels in resolution. Each frame was hand-traced for areas of interest (AOIs), which were demarcated as a box encompassing each character's face as in the prior studies of typical development using the same stimuli.(M. C. Frank et al., 2014a, 2009) Fixations that fell within AOIs were identified using software written in MATLAB (MathWorks, Inc; Natick, MA). The primary dependent measure was percent of fixations directed towards faces.

MRI Data Acquisition. MRI data were acquired on a 3T Siemens Tim Trio scanner using a 12 channel head coil during natural sleep. Parents were instructed to put their infant to sleep using their normal bedtime routine. After the infant was asleep and swaddled, silicon

earplugs were placed over the infant's ear canal, and mini earmuffs were fitted over the entire outer ears. Infants were then placed on a custom-made bed that fit inside the scanner's head coil and secured on the scanner bed with a Velcro strap. To minimize movement, a weighted blanket was used and foam pads were positioned around each infant's head.

A localizer scan was used for graphic prescription. Structural matched bandwidth T2-weighted high-resolution echo planar images are acquired co-planar to the functional scans to ensure identical distortion characteristics to the fMRI scans (TR=5000ms, TE=34ms, matrix size 128x128, FOV=192mm, 34 slices, 1.5mm in-plane resolution, with 4mm-thick axial slices). Resting-state data were collected during an 8-min rs-fMRI scan (TR=2000ms, TE=28ms, matrix size 64x64, FOV=192mm, 34 slices, 3mm in-plane resolution, with 4mm-thick axial slices).

fMRI Data Preprocessing. Functional imaging data were preprocessed and analyzed using FSL version 5.0.8 (fMRIB's Software Library, Jenkinson et al. 2012) Functional images were co-registered to the subject's corresponding T2-weighted high-resolution anatomical scan, registered to an infant brain template (Shi et al., 2011) using 12-parameter affine transformations, and spatially smoothed (Gaussian kernel of 6mm FWHM) to increase signal-to-noise ratio. ICA-AROMA was used to detect and remove motion artifacts from the data (Pruim, Mennes, van Rooij, et al., 2015). This is a validated procedure that uses probabilistic independent component analysis to automatically detect participant-specific motion-related independent components while preserving signal of interest (Pruim, Mennes, Buitelaar, & Beckmann, 2015). HR and LR infants did not differ in the average number of noise components identified by ICA-AROMA [HR: Mean=27.25, SD=9.43; LR: Mean=27.72, SD=11.37; $t(51)$=0.16, p=0.87]; the number of noise components detected were comparable to that 23.2 components reported by Pruim and colleagues (2015). HR and LR infants also did not differ on

average relative motion prior to the denoising with ICA-AROMA (HR: Mean=0.10mm, SD=0.07mm; LR: Mean=0.23 mm, SD=0.82mm; $t(51)$=0.81, p=0.42). Data were then band-pass filtered (0.01Hz—0.1Hz) and mean cerebrospinal fluid, white matter, and global time series were regressed from the data.

Eye-tracking Statistical Analysis. Our primary interest from modeling the eye-tracking data was to estimate each infant's rate of change in attention to faces from 3 to 12 months, such that individual developmental trajectories in social attention could be analyzed as a function of 6-week SN connectivity (see Figure 3.3). Longitudinal changes in percent fixation to faces were analyzed with a Bayesian hierarchical linear model in R (rstanarm package), which uses a Markov chain Monte Carlo simulation to draw a posterior distribution (e.g., a range of probable values for a variable given the data). This aspect of the Bayesian framework allows for greater precision in estimating parameters than by the frequentist approach (J K Kruschke & Vanpaemel, 2015; John K. Kruschke & Liddell, 2017).

Attention to faces was operationalized as percent fixation to characters' faces in each video stimulus. Developmental trajectories were modeled as the linear and quadratic effects of age (i.e., age and age^2, respectively). Risk status (HR versus LR) and video stimulus type (*Charlie Brown* versus *Sesame Street*) were modeled as fixed effects; age and intercept were modeled as random effects to account for individual differences and correlated repeated measures at 3, 6, 9, and 12 months. The inclusion of the change of slope (i.e., the quadratic term age^2) aimed to capture the change in growth rate from 3 to 12 months. Student-t distributions were used as priors for the regression coefficients and standard deviation, and a Gaussian function was used as the identity link function.

The model in equation form is:

$$\text{Level 1: } Fixation_{ij} = intercept_{0j} + B_{1j}(Age)_{ij} + B_{2j}(Age)^2_{ij} + error_{ij}$$

$$\text{Level 2: } Intercept_{0j} = \Upsilon_{00} + \Upsilon_{01}(\text{Risk Status}) + \Upsilon_{02}(\text{Stimulus Type}) + error_{0j}$$

$$\text{Level 2: } B_{1j} = \Upsilon_{10} + \Upsilon_{11}(\text{Risk Status}) + \Upsilon_{12}(\text{Stimulus Type}) + error_{1j}$$

$$\text{Level 2: } B_{2j} = \Upsilon_{20} + \Upsilon_{21}(\text{Risk Status}) + \Upsilon_{22}(\text{Stim Type}) + error_{2j}$$

Pareto k diagnostic values indicated a good model fit (all pareto k estimates were less that 0.7). Diagnostics regarding model fit and visualization of posterior distributions were verified with the shinystan package in R. As expected, there was a significant linear effect of age such that attention to faces increased with age across all participants (95% CI: 1.73—9.39). The rate of change overtime decreased across all infants indicating a quadratic trajectory in face-looking (95% CI: -0.47 — -0.01). There was also a main effect of risk status; HR infants overall attended less to faces than LR infants (95% CI: -40.91— -3.66).

