UNIVERSITY OF CALIFORNIA SAN DIEGO

SAN DIEGO STATE UNIVERSITY

# Brain Development in Toddlers and Preschoolers with Autism Spectrum Disorder: A Multimodal MRI Examination

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

in

Clinical Psychology

by

Bosi Chen

Committee in charge:

San Diego State University

Professor Inna Fishman, Chair
Professor Martin Sereno, Co-Chair
Professor Ruth Carper

University of California San Diego

Professor Timothy T. Brown
Professor Jay Giedd
Professor Lindsey Powell

2023

The Dissertation of Bosi Chen is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

---

---

---

---

Co-chair

---

Chair

University of California San Diego

San Diego State University

2023

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Inna Fishman and Dr. Martin Sereno for their supports and guidance as the chair and co-chair of my committee and help me become a better researcher. I am also very grateful for the other members of my committee, Dr. Ruth Carper, Dr. Timothy Brown, Dr. Jay Giedd, and Dr. Lindsey Powell. Last but not least, I want express my gratitude to Dr. Annika Linke for her guidance throughout my entire graduate study journey. Many thanks to all the staff, fellow graduate students, and research assistants of Brain Development Imaging Labs at SDSU who make this research possible.

Chapter 1, in full is a reprint of the material as it appears in the Journal of Child Psychology and Psychiatry, 2021. Chen, B., Linke, A., Olson, L., Ibarra, C., Reynolds, S., Müller, R. A., Kinnear, M., and Fishman, I., Wiley, 2021. The dissertation author was the primary investigator and author of this paper.

Chapter 2, in full is a reprint of the material as it appears in Developmental Neurobiology, 2022. Chen, B., Linke, A., Olson, L., Kohli, J., Kinnear, M., Sereno, M., Müller, R. A., Carper, R., and Fishman, I., Wiley, 2022. The dissertation author was the primary investigator and author of this paper.

Chapter 3, in full, is currently under review for publication. Chen, B., Olson, L., Rio, A., Salmina, M., Linke, A., and Fishman, I. The dissertation author was the primary investigator and author of this paper.

# VITA

| | |
|---|---|
| 2023 | **Doctor of Philosophy** in Clinical Psychology<br>San Diego State University / University of California San Diego Joint Doctoral Program in Clinical Psychology<br>Major Area of Study: Neuropsychology<br>Emphasis: Child and Adolescent Psychopathology<br>Dissertation Chair and Co-Chair: Drs. Inna Fishman and Martin Sereno<br>Dissertation Title: Brain Development in Toddlers and Preschoolers with Autism Spectrum Disorder: A multimodal MRI Examination |
| 2022-2023 | **Pre-doctoral Internship**<br>Baylor College of Medicine / Texas Children's Hospital<br>Major Area of Study: Pediatric Neuropsychology |
| 2019 | **Master of Science** in Clinical Psychology<br>San Diego State University<br>Thesis Chair: Dr. Inna Fishman<br>Thesis Title: Brain Network Connectivity and Its Relationship to Behavioral Outcomes in Toddlers with Autism Spectrum Disorder |
| 2014 | **Bachelor of Arts** in Psychology and Economics, Magna Cum Laude<br>New York University |

## PEER-REVIEW PUBLICATIONS

**Chen, B**., Linke, A., Olson, L., Kohli, J., Kinnear, M., Müller, R-A., Sereno, M., Müller, R-A., Carper, R., & Fishman, I. (2022). Cortical myelination in toddlers and preschoolers with autism spectrum disorder. *Developmental Neurobiology*. *82*(3), 261-274. https://doi.org/10.1002/dneu.22874

Linke, A., **Chen, B**., Olson, L., Ibarra, C., Fong C., Apostol, M., Kinnear, M., Müller, R-A., & Fishman, I. (2023). Sleep problems in preschoolers with autism spectrum disorder are associated with sensory sensitivities and thalamocortical overconnectivity. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. *8*(1), 21-31. https://doi.org/10.1016/j.bpsc.2021.07.008

**Chen, B**., Linke, A., Olson, L., Ibarra, C., Kinnear, M., & Fishman, I. (2021). Resting state functional networks in 1-to-3-year-old typically developing children. *Developmental Cognitive Neuroscience*, *51*, 100991. https://doi.org/10.1016/j.dcn.2021.100991

Olson, L., **Chen, B**., Ibarra, C., Wang, T., Mash, L., Linke, A., Kinnear, M., & Fishman, I. (2022). Externalizing behaviors are associated with increased parenting stress in caregivers of young children with autism. *Journal of Autism and Developmental Disorders. 52*(3), 975-986. https://doi.org/10.1007/s10803-021-04995-w

Olson, L., Chen, B., & Fishman, I. (2021). Neural correlates of socioeconomic status in early childhood: a systematic review of the literature. Child Neuropsychology, 27(3), 390-423. https://doi.org/10.1080/09297049.2021.1879766

**Chen, B.**, Linke, A., Olson, L., Ibarra, C., Reynolds, S., Müller, R-A., Kinnear, M., & Fishman, I. (2021). Greater functional connectivity between sensory networks is related to symptom severity in toddlers with autism spectrum disorder. *Journal of Child Psychology and Psychiatry, 62*(2), 160-170. https://doi.org/10.1111/jcpp.13268

Olson, L., Kinnear, M., **Chen, B**., Reynolds, S., Ibarra, C., Wang, T., Linke, A., & Fishman, I. (2020). Socioeconomic factors account for variability in language skills in preschoolers with autism spectrum disorders. *Journal of Developmental and Behavior Pediatrics, 42*(2), 101-108. *https://doi.org/10.1097/DBP.0000000000000870*

Di Martino, A., O'Connor, D.*, **Chen, B.*,** Alaerts, K., Anderson, J.S., Assaf, M., Balsters, J.H., … & Milham, M.P. (2017). Enhancing studies of the connectome in autism using the Autism Brain Imaging Data Exchange II. *Scientific Data*, *4*, 170010. https://doi.org/10.1038/sdata.2017.10

Aoki, Y., Yoncheva, Y., **Chen, B.,** Sharp, D., Lazar, M., Castellanos, F.X., Milham, M, P., & Di Martino, A. (2017). Association of white matter structure with autism spectrum disorder and attention deficit/hyperactivity disorder. *JAMA Psychiatry*, *74*(11), 1120-28. https://doi.org/10.1001/jamapsychiatry.2017.2573

*authors contributed equally

ABSTRACT OF THE DISSERTATION

Brain Development in Toddlers and Preschoolers with Autism Spectrum Disorder:
A Multimodal MRI Examination

by

Bosi Chen

Doctor of Philosophy in Clinical Psychology

University of California San Diego, 2023
San Diego State University, 2023

Professor Inna Fishman, Chair
Professor Martin Sereno, Co-Chair

Although symptoms of autism spectrum disorder (ASD) emerge in the first years of life, little is known about the trajectories of brain development and their relation to symptom onset in the first years of life in ASD. Identification of early brain markers of ASD during one of the most dynamic and vulnerable neurodevelopmental periods is critical to developing more targeted intervention programs. This three-paper dissertation used anatomical and functional MRI in combination with clinical and behavioral data that have been collected from toddlers and preschoolers with ASD and typically developing (TD) young children in the context of the

SDSU Toddler MRI Project. Study 1 (Chen et al., 2021) used resting-state fMRI data acquired during natural sleep from 24 children with early diagnosis of ASD and 33 TD children (aged 1.5-3.5 years) to examine intrinsic functional connectivity (iFC) within and between functional networks generated with independent component analysis. Atypically increased iFC between visual and sensorimotor networks was found in young children with ASD, and was linked with greater autism symptoms, suggesting that disrupted connectivity within primary sensory circuits may be implicated in the emergence of autism symptomatology. Building upon these results, Study 2 (Chen et al., 2022) examined intracortical myelination, an aspect of brain maturation essential for establishing and maintaining neural connectivity, using anatomical T1-weighted and T2-weighted MRI data acquired in 21 young children with ASD and 16 TD children (aged 1.5-5.5 years). Although no group differences were found between TD children and those with ASD in intracortical myelin estimated with T1w/T2w ratio, differential associations between T1w/T2w and age were identified in several early myelinated regions in the ASD and TD groups. The atypical age-related effects in intracortical myelin suggested a disruption in myelination in the first years of life in ASD, which may have cascading effects on brain network connectivity development. Study 3 (Chen et al., in preparation) examined multivariate relationships between brain structure and function using morphometric measures (i.e., surface area and cortical thickness) and an index of local spontaneous activity (fractional Amplitude of Low Frequency Fluctuation) in 38 young children with ASD and 31 TD children (aged 1.5-5.5 years) using canonical correlation analysis. Significantly reduced structure-function correlation and differential age-related effects in structure-function covariation were identified in the ASD cohort as compared to children with typical development. Furthermore, the functional composite capturing local spontaneous activity was associated with overall developmental and adaptive

behavior skills in children with ASD. Collectively, Studies 1, 2, and 3 showed that multiple neurodevelopmental processes (i.e., functional network and connectivity, intracortical myelination, cortical morphometry, and local spontaneous activity) are implicated in ASD in early childhood, when autism symptoms first manifest. These findings highlight the importance of integrating multimodal data and examining distinct but complementary anatomical and functional brain measures to elucidate the trajectories of early brain development in ASD.

# INTEGRATED INTRODUCTION

Autism spectrum disorders (ASD) are neurodevelopmental disorders behaviorally characterized by social communication deficits and restricted and repetitive behaviors, which appear early in life and are typically persistent throughout life (APA, 2013). As disorders of development, ASD can have profound, lifelong impacts for affected individuals and their families, with most individuals with ASD requiring long-term support in some form—familial, community, public services, healthcare system, employment, etc. (Lord, Elsabbagh, Baird, & Veenstra-Vanderweele, 2018)—making research in this population a pressing public health priority. Symptoms of ASD emerge early in postnatal life (Johnson, Gliga, Jones, & Charman, 2015; Jones, Gliga, Bedford, Charman, & Johnson, 2014; Palomo, Belinchon, & Ozonoff, 2006; Pierce et al., 2011), can be reliably identified during the second year of life (Corsello, Akshoomoff, & Stahmer, 2013; Ozonoff et al., 2015; Pierce et al., 2019), and early diagnosis of ASD is typically stable throughout life (McCauley, Elias, & Lord, 2020). However, most children with ASD are not diagnosed until after age 4 (and even later in socioeconomically disadvantaged communities), with the median age of diagnosis in the US being 52 months (Maenner et al., 2020), in large part due to the lack of clinically meaningful biomarkers. The implications of delayed identification are significant, given the positive impact of early interventions on both behavior and the developing brain (Dawson et al., 2012; Estes et al., 2015; Landa, 2018).

Although there is no known single etiology for ASD, the current consensus is that ASD is highly heritable (Sandin et al., 2017) and originates prenatally, affecting early building blocks of brain circuit development and function, such as protein synthesis and cellular metabolism, with cascading effects on neuronal proliferation and migration, synaptogenesis and synaptic signaling,

myelination, and network formation (Courchesne et al., 2019; Gordon & Geschwind, 2020). However, the current understanding of these fundamental neurodevelopmental processes, some of which continue to varying degrees throughout life (e.g., synaptic pruning [(Spear, 2013), myelination (Abrahám et al., 2010; Chapman & Hill, 2020)), is limited by the scarcity of brain imaging studies in young children with ASD (before 5 years of age) due to the known challenges of acquiring high quality imaging data early in life (Turesky, Vanderauwera, & Gaab, 2021). While not directly addressing the cellular and molecular mechanisms giving rise to atypical neurodevelopment in ASD, neuroimaging can help understand variability at circuit levels through examination of how different brain systems map onto various developmental trajectories and outcomes in ASD. Thus, investigations of brain development during early childhood, which represents one of the most dynamic and vulnerable neurodevelopmental periods (Gao, Alcauter, Elton, et al., 2015; Gilmore et al., 2012; Johnson et al., 2015), is critical for better understanding of both typical brain maturation and the pathogenic pathways to ASD and other neurodevelopmental disorders.

Magnetic Resonance Imaging (MRI) has become a principled choice for studying the developing brain, including in children with neurodevelopmental disorders, because of its ability to examine both brain structure and function non-invasively (i.e., without ionizing radiation). There has been a significant increase in the number of MRI studies in early childhood due to improved methods in data acquisition (Dean et al., 2014; Reynolds, Long, Paniukov, Bagshawe, & Lebel, 2020) and data processing (Makropoulos, Counsell, & Rueckert, 2018; Zöllei, Iglesias, Ou, Grant, & Fischl, 2020). These advancements are particularly important as the participants are often required to remain still in the MRI scanner for at least 20-30 minutes given the sensitivity of MRI images to motion artifacts. In children older than 4-5 years old,

comprehensive habituation protocols have been developed and implemented by many groups to reduce fear and anxiety to the MRI environment and minimize in-scanner movements through practice in a mock scanner with feedback, in which the child's head movement is monitored by a motion tracker and relayed back to the child as a game (de Bie et al., 2010). Unfortunately, these habituation procedures are less useful for children younger than 5, as they are unlikely to be able to understand and follow instructions given their developmental level. While sedation or general anesthesia are routinely used in clinical MRI to increase compliance and reduce movements when scans are clinically indicated, use of sedation in research studies is unacceptable because of the related risks such as hypoxia and neurotoxic effects on development. Due to these considerations, in research studies, infants and toddlers are usually scanned under natural sleep, without sedation (Chen, Linke, Olson, Ibarra, Kinnear, et al., 2021; Dean et al., 2014; Nordahl et al., 2016; Raschle et al., 2012). Scanning infants (from birth to 1-year-old) during natural sleep is relatively easy to accomplish as they can be fed and swaddled prior to the scan and are likely to be asleep during most of the day (Almli, Rivkin, & McKinstry, 2007). However, scanning toddlers and preschoolers (1-to-4-year-old) during natural sleep presents multiple practical challenges, such as getting the child to fall asleep in a novel environment and to maintain sleep during transfer to the MRI bed or while being scanned, with the loud scanner noise, as well as ensuring that the child remains still during sleep throughout the scan (in the same position, on their back), without physical constraints. Consequently, relatively few imaging studies – in typical development or in neurodevelopmental disorders – have been conducted in this age range.

## 1. Brain maturation in the first years of life in typical development

Early childhood (which typically refers to the first years of life spanning infancy, toddlerhood and preschool age), when ASD symptoms first emerge and fully manifest, is a key period of postnatal brain maturation (Tau & Peterson, 2010), peak neuroplasticity (Kolb & Gibb, 2011), and a critical window for emergence of cognitive and behavioral skills and rapid learning (Johnson, Grossmann, & Farroni, 2008). The time from birth to five years is when many foundational skills across all areas of development are acquired, including gross and fine motor skills, receptive and expressive language skills allowing communication with others, and socioemotional skills enabling the child to learn from others through social learning and to form relationships (Bornstein, 2014). These skills serve as developmental building blocks, laying foundation for acquisition of academic and socioemotional competencies in middle and late childhood, with lifelong effects on one's adaptive functioning, health, and well-being. At the same time, both brain and neurocognitive development in early childhood are particularly responsive to environmental input (Merz, Wiltshire, & Noble, 2019; Tierney & Nelson, 2009), setting the stage for the greatest window of opportunity for modifying developmental trajectories (Dawson et al., 2012).

### 1.1 Structural brain changes in the first years of life

A growing number of cross-sectional and longitudinal studies have begun to characterize brain maturation in the first years of life. Generally, these studies have indicated that brain structure is largely in place by the second year of life, undergoing reorganization and fine-tunning beyond two years of age. Cortical and subcortical gray matter volumes, in particular, grow most rapidly in the first year of life, with growth rates peaking in early childhood (Bethlehem et al., 2022; Gilmore et al., 2012; Haynes et al., 2020; Knickmeyer et al., 2008; Pfefferbaum et al., 1994). This rapid gray matter volume growth likely reflects continuous

synapse formation, albeit with variable rates across different brain regions (Huttenlocher & Dabholkar, 1997). Beyond the volumetric growth, maturational changes in brain structure are indexed by dramatic increases in cortical thickness and expansion of cortical surface area, as well as continued cortical folding, or gyrification observed in the first years of life (Brown & Jernigan, 2012; Li et al., 2013; Li et al., 2014; Lyall et al., 2015). The rapid cortical thickness growth and surface area expansion are thought to reflect postnatal increases in dendritic arborization, axonal elongation and thickening (as a result of continuous myelination, as described below), and synaptogenesis (Huttenlocher & Dabholkar, 1997). While there is a general consensus that expansion of cortical surface area peaks around middle to late childhood, the findings on when cortical thickness (CT) peaks in development are mixed (Frangou et al., 2022; Walhovd, Fjell, Giedd, Dale, & Brown, 2017), with some studies suggesting that it peaks around age 8 or later (Raznahan et al., 2011; Shaw et al., 2008) while others reporting that cortical thinning, or decrease in CT, starts in early childhood, as early as before age 2 (Bethlehem et al., 2022; Brown et al., 2012; Sowell et al., 2004). Generally, across various indices of neurodevelopment, the rate of the growth and maturational changes is nonuniform throughout the brain, with sensory and motor cortices developing earlier and more rapidly than association cortices (Brown et al., 2012; Tau & Peterson, 2010).

In addition to these macro-scale morphometric changes, microstructural changes are also a salient feature of brain maturation. One such neurodevelopmental process shaping the brain structure and function is myelination which is essential for efficient neural communication (Liu, Li, Zhu, Li, & Liu, 2019), as myelinated axons and white matter fibers allow for rapid and reliable propagation of inter-neuronal signals across the brain. Although the majority of major white matter pathways are present at birth, white matter myelination continues over a protracted

developmental period from birth into adulthood (Gilmore, Knickmeyer, & Gao, 2018). Equally critical for brain maturation and connectivity is intracortical myelination found predominately in the deeper cortical layers (Fields, 2014), in part due to propagation of myelin in the white mater into the periphery of cortical neuropil (Shaw et al., 2008; Sowell et al., 2004). Intracortical myelination is essential for establishing and maintaining neuronal circuitry, as it contributes to fine-tuning the timing and synchrony of neural connectivity (Haroutunian et al., 2014). In typical development, the maturational timing of intracortical myelination, which commences at or near birth (Arnold & Trojanowski, 1996), follows a general neurodevelopmental trajectory, with unimodal primary sensory and motor cortices being highly myelinated by 1 year of age, and association areas in frontal and temporal cortices exhibiting more protracted myelination, continuing at least through the third decade of life (Deoni, Dean, Remer, Dirks, & O'Muircheartaigh, 2015; Rowley et al., 2017).

### 1.2 Functional brain changes in the first years of life

The first years of life are also a period of rapid development of brain functional organization. Functionally, the brain is organized into large-scale networks supporting specialized cognitive or physical functions (Petersen & Sporns, 2015; Raichle, 2010). Most functional networks are present at birth and undergo reorganization and fine-tuning throughout early childhood (Gao, Alcauter, Elton, et al., 2015), in parallel with the behavioral and cognitive milestones achieved during this pivotal period in human development (Johnson, 2001). In particular, primary sensory and motor networks – implicated in processing sensory information and supporting motor development – become increasingly more integrated in the first year of life and substantially resemble adult topology by age two (Gao et al., 2015; Lin et al., 2008). In contrast, supra-modal functional networks implicated in higher-order cognitive functions are far

from the adult-like organization in the first postnatal years and undergo prolonged maturation over the first decades of life (Gao, Alcauter, Elton, et al., 2015; Hoff, Van den Heuvel, Benders, Kersbergen, & De Vries, 2013).