Using the coef function in R, we extracted each infant's estimate of developmental increase in face-looking from 3 to 12 months (i.e., each infant's beta coefficient for the age term in the model). These estimates were then used as a covariate of interest in a model of SN connectivity.

fMRI Data Analysis. Resting-state fMRI analyses were conducted with FSL fMRI Expert Analysis Tool (FEAT, version 6.0 www.fmrib.ox.ac.uk/fsl/(Woolrich et al., 2009)). Whole brain connectivity within the SN was examined using a right anterior insula (rAI) seed,(Seeley et al., 2007) which was defined as voxels anterior to the midline of the right insula from a neonatal brain template.(Shi et al., 2011) Region-of-interest (ROI) time-series from each infant's processed residuals in standard space were extracted and correlated with every other voxel in the brain to generate SN functional connectivity maps. Individual SN maps were converted into z-statistic maps using Fischer's r-to-Z transformation. At the group level, we

modeled a 2-sample mixed-effects design, cluster corrected for multiple comparisons with Gaussian random field theory at $Z>2.3$, $p<0.01$ using the FSL FLAME (FMRIB's Local Analysis of Mixed Effects State) Stage 1+2. Family income, gestational age, and birth order were examined as potential covariates; as none contributed significantly they were excluded from the final analysis. Subsequent between-group comparisons and correlation analyses with estimates of increases in face-looking from 3 to 12 months, AOSI, ESCS, and Infant/Toddler Sensory Profile were restricted to (i.e., masked by) the joint [HR + LR] SN connectivity maps. While these maps were more liberally thresholded at $Z>1.7$, cluster-corrected for multiple comparisons ($p<0.05$) using Gaussian random field theory, we regarded as significant and report only clusters that exceeded $Z>2.3$ ($p<0.01$) (see Table 3.2). Parameter estimates of connectivity strength for between-group comparisons were extracted from significant clusters using FMRIB and evaluated using Pearson correlation.

Results

Behavioral Characterization of Development. We examined core autism symptomatology with the Autism Observation Scale for Infants (AOSI) and nonverbal social-communication with the Early Social Communication Scale (ESCS) at 12 months (see Table 3.1), as well as sensory sensitivity with the Infant/Toddler Sensory Profile (ITSP) from 6- to 12-months. The Mullen Scales of Early Learning (MSEL), a play-based standardized measure of developmental level, was also measured at 12 months. In line with prior research (e.g., Tager-Flusberg 2010), at 12 months HR and LR infants were comparable across all measures of development. Cognitive developmental level did not differ by risk status (Mullen ELC: $t_{49}= 0.87$, $p = 0.39$), nor did total number of risk markers of the AOSI ($t_{46}=0.85$, $p=0.40$), parental report of

sensory sensitivities (t_{42}=0.65, p = 0.52), and joint attention skills (IJA: t_{49}=1.90, p=0.06; RJA: t_{49}=1.04, p = 0.30).

Six-Week Salience Network Maps. We used a right anterior insula (rAI) seed derived from a neonatal brain template to characterize SN connectivity across the whole brain.(S. A. Green et al., 2016; Seeley et al., 2007) Both HR and LR infants showed evidence of a canonical SN (Figure 3.1). We then examined between-group differences in SN connectivity (Figure 3.2). Compared to LR infants, HR infants showed *greater* connectivity between the hub of the SN (i.e., the rAI) and sensorimotor regions, including bilateral precentral gyrus and left postcentral gyrus, thalamus, hippocampus, caudate and putamen. In contrast, compared to LR infants, HR infants showed *less* connectivity between the rAI and prefrontal regions associated with social and language processing and attentional control, including right orbito-frontal cortex (OFC), inferior and middle frontal gyrus (IFG, MFG), and anterior cingulate. Importantly, we found an inverse relationship in SN connectivity with sensorimotor versus prefrontal regions (r=*-0.37, p* = 0.007) –infants with the strongest SN connectivity with sensorimotor regions showed the weakest connectivity with higher-order prefrontal regions. This direct tradeoff between neural resources allocated towards sensorimotor processing versus social attention could thus explain the co-emergence of both core sensory and social ASD symptoms.

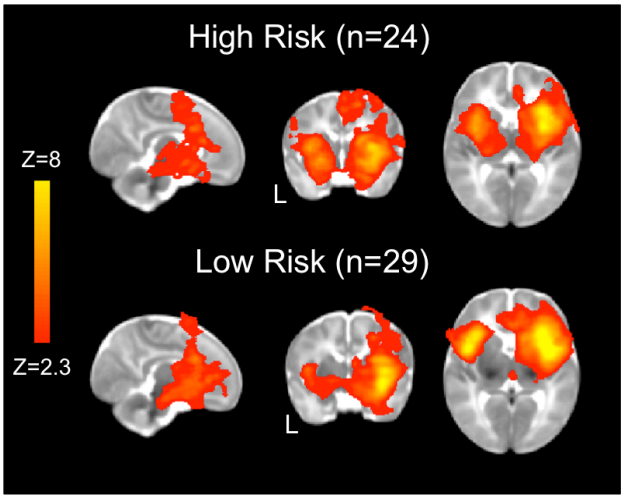

Figure 3.1: The Salience Network was detected in both HR and LR infants using the right anterior insula (rAI) as the seed.