Current understanding of brain functional networks has been primarily informed by resting-state functional connectivity studies, which use fMRI blood oxygen level-dependent (BOLD) signal to estimate synchronicity in neural activity across different brain regions. The inter-regional synchronicity or coupling is thought to reflect intrinsically organized functional networks formed by a history of co-activation in the course of development or individual experience associated with functional specialization. Validity of this interpretation is supported by the observation that most of the resting-state functional connectivity patterns tend to occur between brain regions that overlap in function and have plausible neuroanatomical links, for example regions known to comprise motor, visual, or auditory circuitry (e.g., (Biswal, Yetkin, Haughton, & Hyde, 1995; Damoiseaux et al., 2006; van den Heuvel & Hulshoff Pol, 2010). Further, because functional networks reflect ongoing neural activity synchronized between different brain regions as a result of co-activation, these patterns are present and can be detected at rest, during so-called resting state, as well as under various states of arousal, including sleep and anesthesia (Power, Fair, Schlaggar, & Petersen, 2010; Raichle, 2010). Methodologically, a common data-driven approach for identifying resting-state functional networks (RFNs) is independent component analysis (ICA), which statistically decomposes fMRI data into a set of spatial components with maximally independent time courses (Beckmann, 2012), with resultant components showing close correspondence to known neuroanatomy and canonical brain functional activation maps (Smith et al., 2009). Based on their spatial and temporal characteristics, independent components are classified as RFNs (e.g., visual network spanning

regions in the occipital visual cortex, motor network spanning the motor strip, etc.) or non-neuronal noise or artifact components (e.g., generated by motion during scanning, or corresponding to physiological variables, such as breathing or pulsation in large blood vessels (Griffanti et al., 2017)).

Utilizing ICA approach, distinct RFNs have been reliably described in children and adults, including primary, unimodal sensory networks (i.e., visual, auditory, and sensorimotor networks) and higher-order or supra-modal networks, such as self-reference (default mode), language, attention, and executive networks (Damoiseaux et al., 2006; Smith et al., 2009). Comparable functional brain networks have also been reliably identified early in life in young children (Chen, Linke, Olson, Ibarra, Kinnear, et al., 2021; Fransson et al., 2007; Gao, Alcauter, Elton, et al., 2015; Gao, Alcauter, Smith, Gilmore, & Lin, 2015), with some observed even prenatally with fetal imaging methods (Thomason et al., 2013). As mentioned above, brain functional networks mature along the general neurodevelopmental sequence, with primary sensory networks maturing first (Gao, Alcauter, Smith, et al., 2015; Lin et al., 2008) and association or supra-modal functional networks undergoing prolonged maturation over the first decades of life (Dosenbach et al., 2010; Fair et al., 2008; Gao, Alcauter, Elton, et al., 2015), resembling the order in which behavioral and cognitive skills emerge and develop in early childhood (Johnson, 2001).

Since the seminal studies by Gao and colleagues describing the development of RFNs in infants, the maturational trajectories of RFNs in toddlerhood and preschool age (1 to 4 years) remain understudied. Only a few studies have examined brain activation or functional connectivity in typically developing toddlers and preschoolers, with a particular focus on speech perception and language circuitry, either through passive auditory stimulation or resting-state

during sleep (Hutton et al., 2015; Redcay, Haist, & Courchesne, 2008; Xiao et al., 2016). Examining local and global functional connectivity features (i.e., amplitude of low frequency fluctuation, regional homogeneity, and eigenvector centrality mapping) in awake 2.5-6 year old children during passive viewing fMRI, Long and colleagues (Long, Benischek, Dewey, & Lebel, 2017) found age-related increase in local and global connectivity in regions within the default mode and frontoparietal networks, as well as age-related shift from more global to local connectivity in the superior parietal and fusiform gyri, and a local-to-global shift in the superior temporal area, suggesting that functional connectivity undergoes substantial reorganization during toddler and preschool years. More recently, Bruchhage and colleagues (2020) also reported a cross-age shift in functional connectivity toward networks involved in higher-order cognitive processes (e.g., default mode, attention, and salience networks) and links between functional connectivity indices and developmental skills in a cohort spanning a relatively broad age range from infancy to early school age (3 months to 6 years). Similarly, in our own work (Chen, Linke, Olson, Ibarra, Kinnear, et al., 2021) we observed age-related increase in network homogeneity in several higher-order networks (e.g., default mode, salience, and language networks), which was associated with more advanced developmental skills in typically developing toddlers between ages 1.5 and 3.5 years, suggesting that greater network cohesiveness or functional specialization may be critical for, or at least coupled with development of behavioral skills in early childhood.

## 2. Early brain development in ASD: Review of neuroimaging findings to date

Although much of the neurobiology of ASD remains unknown, a few consistent findings have emerged from the relatively limited but growing number of MRI studies focusing on ASD in infancy and toddlerhood, including prospective studies of infant siblings of older children with

ASD who themselves are at a high risk for developing ASD. These studies increasingly support a picture of early neurodevelopmental abnormalities, including atypical brain development and widespread alterations in brain connectivity. The most consistently reported finding to date is enlargement of brain volume early in life reported at the group-level, in comparisons of young children with, or at familial risk for ASD to TD peers (Courchesne et al., 2001; Hazlett et al., 2005; Hazlett et al., 2011; Nordahl et al., 2011). This early brain overgrowth appears to reflect accelerated growth rate between one and two years of age (Hazlett et al., 2011), affecting both white and gray matter volumes (Hazlett et al., 2005), and being possibly driven by cortical surface area hyper-expansion between 6 and 12 months of age (Hazlett et al., 2017). Additionally, a number of cross-sectional and longitudinal diffusion-weighted imaging (DWI) studies have reported atypical increases in structural connectivity (indexed by greater fractional anisotropy [FA]) across multiple white matter tracts (e.g., corpus callosum, cingulum, arcuate fasciculus) in infants and toddlers who either have been or are later diagnosed with ASD (Conti et al., 2017; Solso et al., 2016; Wolff et al., 2012; Xiao et al., 2014). The early increase in structural connectivity – contrasted with generally reduced structural connectivity (i.e., lower FA) reported in older children and adults with ASD (Travers et al., 2012) – is thought to indicate an accelerated white matter growth in the first years of life in ASD, consistent with the aforementioned accelerated volumetric growth.

Besides these findings of early structural brain abnormalities in ASD detected with anatomical and diffusion MRI, a growing number of studies using functional MRI (fMRI) acquired during natural sleep have contributed unique information on the functional architecture of neural networks in young children with ASD. Earlier studies using fMRI activation to speech stimuli delivered during natural sleep have largely focused on brain function in putative language

regions, given that language delay had been one of the core symptoms required for an ASD diagnosis prior to the most recent DSM-5 diagnostic conceptualization of ASD. These studies have found reduced neural activity in response to speech sounds, absent or reversed hemispheric lateralization for language processing, and diminished interhemispheric synchronization of language region activation patterns in young children (1–4 years) with ASD (Dinstein et al., 2011; Eyler, Pierce, & Courchesne, 2012; Lombardo et al., 2015; Redcay & Courchesne, 2008). More recently, studies have begun examining functional connectivity patterns in infants and toddlers who have been, or are later diagnosed with ASD. While Shen and colleagues (Shen et al., 2016) reported weaker cortical and subcortical amygdala connectivity in preschoolers with ASD (mean age 3.5 years) compared to typically developing controls (which is significant in the context of amygdala's critical involvement in social cognition), most other studies to date have utilized data acquired in infants with familial risk for ASD (i.e., those with an older sibling with autism) followed prospectively (e.g., the Infant Brain Imaging Study [IBIS]). The evidence beginning to emerge from these studies suggests that (a) whole-brain functional connectivity patterns at 6 months appear to predict clinical best-estimate diagnosis of autism at two years (Emerson et al., 2017), and (b) distinct functional brain networks identified in both low- and high-risk infants at 12 months are associated with the emergence of gross motor (walking; Marrus et al., 2018) and fundamental social skills (initiation of joint attention; Eggebrecht et al., 2017), as well as with the core ASD symptoms such as restricted and repetitive behaviors (McKinnon et al., 2019), both at 12 and 24 months. Notably, while these prospective studies provided important insights into the development of functional brain networks underlying specific behavioral outcomes (i.e., walking and joint attention), most (Eggebrecht et al., 2017; Emerson et al., 2017; Marrus et al., 2018) focused on outcomes *shared* by all high- and low-risk

children. Thus, still little is known about the organization and development of functional brain networks in ASD most proximal to the onset of its core symptomatology. This gap in knowledge is critical because studies in older children with ASD have provided robust evidence of disrupted brain connectivity in school age and adolescence (Fishman, Datko, Cabrera, Carper, & Müller, 2015; Fishman, Keown, Lincoln, Pineda, & Müller, 2014; Fishman, Linke, Hau, Carper, & Müller, 2018), which persists into adulthood (cf. (Hull et al., 2016) for review). This life-long broad dysconnectivity likely reflects cascading and pervasive effects of early neurodevelopmental abnormalities of brain function and organization; however, the extent to which it is present early in life – when the defining behavioral features of ASD first emerge and are identifiable – remains unknown. Understanding the full developmental course of brain network abnormalities in ASD is needed in order to make any causal or mechanistic inferences about neurodevelopmental origins of ASD.

Yet, other brain maturational trajectories remain even more opaque in ASD. For instance, to our knowledge, intracortical myelination has not been directly studied in ASD, despite its central role in brain maturation and neural wiring and connectivity (Barbas, 2015). A few recent studies have examined the gray-white matter boundary contrast (GWC) in ASD (although not in the first years of life), which is methodologically related to in-vivo ascertainment of myelin content from MRI data. Specifically, lower GWC indicates that gray matter (GM) and white matter (WM) intensities are more similar, while a higher contrast reflects a sharper GM-WM transition. Yet, the biological interpretation of GWC is more complex as it can be driven by a number of neurodevelopmental processes such as neuronal migration and myelination. Andrews and colleagues (2017) first reported reduced GWC in adults with ASD indicating a less distinct gray-white matter boundary in ASD, consistent with prior postmortem findings (Avino &

Hutsler, 2010). Further, examination of gray matter intensity (GMI) across the cortical layers at different depths into the cortical sheet has revealed increased GMI in many regions with reduced GWC, suggesting that ASD may be primarily associated with disruptions to the GM rather than the WM (Andrews et al., 2017). It has been proposed that the increased GMI may be driven by atypical myelination (Sowell et al., 2004) and/or differences in cytoarchitectural organization (Casanova, Buxhoeveden, Switala, & Roy, 2002). A subsequent study investigating age-related changes of GWC in children and young adults with ASD found that the most prominent decreases in GWC occur during childhood (Mann et al., 2018), which suggests that the disrupted GWC in ASD may not be exclusively driven by atypical GM cytoarchitecture (typically set around birth) but rather reflects atypical myelination. Although GWC is not a specific index of intracortical myelin, these recent studies on atypical GWC in ASD suggest that the developmental trajectory of intracortical myelin may be disrupted in ASD. This is further supported by a recent study (Olafson et al., 2021), which employed a novel metric capturing cortical blurring, boundary sharpness coefficient (BSC), in a large multisite dataset of individuals with ASD spanning a wide age range (4 – 65 years). The results revealed increased BSC, indicating sharper transition from gray to white matter, in lateral frontal and temporal regions, which may reflect a reduction of intracortical myelin content in ASD. The validity of this interpretation is supported by the general agreement between the BSC map in this cohort and the intracortical myelin maps indexed by T1w/T2w ratio obtained in a large cohort of healthy adults by Glasser et al (2011). However, given the lack of direct investigations of intracortical myelin content in ASD, and during early childhood in particular, studies examining intracortical myelination in young children with ASD are warranted to enhance our understanding of disrupted neurodevelopment in autism.

## 3. General Aims

This staple dissertation aimed to use multimodal MRI data to comprehensively examine multiple aspects of brain development in the first years of life in ASD. Generally, we sought to understand how (and potentially when) the postnatal brain maturation deviates from typical development, and whether early brain markers are related to ASD symptomatology and overall developmental skills in the first years of life. The main objectives were as follows: 1) to better characterize the large-scale resting-state functional networks in toddlers and preschoolers with ASD, 2) to examine intracortical myelination in young children with ASD, 3) to investigate multimodal covariation patterns between brain morphometry including surface area and cortical thickness and local spontaneous activity in young children with ASD, and 4) to relate brain indices derived across the abovementioned modalities to overall developmental abilities and ASD symptomatology. Overall, these 3 studies combined improve our understanding of multiple aspects of structural and functional brain development in ASD between 1 and 5 years of age, when autism behavioral symptoms first emerge and manifest.

# CHAPTER 1: STUDY 1

The content within this section, titled "Chapter 1: Study 1," reflects material from a paper that has been published in the *Journal of Child Psychology and Psychiatry*. The full citation is as follows:

Chen, B., Linke, A., Olson, L., Ibarra, C., Reynolds, S., Müller, R. A., Kinnear, M., & Fishman, I. (2021). Greater functional connectivity between sensory networks is related to symptom severity in toddlers with autism spectrum disorder. *Journal of Child Psychology and Psychiatry*, *62*(2), 160–170. https://doi.org/10.1111/jcpp.13268

## ABSTRACT

Symptoms of autism spectrum disorder (ASD) emerge in the first years of life. Yet, little is known about the organization and development of functional brain networks in ASD proximally to the symptom onset. Further, the relationship between brain network connectivity and emerging ASD symptoms and overall functioning in early childhood is not well understood. Resting-state fMRI data were acquired during natural sleep from 24 young children with ASD and 23 typically developing (TD) children, ages 17 to 45 months. Intrinsic functional connectivity (iFC) within and between resting-state functional networks was derived with independent component analysis (ICA). Increased iFC between visual and sensorimotor networks was found in young children with ASD compared to TD participants. Within the ASD group, the degree of overconnectivity between visual and sensorimotor networks was associated with greater autism symptoms. Age-related weakening of the visual-auditory between-network connectivity was observed in the ASD but not the TD group. Taken together, these results provide evidence for disrupted functional network maturation and differentiation, particularly involving visual and sensorimotor networks, during the first years of life in ASD. The observed pattern of greater visual-sensorimotor between-network connectivity associated with poorer clinical outcomes suggests that disruptions in multisensory brain circuitry may play a critical role for early development of behavioral skills and autism symptomatology in young children with ASD.

## Introduction

Autism spectrum disorders (ASD) comprise a group of neurodevelopmental disorders clinically characterized by social communication deficits and restricted and repetitive behaviors (American Psychiatric Association, 2013). These impairments affect the individual's ability to function socially, at school, at work, or in other areas of life, often throughout the lifespan. Symptoms of ASD emerge in the first years of life, and can be reliably identified during the second year of life (Chlebowski, Robins, Barton, & Fein, 2013; Pierce et al., 2019). Yet, most children with ASD are not diagnosed until approximately 4 years of age (Baio et al., 2018) creating a missed opportunity for early implementation of interventions shown to be most effective in the first years of life, at the time when the brain undergoes profound maturational changes providing a fertile ground for maximal learning and improvements. In typical neurodevelopment, the early years are marked by major morphological changes in cortical lamination (Petanjek, Judas, Kostovic, & Uylings, 2008), neuronal differentiation and axon myelination (Huttenlocher, 1984; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008), synaptogenesis and synapse elimination (Huttenlocher & Dabholkar, 1997), and intragyral connectivity (Mrzljak, Uylings, Van Eden, & Judas, 1990). These progressive and regressive processes contribute to the increasing cortical differentiation and specialization of neural pathways, through continuous activity-dependent interactions between brain regions (Fair et al., 2009; Johnson, 2000, 2003), characterizing a typical maturational course for brain circuits.

The extent to which in ASD these neurodevelopmental processes take an aberrant path in the early years, proximally to the symptom onset, remains largely unknown. Despite the sizeable (albeit often inconsistent) evidence of atypical brain structure, function, and connectivity in older children, adolescents and, to a lesser degree, adults with ASD (Ecker, Bookheimer, & Murphy,

2015; Hull et al., 2016), the current understanding of brain network organization and connectivity in the first years of life in autism is limited by the scarcity of brain imaging studies in young children (before 4-5 years of age) due to the challenges of acquiring usable imaging data at that age. Within this more limited MRI literature on infants and toddlers with ASD, a relatively well-established finding is atypically increased brain growth in the first years of life (Courchesne et al., 2001; Hazlett et al., 2005; Hazlett et al., 2011; although see Nordahl et al., 2011). This early brain overgrowth appears to affect both white and gray matter volumes (Hazlett et al., 2005), is driven by surface area rather than cortical thickness expansion and, based on longitudinal evidence, reflects accelerated growth rate between ages one and two years (Hazlett et al., 2011). Additionally, a number of cross-sectional and longitudinal diffusion-weighted imaging (DWI) studies have reported increased fractional anisotropy (FA) across multiple white matter tracts (e.g., corpus callosum, cingulum, arcuate fasciculus) in infants and toddlers who either have been or are later diagnosed with ASD (Conti et al., 2017; Solso et al., 2016; Wolff et al., 2012; Xiao et al., 2014). Notably, these findings of increased structural connectivity indices (i.e., increased FA) in the first years of life in autism are in contrast with generally reduced connectivity (i.e., lower FA) reported in older children and adults with ASD (Travers et al., 2012), suggesting an accelerated white matter growth in the first years of life, consistent with the aforementioned atypical volumetric growth trajectories.

Besides these findings of early structural brain abnormalities in ASD detected with anatomical and diffusion MRI, a growing number of studies using functional MRI (fMRI) acquired during natural sleep (Dean et al., 2014; Nordahl et al., 2016) have contributed unique information on the functional architecture of neural networks in infants and toddlers with ASD. Studies using fMRI activation to speech stimuli delivered during natural sleep have largely

focused on brain function in putative language regions and found reduced neural activity in response to speech sounds, absent or reversed hemispheric lateralization typically associated with language processing, as well as diminished inter-hemispheric synchronization of language region activation patterns in young children (1 to 4 years) with ASD (Dinstein et al., 2011; Eyler, Pierce, & Courchesne, 2012; Lombardo et al., 2015; Redcay & Courchesne, 2008). Additionally, a handful of studies have examined intrinsic functional connectivity (iFC) in infants and toddlers who have been, or are later diagnosed with ASD. While Shen and colleagues (Shen et al., 2016) reported weaker cortical and subcortical amygdala connectivity in preschoolers with ASD (mean age 3.5 years) compared to typically developing controls, all the other studies to date have utilized data acquired in infants with high familial risk for ASD, due to having an older sibling with autism (i.e., the Infant Brain Imaging Study [IBIS]). The evidence beginning to emerge from this cohort of at-risk infants followed prospectively suggests that (a) whole-brain iFC patterns at 6 months appear to predict clinical best-estimate diagnosis of autism at two years (Emerson et al., 2017), and (b) distinct functional brain networks identified in both low- and high-risk infants at 12 months are associated with the emergence of gross motor (walking) (Marrus et al., 2018) and fundamental social skills (initiation of joint attention) (Eggebrecht et al., 2017), as well as with restricted and repetitive behaviors (McKinnon et al., 2019), both at 12 and 24 months. Notably, while these prospective studies provided important insights into the development of functional brain networks underlying specific behavioral outcomes (i.e., walking and joint attention), three (Eggebrecht et al., 2017; Emerson et al., 2017; Marrus et al., 2018) out of the four studies focused on outcomes shared by all high- and low-risk children.