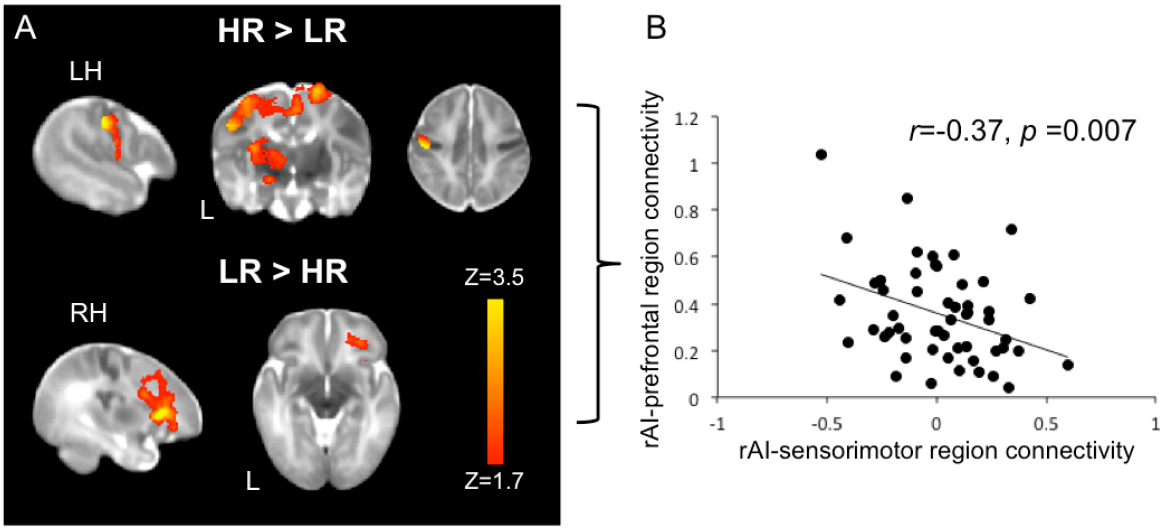

Figure 3.2: A) Relative to LR infants, HR infants showed greater connectivity between the rAI and left pre- and post-central gyri, thalamus, and caudate – regions associated with sensorimotor processing. In contrast, relative to HR infants, LR infants showed greater connectivity between the right anterior insula and right orbitalfrontal cortex and inferior frontal gyrus—frontal regions associated with social processing. B) Indices of functional connectivity were extracted from these between-group SN connectivity maps (as shown in A) and correlated with each other. This analysis revealed an inverse relationship such that, across all infants, stronger rAI connectivity with sensorimotor regions was associated with weaker rAI connectivity with prefrontal regions.

Six-Week SN Connectivity Predicts Developmental Increases in Attention to Faces.

To directly examine the downstream effects of brain connectivity on visual social attention,

which has been shown to be altered in HR infants (Chawarska et al. 2013; Shic et al. 2014; Jones & Klin 2013), we evaluated whether early SN connectivity predicted individual trajectories in attention to faces during the first postnatal year. Infants were eye-tracked while they watched video excerpts from *Charlie Brown* and *Sesame Street* at 3, 6, 9, and 12-months of age (see Methods, Figure 3.3A). These video excerpts have been used in other studies of visual social attention in typically developing infants (M. C. Frank et al., 2014b, 2009). Percent looking time to the characters' faces from the eye-tracking data was measured and longitudinally modeled with a Bayesian hierarchical linear model. Estimates of each infant's rate of increased attention to faces from 3- to 12-months were derived from the longitudinal model and then used as a covariate of interest in our analysis of SN connectivity at 6 weeks.

HR infants did not show any significant association between 6-week SN connectivity and subsequent trajectories in attention to faces. In LR infants, however, greater connectivity between the two major hubs of the SN—rAI and the anterior cingulate cortex (ACC)—and right lateral OFC at 6 weeks predicted greater increases in attention to faces across the first postnatal year (Figure 3.3). Importantly, these prefrontal regions predicting increased social attention overlapped with those exhibiting greater SN connectivity in LR infants relative to HR infants (i.e., right OFC). This suggests that connectivity with prefrontal regions within the Salience Network support the normative development of social attention.