In the face of this evidence, still little is known about the organization and development of functional brain networks in ASD proximally to the onset of core symptomatology. The

human brain is intrinsically organized into large-scale, coherent functional networks, which reflect strong coupling of the ongoing brain activity fluctuations in different brain regions, robustly detected under different mental states, including wakefulness, sleep and anesthesia (Power, Fair, Schlaggar, & Petersen, 2010; Raichle, 2010). Functional brain networks, including primary sensory and, more variably, higher-order supramodal networks, such as default mode and frontoparietal networks, have been reliably identified early in life in typical development (Fransson et al., 2007; Gao, Alcauter, Elton, et al., 2015; Gao, Alcauter, Smith, Gilmore, & Lin, 2015), with some observed even prenatally with fetal imaging methods (Thomason et al., 2013). While the primary sensory networks undergo subtle refinement and strengthening over the first two years of life, and substantially resemble adult topology by age two (Gao, Alcauter, Elton, et al., 2015; Lin et al., 2008), higher-order functional networks are far from the adult-like organization in the first postnatal years, and undergo prolonged development over the first decades of life (Dosenbach et al., 2010; Fair et al., 2008; Gao, Alcauter, Elton, et al., 2015), in parallel with the order in which behavioral and cognitive skills emerge (Johnson, 2001). These trajectories of functional network integration and differentiation have not been mapped in ASD, and it remains unknown how or when the network maturation in the first years of life in ASD deviates from typical development, and whether it is related to ASD symptomatology and overall functioning.

In the current study, we examined the large-scale functional networks in toddlers and preschoolers with ASD, compared to typically developing (TD) controls, between the ages of 1.5 and 3.5 years, using resting-state fMRI acquired during natural nocturnal sleep. We utilized independent component analysis (ICA), a data-driven approach, to derive resting-state functional networks (RFNs), and compared the iFC within and between RFNs in the ASD and TD cohorts.

We hypothesized that, when compared to matched TD controls, young children with ASD would exhibit atypical iFC patterns involving primary sensorimotor networks, and that the atypical connectivity would be associated with more severe autism symptoms and impaired developmental outcomes.

## Methods

### Participants

Participants were enrolled in the San Diego State University (SDSU) Toddler MRI Project, an ongoing longitudinal study of early brain markers of ASD. Children between the ages of 16 and 48 months with a diagnosis of ASD or behavioral concerns consistent with ASD symptoms were referred to the Toddler MRI Project from specialty autism clinics, state-funded early education and developmental evaluation programs, local pediatricians, service providers, and community clinics. Typically developing (TD) children were recruited from the community. Participants in either group were screened and excluded for any comorbid neurological disorders (e.g., cerebral palsy), history of perinatal CNS infection or gross CNS injury, non-febrile seizures, contraindications for MRI. Participants with known syndromic forms of ASD (e.g., fragile X or Rett syndrome), as ascertained from parent report, were also excluded. To limit known risk factors for developmental delays among children enrolled in the TD group, TD participants were also screened and excluded for prematurity (<36 weeks of gestation), family history (in first-degree relatives) of ASD, intellectual disability, or other heritable psychiatric or neurological disorders. The research protocol was approved by the institutional review boards of SDSU and University of California San Diego (UCSD), and the County of San Diego Health and Human Services Agency. Written informed consent was obtained from the caregivers. This

report includes cross-sectional data only from 24 children with ASD and 23 TD participants, matched at the group level on age and gender distribution (see Table 2.1).

**Diagnostic and Developmental Assessment**

Upon enrollment, diagnoses of ASD (or clinical best estimate (Ozonoff et al., 2015) in children younger than age 3 years) were established in all participants in the ASD group in a specialty clinic (SDSU Center for Autism and Developmental Disorders) based on the DSM-5 (American Psychiatric Association, 2013) criteria, supported by the Autism Diagnostic Observation Schedule-Second Edition (ADOS-2; Lord et al., 2012) administered by research-reliable clinicians, the Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & Le Couteur, 1994), in children older than 36 months), and expert clinical judgment (by two senior authors). Developmental skills were assessed in all TD and ASD participants with the Mullen Scales of Early Learning (MSEL) (Mullen, 1995), a clinician-administered assessment of cognitive, language, and motor development. Parents also completed the Social Communication Questionnaire (SCQ, Current form; Lord & Rutter, 2003), a screener for autism spectrum disorders, with no TD participants exceeding the cut-off score of 15 (all TD scores ≤ 10; see Table 2.1).

**MRI Data Acquisition**

MRI data were collected during natural nocturnal sleep on a GE Discovery MR750 3T MRI scanner at the UCSD Center for Functional Magnetic Resonance Imaging, using a Nova Medical 32-channel head coil. A multiband multi-echo planar imaging (EPI) sequence allowing simultaneous acquisition of multiple slices was used to acquire two fMRI runs (400 volumes per each 6-minute run) with high spatial resolution and fast acquisition (TR=800ms, TE=35ms, flip angle=52°, 72 slices, multiband acceleration factor=8, 2mm isotropic voxel size,

matrix=104x104, FOV=20.8cm). Two separate 20s spin-echo EPI sequences with opposing phase encoding directions were also acquired using the same matrix size, FOV and prescription to correct for susceptibility-induced distortions. High-resolution anatomical images were acquired with a fast 3D spoiled gradient recalled (FSPGR) T1-weighted sequence (0.8mm isotropic voxel size, NEX=1, TE/TI=min full/1060ms, flip angle=8°, FOV=25.6cm, matrix=320x320, receiver bandwidth 31.25hz). Motion during anatomical scans was corrected in real-time using three navigator scans and real-time prospective motion correction (PROMO) (White et al., 2010), and images were bias corrected using the GE PURE option.

In preparation for the scan night, and to optimize MRI data acquisition, a comprehensive habituation protocol was implemented. An individualized scan night sleep strategy (e.g., time of arrival, approximating home-like sleeping arrangements, including access to a double MRI bed for co-sleeping families, rocking chair, modular playpen mounted on the MRI bed, lighting in the MRI suite, etc.) was developed for each child, based on the typical bedtime routines and habits assessed in advance with an in-house Sleep Habits Questionnaire. To habituate the child to the scanning environment, the parents were instructed to practice nightly inserting soft foam child-size earplugs after the child had fallen asleep, and to play an mp3 file containing the MRI sounds of the scan sequences employed in the study at progressively louder volumes for a week. On the night of the scan, noise protection was achieved with MRI compatible sound reducing headphones and earplugs. In an attempt to standardize sleep stage during scans, scanning always commenced after approximately 15-30min of sleep.

**Data Analysis**

*MRI data pre-processing*

MRI data were preprocessed with FMRIB's Software Libraries (FSL v5.0.10) (Smith et al., 2004), Matlab 2015b (Mathworks Inc., Natick, MA, USA) using SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, UK), and the CONN toolbox v17f (Whitfield-Gabrieli & Nieto-Castanon, 2012; http://www.nitrc.org/projects/conn). Preprocessing steps included correction for susceptibility-induced distortions using the two spin-echo EPI acquisitions with opposite phase encoding directions and FSL's TOPUP tools; motion correction using rigid-body realignment as implemented in SPM12; spatial smoothing using a 6mm Gaussian kernel at full-width half maximum; outlier detection using the Artifact Detection Toolbox as installed with CONN v17f (ART; https://www.nitrc.org/projects/artifact_detect) to identify outlier volumes with frame-wise displacement (FD) >0.5mm and/or changes in signal intensity >3 standard deviations; nuisance regression including censoring of outliers detected by the ART toolbox, regression of the 6 motion parameters and their derivatives, and the first five PCA components derived from the CSF and white matter compartments using aCompCor (Behzadi, Restom, Liau, & Liu, 2007); and band-pass temporal filtering (0.008-0.08 Hz).

The structural images were co-registered to the mean functional image, segmented and normalized to the Montreal Neurological Institute (MNI) atlas space using non-linear registration and the default tissue probability maps included with SPM12 (see Supplementary Methods for details). The white matter (WM) and CSF probability maps obtained from segmentation of the structural image for each subject were thresholded at 0.95 and eroded by 1 voxel. These thresholded and eroded masks were applied to functional images to extract WM and CSF time courses, which were submitted to a principal component analysis with aCompCor (Behzadi et al., 2007) for subsequent nuisance regression. Functional images were directly normalized to MNI space with the same non-linear registration as used for the structural images.

In order to ensure that the findings were not affected by group differences in motion, ASD and TD groups were matched, at the group level, on mean head motion indexed by root mean square of displacement (RMSD) across two fMRI runs, calculated from rigid-body realignment of the raw data prior to TOPUP correction, on the percentage of censored volume across two fMRI runs, and on the mean temporal signal to noise ratio (tSNR) across two fMRI runs (Table 2.1). Mean RMSD was also included as a covariate for all imaging analyses.

*Independent component analysis*

Preprocessed fMRI data (concatenated across two runs) from all ASD and TD participants combined were entered into group independent component analysis (ICA) using CONN's ICA implementation (Calhoun, Adali, Pearlson, & Pekar, 2001) to generate maximally independent intrinsic functional networks. Each subject contributed 800 volumes (across two runs) to the group ICA for a total of 37,600 3D volumes. Twenty independent components (ICs) were extracted, and each component's spatial distribution and time course were visually inspected by two raters. ICs identified as noise (Beckmann, 2012) (i.e., motion, cerebral spinal fluid pulsations, signal from large blood vessels) were excluded from further analyses. The remaining ICs were compared to the 20 components generated by Smith et al. (2009) and the 8 components generated from the Human Connectome Project Consortium's 500 Subjects Release (https://db.humanconnectome.org/data/projects/HCP_500), both based on adult data, as well as to published pediatric RFNs (Manning, Courchesne, & Fox, 2013; Thornburgh et al., 2017). This resulted in 10 ICs being classified as RFNs and retained for functional connectivity analyses (for details on the three-step IC identification and selection process, see Supplementary Methods).

*Functional connectivity analyses*

The retained ICs were thresholded at z>3.0 and extracted as group RFN maps. Within these thresholded RFN maps clusters exceeding 200 voxels (see Supplementary Table S1 for detailed cluster description) were extracted as regions of interest (20 ROIs) and entered into ROI-to-ROI connectivity analysis. Specifically, for each participant, the average time series of all voxels within each ROI was computed and then correlated with the average time series computed for every other ROI. The resulting Pearson's correlation coefficients (190 ROI-to-ROI pairs: (20x19)/2) were converted to normally distributed z values (using Fisher r-to-z transformation) and entered into two-sample t-tests (including RMSD as covariate) to examine between-group (ASD vs. TD) functional connectivity patterns. Two-sided cluster-level False Discovery Rate (FDR) correction implemented in the CONN toolbox was applied (corrected $p_{FDR}<.05$).

*Correlations with developmental and diagnostic indices*

Pearson's partial correlation analyses were conducted to examine relationships between connectivity indices emerging from the above FC analysis and autism symptoms (ADOS-2), controlling for head motion and overall developmental level indexed by MSEL Early Learning Composite. Due to the limited range and ordinal scale of the ADOS-2 Calibrated Severity Scores (CSS), not suited for correlational analyses, ADOS-2 Total scores yielding a wider range and more continuous distribution were utilized in behavioral correlations, while controlling for ADOS-2 Module.

## Results

Participant demographic, diagnostic, and behavioral characteristics are presented in Table 1.1. As expected, groups differed on indices of cognitive, language, and, motor development measured with MSEL, with significantly lower scores observed in toddlers and preschoolers with

ASD. No TD participants had a MSEL Early Learning Composite score < 80, which is equivalent to no more than 1.3 SD below the normative mean.

*Resting-state functional networks (RFNs) identified in the combined ASD-TD sample*

The 10 ICs classified as non-artifact functional networks (RFNs) largely resembled the previously reported networks in adults and children, including the visual, sensorimotor, auditory, multimodal-sensory, and salience networks (see Figure 1.1 for spatial maps and Supplementary Table S1 for detailed clusters description).

*Group differences in functional connectivity*

Connectivity matrices of mean ROI-to-ROI connectivity (z values) within TD and ASD groups are shown in Figure 1.2A. Direct group comparisons revealed significantly greater connectivity (corrected $p_{FDR}$ <0.05) between ROIs in visual and sensorimotor networks in the ASD group compared to the TD group, after controlling for RMSD (see Figure 1.2B). Because the ASD group included two children born prematurely (see Table 1.1), these analyses were repeated after excluding these two participants, with the results remaining largely unchanged. In order to further examine iFC differences, detection of which may have been impeded by the large number of comparisons, a post-hoc analysis grouping RFNs and corresponding ROIs into five overarching functional domains (visual, sensorimotor, auditory, multimodal-sensory, and salience; see Supplementary Figure S1) was conducted. Direct group comparisons of the within-domain network connectivity, calculated as the mean iFC within all-domain ROIs, and between-domain network connectivity, calculated for all between-domain pairs (10 between-domain comparisons: (5x4)/2), revealed no significant group differences after FDR correction (at $p_{FDR}$<.05; see Supplementary Figure S1), although greater connectivity between all-visual and

salience networks in the ASD group was observed at an uncorrected $p = 0.057$ with medium effect size (Cohen's $d = 0.55$).

*Between-network connectivity and its links with developmental and clinical indices*

Given that both ROI-ROI analyses and comparisons at the level of functional domains pointed to group differences in between-network connectivity involving visual, sensorimotor and salience networks (Figure 1.2 and Supplementary Figure S1), partial correlational analyses were conducted to examine whether between-network iFC was associated with developmental outcomes within the ASD group. Because, as expected in young children with ASD, there was a significant, negative correlation between autism symptoms measured with the ADOS-2 Total score and the overall developmental level indexed with the MSEL Early Learning Composite (ELC) score ($r = -0.53$, $p = 0.008$), partial correlation analyses between 10 between-domain network connectivity indices (mean z-scores for 10 between-domain comparisons: Vis-SM, Vis-Aud, Vis-MSen, Vis-SN, SM-Aud, SM-MSen, SM-SN, Aud-MSen, Aud-SN, MSen-SN) and autism symptomatology were conducted while controlling for ELC (as well as for RMSD and ADOS-2 module). Results revealed a significant positive correlation between autism symptoms (ADOS-2 Total scores) and iFC between visual and sensorimotor domains ($r = 0.60$, $p_{FDR} < 0.05$), controlling for RMSD, MSEL ELC, and ADOS-2 module, such that greater visual-sensorimotor between-domain connectivity was associated with greater ASD symptoms (see Figure 2.3). Results of the supplementary correlational analyses between iFC and MSEL developmental indices are depicted in Supplementary Figures S2 and S3.

*Age-related effects on between-network iFC*

Partial correlation analyses of between-domain network connectivity and age, in months, were performed to examine whether age moderated between-group iFC effects, controlling for

head motion. In children with ASD, a negative correlation with age was observed for the connectivity between visual and auditory networks after controlling for RMSD ($r$ = -0.54, $p$ = 0.01), with visual-auditory between-domain iFC weakening with age (Supplementary Figure S4). This relationship was not present in TD toddlers ($r$ = -0.22, $p$ = 0.33), and there was no significant group by age interaction.

## Discussion

We used resting-state fMRI data acquired during natural sleep to examine large-scale resting-state functional networks in toddlers and preschoolers with ASD compared to matched TD controls. A set of RFNs, identified through data-driven group-ICA, largely corresponded with RFNs previously reported in studies of older children and adults (e.g., visual, auditory, sensorimotor, salience networks). Functional connectivity analyses of within- and between-network connectivity revealed increased between-network connections in the ASD group, specifically between regions in the visual and sensorimotor networks. Critically, among the children with ASD, greater connectivity between the visual and sensorimotor functional domains was associated with increased autism symptomatology, while controlling for the overall developmental level.

### *Overconnectivity between sensory circuits in the first years of life in ASD*

The finding of overconnectivity observed between visual and sensorimotor networks in young children with ASD is remarkable in the context of sensory processing abnormalities and multisensory integration deficits frequently reported in ASD. Prevalence estimates of abnormal sensory processing in children with ASD range from 60 to 96% (Dawson & Watling, 2000; Dunn, Myles, & Orr, 2002; Klintwall et al., 2011; Lane, Dennis, & Geraghty, 2011) and sensory disturbances are now recognized as part of the core symptoms of ASD in the DSM-5. Besides

hypo- and/or hyper-sensitivity to sensory stimuli within single modality (e.g., visual, auditory, tactile, olfactory), children with ASD often exhibit impairments in integrating sensory information across different modalities (Baum, Stevenson, & Wallace, 2015; Iarocci & McDonald, 2006; Stevenson, Siemann, Schneider, et al., 2014; Stevenson, Siemann, Woynaroski, et al., 2014). These sensory symptoms typically manifest early in development (Baranek et al., 2013; Estes et al., 2015; Germani et al., 2014), as early as infancy, as demonstrated with prospective studies of infant siblings with high familial risk for ASD (Ozonoff et al., 2010). The early emerging sensory abnormalities are likely to have cascading effects on higher-order cognitive, social and communicative impairments in ASD (Thye, Bednarz, Herringshaw, Sartin, & Kana, 2018) because of the close interconnections between motor, cognitive, social, and language development at this age (Oudgenoeg-Paz, Leseman, & Volman, 2015; Oudgenoeg-Paz, Volman, & Leseman, 2012; Walle & Campos, 2014).

Functional connectivity involving primary sensorimotor networks has been implicated in the development of motor skills as well as core symptoms of ASD (e.g., social deficits and restricted and repetitive behaviors) in prospective studies of infant siblings. Specifically, functional connectivity within and between motor and DMN networks was correlated with walking onset and gross motor function (Marrus et al., 2018), while connectivity between visual and higher-order networks, including dorsal attention network and posterior DMN, was associated with initiation of joint attention (Eggebrecht et al., 2017). Finally, functional connectivity between visual and DMN as well as frontoparietal control network appeared to be related to certain aspects of restricted and repetitive behaviors (McKinnon et al., 2019). Although highlighting the role of primary sensory networks in the emergence of key developmental skills, including those associated with core symptoms of ASD (e.g., joint attention, restricted and

repetitive behaviors), these findings are not specific to children with ASD, having been observed *across* both high- and low-risk infants. In a cohort more similar to ours (i.e., preschoolers with ASD, albeit somewhat older, with mean age reported as 3.5 years), Shen and colleagues (2016) observed reduced connectivity between primary visual cortex and sensorimotor regions that was related to sensory hypersensitivity in preschoolers with ASD. While at first this appears inconsistent with our finding of atypically increased connectivity between visual and sensorimotor networks, the disparity can likely be attributed to methodological differences between the studies, with ours focusing on comparisons at broader, network- and functional-domain levels, vs. more targeted, seed-based analyses spotlighting connectivity of primary visual cortex, the earliest cortical area to process incoming visual information (in contrast to all primary and visual association cortices, with manifestly different connectivity fingerprints). This distinction, nonetheless, further highlights the scarcity of published data in this age group, and the need for additional studies on brain network development and organization at this critical stage in young children with ASD.