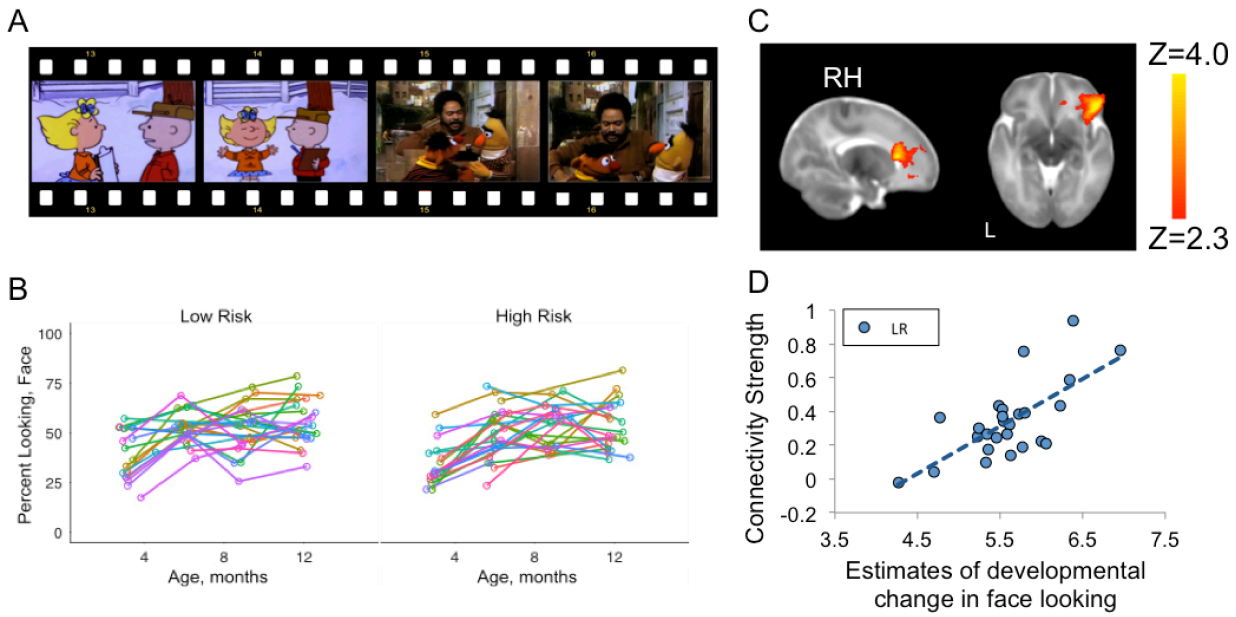

Figure 3.3: A) Example stills from the free-viewing eye-tracking stimuli. B) Raw percent looking at faces are plotted; individual data points were analyzed in a Bayesian hierarchical linear model. Estimates of each infant's change in looking time to faces across age were derived from the hierarchical linear model and included as a covariate of interest in a model of SN connectivity. C) Relative to HR infants, greater connectivity between the rAI and anterior cingulate cortex and right lateral orbitofrontal cortex was associated with greater increases in attention to faces from 3-to12-months of age. D) Estimates of connectivity strength of significant clusters depicted in C) are plotted against estimates of increased rates of looking at faces.

Six-Week SN Connectivity Predicts Social Communicative Skills and Sensory Sensitivity. We next examined the relation between SN connectivity and standardized measures of communicative development (ESCS), ASD-related behaviors (AOSI), and sensory processing atypicalities (ITSP). In LR infants, greater connectivity between the SN hub (i.e., rAI) and both prefrontal (i.e., right IFG, MFG, and OFC) and subcortical regions associated with reward and learning (i.e., bilateral caudate, pallidum, and nucleus accumbens) predicted higher rates of initiating joint attention – nonverbal communicative behaviors associated with better language functioning and social competence (Mundy & Newell, 2007)– at 12 months (Figure 3.4A). This provides further evidence that normative patterns of SN connectivity in early infancy support

attention to socially relevant stimuli, thereby scaffolding the development of social communication skills.

Specific patterns of 6-week SN connectivity also predicted subsequent behavioral markers of ASD risk in the HR infants (Figure 4B and 4C). Greater SN connectivity with regions associated with primary auditory and sensory processing (right superior temporal gyrus and thalamus), as well as attention and emotional valence (right amygdala), predicted higher parental ratings of sensory hypersensitivity at 6-12 months. This finding is consistent with observed associations between SN hyperconnectivity and sensory over-responsivity in older children with ASD (S. A. Green et al., 2016). HR infants may be predisposed to attend to extraneous sensory inputs quite early in development at the expense of socially relevant information. Furthermore, stronger connectivity between the SN hub and regions involved in implicit learning and reward processing (left putamen, IFG, caudate, amygdala), and memory (left hippocampus) predicted fewer ASD risk markers on the AOSI at 12 months. Better integration between the SN and both reward and emotional systems (e.g., regions associated with the social brain; Insel & Fernald 2004) may be protective for social cognitive development in HR infants; conversely, reduced SN connectivity with social brain regions in early infancy may negatively impact social learning.

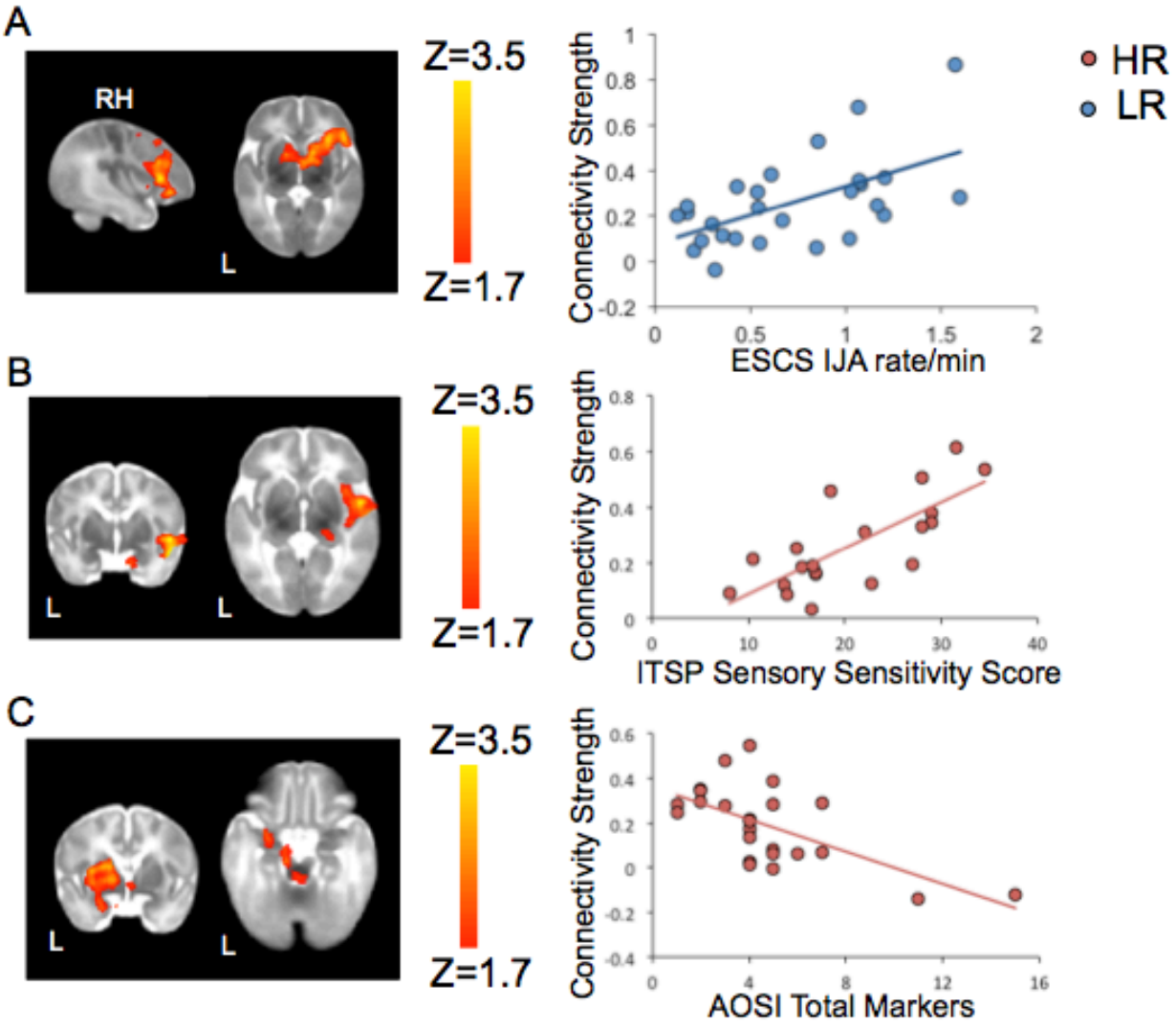

Figure 3.4: A) Greater connectivity with rAI and right orbitalfrontal cortex, reward-processing regions, and prefrontal regions predicted greater rates of initiating joint attention in LR infants at 12 months (ESCS IJA: Early Social Communication Skills, Initiating Joint Attention). B) Greater connectivity with the rAI and right superior temporal gyrus, amygdala and thalamus at 6 weeks predicted greater level of parent-reported sensory sensitivity in HR infants (ITSP: Infant Toddler Sensory Checklist). C) Greater connectivity with the rAI and left basal ganglia, thalamus, and amygdala at 6 weeks predicted lower level of social impairment on the AOSI at 12 months in HR infants (AOSI: Autism Observation Scale for Infants).

Table 3.2: Coordinates of regions with significant functional connections with the right anterior insula in the Salience Network

Region	Side	Peak mm (x,y,z)	Max Z
SN connectivity map between group comparison: HR>LR			
Precentral Gyrus	L	(-14, -17, 40)	3.41
Postcentral Gryus	L	(-35, -15, 27)	3.25
SN connectivity map between group comparison: LR>HR			
Inferior Frontal Gyrus (triangular)	R	(26, 11, 4)	3.58
Orbitofrontal Cortex	R	(16, 18, -3)	2.43
Positive correlations between right anterior insula and Face-looking in LR group			
Orbitofrontal Cortex	R	(35, 21, -5)	4.61
Inferior Frontal Gyrus (opercular)	R	(29, 8, 7)	4.36
Anterior Cingulate Gyrus	R	(11, 13, 10)	4.08
Positive correlations between right anterior insula and ESCS IJA in LR group			
Orbitofrontal Cortex	R	(33, 19, -4)	3.25
Inferior Frontal Gyrus (triangular)	R	(23, 13, 5)	3.01
Positive correlations between right anterior insula and ITSP Sensory Sensitivity in HR group			
Superior Temporal Gyrus	R	(35, -4, 8)	3.85
Negative correlations between right anterior insula and AOSI Total Markers in HR group			
Putamen	L	(-14, 0 11)	4.10
Insula	L	(-19, 0, 10)	3.41
Hippocampus	L	(-16, -16, -2)	3.41

Notes: SN, Salience Network; LR, Low Risk; HR, High Risk; L, left; R, right; ESCS IJA, Early Social Communication Scales Initiation of Joint Attention; ITSP, Infant Toddler Sensory Profile; AOSI, Autism Observation Scale for Infants

Discussion

The current study examined SN connectivity in 6-week-old infants at high versus low risk for ASD, and its association with the emergence of early behavioral markers of ASD risk, including facets of the development of social attention and sensory processing. We found that at six weeks, infants with familial risk for ASD already differ from low-risk infants in SN connectivity, such that they exhibit hyperconnectivity between the hub of the SN and sensorimotor regions. The observed tradeoff in functional connectivity between sensorimotor and higher-order prefrontal regions moreover suggests that greater salience allocation to nonsocial sensory stimuli likely comes at the expense of attention to socially-relevant stimuli, and vice versa. Notably, our data highlight some downstream behavioral effects of these early neural profiles; for instance, greater connectivity with basic sensory processing regions in 6-week HR infants predicted greater parent-reported sensory sensitivities across the first postnatal year, and greater connectivity with the orbitofrontal cortex and anterior cingulate—regions associated with attention, reward processing, and social processing—in 6-week LR infants predicted greater rates in increased attention to faces across the first postnatal year. The associations between SN connectivity and both social and nonsocial dimensions of the ASD symptomatology provide a parsimonious, mechanistic account for the emergence of the social impairments and repetitive, restrictive behaviors diagnostic to ASD. Altogether, this study indicates that resting-state functional connectivity can index developmental vulnerabilities conferred by familial risk for ASD shortly after birth, and corroborates cytoarchitectural and genetic data implicating prenatal neural development as a neurobiological risk factor for ASD (Stoner et al., 2014).