There is some evidence that functional connectivity involving visual, motor, and somatosensory networks is decreased (Nebel et al., 2016; Oldehinkel et al., 2019), rather than increased in older (school-age) children and young adults with ASD. In line with this evidence, we have detected an age-related effect showing that, among young children with ASD, connectivity between visual and auditory circuits is decreasing with age (albeit cross-sectionally) across the sampled age range. Because this age-related decrease in iFC was absent in the TD group, these results may indicate a distinct developmental trajectory of sensory network maturation and differentiation in ASD, with greater "cross-talk" between different sensory networks early in life, followed by a more protracted weakening of the between-network

functional connectivity, as compared to neurotypical trajectories. While direct comparisons between brain morphometric and iFC indices are, at best, tenuous, this trajectory of early functional overconnectivity followed by later underconnectivity appears to echo the account of the accelerated early brain overgrowth observed in autism during infancy and toddler years, reflecting disrupted neurodevelopmental pathways manifest across different scales and measurements of brain structure and function.

*Early between-network overconnectivity in ASD associated with poorer developmental outcomes and increased symptomatology*

The relationship observed between visual-sensorimotor overconnectivity and greater autism symptoms suggests that brain connections between primary sensory and motor circuits may play an important role in the development of early behavioral skills and autism symptomatology in children with ASD. There is extensive evidence that multisensory processing is crucial for developing fundamental communication and social skills. For example, the ability to integrate auditory and visual information on multisensory perceptual tasks has been linked to greater communication and social skills in children with ASD (Mongillo et al., 2008; Woynaroski et al., 2013). Thus, greater connectivity between visual and sensorimotor networks may indicate inadequate integration of visual and somatosensory input into the socio-affective circuits, as shown in older, school-age children with ASD (Green, Hernandez, Bookheimer, & Dapretto, 2016). Overall, the increased cross-talk between visual and sensorimotor networks in the first years of life and its links to greater autism symptomatology may signify that dysfunctional connectivity within primary sensory circuits has broad effects and may be implicated in the emerging autism symptomatology.

*Potential limitations*

One limitation of the present study is its relatively small sample size (due to the challenges of acquiring usable imaging data in this age group), and the use of cross-sectional data to investigate age-related FC effects. In the future, the analyses presented here may be extended to longitudinal data to elucidate within-subject trajectories of functional network development and its relationship with symptoms and developmental skills. Another limitation of the study is the lack of appropriate measures of sensory processing abnormalities in ASD. Finally, because fMRI data were acquired during natural sleep and sleep stage was not monitored with EEG, potential differences in sleep stage between ASD and TD groups could not be ruled out. Although precise sleep staging would be desirable, it is not feasible in this age group without risk of severe data loss; indeed, no studies to date have reported sleep staging with EEG during sleep MRI scanning in young children.

Lastly, it is also worth noting that, outside of the observed differences pertaining to the increased connectivity between visual and sensorimotor circuits in children with ASD, the patterns of functional connectivity within and between other networks examined in this study were largely comparable in the two groups. While this could be interpreted as evidence of broadly "typical" neurodevelopment of functional brain networks in young children with ASD, a more plausible explanation involves a number of other neurobiological mechanisms not captured by BOLD signal but likely at play, reflecting atypical brain maturation processes in autism. Finally, it is worth considering that the additional fundamental group differences in network connectivity may have been masked by differential maturational trajectories across the sampled age range characterizing typically developing children and those with ASD (as evidenced by at least one connectivity effect with divergent age-related trajectories in ASD and TD children; see Supplementary Figure S4).

*Conclusions*

Taken together, our results are the first to characterize the large-scale resting-state functional networks in toddlers and preschoolers with ASD and to demonstrate increased connectivity between visual and sensorimotor networks in the first years of life in ASD. This greater between-network connectivity involving visual and sensorimotor networks was correlated with less favorable clinical outcomes (i.e., greater autism symptoms), highlighting the role of primary sensory circuits in the emergence of autism symptomatology.

**Acknowledgements**

This research was supported by the National Institutes of Health (R01 MH107802 to IF). The funding sources had no role in study design, writing of the report, or the decision to submit the article for publication.

The authors are grateful to Chris Fong, M.A. and Lisa Mash, M.A., of San Diego State University, and Tiffany Wang, M.S., of University of California, San Diego, for invaluable assistance with data collection. The authors' strongest gratitude goes to the children and families who so generously dedicated their time and effort to this research.

Chapter 1, in full is a reprint of the material as it appears in the Journal of Child Psychology and Psychiatry, 2021. Chen, B., Linke, A., Olson, L., Ibarra, C., Reynolds, S., Müller, R. A., Kinnear, M., and Fishman, I., Wiley, 2021. The dissertation author was the primary investigator and author of this paper.

**Table 1.1 Participant Characteristics**

| | ASD (n = 24) | TD (n = 23) | ASD vs. TD | |
|---|---|---|---|---|
| | **Mean ± SD (Min - Max)** | **Mean ± SD (Min - Max)** | **t/X²** | ***p* value** |
| Age (months) | 30.0 ± 7.3 (18 - 45) | 28.9 ± 8.5 (17 - 44) | t(45) = 0.45 | 0.65 |
| Gender (M/F)[a] | 15/9 | 14/9 | $\chi^2(2) = 0.01$ | 0.91 |
| Ethnicity (Hispanic/Non-Hispanic)[a] | 12/12 | 5/18 | $\chi^2(2) = 4.06$ | 0.04 |
| Race (White/Black/More-than-one/Asian/Unknown) | 13/0/8/1/2 | 14/2/4/2/1 | -- | -- |
| Gestational Age (weeks)[b] | 38.7 ± 2.4 (31 - 43) | 39.5 ± 1.3 (37 - 42) | t(43) = -1.45 | 0.16 |
| Birth Weight (grams)[c] | 3429 ± 617 (2098 - 4600) | 3410 ± 342 (2806 - 4026) | t(42) = 0.12 | 0.90 |
| Mullen Scales of Early Learning (MSEL) | | | | |
| Visual Reception, *T-Score* | 41.3 ± 16.0 (20 - 69) | 56.9 ± 11.8 (36 - 79) | t(45) = -3.78 | <0.001 |
| Visual Reception, *Age Equivalent* | 25.4 ± 11.1 (11 - 57) | 31.6 ± 9.2 (16 - 50) | t(45) = -2.06 | 0.05 |
| Fine Motor, *T-Score* | 35.0 ± 11.1 (20 - 54) | 51.3 ± 13.0 (23 - 80) | t(45) = -4.63 | <0.001 |
| Fine Motor, *Age Equivalent* | 23.2 ± 7.0 (13 - 45) | 28.7 ± 9.7 (16 - 47) | t(45) = -2.22 | 0.03 |
| Receptive Language, *T-Score* | 32.4 ± 13.5 (20 - 61) | 54.9 ± 9.3 (30 - 76) | t(45) = -6.63 | <0.001 |
| Receptive Language, *Age Equivalent* | 20.0 ± 10.9 (7 - 44) | 30.8 ± 7.7 (15 - 47) | t(45) = -3.91 | <0.001 |
| Expressive Language, *T-Score* | 32.6 ± 12.1 (20 - 62) | 49.3 ± 10.2 (28 - 68) | t(45) = -5.10 | <0.001 |
| Expressive Language, *Age Equivalent* | 18.9 ± 10.2 (4 - 47) | 28.0 ± 9.7 (13 - 42) | t(45) = -3.14 | 0.003 |
| Early Learning Composite, *Standard Score* | 74.5 ± 19.8 (49 - 111) | 106.4 ± 16.3 (80 - 136) | t(45) = -6.02 | <0.001 |
| Social Communication Questionnaire (SCQ) | 17.5 ± 8.0 (3 - 35) | 5.1 ± 2.6 (0 - 10) | t(41) = 6.77 | <0.001 |

**Table 1.1 Participant Characteristics – continued**

| | ASD (n = 24) | TD (n = 23) | ASD vs. TD | |
|---|---|---|---|---|
| | **Mean ± SD (Min - Max)** | **Mean ± SD (Min - Max)** | **t/X²** | ***p* value** |
| ADOS-2 | | | | |
| Toddler Module, *Total*[d] | 17.6 ± 5.2 (9 - 25) | -- | -- | -- |
| | **Mean ± SD (Min - Max)** | **Mean ± SD (Min - Max)** | **t/X²** | ***p* value** |
| Module 1, *Total*[d] | 13.4 ± 4.9 (7 - 21) | -- | -- | -- |
| Module 2, *Total*[d] | 12.7 ± 4.2 (8 - 16) | -- | -- | -- |
| Calibrated Severity Score (*across Modules*) | 6.75 ± 2.0 (3 - 10) | -- | -- | -- |
| fMRI motion and SNR indices[e] | | | | |
| Mean RMSD[e] | 0.12 ± 0.04 (0.05 - 0.21) | 0.10 ± 0.03 (0.05 - 0.18) | t(45) = 1.46 | 0.15 |
| % of Volumes Censored[e] | 2.41 ± 2.72 (0 - 8.63) | 1.41 ± 1.64 (0 - 5.50) | t(45) = 1.49 | 0.15 |
| Mean temporal SNR[e] | 30.2 ± 4.4 (20.0 - 36.5) | 30.8 ± 4.1 (18.2 - 37.8) | t(45) = -0.56 | 0.58 |

Note: M = male; F = female; ADOS-2 = Autism Diagnostic Observation Schedule, 2nd Edition; RMSD = root mean squared displacement; SNR = signal to noise ratio

[a] Values denote counts and corresponding $\chi^2$ *p* values. Remaining comparisons reflect two-sample *t*-tests and corresponding *p* values.

[b] Gestational age data are missing for 1 ASD participant. Two ASD participants were born before 36 weeks of gestation (at 31 and 35 weeks).

[c] Birth weight data are missing for 1 TD and 2 ASD participants.

[d] Because the choice of ADOS-2 Module depends on the child's age and language level, 14 ASD participants completed the ADOS-2 Toddler Module; 7 completed the ADOS-2 Module 1; 3 completed the ADOS-2 Module 2.

[e] Mean RMSD, # of volumes censored, and mean temporal SNR were calculated across two fMRI runs.

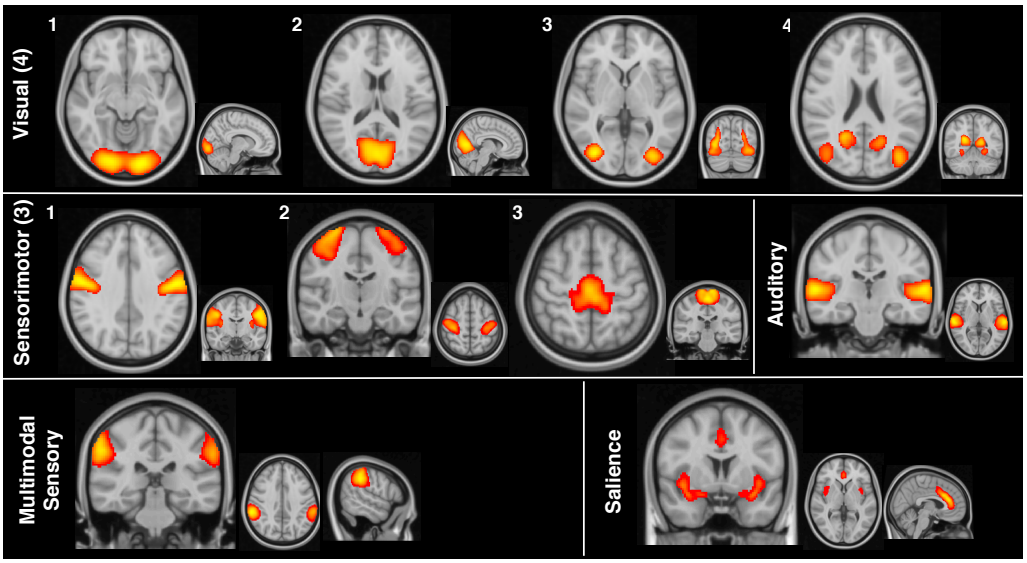


**Figure 1.1 Intrinsic functional connectivity networks in toddlers with and without ASD**
Results of the 20-dimensional group ICA; images are z statistics thresholded at z=3.0 (p<.001) grouped into functional domain categories as depicted. IC labels: Vis1=Occipital pole visual, Vis2=Medial visual, Vis3=Lateral visual, Vis4=Higher order visual, SM1=Primary motor, SM2=Lateral sensorimotor, SM3=Medial sensorimotor, Aud=Auditory, MSen=Multimodal-sensory, SN=Salience. Images are presented in the Montreal Neurological Institute (MNI) space, in neurological convention (with the left side of the brain represented on the left).

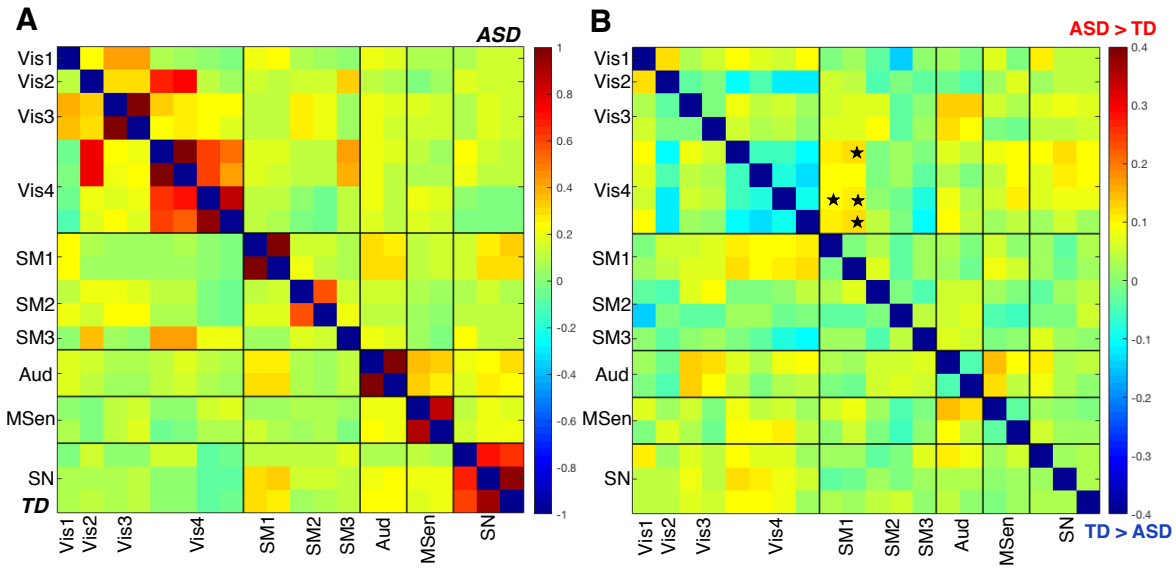


**Figure 1.2 Connectivity matrices between RFN cluster time courses**
(A) Normalized pairwise ROI-ROI (RFN clusters) correlation coefficients (z-values) are presented separately for the ASD (upper triangle) and TD (lower triangle) groups. Both axes represent the 20 RFN clusters (see Supplementary Table S1 for detailed cluster description). Pixel color of each cell represents the magnitude of correlation for each region of interest (ROI) pair, with warmer colors indicating greater correlation coefficient values. (B) Difference connectivity matrix for ASD vs. TD (ASD>TD) comparison. ★ denotes ROI-ROI pairs with significantly stronger connections at FDR corrected $p<0.05$, after controlling for mean RMSD.

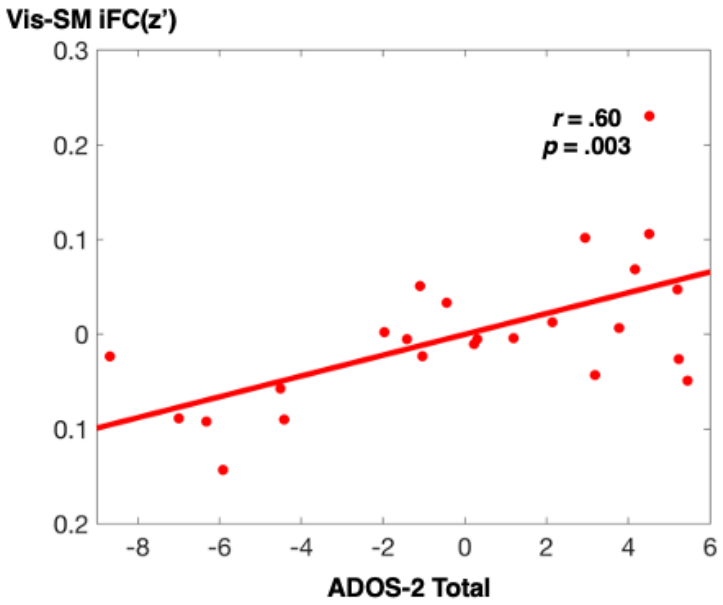


**Figure 1.3 Relationship between autism symptomatology and visual-sensorimotor connectivity in the ASD group**

Partial correlation between connectivity (z scores) between all visual and sensorimotor networks and ADOS-2 Total scores (pFDR <0.05). Increasing ADOS-2 Total values indicate greater symptom count and, hence, greater impairment. The values on the X and Y axes reflect residuals of ADOS-2 and z scores, respectively, after controlling for RMSD, MSEL ELC, and ADOS-2 module.

## References


American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders* (5th ed). Washington, DC: Author.

Baio, J., Wiggins, L., Christensen, D.L., Maenner, M.J., Daniels, J., Warren, Z., … & Dowling, N.F. (2018). Prevalence of Autism Spectrum Disorder among children aged 8 years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR Surveill Summ, 67,* 1-23.

Baranek, G.T., Watson, L.R., Boyd, B.A., Poe, M.D., David, F.J., & McGuire, L. (2013). Hyporesponsiveness to social and nonsocial sensory stimuli in children with autism, children with developmental delays, and typically developing children. *Dev Psychopathol, 25,* 307-320.

Baum, S.H., Stevenson, R.A., & Wallace, M.T. (2015). Behavioral, perceptual, and neural alterations in sensory and multisensory function in autism spectrum disorder. *Prog Neurobiol, 134,* 140-160.

Beckmann, C.F. (2012). Modelling with independent components. *Neuroimage, 62*, 891-901.

Behzadi, Y., Restom, K., Liau, J., & Liu, T.T. (2007). A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *Neuroimage, 37,* 90-101.

Calhoun, V.D., Adali, T., Pearlson, G.D., & Pekar, J.J. (2001). A method for making group inferences from functional MRI data using independent component analysis. *Hum Brain Mapp, 14,* 140-151.

Chlebowski, C., Robins, D.L., Barton, M. L., & Fein, D. (2013). Large-scale use of the modified checklist for autism in low-risk toddlers. *Pediatrics, 131,* e1121-1127.

Conti, E., Mitra, J., Calderoni, S., Pannek, K., Shen, K.K., Pagnozzi, A., … & Guzzetta, A. (2017). Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: A diffusion MRI study. *Hum Brain Mapp, 38,* 2333-2344.

Courchesne, E., Karns, C.M., Davis, H.R., Ziccardi, R., Carper, R.A., Tigue, Z.D., … Courchesne, R.Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology, 57,* 245-254.

Dawson, G., & Watling, R. (2000). Interventions to facilitate auditory, visual, and motor integration in autism: a review of the evidence. *J Autism Dev Disord, 30,* 415-421.

Dean, D.C., Dirks, H., O'Muircheartaigh, J., Walker, L., Jerskey, B.A., Lehman, K., … & Deoni, S.C. (2014). Pediatric neuroimaging using magnetic resonance imaging during non-sedated sleep. *Pediatr Radiol, 44,* 64-72.

Dinstein, I., Pierce, K., Eyler, L., Solso, S., Malach, R., Behrmann, M., & Courchesne, E. (2011). Disrupted neural synchronization in toddlers with autism. *Neuron, 70,* 1218-1225.

Dosenbach, N.U., Nardos, B., Cohen, A.L., Fair, D.A., Power, J.D., Church, J.A., … & Schlaggar, B.L. (2010). Prediction of individual brain maturity using fMRI. *Science, 329,* 1358-1361.

Dunn, W., Myles, B.S., & Orr, S. (2002). Sensory processing issues associated with Asperger syndrome: a preliminary investigation. *Am J Occup Ther, 56,* 97-102.

Ecker, C., Bookheimer, S.Y., & Murphy, D.G. (2015). Neuroimaging in autism spectrum disorder: brain structure and function across the lifespan. *Lancet Neurol, 14,* 1121-1134.

Eggebrecht, A.T., Elison, J.T., Feczko, E., Todorov, A., Wolff, J.J., Kandala, S., … & Pruett J.R. (2017). Joint attention and brain functional connectivity in infants and toddlers. *Cereb Cortex, 27,* 1709-1720.

Emerson, R.W., Adams, C., Nishino, T., Hazlett, H.C., Wolff, J.J., Zwaigenbaum, L., … & Piven, J. (2017). Functional neuroimaging of high-risk 6-month-old infants predicts a diagnosis of autism at 24 months of age. *Science translational medicine, 9,* eaag2882.

Estes, A., Zwaigenbaum, L., Gu, H., St John, T., Paterson, S., Elison, J.T., … & Piven, J. (2015). Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life. *J Neurodev Disord, 7,* 24.

Eyler, L.T., Pierce, K., & Courchesne, E. (2012). A failure of left temporal cortex to specialize for language is an early emerging and fundamental property of autism. *Brain, 135,* 949-960.

Fair, D.A., Cohen, A.L., Dosenbach, N.U., Church, J.A., Miezin, F.M., Barch, D.M., … & Schlaggar, B.L. (2008). The maturing architecture of the brain's default network. *Proc Natl Acad Sci, 105,* 4028-4032.

Fair, D.A., Cohen, A.L., Power, J.D., Dosenbach, N.U., Church, J.A., Miezin, F.M., … & Petersen, S.E. (2009). Functional brain networks develop from a "local to distributed" organization. *PLoS Comput Biol, 5,* e1000381.

Fransson, P., Skiöld, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., & Åden, U. (2007). Resting-state networks in the infant brain. *Proc Natl Acad Sci, 104,* 15531-15536.

Gao, W., Alcauter, S., Elton, A., Hernandez-Castillo, C.R., Smith, J.K., Ramirez, J., & Lin, W. (2015). Functional network development during the first year: relative sequence and socioeconomic correlations. *Cereb Cortex, 25,* 2919-2928.

Gao, W., Alcauter, S., Smith, J.K., Gilmore, J.H., & Lin, W. (2015). Development of human brain cortical network architecture during infancy. *Brain Struct Funct, 220,* 1173-1186.

Germani, T., Zwaigenbaum, L., Bryson, S., Brian, J., Smith, I., Roberts, W., … & Vaillancourt, T. (2014). Brief report: assessment of early sensory processing in infants at high-risk of autism spectrum disorder. *J Autism Dev Disord, 44,* 3264-3270.

Green, S.A., Hernandez, L., Bookheimer, S.Y., & Dapretto, M. (2016). Salience network connectivity in autism is related to brain and behavioral markers of sensory overresponsivity. *J Am Acad Child Adolesc Psychiatry, 55,* 618-626.e611.

Hazlett, H.C., Poe, M., Gerig, G., Smith, R.G., Provenzale, J., Ross, A., … & Piven, J. (2005). Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch Gen Psychiatry, 62,* 1366-1376.

Hazlett, H.C., Poe, M.D., Gerig, G., Styner, M., Chappell, C., Smith, R.G., … & Piven, J. (2011). Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Archives of general psychiatry, 68,* 467-476.

Hull, J.V., Dokovna, L.B., Jacokes, Z.J., Torgerson, C.M., Irimia, A., & Van Horn, J.D. (2016). Resting-state functional connectivity in autism spectrum disorders: a review. *Front Psychiatry, 7,* 205.

Huttenlocher, P.R. (1984). Synapse elimination and plasticity in developing human cerebral cortex. *Am J Ment Defic, 88,* 488-496.

Huttenlocher, P.R., & Dabholkar, A.S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol, 387,* 167-178.

Iarocci, G., & McDonald, J. (2006). Sensory integration and the perceptual experience of persons with autism. *J Autism Dev Disord, 36,* 77-90.

Johnson, M.H. (2000). Functional brain development in infants: elements of an interactive specialization framework. *Child Dev, 71,* 75-81.

Johnson, M.H. (2001). Functional brain development in humans. *Nat Rev Neurosci, 2,* 475-483.

Johnson, M.H. (2003). Development of human brain functions. *Biol Psychiatry, 54,* 1312-1316.

Klintwall, L., Holm, A., Eriksson, M., Carlsson, L.H., Olsson, M.B., Hedvall, A., … & Fernell, E. (2011). Sensory abnormalities in autism. A brief report. *Res Dev Disabil, 32,* 795-800.

Lane, A.E., Dennis, S.J., & Geraghty, M.E. (2011). Brief report: Further evidence of sensory subtypes in autism. *J Autism Dev Disord, 41,* 826-831.

Lebel, C., Walker, L., Leemans, A., Phillips, L., & Beaulieu, C. (2008). Microstructural maturation of the human brain from childhood to adulthood. *Neuroimage, 40,* 1044-1055.

Lin, W., Zhu, Q., Gao, W., Chen, Y., Toh, C.H., Styner, M., … & Gilmore, J.H. (2008). Functional connectivity MR imaging reveals cortical functional connectivity in the developing brain. *AJNR Am J Neuroradiol, 29,* 1883-1889.

Lombardo, M.V., Pierce, K., Eyler, L.T., Barnes, C.C., Ahrens-Barbeau, C., Solso, S., … & Courchesne, E. (2015). Different functional neural substrates for good and poor language outcome in autism. *Neuron, 86,* 567-577.

Lord, C., & Rutter, M. (2003). *Social communication questionnaire* (SCQ). Torrance, CA: WPS.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord, 24,* 659-685.

Lord, C., Rutter, M., DiLavore, P.C., Risi, S., Gotham, K., & Bishop, S.L. (2012). *Autism diagnostic observation schedule, second edition* (ADOS-2). Torrence, CA: Western Psychological Services.

Manning, J.H., Courchesne, E., & Fox, P.T. (2013). Intrinsic connectivity network mapping in young children during natural sleep. *Neuroimage, 83,* 288-293.

Marrus, N., Eggebrecht, A.T., Todorov, A., Elison, J.T., Wolff, J.J., Cole, L., … & Pruett J.R. (2018). Walking, gross motor development, and brain functional connectivity in infants and toddlers. *Cereb Cortex, 28,* 750-763.

McKinnon, C.J., Eggebrecht, A.T., Todorov, A., Wolff, J.J., Elison, J.T., Adams, C.M., … & Pruett, J.R. (2019). Restricted and repetitive behavior and brain functional connectivity in infants at risk for developing autism spectrum disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging, 4,* 50-61.

Mongillo, E.A., Irwin, J.R., Whalen, D.H., Klaiman, C., Carter, A.S., & Schultz, R.T. (2008). Audiovisual processing in children with and without autism spectrum disorders. *J Autism Dev Disord, 38,* 1349-1358.

Mrzljak, L., Uylings, H.B., Van Eden, C.G., & Judas, M. (1990). Neuronal development in human prefrontal cortex in prenatal and postnatal stages. *Prog Brain Res, 85,* 185-222.

Mullen, E.M. (1995). Mullen scales of early learning. Circle Pines, MN: American Guidance Service.

Nebel, M.B., Eloyan, A., Nettles, C.A., Sweeney, K.L., Ament, K., Ward, R.E., … & Mostofsky, S.H. (2016). Intrinsic visual-motor synchrony correlates with social deficits in autism. *Biol Psychiatry, 79,* 633-641.

Nordahl, C.W., Lange, N., Li, D.D., Barnett, L.A., Lee, A., Buonocore, M.H., … & Amaral, D.G. (2011). Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. *Proc Natl Acad Sci, 108,* 20195-20200.

Nordahl, C.W., Mello, M., Shen, A.M., Shen, M.D., Vismara, L.A., Li, D., … & Amaral, D.G. (2016). Methods for acquiring MRI data in children with autism spectrum disorder and intellectual impairment without the use of sedation. *Journal of neurodevelopmental disorders, 8,* 20-20.

Oldehinkel, M., Mennes, M., Marquand, A., Charman, T., Tillmann, J., Ecker, C., … & Buitelaar, J.K. (2019). Altered connectivity between cerebellum, visual, and sensory-motor networks in autism spectrum disorder: results from the EU-AIMS Longitudinal European Autism Project. *Biol Psychiatry Cogn Neurosci Neuroimaging, 4,* 260-270.

Oudgenoeg-Paz, O., Leseman, P.P., & Volman, M.C. (2015). Exploration as a mediator of the relation between the attainment of motor milestones and the development of spatial cognition and spatial language. *Dev Psychol, 51,* 1241-1253.

Oudgenoeg-Paz, O., Volman, M.C., & Leseman, P.P. (2012). Attainment of sitting and walking predicts development of productive vocabulary between ages 16 and 28 months. *Infant Behav Dev, 35,* 733-736.

Ozonoff, S., Iosif, A.M., Baguio, F., Cook, I.C., Hill, M.M., Hutman, T., … & Young, G.S. (2010). A prospective study of the emergence of early behavioral signs of autism. *J Am Acad Child Adolesc Psychiatry, 49,* 256-266.e251-252.

Ozonoff, S., Young, G.S., Landa, R.J., Brian, J., Bryson, S., Charman, T., … & Iosif, A.M. (2015). Diagnostic stability in young children at risk for autism spectrum disorder: a baby siblings research consortium study. *J Child Psychol Psychiatry, 56,* 988-998.

Petanjek, Z., Judas, M., Kostovic, I., & Uylings, H.B. (2008). Lifespan alterations of basal dendritic trees of pyramidal neurons in the human prefrontal cortex: a layer-specific pattern. *Cereb Cortex, 18,* 915-929.

Pierce, K., Gazestani, V.H., Bacon, E., Barnes, C.C., Cha, D., Nalabolu, S., … & Courchesne, E. (2019). Evaluation of the diagnostic stability of the early Autism Spectrum Disorder phenotype in the general population starting at 12 months. *JAMA Pediatr, 173,* 578-587.

Power, J.D., Fair, D.A., Schlaggar, B.L., & Petersen, S.E. (2010). The development of human functional brain networks. *Neuron, 67,* 735-748.

Raichle, M.E. (2010). Two views of brain function. *Trends Cogn Sci, 14,* 180-190.

Redcay, E & Courchesne, E. (2008). Deviant functional magnetic resonance imaging patterns of brain activity to speech in 2-3-year-old children with autism spectrum disorder. *Biol Psychiatry, 64,* 589-598.

Shen, M.D., Li, D.D., Keown, C.L., Lee, A., Johnson, R.T., Angkustsiri, K., … & Nordahl, C.W. (2016). Functional connectivity of the amygdala is disrupted in preschool-aged children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry, 55,* 817-824.

Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., Mackay, C.E., … & Beckmann, C.F. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci, 106,* 13040-13045.

Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E, Johansen-Berg, H., … & Matthews, P.M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage, 23,* S208-219.

Solso, S., Xu, R., Proudfoot, J., Hagler, D.J, Jr., Campbell, K., Venkatraman, V., … & Courchesne, E. (2016). Diffusion tensor imaging provides evidence of possible axonal overconnectivity in frontal lobes in autism spectrum disorder toddlers. *Biol Psychiatry, 79,* 676-684.

Stevenson, R.A., Siemann, J.K., Schneider, B.C., Eberly, H.E., Woynaroski, T.G., Camarata, S.M., & Wallace, M.T. (2014). Multisensory temporal integration in autism spectrum disorders. J *Neurosci, 34,* 691-697.

Stevenson, R.A., Siemann, J.K., Woynaroski, T.G., Schneider, B.C., Eberly, H.E., Camarata, S.M., & Wallace, M.T. (2014). Evidence for diminished multisensory integration in autism spectrum disorders. *J Autism Dev Disord, 44,* 3161-3167.

Thomason, M.E., Dassanayake, M.T., Shen, S., Katkuri, Y., Alexis, M., Anderson, A.L., … & Romero, R. (2013). Cross-hemispheric functional connectivity in the human fetal brain. *Sci Transl Med, 5,* 173ra124.

Thornburgh, C.L., Narayana, S., Rezaie, R., Bydlinski, B.N., Tylavsky, F.A., Papanicolaou, A.C., & Volgyi, E. (2017). Concordance of the resting state networks in typically developing, 6-to 7-year-old children and healthy adults. *Front Hum Neurosci, 11,* 199.

Thye, M.D., Bednarz, H.M., Herringshaw, A.J., Sartin, E.B., & Kana, R.K. (2018). The impact of atypical sensory processing on social impairments in autism spectrum disorder. *Dev Cogn Neurosci, 29,* 151-167.

Travers, B.G., Adluru, N., Ennis, C., Tromp, D.P.M, Destiche, D., Doran, S., … & Alexander, A.L. (2012). Diffusion tensor imaging in autism spectrum disorder: a review. *Autism Res, 5,* 289-313.

Walle, E.A., & Campos, J.J. (2014). Infant language development is related to the acquisition of walking. *Dev Psychol, 50,* 336-348.

White, N., Roddey, C., Shankaranarayanan, A., Han, E., Rettmann, D., Santos, J., … & Dale, A. (2010). PROMO: Real-time prospective motion correction in MRI using image-based tracking. *Magnetic resonance in medicine, 63,* 91-105.

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect, 2,* 125-141.

Wolff, J.J., Gu, H., Gerig, G., Elison, J.T., Styner, M., Gouttard, S., … & Piven, J. (2012). Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. *Am J Psychiatry, 169,* 589-600.

Woynaroski, T.G., Kwakye, L.D., Foss-Feig, J.H., Stevenson, R.A., Stone, W.L., & Wallace, M.T. (2013). Multisensory speech perception in children with autism spectrum disorders. *J Autism Dev Disord, 43,* 2891-2902.

Xiao, Z., Qiu, T., Ke, X., Xiao, X., Xiao, T., Liang, F., … & Liu, Y. (2014). Autism spectrum disorder as early neurodevelopmental disorder: evidence from the brain imaging abnormalities in 2-3 years old toddlers. *J Autism Dev Disord, 44,* 1633-1640.

# CHAPTER 2: STUDY 2

The content within this section, titled "Chapter 2: Study 2," reflects material from a paper that has been published in *Developmental Neurobiology*. The full citation is as follows:

Chen, B., Linke, A., Olson, L., Kohli, J., Kinnear, M., Sereno, M., Müller, R. A., Carper, R., & Fishman, I. (2022). Cortical myelination in toddlers and preschoolers with autism spectrum disorder. *Developmental Neurobiology*, *82*(3), 261–274. https://doi.org/10.1002/dneu.22874

## ABSTRACT

Intracortical myelin is thought to play a significant role in the development of neural circuits and functional networks, with consistent evidence of atypical network connectivity in children with autism spectrum disorders (ASD). However, little is known about the development of intracortical myelin in the first years of life in ASD, during the critical neurodevelopmental period when autism symptoms first emerge. Using T1-weighted (T1w) and T2-weighted (T2w) structural magnetic resonance imaging (MRI) in 21 young children with ASD and 16 typically developing (TD) children, ages 1.5 to 5.5 years, we demonstrate the feasibility of estimating intracortical myelin *in vivo* using the T1w/T2w ratio as a proxy. The resultant T1w/T2w maps were largely comparable with those reported in prior T1w/T2w studies in typically developing children and adults, and revealed no group differences between TD children and those with ASD. However, differential associations between T1w/T2w and age were identified in several early myelinated regions (e.g., visual, posterior cingulate, precuneus cortices) in the ASD and TD groups, with age-related increase in estimated myelin content across the toddler and preschool years detected in TD children, but not in children with ASD. The atypical age-related effects in intracortical myelin, suggesting a disrupted myelination in the first years of life in ASD, may be related to the aberrant brain network connectivity reported in young children with ASD in some of the same cortical regions and circuits.

## Introduction

Although symptoms of autism spectrum disorder (ASD) emerge early in postnatal life (Pierce et al., 2011) and can be reliably identified during the second year of life (Corsello, Akshoomoff, & Stahmer, 2013; Ozonoff, Heung, Byrd, Hansen, & Hertz-Picciotto, 2008; Ozonoff et al., 2015; Pierce et al., 2019; Sacrey et al., 2018), most children with ASD are not diagnosed until they are 4-5 years old (Maenner et al., 2020), in large part due to the lack of clinically meaningful biomarkers. The implications of delayed identification are significant, given the positive impact of early interventions on both behavior and the developing brain (Dawson et al., 2012; Estes et al., 2015; Landa, 2018). The current consensus on the underlying neurobiology is that ASD originates prenatally, affecting early building blocks of brain circuit development and function, such as protein synthesis and cellular metabolism, with cascading effects on neuronal proliferation and migration, synaptogenesis and synaptic signaling, myelination, and network formation (Courchesne et al., 2019; Gordon & Geschwind, 2020). However, the current understanding of these fundamental neurodevelopmental processes (e.g., synaptic pruning (Spear, 2013) or myelination (Abrahám et al., 2010; Chapman & Hill, 2020) is limited by the scarcity of brain imaging studies in young children with ASD (before 4-5 years of age). This is in part due to the known practical and methodological challenges of acquiring high quality imaging data early in life (Turesky, Vanderauwera, & Gaab, 2021).