Prior research in youth with ASD have similarly found hyperconnectivity in the SN (S. A. Green et al., 2016; L. Q. Uddin, 2013), and, in particular, greater SN connectivity with sensory processing regions was associated with higher levels of sensory symptoms (S. A. Green et al., 2016). Here, we demonstrate developmental continuity for later altered SN connectivity and symptom severity in youth with ASD. This is quite notable because it suggests that early deviations in SN connectivity become crystallized over development through a feed-forward look of brain-behavior interactions. Furthermore, our findings extend a growing body of literature implicating early structural and functional differences in brain development in ASD (Shen & Piven, 2014). Prior imaging studies have found large-scale atypicalities in brain development that are apparent at 6 months of age and predictive of a subsequent ASD diagnosis, including brain overgrowth (Hazlett et al., 2017), increased extra-axial cerebral spinal fluid (Shen et al., 2013), aberrant development of white matter tracts (Wolff et al., 2012) and whole-brain functional connectivity (Emerson et al., 2017). Here we show that functional brain differences are not only detectable much earlier than previously reported within the first few weeks of postnatal life—much earlier than previously reported, but also map onto converging measures of early symptom-based markers of ASD risk. This bridges neuroimaging work with a rich body of behavioral work cataloguing developmental profiles of infants at risk for ASD (Bryson et al., 2008; E. J. H. Jones et al., 2014; Zwaigenbaum et al., 2005). The associations between early SN connectivity and subsequent social and nonsocial symptoms of ASD suggest that differences in SN connectivity in early infancy may eventually lead to divergent developmental outcomes between infants at high versus low risk for ASD.

Notably, the inverse relationship between SN connectivity with sensorimotor regions versus SN connectivity with prefrontal regions provides the first empirical support for recent

theoretical frameworks positing that initial deviations in attentional biases and/or sensorimotor processing may account for the emergence of ASD-related behaviors by altering the experience-dependent brain changes that typically guide social development (J Piven et al., 2017). Indeed, specific patterns of SN connectivity predicted individual differences in social functioning and sensory processing in HR and LR infants, possibility by predisposing these infants to be more attuned to one class of stimuli over the other. It is also important to note that despite detectable differences in functional brain connectivity at 6 weeks of age, HR and LR infants exhibited comparable behavioral profiles at 12 months, which is consistent with prior behavioral studies (Zwaigenbaum et al., 2013). This highlights a protracted prodromal period for overt behavioral symptoms of ASD to emerge, which promotes a promising temporal opportunity to intervene and prevent the crystallization of atypical social communicative development in ASD.

Our data also inform normative processes that underlie social cognitive development. Relative to HR infants, LR infants showed greater connectivity with frontal regions including the right medial and lateral OFC. Moreover, rAI and right OFC connectivity within the SN for LR infants predicted both increased attention to faces and higher rate of initiating joint attention. In adults, the OFC is an integral region within the "social brain" (Grossmann & Johnson, 2007) and is also involved in learning and reward processing (Schoenbaum et al., 2011). The predictive relation between these prefrontal neural regions and subsequent social functioning in our data may support the notion of interactive specialization of human brain development (M. H. Johnson, 2011). The OFC may become functionally tuned to subserve social processes as attention is directed towards social stimuli, such as faces, that are perceived to be salient. Given that the OFC is involved in social reinforcement and value-guided behaviors (Pelphrey, Shultz, Hudac, & Vander Wyk, 2011), developmental increases in visual attention to faces (M. C. Frank

et al., 2009; M. Frank et al., 2011) may reflect a behavior embedded in social reward. That is, the association between rAI and OFC connectivity within the SN at rest early in infancy may signify an aptitude to perceive social stimuli as rewarding, which becomes solidified with development. Deviations in normative patterns of social attention to faces in infants who develop ASD (Jones & Klin 2013) may in part be attributed to a lack of association between the Salience Network connectivity and development of visual social attention. Although HR infants look at faces, they do so for reasons different than LR infants (Chawarska & Shic, 2009).

Examining early functional brain connectivity can inform the impact of genetic and environmental risk factors on subsequent development by highlighting the intermediary neural networks that ultimately underlie behavior. While a primary aim of the current study was to elucidate the role of SN connectivity in conferring ASD risk, infants with a family history of ASD are also more likely to exhibit atypical development, including subclinical ASD symptomatology (i.e., broader autism phenotype), speech/language delays, global developmental delay, and ADHD during school-age years (Miller, Iosif, et al., 2015). Thus, atypical patterns of SN connectivity may reflect a more general developmental vulnerability—a possibility that should be examined in large-scale longitudinal studies. In sum, our findings demonstrate that aberrant patterns of *functional* brain connectivity can be detected in infants at high risk for developing ASD shortly after birth, and that these alterations predict ASD-related behaviors a year later. Identifying abnormal brain connectivity in infancy may pave the way for early interventions that can effectively redirect attention to social versus nonsocial inputs and promote development along normative trajectories.

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General Discussion

Autism is an incredibly complex neurodevelopmental disorder that impacts development across social, cognitive, and perceptual domains. It is recognized that a variety of genetic and environmental factors contribute to ASD risk; however, little is known about the developmental origins of the disorder. Using an infant sibling paradigm to examine the ontology of ASD, as opposed to other models of ASD risk (e.g., Fragile X, TSC, William's Syndrome) presumably combines aspects of both genetic and environmental risk with, the family unit being a source of both genetic heritability and early social learning opportunities (e.g., Hallmayer et al., 2011). This likely yields a heterogeneous group and, indeed, infants with familial risk for ASD exhibit a wide range of developmental outcomes (Miller, Iosif, et al., 2015).

Yet, in spite of this heterogeneity, the data presented here illustrate a remarkable convergence in both neural and behavioral profiles associated with familial risk for ASD. For instance, as young as 6 weeks of age, HR infants already exhibit altered Salience Network connectivity that predisposes them to attend to basic sensory stimuli over socially-relevant information, as well as suboptimal routes for early language processing that impact subsequent lexical development. While the observed effects may in part be influenced by the HR infants who ultimately develop ASD, these findings are still notable because they capture developmental vulnerabilities in domains known to be impacted in younger siblings who do not develop ASD (Miller, Iosif, et al., 2015; Miller, Young, et al., 2015; Tsang, Gillespie-Lynch, & Hutman, 2016). Thus, not only does this work advance our understanding of the mechanisms by which familial risk for ASD impacts development, but it also builds a foundation for an overarching goal of this line of research: to identify objective biomarkers that index ASD risk in early infancy. This may eventually aid in the development of targeted interventions that can potentially

prevent the full onset of ASD, rather than ameliorate its effects.