Although much of the developmental neurobiology of ASD remains unknown, a few consistent findings have emerged from the relatively limited but growing number of MRI studies in infants and toddlers at risk for, or with first symptoms of autism, including prospective studies of infant siblings of older children with ASD. The most consistently reported finding to date is enlargement of brain volume early in life (Courchesne et al., 2001; Hazlett et al., 2005; Hazlett et

al., 2011; Nordahl et al., 2011). This early brain overgrowth appears to reflect accelerated growth rate particularly between one and two years of age (Hazlett et al., 2011), affecting both white and gray matter volumes (Hazlett et al., 2005), and being possibly driven by cortical surface area hyper-expansion between 6 and 12 months of age (Hazlett et al., 2017). Additionally, a number of cross-sectional and longitudinal diffusion-weighted imaging studies have reported increased structural connectivity (indexed by greater fractional anisotropy [FA]) across multiple white matter tracts (e.g., corpus callosum, cingulum, arcuate fasciculus) in infants and toddlers who either have been or are later diagnosed with ASD (Conti et al., 2017; Solso et al., 2016; Wolff et al., 2012; Xiao et al., 2014). The early increase in structural connectivity – contrasted with broadly reduced structural connectivity (i.e., lower FA) in older children and adults with ASD (Travers et al., 2012) – is thought to indicate an accelerated white matter growth in the first years of life in ASD, consistent with the aforementioned accelerated trajectory of volumetric growth.

While these findings provide indirect evidence of altered brain maturation early in life in ASD, our understanding of the specific neurodevelopmental processes contributing to atypical early growth and structural connectivity remains limited. Among such fundamental processes shaping the brain structure and function is myelination which is essential for efficient neural communication (Liu, Li, Zhu, Li, & Liu, 2019), as myelinated axons allow for rapid and reliable propagation of neuronal signals across the brain. Equally critical for brain maturation and connectivity is intracortical myelination found predominately in the deeper cortical layers (Fields, 2014), in part due to spread of the white matter myelin into the periphery of cortical neuropil (Shaw et al., 2008; Sowell et al., 2004). Intracortical myelination is essential for establishing and maintaining neural circuitry, as it contributes to fine-tuning the timing and synchrony of neural networks (Haroutunian et al., 2014). In typical development, the

maturational timing of intracortical myelination, which commences at or near birth (Arnold & Trojanowski, 1996), follows a general primary-to-association cortices gradient (Sydnor et al., 2021), with unimodal primary sensory and motor cortices being highly myelinated by 1 year of age, and transmodal association areas in frontal and temporal cortices exhibiting more protracted myelination, continuing at least through the third decade of life (Deoni, Dean, Remer, Dirks, & O'Muircheartaigh, 2015; Grydeland et al., 2019; Rowley et al., 2017; Shafee, Buckner, & Fischl, 2015). Critically, this maturational principle parallels the development of brain network connectivity in healthy development (Chen, Linke, Olson, Ibarra, Kinnear, et al., 2021; Dong, Margulies, Zuo, & Holmes., 2021; Gao et al., 2015), with recent evidence suggesting that the timing of functional network maturation and differentiation may be disrupted in toddlers with ASD (Chen, Linke, Olson, Ibarra, Reynolds, et al., 2021).

Although no neuroimaging techniques allow direct measurement of myelin in the human brain *in vivo*, advanced MRI acquisition methods permit estimation of myelin content through either quantitative imaging, such as voxel-wise mapping of longitudinal or transverse relaxation times (Bock et al., 2013; Geyer, Weiss, Reimann, Lohmann, & Turner, 2011), or semi-quantitative ratio of T1-weighted (T1w) and T2-weighted (T2w) signal intensity (Glasser et al., 2013; Glasser & Van Essen, 2011). The T1w/T2w ratio has been shown to successfully map the regional differences in myelin content (Glasser & Van Essen, 2011) and has been incorporated in the multimodal minimal preprocessing pipelines for the Human Connectome Project (Glasser et al., 2013). Investigating the change in the T1w/T2w-estimated myelin content over much of the human life span in a large cross-sectional cohort of neurotypical children and adults between ages 8 to 83 years, Grydeland et al. (2013) reported linear increases in the T1w/T2w-estimated intracortical myelin content through the late 30s, followed by about 20 stable years and a gradual

decline from the late 50s. The T1w/T2w ratio has also been associated with cognitive performance, especially on tasks of cognitive control (Grydeland, Walhovd, Tamnes, Westlye, & Fjell, 2013; Grydeland, Westlye, Walhovd, & Fjell, 2016). Thus, the T1w/T2w ratio may be a well-suited MRI-accessible proxy for investigating the development of intracortical myelin in young children with ASD, which, to our knowledge, has yet to be evaluated.

To enhance our understanding of early neurodevelopment in autism, the current study set out to examine age-related effects in intracortical myelin in young children with ASD, compared to typically developing (TD) age-matched peers, using both T1w and T2w structural MRI data acquired during natural nocturnal sleep. Given the lack of previous studies using the T1w/T2w ratio in young children with ASD, we expected to find main effects of diagnosis, but had no a priori hypotheses regarding the direction of potential effects.

## Methods

### Participants

This study includes data from young children enrolled in the San Diego State University (SDSU) Toddler MRI Project, an ongoing longitudinal study of early brain markers of ASD. Children between the ages of 18 and 42 months with a diagnosis of ASD (or behavioral concerns consistent with ASD symptoms) were referred to the study from specialty autism clinics, state-funded early education and developmental evaluation programs, local pediatricians, service providers, and community clinics, and are being followed up through age 5 years. TD children were recruited from the community, including early head start programs, and via print and social media advertisements. Participants in either group were screened and excluded for any co-occurring neurological disorders (e.g., cerebral palsy), history of perinatal CNS infection or gross CNS injury, non-febrile seizures, and contraindications for MRI. Participants with known

syndromic forms of ASD (e.g., fragile X or Rett syndrome), as ascertained from parent report, were also excluded. To limit known risk factors for developmental delays among children enrolled in the TD group, TD participants were also screened and excluded for prematurity (<36 weeks of gestation), family history (in first-degree relatives) of ASD, intellectual disability, or other heritable psychiatric or neurological disorders. The research protocol was approved by the institutional review boards of SDSU and University of California San Diego (UCSD), and the County of San Diego Health and Human Services Agency. Written informed consent was obtained from the caregivers.

This report includes cross-sectional data from 21 children with ASD and 16 TD participants, ages 1.5 - 5 years, for whom high quality T1w and T2w anatomical MRI data acquired in the same session (during natural sleep; see *MRI Data Acquisition* below for details) were available. While 32 children with ASD and 23 TD children had completed the full imaging protocol, data from 11 children with ASD and 7 TD children were excluded following stringent data quality assessment, as detailed below in *MRI Data Preprocessing and Quality Assessment*. Participants with ASD and TD children were matched at the group level on age and gender distribution (see Table 3.1 for demographic characteristics of the sample).

**Diagnostic and Developmental Assessment**

Upon enrollment, diagnoses of ASD (or clinical best estimate (Ozonoff et al., 2015) in children younger than age 3 years) were established at a specialty clinic (SDSU Center for Autism and Developmental Disorders) using standardized measures in combination with clinical judgment, in accordance with the current recommendations by the American Academy of Pediatrics and Society for Developmental and Behavioral Pediatrics (Weitzman & Wegner, 2015). Only participants who met diagnostic criteria for ASD, or clinical best estimate, on the

DSM-5 (APA, 2013) were included in the ASD group. Because diagnostic evaluation is repeated at follow-up visits in the context of the larger longitudinal study, only data from children with confirmed diagnosis were included in the current dataset. The diagnoses were supported by the Autism Diagnostic Observation Schedule-Second Edition (Lord et al., 2012) administered by research-reliable clinicians, the Social Communication Questionnaire (Lord & Rutter, 2003) or the Autism Diagnostic Interview-Revised (Lord, Rutter, & Le Couteur, 1994) administered to caregivers of children 36-month-old and older, and expert clinical judgment (by two senior authors). Developmental skills were assessed in all TD and ASD participants with the Mullen Scales of Early Learning (Mullen, 1995), a clinician-administered standardized assessment of cognitive, language, and motor development. The Vineland Adaptive Behavior Scales, 2nd Edition, Survey Interview (Sparrow, Cicchetti, & Balla, 2005), a semi-structured interview, was administered to caregivers to assess the child's adaptive development skills demonstrated at home and other settings; the Vineland scores were utilized to support the diagnostic and developmental classification, and are not used as variables of interest in the current analyses. The Social Communication Questionnaire (Lord & Rutter, 2003), a screener for autism spectrum disorder, was administered to caregivers of all participants, with no TD participants exceeding the clinical cut-off score of 15 (all TD scores ≤ 10; see Table 3.1).

**MRI Data Acquisition**

MRI data were collected during natural nocturnal sleep on a 3T GE Discovery MR750 MRI scanner, using a Nova Medical 32-channel head coil. Whole-brain high-resolution anatomical images were obtained using a fast 3D spoiled gradient recalled (FSPGR) T1-weighted sequence (voxel size=0.8mm$^3$, NEX=1, TE/TI=min full/1060ms, flip angle=8°, FOV=25.6cm, matrix=320x320, receiver bandwidth 31.25htz) and Cube T2-weighted sequence

(voxel size=0.8mm$^3$, NEX=1, TR=3200ms, TE=minimum, FOV=25.6cm, matrix=320x320, bandwidth 125htz). Motion during T1w and T2w scans was corrected in real-time using three navigator scans and prospective motion correction (White et al., 2010), and images were bias corrected using the GE PURE option. Other MRI data, including functional and diffusion MRI, were also acquired but are not included in this study.

In preparation for the scan night, and to optimize MRI data acquisition, a comprehensive habituation protocol was implemented. An individualized scan night sleep strategy (e.g., time of arrival, approximating home-like sleeping arrangements, including access to a double MRI bed for co-sleeping families, rocking chair, modular playpen mounted on the MRI bed resembling a crib, lighting in the MRI suite, etc.) was developed for each child, based on the typical bedtime routines assessed in advance with an in-house Sleep Habits Questionnaire. To habituate the child to the scanning environment, the parents were instructed to practice nightly inserting soft foam child-size earplugs after the child had fallen asleep, and to play an mp3 file containing the MRI sounds of the scan sequences employed in the study at progressively louder volumes for a week. On the night of the scan, noise protection was achieved with MRI compatible headphones (MR Confon) and earplugs. Scanning commenced after approximately 30-50 minutes of sleep, with the T1w sequence acquired about 15 minutes into the scanning session and the T2w scan being the last sequence acquired approximately 40 minutes after the start of scanning.

**MRI Data Preprocessing and Quality Assessment**

All structural images were visually inspected for motion-related and other artifacts. Whole-brain average gray/white contrast-to-noise ratio (CNR) was calculated for each participant's T1w image (see Table 3.1). The Human Connectome Project minimal preprocessing structural pipelines (*PreFreeSurfer, FreeSurfer,* and *PostFreeSurfer*) were

employed to perform cortical reconstruction and to generate cortical myelin maps (Glasser et al., 2013). Briefly, the *PreFreeSurfer* pipeline was used to correct for gradient nonlinearity distortion, to align the T1w and T2w images with a 6 degrees of freedom rigid body transformation, and to correct for intensity inhomogeneity, including correction for B1-bias and some B1+bias in the T1w and T2 images by estimating the bias field *F* from the square root of the product of the T1w and T2w images after thresholding out non-brain tissues.

The *FreeSurfer* pipeline used a modified FreeSurfer's (v.5.3.0-HCP) recon-all pipeline (Dale, Fischl, & Sereno, 1999; Fischl, Sereno, & Dale, 1999; Fischl, Sereno, Tootell, & Dale, 1999) to perform brain extraction, automated tissue segmentation, surface topology correction, and white and pial surface generation on the distortion- and bias-corrected T1w images in native volume space derived from the *PreFreeSurfer* pipeline. The T2w to T1w registration was further improved using FreeSurfer's BBRegister (Greve & Fischl, 2009).

Surface myelin maps were generated with the *PostFreeSurfer* pipeline, using the methods described in Glasser & Van Essen (2011) and Glasser, Goyal, Preuss, Raichle, & Van Essen (2014). The T1w/T2w ratio images were obtained by dividing the T1w images by the aligned T2w images, and were sampled at mid-thickness between the white and pial surfaces as a proxy of intracortical myelin. Residual bias field in the T1w/T2w images was corrected by modeling the expected low spatial frequency distribution of T1w/T2w intensities across the surface and subtracting it from the individual T1w/T2w-estimated myelin maps (Glasser & Van Essen, 2011; Glasser, Goyal, Preuss, & Van Essen, 2014).

All T1w and T2w images and FreeSurfer outputs were examined slice-by-slice by two independent raters to assess overall image quality and identify any inaccuracies in surface placement. Of the 32 datasets from children with ASD and 23 datasets from TD children with

both T1w and T2w images available, data from 10 ASD and 7 TD children were excluded due to major artifacts in the T1w or T2w image, such as ghosting and ringing, and/or surface placement inaccuracies on the FreeSurfer output of the T1w image. All T1w/T2w-estimated myelin maps were also visually inspected and whole brain mean T1w/T2w ratio was calculated for each participant. Data from one child with ASD with the whole brain mean T1w/T2w ratio greater than two standard deviations above the group mean was identified as an outlier and excluded from the analysis. The excluded children (n = 18) did not significantly differ from those included in the study with regard to their age ($p = 0.63$), sex distribution ($p = 0.73$), overall developmental skills ($p = 0.33$) or, among children with ASD, autism symptom severity ($p = 0.27$).

**Statistical Analysis**

*Regions of interest*

The average T1w/T2w ratio values were extracted from 34 cortical regions of interest (ROIs) per hemisphere from the Desikan-Killiany atlas (Desikan et al., 2006). In order to reduce the number of comparisons, we focused on the regions known to undergo rapid myelination in the first years of life (Deoni et al., 2015). Thus, only ROIs with the T1w/T2w ratio greater than the average whole-brain T1w/T2w for this cohort (T1w/T2w ratio > 2.2) were selected for subsequent analyses, resulting in 11 ROIs. These highly myelinated cortical regions included pericalcarine, cuneus, lingual, isthmus cingulate, transverse temporal, lateral occipital, postcentral, posterior cingulate, paracentral, precentral, and precuneus cortices, encompassing the primary somatosensory, motor, visual, auditory, and posterior parts of the cingulate cortices. Given the similar rate of myelin development in the left and right hemisphere reported in a large cohort of 1-to-6-year-old children (Deoni et al., 2015), we averaged between the left and right homologous ROIs to calculate the mean T1w/T2w ratio for each ROI.

*T1w/T2w ratio analyses*

Linear regression models were used with T1w/T2w ratio as the outcome variable and diagnostic group, age, and age by group interaction as predictors, for each of the selected ROIs. Sex, gray/white CNR, and total brain volume (TBV) were included as covariates in all regression models during model specification state, and retained only if revealed to be significant predictors. Gray/white CNR did not differ between groups (see Table 3.1) nor accounted for significant variance ($ps > 0.2$) in any of the models; as a result, it was removed from the final models. Corrections for multiple comparisons were conducted using Benjamini-Hochberg False Discovery Rate (FDR) at $q < 0.1$. Follow-up partial correlations were calculated between age and T1w/T2w ratio for each group for those ROIs with significant age by group interactions, while controlling for covariates revealed to be significant predictors.

*Correlations with autism symptoms*

Associations between estimated myelin content and autism symptoms (in children with ASD only) were examined with linear regression models with T1w/T2w ratio as the outcome variable, and ADOS-2 Calibrated Severity Scores (CSS, an index of ASD symptom severity, which allows comparisons across ages and language abilities) as predictor, controlling for age, sex, and MSEL Early Learning Composite (ELC, a standard score indexing child's overall developmental level). These models were only applied for the ROIs showing significant diagnostic group, or age by group interaction effects in the main analysis. Benjamini-Hochberg FDR at $q < 0.1$ was used to correct for multiple comparisons.

## Results

The resultant T1w/T2w maps for the ASD and TD groups are shown in Figure 3.1A. Overall, the T1w/T2w spatial patterns and distribution were very similar for children in the ASD

and TD groups, with the motor/somatosensory strip in the central sulcus, visual cortex in the occipital lobe, primary auditory areas in Heschl's gyrus, and posterior aspects of the cingulate cortex showing the highest T1w/T2w values. Regions with the lowest myelination included the temporal pole, medial prefrontal cortex, and the anterior cingulate cortex. These patterns are highly consistent with those reported in adults (Glasser & Van Essen, 2011) and in typically developing young children in the same age range (obtained with a different myelin mapping method; Deoni et al., 2015).

*Group comparisons and age-related effects on T1w/T2w ratio*

Results of the regression analyses revealed no significant group differences (ASD v. TD) in the average T1w/T2w ratio in the selected 11 ROIs (Figure 3.1B, 3.1C). However, significant age by group interaction effects ($q < 0.1$) were identified in 7 out of the 11 ROIs (including the pericalcarine, cuneus, lingual, isthmus cingulate, lateral occipital, posterior cingulate, and precuneus cortices), with a consistent pattern of positive associations between T1w/T2w and age in TD children (correlation coefficients $r$ = [0.32 to 0.58]) and a general lack of such relationship with age in the ASD group (correlation coefficients $r$ = [–0.28 to –0.04]; see scatterplots in Figure 3.2). Sex and TBV did not account for significant variance in these models.

*Links with autism symptoms*

There were no significant associations between estimated myelin content (T1w/T2w ratio) and autism symptoms (ADOS-2 CSS) after controlling for age, sex, and overall developmental skills (Mullen ELC) in the 7 ROIs identified in the main analyses.

*Post-hoc examination of links between T1w/T2w ratio and other morphometric indices of cortical maturation*

Given the rapid brain volume growth and prominent morphometric changes in the first years of life (Gilmore et al., 2012; Li et al., 2013; Lyall et al., 2015), we conducted post-hoc analyses to explore whether T1w/T2w ratio relates to gray matter volume in the cortical ROIs showing significant age by group interaction effects in the main analysis. Linear regression models with T1w/T2w ratio as the outcome variable, and diagnostic group, cortical volume, and group by volume interaction as predictors, while controlling for age, sex, and TBV were employed (cortical volumes were automatically calculated at each FreeSurfer surface vertex during preprocessing, and were averaged across all vertices within each ROI). Corrections for multiple comparisons were conducted using Benjamini-Hochberg FDR at $q < 0.1$.