The three studies presented in this dissertation employed eye-tracking, resting-state and stimulus-evoked fMRI, as well as behavioral measures to take an innovative, multimodal approach to prospectively examine early markers of ASD risk. Notably, we examined social attention in both visual and auditory domains, as well as an underlying resting-state network that is associated with the non-social symptoms of ASD. Altogether, the data suggest that a multi-method approach is indeed sensitive at not only detecting early alterations in functional brain development, but also highlighting environmental factors that affect the emergence of symptom-based markers of ASD.

In Study 1, I used eye-tracking methods in conjunction with measures of social communicative functioning in both infants and parents to examine visual social attention in HR infants. I found that parental affectedness of ASD-related behaviors moderated developmental trajectories of attention to faces, and that increased attention to faces in HR infants from 3- to 12-months was associated with better concurrent and subsequent social functioning. Thus, these findings have clinical implications for developing parent-mediated interventions for young infants at risk for ASD.

In Study 2, I used a passive listening stimulus-evoked fMRI paradigm to evaluate language processing (e.g., auditory social attention). I found that at 1.5 months/6 weeks of age HR infants showed greater amygdala activity for their native versus nonnative language, which suggests an atypical neural substrate for language processing. Moreover, at 9 months of age, HR infants showed attenuated responses to both native and nonnative language, particularly in regions associated with reward processing (e.g., caudate, basal ganglia, medial prefrontal regions). Importantly, this attenuated neural response for English and Japanese was especially so

for HR infants with subsequent delayed language development, who also showed more social symptoms than HR infants without delayed language. These results suggest that examining the neural correlates of early language processing can serve as a means to gauge auditory social attention; our data then suggest that early in development speech may not be perceived as a special class of stimuli in HR infants. Subtle deviations in attention to speech early in development appear to iteratively result in larger discrepancies in developmental trajectories towards the end of the first postnatal year.

In Study 3, I used resting-state fMRI in conjunction with eye-tracking and behavioral measures to examine early Salience Network connectivity and its association with symptom-based markers of ASD, including indices of social functioning and sensory processing. Here we found that early deviations in Salience Network connectivity may predispose HR infants to attend to non-social sensory inputs over social information. This pattern of results presents a parsimonious model for the emergence of both social and non-social symptom dimensions of ASD and lend support to the hypothesis that initial disruptions in brain systems involved in sensory/attentional processes is a critical antecedent to the later-emerging core symptoms of ASD.

ASD as a developmental disorder

The data presented here report the earliest detectable differences in functional brain development in HR infants. The significance of this cannot be understated. Across all three studies, behavioral differences in social communicative functioning were only apparent at 18 months. This marks a protracted prodromal period during which deviations in brain function and connectivity – likely conferred prenatally for them to be apparent by 6 weeks of age – slowly crystallize as atypical interactions with the social environment and reciprocally influence further

brain development. Indeed, data from the native language fMRI study provides evidence for this hypothesis: Relative to 6 weeks, between group-differences in neural response to language are more widespread at 9 months. Moreover, neural differences in language processing in HR infants with and without delayed language were apparent at 9 months, but not 6 weeks of age, suggesting a feed-forward loop by which early deviations iteratively cascade to both behavioral and functional brain differences. These examples illustrate the *developmental* aspect of ASD. Oftentimes, the description that ASD is a developmental disorder is understood to mean that ASD is a disorder emerging in early childhood. However, an alternative conceptualization is that overt behavioral symptoms of ASD unfold overtime in a probabilistic fashion; initial genetic/molecular risk factors may set into motion deviations in prenatal brain development that are postnatally reinforced at both the neural and behavioral level.

The findings presented here lend support a recent theory whereby the development of ASD stems from early disruptions in sensory processing that compromise the development of higher-order networks integrating sensory experiences for social processes (Piven, Elison, & Zylka, 2017). The lack of between-group differences in visual social attention between HR and LR infants in Study 1, in conjunction with the developmental findings from Studies 2 and 3, suggest that the social impairments associated with ASD are likely secondary to earlier atypicalities in systems involved in basic sensory processing. Our data indicate that early sensorimotor/attentional experiences are disrupted in HR infants as early as 6 weeks. By 9 months, we have evidence for altered experience-dependent neural development in HR infants, at least with regards to language processing. Thus, although HR and LR infants are similarly attending to faces from 3- to 12-months of age in Study 1, the social learning experiences afforded to HR versus LR infants may be different (e.g., Chawarska & Shic, 2009). At then rural

level, we see some evidence of the converse in Study 2, whereby HR infants with seemingly normative trajectories of language acquisition exhibit different patterns of activity for English and Japanese than LR infants.

Normative social development

By virtue of comparing development between HR and LR infants, the data presented here also inform aspects of normative social development. Our data imply that the developing brain is predisposed to perceive social information as intrinsically salient. In LR infants, we observed that attention to faces increased during the first postnatal year, which appears to be mediated by SN connectivity with prefrontal regions. Notably, SN connectivity supporting both increased attention to faces and joint attention behaviors included the medial prefrontal cortex—a neural region involved in reward processing and social orienting in adults. Furthermore, we observed neural differentiation between native and non-native language in regions association with speech and voice-selective processing and attention at 6 weeks (Gervain & Mehler, 2010), as well as widespread activity in response to language in regions involved in language, social motivation/reward processing, and learning/memory at 9 months of age.

These findings are in line with two aspects of social brain development. First, early orienting mechanisms are apparent shortly after birth for socially adaptive behaviors, including attention to faces and the social signals they impart (e.g., speech). These supposedly reflexive orienting mechanisms might lay the foundation for social learning. Second, the data also support the notion of interactive specialization (Johnson, 2011). The fact that brain activity and/or connectivity at 6 weeks can be associated with behaviors emerging during the first postnatal year indicates early functional specialization for social orienting and language processing. This very early specialization highlights the evolutionary adaptive function of orienting to conspecifics

(Shultz, Klin, & Jones, 2018). One of the hypotheses set forth by interactive specialization theory is that the plasticity of a brain region will relate to the degree of its functional specialization (i.e., if a region is already specialized for some specific functions, it will not likely assume other ones). To some extent, this explains the functional correspondence between anatomical regions in infancy and adulthood (e.g., temporal regions appear to be associated with auditory and language processing across the lifespan). Another hypothesis from the interactive specialization theory is that developmental change in behavior will also be reflected in widespread changes at the neural level. The larger recruitment of neural regions involved in language processing at 9 months versus 6 weeks of age is likely a reflection of the dynamic process of acquiring language – one that integrates auditory processing, learning/memory, and reward processing. In the context of the interactive specialization theory, the pattern of SN connectivity and neural response to language in LR infants inform why socially directed behaviors may be reinforced – they include regions involved in social motivation and reward processing.

Limitations and Future Directions

The current set of studies primarily focused on development in the first postnatal year when diagnostic outcomes are not available. Thus, the findings here can only speak to ASD risk, and should not be regarded as veridical markers of the disorder. Given the aforementioned implications of a protracted prodromal period, these brain-based and attentional markers of ASD risk are probabilistic in their long-term impact on development. It is crucial to follow HR infant siblings at least through school-age years to understand how early patterns in visual and auditory social attention, and salience network connectivity may be associated with other suboptimal development outcomes aside from ASD (e.g., ADHD, speech-language delays, anxiety).

At the same time, the long-term prospective study of these infant siblings can inform

aspects of resilience. In light of developmental vulnerabilities, what are the protective factors that lead to normative developmental outcomes in infants with familial risk for ASD? Here we allude to the possibility that parental attunement to social communicative cues may support social development. Another observation is that HR siblings appear to be high-functioning both in cognitive and verbal skills (Miller, Iosif, et al., 2015; Tsang et al., 2016); perhaps the close monitoring of these infants' development not only further educates parents on expected developmental milestones but also serves as an enhancing experience for the infants themselves. Future research should incorporate measures of the infants' social environment (e.g., parent-child, sibling, or peer interactions) to gain a transactional understanding of how these factors may interact with ASD risk to ultimately shape developmental trajectories.

Another noted caveat about these data is that a family history of ASD was used to index elevated ASD risk. The genetic architecture of ASD is complex and a number of genetic syndromes and variants are associated with the clinical profile of ASD including tuberous sclerosis, dup22, Fragile X, and SCN2 (Devlin & Scherer, 2012). Comparing the development of infants with familial risk versus other genetic risk conditions will further inform ASD pathogenesis, and potentially identify subgroups of ASD with distinct developmental pathways and symptom profiles (e.g., with/without intellectual disability, seizures, delayed language, minimally verbal, etc.). Nonetheless, the present findings are notable because despite the likely genetic heterogeneity associated with familial risk, we still see convergence in aspects of brain function and connectivity that predict symptom-based markers of ASD.

Related to factors that may confer risk versus resilience, prospective studies of infant siblings present the opportunity to examine the gender bias associated with ASD. Boys are four times more likely than girls to be affected by ASD, and there is mounting evidence that ASD

may present differently in girls than in boys (Jeste & Geschwind, 2014). Our sample was too small to be sufficiently powered to examine potential sex differences in development; however, larger multisite studies could evaluate whether girls require a higher ASD-related genetic load than boys to reach clinical impairment (e.g., the female protective factor; Jacquemonet, Coe, Hersch et al., 2014), and whether girls' developmental profiles differ from those observed in boys.

Corroborating a small but growing literature, the present studies demonstrate that neuroimaging methods are sensitive at detecting early markers of ASD risk (Shen & Piven, 2014). While some aspect of atypical brain development are observed shortly after birth (e.g., SN connectivity), other functional brain differences become more apparent with development (e.g., language processing). Sampling across more developmental time points during infancy can further track changes in brain development and how these relate to changes in the infants' behavioral repertoires, as well as identify developmental atypicalities for different neural systems. Subsequent work that examines the development of other resting-state network and their inter-connectivity will provide a more comprehensive account of the functional architecture associated with ASD risk.

Finally, and most importantly, future studies should strive to inform individual clinical outcomes. Notably, data driven approaches (e.g., latent-variable modeling, machine learning) can revolutionize not only our understanding of how the early brain-based measures observed here are associated with one another, but also our ability to build predictive models to identify which infant may be at greatest risk for ASD. This effort will require large data sets that can only become available through collaboration across multiple institutions.

Concluding Remarks

Autism Spectrum Disorder is a highly prevalent disorder and carries a large societal impact. Although risk factors associated with ASD are likely prenatally determined, the emergence of the overt behavioral symptoms overlaps with a period of dynamic brain development. This dissertation used an innovative and integrative multimodal approach to connect early aspects of functional brain development with behaviors associated with ASD risk. I found that familial risk for ASD confers developmental vulnerabilities in neural systems associated with attention and salience detection as early at 6 weeks of age, and that these alterations are predictive of social communicative functioning and sensory processing throughout the first postnatal year. This work advances our understanding of early brain-based mechanisms underlying atypical social communicative and sensory development. Moreover, the findings here provide the foundation for future studies to detect infants most vulnerable for atypical social communicative development, and for the creation of individualized interventions to promote optimal outcomes.

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