These analyses revealed significant group by volume interaction effects in the cuneus, isthmus cingulate, and precuneus cortices, after correcting for multiple comparisons ($q < 0.1$). Age, sex, and TBV did not account for significant variance in any of these models. Follow-up correlational analyses between T1w/T2w and ROI volume in each group revealed a significant positive association between T1w/T2w and volume in the cuneus in the TD group ($r = 0.74$, $p < 0.001$), indicating that the two indices of cortical maturation co-vary in young TD children, while such relationship was not present in the ASD group (see Figure 3.3). Because cortical volume is, by and large, a product of cortical thickness and surface area, we conducted an additional exploratory analysis to examine whether the atypical relationship between cortical myelin and gray matter volume observed in the ASD group may be related to atypical cortical thinning or surface area expansion (thought to have different genetic origins and distinct developmental trajectories; Wierenga, Langen, Oranje, & Durston, 2014). Follow-up correlational analyses between T1w/T2w and surface area/cortical thickness conducted for the three ROIs (cuneus, isthmus cingulate, and precuneus) revealed no significant relationships between T1w/T2w ratio

and cortical thickness, in either the ASD or TD groups. However, positive associations between T1w/T2w and surface area were identified in all 3 ROIs in the TD but not in the ASD group, mirroring the relationship between T1w/T2w and cortical volume in the same ROIs (Figure 3.3).

## Discussion

To our knowledge, this is the first study to examine intracortical myelin in toddlers and preschoolers with ASD. Our primary aim was to test the feasibility of using the T1w/T2w ratio as an estimate of intracortical myelin content in young children with ASD, and to examine the age-related effects on T1w/T2w across early childhood in ASD (cross-sectionally), as compared to typical development. We also set out to explore whether, in children with ASD, estimated intracortical myelin content in the rapidly myelinated regions was associated with autism symptoms, and whether it was related to other indices of cortical maturation. Results revealed that the overall spatial patterns of intracortical myelin distribution estimated with T1w/T2w in young children with ASD were largely comparable to the patterns observed in the TD group, as well as to those reported in prior studies in TD children (Deoni et al., 2015) and adults (Glasser & Van Essen, 2011). Although direct between-group comparisons revealed no group differences in T1w/T2w between TD children and those with ASD, differential associations with age in the early-myelinated areas, including visual, posterior cingulate, and precuneus cortices, were observed in the ASD and TD groups. Specifically, a consistent pattern of positive associations between intracortical myelin in these regions and age was detected in the TD group (cross-sectionally), indicating age-related increase in estimated myelin content across the toddler and preschool years. In contrast, such age-related effects were generally absent in the ASD group. Furthermore, differential relationships between intracortical myelin and cortical volumes and surface area in posterior cortices were detected in the ASD and TD groups, with estimated

myelin content positively associated with volume and surface area in TD children, whereas such relationship was not present in young children with ASD. Finally, no significant association between cortical myelin and symptoms of autism was detected among children with ASD.

Our finding of aberrant age-related trajectories of estimated intracortical myelin content in young children with ASD, relative to TD children, suggests that this fundamental neurodevelopmental process is altered in the first years of life in autism. Although no diagnostic group differences (ASD v. TD) in intracortical myelin content were detected, significant group by age interaction effects observed across several posterior cortical regions indicate that the developmental timing of myelination may be disordered in young children with ASD, in comparison to TD children who showed expected age-related increase in intracortical myelination. Across the human lifespan, intracortical myelination follows an inverted U-shape trajectory with an initial increase in intracortical myelin across most of the cortex continuing through at least the middle of the third decade of life, with the first wave of maturation in primary sensory and motor cortices followed by a second wave of maturation in association, limbic, and insular cortices (Grydeland et al., 2019; Grydeland et al., 2013; Rowley et al., 2017; Shafee et al., 2015). Based on our findings, albeit in a relatively modest-size sample, this trajectory appears to be mis-timed (as assessed with cross-sectional design) in early childhood in ASD.

Although T1w/T2w had not been previously investigated in young children with ASD, a recent report (Darki, Nyström, McAlonan, Bölte, & Falck-Ytter, 2021) described lower T1w/T2w values in 5-month-old infants at familial risk for ASD in both white and gray matter in broadly distributed brain regions, compared to infants with no familial risk. Notably, some of the gray matter regions where significant group differences in T1w/T2w were observed in infants at

risk for ASD overlap with the ROIs with significant group by age interaction effects in our cohort, including the cingulate, precuneus, and lateral occipital cortices. However, considerable methodological differences between the two studies preclude any further inferences (e.g., a volume-based approach for tissue segmentation, which limits the accuracy of delineation of gray and white matter, and voxel-wise calculation of T1w/T2w across the cortex, limiting the specification of the underlying neurobiological processes, used by Darki and colleagues, vs. a surface-based approach for tissue segmentation and estimation of T1w/T2w at mid-thickness between the white and pial surfaces used in the current study). Additionally, it is unclear if these findings are specific to children with ASD given the lack of subsequent diagnostic confirmation for infants at familial risk. Nonetheless, considered together with these results, our findings highlight the developmental significance of the T1w/T2w ratio as an index of aberrant neurodevelopment characterizing young children with, or at risk for autism spectrum disorders.

Notably, studies using other methodologically-related MRI metrics (also dependent on image intensity variations and contrast, similarly to T1w/T2w) in ASD have shown blurring of the boundary between cerebral gray and white matter, where intracortical myelin is predominately found. Andrews and colleagues (2017) first reported reduced gray-white matter boundary contrast (GWC) in adults with ASD, consistent with earlier postmortem histological findings (Avino & Hutsler, 2010). The reduced GWC values in adults with ASD were driven primarily by increased gray matter intensity (GMI) across the cortical layers at different depths into the cortical sheet. This is pertinent because increased GMI may be driven by atypical myelination (Sowell et al., 2004) and/or differences in cytoarchitectural organization (Casanova, Buxhoeveden, Switala, & Roy, 2002). A subsequent study investigating age-related changes of GWC in youth and young adults with ASD (ages 7-25 years) found that the most prominent

changes in GWC occur during childhood (Mann et al., 2018), suggesting that the disrupted GWC in ASD may not be exclusively driven by atypical gray matter cytoarchitecture (which is largely set around birth) but rather reflects ongoing, age-dependent changes in myelination. Finally, a recent longitudinal study in toddlers with familial risk of ASD reported that atypically increased GWC in the second year of life (in the context of the normative increase observed at this age in typical development) was associated with ASD diagnosis and symptom severity at age 3 years (Godel et al., 2021). Overall, although the GWC index is not specific to myelin content, its methodological interdependence with estimated myelin content (through similar reliance on image intensity variations and contrast in estimating the gray-white cortical boundary, where intracortical myelin is predominately found) makes these findings relevant to the pattern of results observed in young children with ASD in our study.

Broadly, the early disruption in intracortical myelination is significant in the context of its effects on the development of brain circuits and functional networks, including the inhibitory effects of myelin on axon sprouting and synapse formation and dendritic plasticity (McGee, Yang, Fischer, Daw, & Strittmatter, 2005; Tomassy et al., 2014) thought to help stabilize the architecture of developing neural networks. The cortical regions where atypical age-related effects in intracortical myelin were observed in our cohort encompassed visual cortices (i.e., pericalcarine, cuneus, lingual, lateral occipital) and posterior nodes of the Default Mode Network (DMN; Buckner, Andrews-Hanna, & Schacter, 2008; Raichle et al., 2001) (i.e., isthmus cingulate, posterior cingulate, precuneus), with consistent reports of atypical functional connectivity in those circuits in ASD (Assaf et al., 2010; Keehn, Shih, Brenner, Townsend, & Müller, 2013; Wang et al., 2021; Yerys et al., 2015). The aberrant brain connectivity and network organization involving visual and DMN networks have been reported in young children

with (or at risk for) ASD in particular, with atypical connectivity linked to autism symptoms or early behavioral signs of ASD (e.g., joint attention or core autism symptoms; Chen, Linke, Olson, Ibarra, Reynolds, et al., 2021; Eggebrecht et al., 2017; McKinnon et al., 2019).

Further, the atypical relationships between T1w/T2w ratio and gray matter volume / surface area in young children with ASD, as compared to TD children, revealed in the post-hoc analyses, suggest that the early disruption in intracortical myelination may be one of several aberrant cortical maturational processes underlying the atypical neurodevelopment in the first years of life in ASD. Namely, we found that, in posterior midline cortices, including posterior cingulate, precuneus, and cuneus, estimated intracortical myelin content and cortical volumes / surface area are robustly linked within individuals in TD children, suggesting effectively concomitant maturation of these indices of neurodevelopment. However, intracortical myelin and cortical volumes / surface area were not associated in children with ASD, indicating that the two may be uncoupled in early development in autism. As briefly discussed in the introduction, early accelerated growth in gray matter volume, possibly driven by surface area hyper-expansion, has been consistently reported in young children with ASD (Hazlett et al., 2017). Our findings expand on this literature by highlighting an additional aspect of atypical cortical development in autism, intracortical myelination, that can inform our understanding of the neurobiology of the ASD.

*Limitations and Perspectives*

While this work reports the first *in vivo* description of intracortical myelination in young children with ASD, some methodological limitations need to be acknowledged. The primary limitation is that the T1w/T2w ratio is not a direct measure of intracortical myelin content but rather a proxy that has been shown to successfully map the myeloarchitectonic properties in

adults and typically developing children. Other factors can also contribute to the T1w/T2w measure such as iron content, which affects MRI signal contrast (Fukunaga et al., 2010), head motion affecting image quality which can indirectly affect the accurate placement of the cortical surface, as well as maturation of the local white matter (e.g., Giedd 2004). Given the rigorous quality assurance of all structural images and exclusion of scans with major motion artifacts or surface placement inaccuracies, and the use of gray/white CNR as a measure of the overall image quality (which did not account for significant variance in any of the analyses), head motion is unlikely to be a major contributor for the current results. Additionally, although HCP preprocessing pipeline includes an ad-hoc correction method to minimize the residual B1+ bias in the T1w/T2w maps, this method works well for localizing cortical areas in individual scans, but may potentially attenuate individual differences and reduce sensitivity for detecting cross-participant differences (Ganzetti et al., 2014). An improved method for B1+ transmit field correction on the T1w/T2w-estimated myelin maps may be needed in future studies utilizing the T1w/T2w ratio measure. Other limitations include the relatively modest sample size limiting our study to an exploratory purpose only and the use of cross-sectional data to explore age-related effects. Future studies with larger samples and longitudinal data are necessary to map the developmental trajectories of intracortical myelination in the first years of life in ASD.

Critically, while the current study design does not allow inferences about whether the observed atypical maturational trajectories of intracortical myelin in young children with ASD reflect causation (i.e., pertain to the underlying etiology) or compensatory effects, these findings are nonetheless crucial for translational efforts given the recent evidence of adaptive myelination, modifiable by environmental experience (Fields, 2015; Forbes & Gallo, 2017). Specifically, the prolonged plasticity of intracortical myelin, especially in transmodal,

association cortices, provides an extended window of opportunity for modifications, through early interventions or other critical changes in an individual's socioemotional, educational, and other environmental experiences, to promote experience-dependent plasticity early in life in children with ASD (e.g., Rosen, Amso, & McLaughlin, 2019).

**Acknowledgements**

This research was supported by the National Institutes of Health (R01 MH107802 to IF). The funding sources had no role in study design, writing of the report, or the decision to submit the article for publication. The authors are grateful to Chris Fong, M.A. and Lisa Mash, Ph.D., of San Diego State University, and Tiffany Wang, M.S., of University of California, San Diego, for invaluable assistance with data collection. The authors' strongest gratitude goes to the children and families who so generously dedicated their time and effort to this research.

Chapter 2, in full is a reprint of the material as it appears in Developmental Neurobiology, 2022. Chen, B., Linke, A., Olson, L., Kohli, J., Kinnear, M., Sereno, M., Müller, R. A., Carper, R., and Fishman, I., Wiley, 2022. The dissertation author was the primary investigator and author of this paper.

**Table 2.1 Participant Characteristics**

| | ASD (n = 21) | TD (n = 16) | ASD vs. TD | |
|---|---|---|---|---|
| | Mean ± SD (Min - Max) | Mean ± SD (Min - Max) | $t/X^2$ | *p* value |
| Age at scan (months)* | 42.7 ± 12.8 (21 - 62) | 41.8 ± 15.4 (20 - 65) | $t(35) = 0.19$ | 0.85 |
| Gender (M/F) | 16/5 | 9/7 | $\chi^2(1) = 1.65$ | 0.20 |
| Ethnicity (Hispanic/Non-Hispanic)[a] | 9/10 | 6/10 | $\chi^2(1) = 0.35$ | 0.56 |
| Race (White/Black/More-than-one/Asian)[b] | 16/0/3/1 | 13/2/0/0 | -- | -- |
| Gestational age (weeks)[c] | 39.1 ± 1.9 (35 - 43) | 39.6 ± 1.2 (37 - 42) | $t(34) = -1.09$ | 0.29 |
| Birth weight (grams)[d] | 3276.2 ± 583.1 (2098 - 4394) | 3451.6 ± 363.0 (2863 - 4082) | $t(33) = -1.04$ | 0.30 |
| Delivery method (Vaginal/C-section) | 13/8 | 13/3 | $\chi^2(1) = 1.63$ | 0.20 |
| Maternal education level (%) | | | | |
| High school or some college credit, but <1 year | 38% | 6% | -- | -- |
| Associate degree | 10% | 0 | -- | -- |
| Bachelor's degree | 10% | 44% | -- | -- |
| Master's degree | 29% | 44% | -- | -- |
| Professional degree (MD, PhD, JD) | 14% | 6% | -- | -- |
| MSEL Early Learning Composite, Standard Score | 75.3 ± 17.6 (49 - 105) | 103.1 ± 16.1 (80 - 136) | $t(35) = -4.95$ | <0.001 |
| SCQ Total Score[e] | 14.3 ± 9.2 (3 - 35) | 4.7 ± 2.4 (1 - 9) | $t(33) = 4.02$ | <0.001 |
| ADOS-2 Calibrated Severity Score | 5.9 ± 2.2 (2 - 9) | -- | -- | -- |
| Total Brain Volume ($cm^3$) | 1099.8 ± 99.5 (910.0 - 1282.7) | 1052.5 ± 100.9 (813.9 - 1202.3) | $t(35) = 1.42$ | 0.16 |
| Gray/White CNR | 2.0 ± 0.2 (1.7 - 2.5) | 1.9 ± 0.1 (1.7 - 2.1) | $t(35) = 1.50$ | 0.14 |

Note: M = male; F = female; MSEL = Mullen Scales of Early Learning; SCQ = Social Communication Questionnaire; ADOS-2 = Autism Diagnostic Observation Schedule 2nd Edition; CNR = contrast-to-noise ratio.

*MRI data were acquired within 3 weeks of the diagnostic and behavioral evaluation.

[a]Ethnicity data are missing for 2 ASD subjects

[b]Race data are missing for 1 ASD and 1 TD subjects

[c]Gestational age data are missing for 1 ASD subject

[d]Birth weight data are missing for 2 ASD subjects

[e]SCQ data are missing for 2 ASD subjects

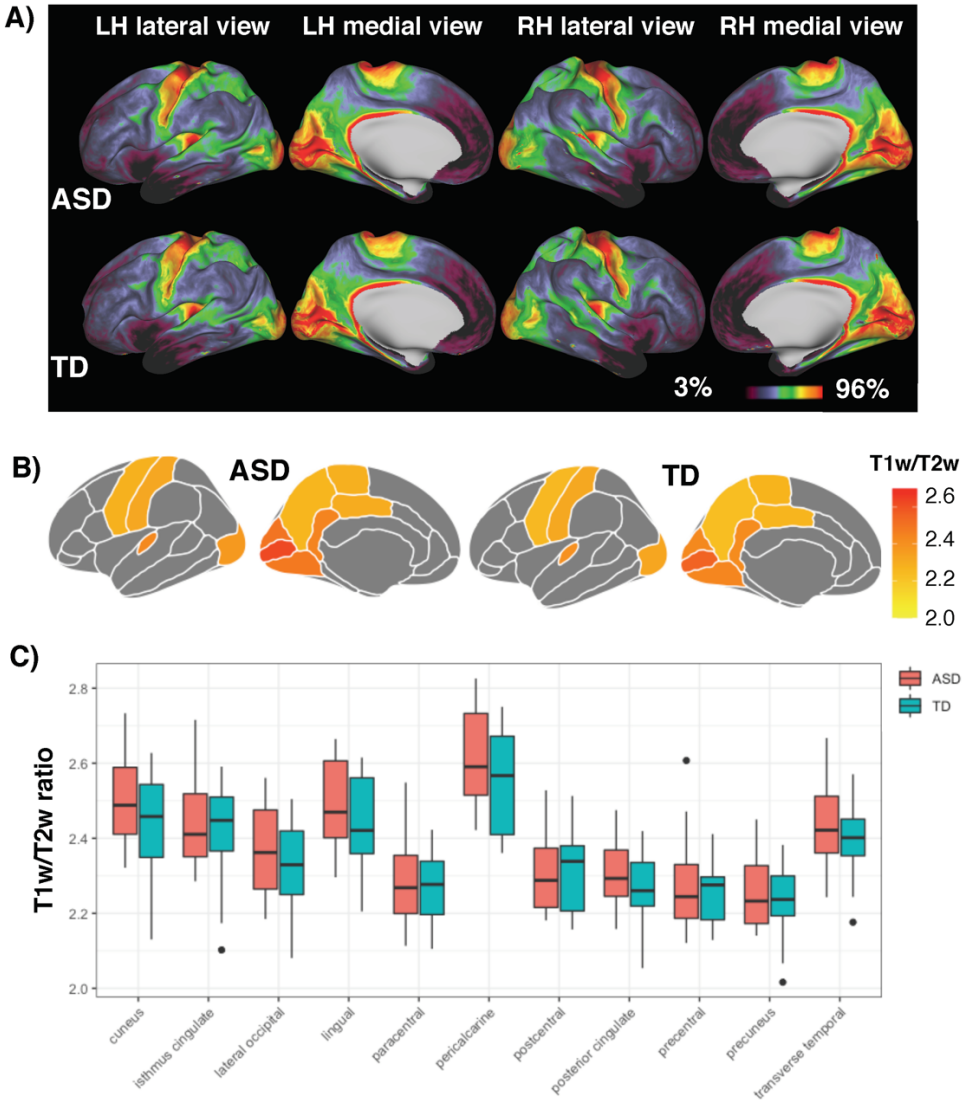


**Figure 2.1 Group comparisons of estimated myelin content**
(A) Average T1w/T2w ratio maps in the ASD and TD groups. Group average T1w/T2w ratio projected on the inflated surface. In all medial surface panels, the medial wall is masked. The color palette reflects T1w/T2w ratio percentile rank indexing lightly myelinated cortex in purple and more highly myelinated cortex in red. (B, C) Estimated myelin content (T1w/T2w ratio, averaged across hemispheres) for the 11 regions of interest (ROIs), in the ASD and TD groups. The 11 ROIs with the highest estimated myelin content (T1w/T2w ratio greater than the average whole-brain T1w/T2w ratio for the whole cohort) include pericalcarine, cuneus, lingual, isthmus cingulate, transverse temporal, lateral occipital, postcentral, posterior cingulate, paracentral, precentral, and precuneus cortices. Panel B shows average T1w/T2w ratio per group, and panel C shows distribution of the T1w/T2w values within each group, for each ROI.

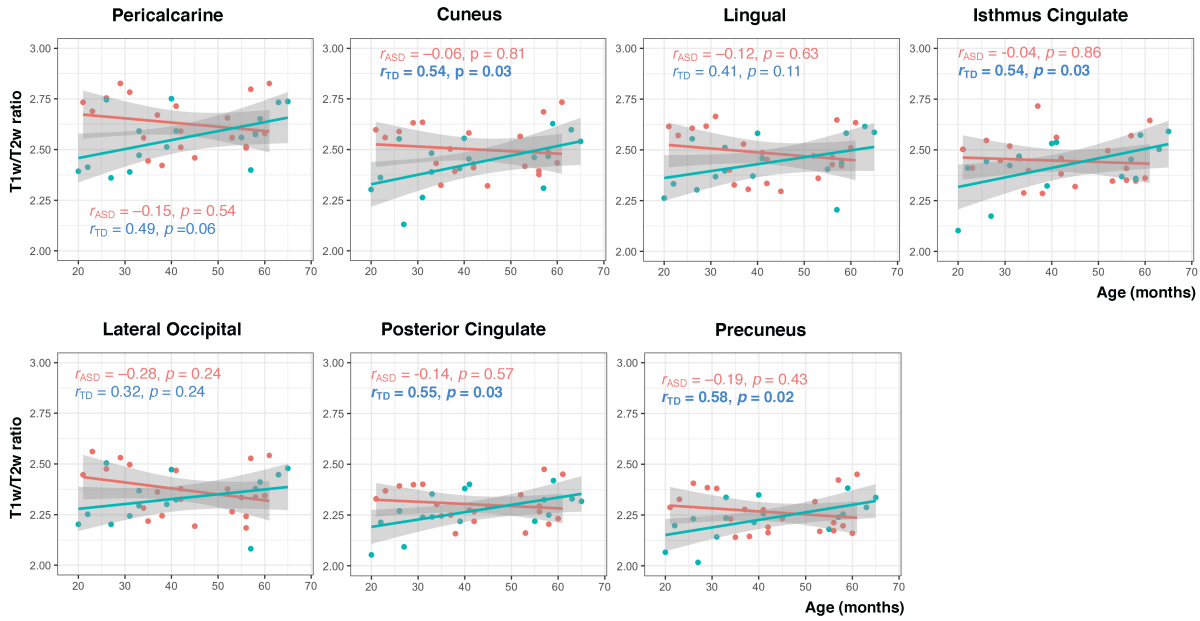


**Figure 2.2 Correlations between estimated myelin content and age**
Scatterplots of correlations between T1w/T2w ratio and age, in 7 out of 11 ROIs where significant age by diagnostic group interaction effects were detected. *r* values denote bivariate (zero-order) correlation coefficients for the ASD and TD groups, with significant within-group correlations indicated in bold font. Bivariate correlations are presented because covariates (sex, CNR, and TBV) did not account for significant variance in these models.

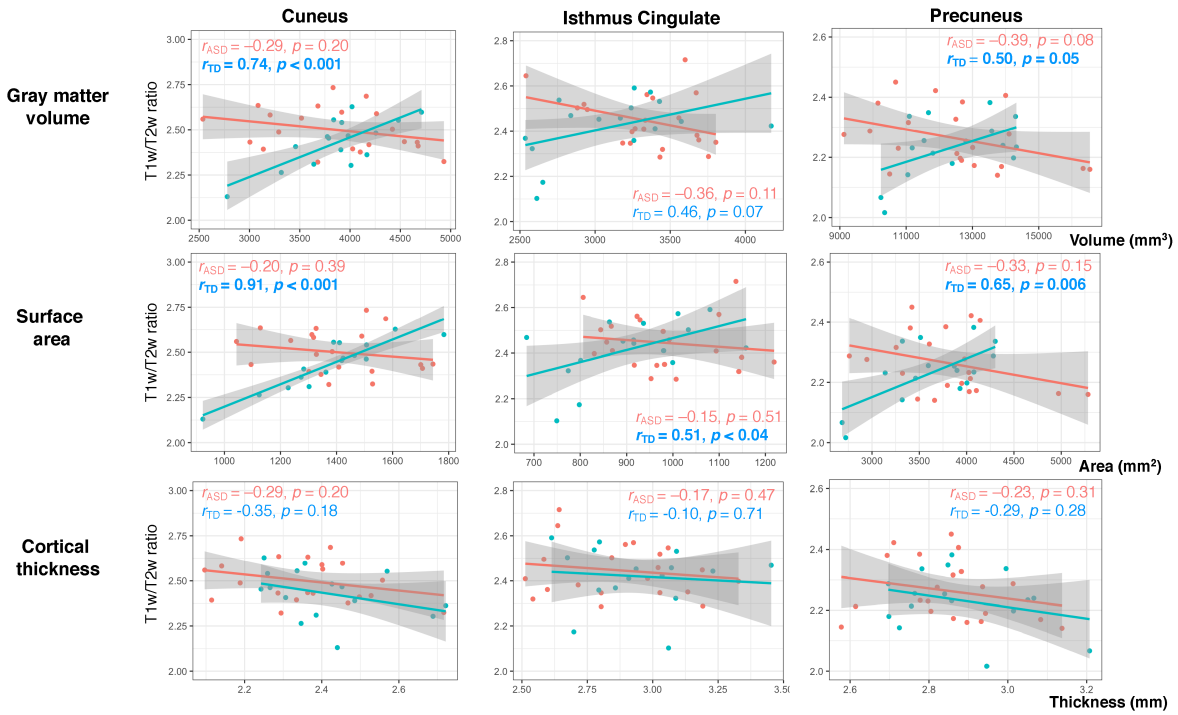


**Figure 2.3 Correlations. Between estimated myelin content and morphometric indices of brain maturation**

Scatterplots of correlations between T1w/T2w ratio and gray matter volume (top panel), surface area (middle panel), and cortical thickness (bottom panel) in 3 out of 7 ROIs where significant T1w/T2w by volume by diagnostic group interaction effects were detected (cuneus, isthmus cingulate, and precuneus cortices). *r* values denote correlation coefficients for the ASD and TD groups, with significant within-group correlations indicated in bold font.

## References


Abrahám, H., Vincze, A., Jewgenow, I., Veszprémi, B., Kravják, A., Gömöri, E., & Seress, L. (2010). Myelination in the human hippocampal formation from midgestation to adulthood. *Int J Dev Neurosci, 28*(5), 401-410. doi:10.1016/j.ijdevneu.2010.03.004

Andrews, D. S., Avino, T. A., Gudbrandsen, M., Daly, E., Marquand, A., Murphy, C. M., Lai, M. C., Lombardo, M. V., Ruigrok, A. N., Williams, S. C., Bullmore, E. T., The Mrc Aims Consortium, Suckling, J., Baron-Cohen, S., Craig, M. C., Murphy, D. G., & Ecker, C. (2017). In Vivo Evidence of Reduced Integrity of the Gray-White Matter Boundary in Autism Spectrum Disorder. *Cerebral Cortex*, *27*(2), 877–887. doi: 10.1093/cercor/bhw404

APA. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5)*: American Psychiatric Pub.

Arnold, S. E., & Trojanowski, J. Q. (1996). Human fetal hippocampal development: I. Cytoarchitecture, myeloarchitecture, and neuronal morphologic features. *J Comp Neurol, 367*(2), 274-292. doi: 10.1002/(SICI)1096-9861(19960401)367:2<274::AID-CNE9>3.0.CO;2-2

Assaf, M., Jagannathan, K., Calhoun, V. D., Miller, L., Stevens, M. C., Sahl, R., . . . Pearlson, G. D. (2010). Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *Neuroimage, 53*(1), 247-256. doi:10.1016/j.neuroimage.2010.05.067

Avino, T. A., & Hutsler, J. J. (2010). Abnormal cell patterning at the cortical gray-white matter boundary in autism spectrum disorders. *Brain Res, 1360*, 138-146. doi:10.1016/j.brainres.2010.08.091

Bock, N. A., Hashim, E., Janik, R., Konyer, N. B., Weiss, M., Stanisz, G. J., . . . Geyer, S. (2013). Optimizing T1-weighted imaging of cortical myelin content at 3.0 T. *Neuroimage, 65*, 1-12. doi:10.1016/j.neuroimage.2012.09.051

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci, 1124*, 1-38. doi:10.1196/annals.1440.011

Casanova, M. F., Buxhoeveden, D. P., Switala, A. E., & Roy, E. (2002). Neuronal density and architecture (Gray Level Index) in the brains of autistic patients. *J Child Neurol, 17*(7), 515-521. doi:10.1177/088307380201700708

Chapman, T. W., & Hill, R. A. (2020). Myelin plasticity in adulthood and aging. *Neurosci Lett, 715*, 134645. doi:10.1016/j.neulet.2019.134645

Chen, B., Linke, A., Olson, L., Ibarra, C., Kinnear, M., & Fishman, I. (2021). Resting state functional networks in 1-to-3-year-old typically developing children. *Dev Cogn Neurosci, 51*, 100991. doi:10.1016/j.dcn.2021.100991

Chen, B., Linke, A., Olson, L., Ibarra, C., Reynolds, S., Müller, R. A., . . . Fishman, I. (2021). Greater functional connectivity between sensory networks is related to symptom severity in toddlers with autism spectrum disorder. *J Child Psychol Psychiatry, 62*(2), 160-170. doi:10.1111/jcpp.13268

Conti, E., Mitra, J., Calderoni, S., Pannek, K., Shen, K. K., Pagnozzi, A., . . . Guzzetta, A. (2017). Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: A diffusion MRI study. *Hum Brain Mapp, 38*(5), 2333-2344. doi:10.1002/hbm.23520

Corsello, C. M., Akshoomoff, N., & Stahmer, A. C. (2013). Diagnosis of autism spectrum disorders in 2-year-olds: a study of community practice. *J Child Psychol Psychiatry, 54*(2), 178-185. doi:10.1111/j.1469-7610.2012.02607.x

Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., . . . Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology, 57*(2), 245-254.

Courchesne, E., Pramparo, T., Gazestani, V. H., Lombardo, M. V., Pierce, K., & Lewis, N. E. (2019). The ASD living biology: from cell proliferation to clinical phenotype. *Mol Psychiatry, 24*(1), 88-107. doi:10.1038/s41380-018-0056-y

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage, 9*(2), 179-194. doi:10.1006/nimg.1998.0395

Darki, F., Nyström, P., McAlonan, G., Bölte, S., & Falck-Ytter, T. (2021). T1-weighted/T2-weighted ratio mapping at 5 months captures individual differences in behavioral development and differentiates infants at familial risk for autism from controls. *Cereb Cortex, 31*(9), 4068-4077. doi:10.1093/cercor/bhab069

Dawson, G., Jones, E. J., Merkle, K., Venema, K., Lowy, R., Faja, S., . . . Webb, S. J. (2012). Early behavioral intervention is associated with normalized brain activity in young children with autism. *J Am Acad Child Adolesc Psychiatry, 51*(11), 1150-1159. doi:10.1016/j.jaac.2012.08.018

Deoni, S. C., Dean, D. C., 3rd, Remer, J., Dirks, H., & O'Muircheartaigh, J. (2015). Cortical maturation and myelination in healthy toddlers and young children. *Neuroimage, 115*, 147-161. doi:10.1016/j.neuroimage.2015.04.058

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., . . . Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage, 31*(3), 968-980. doi:10.1016/j.neuroimage.2006.01.021

Dong, H. M., Margulies, D. S., Zuo, X. N., & Holmes, A. J. (2021). Shifting gradients of macroscale cortical organization mark the transition from childhood to adolescence. *Proceedings of the National Academy of Sciences*, *118*(28). doi: 10.1073/pnas.2024448118

Eggebrecht, A. T., Elison, J. T., Feczko, E., Todorov, A., Wolff, J. J., Kandala, S., . . . Pruett, J. R., Jr. (2017). Joint attention and brain functional connectivity in infants and toddlers. *Cereb Cortex, 27*(3), 1709-1720. doi:10.1093/cercor/bhw403

Estes, A., Munson, J., Rogers, S. J., Greenson, J., Winter, J., & Dawson, G. (2015). Long-Term Outcomes of early intervention in 6-year-old children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry, 54*(7), 580-587. doi:10.1016/j.jaac.2015.04.005

Fields, R. D. (2014). Neuroscience. Myelin--more than insulation. *Science, 344*(6181), 264-266. doi:10.1126/science.1253851

Fields, R. D. (2015). A new mechanism of nervous system plasticity: activity-dependent myelination. *Nat Rev Neurosci, 16*(12), 756-767. doi:10.1038/nrn4023

Fischl, B., Sereno, M. I., & Dale, A. M. (1999). Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage, 9*(2), 195-207. doi:10.1006/nimg.1998.0396

Fischl, B., Sereno, M. I., Tootell, R. B., & Dale, A. M. (1999). High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum Brain Mapp, 8*(4), 272-284. doi:10.1002/(sici)1097-0193(1999)8:4<272::aid-hbm10>3.0.co;2-4

Forbes, T. A., & Gallo, V. (2017). All wrapped up: Environmental effects on myelination. *Trends Neurosci, 40*(9), 572-587. doi:10.1016/j.tins.2017.06.009

Fukunaga, M., Li, T. Q., van Gelderen, P., de Zwart, J. A., Shmueli, K., Yao, B., . . . Duyn, J. H. (2010). Layer-specific variation of iron content in cerebral cortex as a source of MRI contrast. *Proceedings of the National Academy of Sciences of the United States of America, 107*(8), 3834-3839. doi:10.1073/pnas.0911177107

Ganzetti, M., Wenderoth, N., & Mantini, D. (2014). Whole brain myelin mapping using T1- and T2-weighted MR imaging data. *Front Hum Neurosci*, *8*, 671. doi: 10.3389/fnhum.2014.00671

Gao, W., Alcauter, S., Elton, A., Hernandez-Castillo, C. R., Smith, J. K., Ramirez, J., & Lin, W. (2015). Functional network development during the first year: Relative sequence and socioeconomic correlations. *Cereb Cortex, 25*(9), 2919-2928. doi:10.1093/cercor/bhu088

Geyer, S., Weiss, M., Reimann, K., Lohmann, G., & Turner, R. (2011). Microstructural Parcellation of the human cerebral cortex - From Brodmann's post-mortem map to in vivo mapping with high-field Magnetic Resonance Imaging. *Front Hum Neurosci, 5*, 19. doi:10.3389/fnhum.2011.00019

Gilmore, J. H., Shi, F., Woolson, S. L., Knickmeyer, R. C., Short, S. J., Lin, W., . . . Shen, D. (2012). Longitudinal development of cortical and subcortical gray matter from birth to 2 years. *Cereb Cortex, 22*(11), 2478-2485. doi:10.1093/cercor/bhr327

Glasser, M. F., Goyal, M. S., Preuss, T. M., Raichle, M. E., & Van Essen, D. C. (2014). Trends and properties of human cerebral cortex: correlations with cortical myelin content. *Neuroimage, 93 Pt 2*, 165-175. doi:10.1016/j.neuroimage.2013.03.060

Glasser, M. F., Sotiropoulos, S. N., Wilson, J. A., Coalson, T. S., Fischl, B., Andersson, J. L., . . . Jenkinson, M. (2013). The minimal preprocessing pipelines for the Human Connectome Project. *Neuroimage, 80*, 105-124. doi:10.1016/j.neuroimage.2013.04.127

Glasser, M. F., & Van Essen, D. C. (2011). Mapping human cortical areas in vivo based on myelin content as revealed by T1- and T2-weighted MRI. *J Neurosci, 31*(32), 11597-11616. doi:10.1523/jneurosci.2180-11.2011

Godel, M., Andrews, D. S., Amaral, D. G., Ozonoff, S., Young, G. S., Lee, J. K., . . . Schaer, M. (2021). Altered gray-white matter boundary contrast in toddlers at risk for autism relates to later diagnosis of autism spectrum disorder. *Front Neurosci, 15*, 669194. doi:10.3389/fnins.2021.669194

Gordon, A., & Geschwind, D. H. (2020). Human in vitro models for understanding mechanisms of autism spectrum disorder. *Mol Autism, 11*(1), 26. doi:10.1186/s13229-020-00332-7

Greve, D. N., & Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. *Neuroimage, 48*(1), 63-72.

Grydeland, H., Vértes, P. E., Váša, F., Romero-Garcia, R., Whitaker, K., Alexander-Bloch, A. F., . . . Bullmore, E. T. (2019). Waves of maturation and senescence in micro-structural MRI markers of human cortical myelination over the lifespan. *Cereb Cortex, 29*(3), 1369-1381. doi:10.1093/cercor/bhy330

Grydeland, H., Walhovd, K. B., Tamnes, C. K., Westlye, L. T., & Fjell, A. M. (2013). Intracortical myelin links with performance variability across the human lifespan: results from T1- and T2-weighted MRI myelin mapping and diffusion tensor imaging. *J Neurosci, 33*(47), 18618-18630. doi:10.1523/jneurosci.2811-13.2013

Grydeland, H., Westlye, L. T., Walhovd, K. B., & Fjell, A. M. (2016). Intracortical posterior cingulate myelin content relates to error processing: Results from T1- and T2-weighted MRI myelin mapping and electrophysiology in healthy adults. *Cereb Cortex, 26*(6), 2402-2410. doi:10.1093/cercor/bhv065

Haroutunian, V., Katsel, P., Roussos, P., Davis, K. L., Altshuler, L. L., & Bartzokis, G. (2014). Myelination, oligodendrocytes, and serious mental illness. *Glia, 62*(11), 1856-1877. doi:10.1002/glia.22716

Hazlett, H. C., Gu, H., Munsell, B. C., Kim, S. H., Styner, M., Wolff, J. J., . . . Piven, J. (2017). Early brain development in infants at high risk for autism spectrum disorder. *Nature, 542*(7641), 348-351. doi:10.1038/nature21369

Hazlett, H. C., Poe, M., Gerig, G., Smith, R. G., Provenzale, J., Ross, A., . . . Piven, J. (2005). Magnetic resonance imaging and head circumference study of brain size in autism: birth

through age 2 years. *Arch Gen Psychiatry, 62*(12), 1366-1376. doi:10.1001/archpsyc.62.12.1366

Hazlett, H. C., Poe, M. D., Gerig, G., Styner, M., Chappell, C., Smith, R. G., . . . Piven, J. (2011). Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Arch Gen Psychiatry, 68*(5), 467-476. doi:10.1001/archgenpsychiatry.2011.39

Keehn, B., Shih, P., Brenner, L. A., Townsend, J., & Müller, R. A. (2013). Functional connectivity for an "island of sparing" in autism spectrum disorder: an fMRI study of visual search. *Hum Brain Mapp, 34*(10), 2524-2537. doi:10.1002/hbm.22084

Landa, R. J. (2018). Efficacy of early interventions for infants and young children with, and at risk for, autism spectrum disorders. *Int Rev Psychiatry, 30*(1), 25-39. doi:10.1080/09540261.2018.1432574

Li, G., Nie, J., Wang, L., Shi, F., Lin, W., Gilmore, J. H., & Shen, D. (2013). Mapping region-specific longitudinal cortical surface expansion from birth to 2 years of age. *Cereb Cortex, 23*(11), 2724-2733. doi:10.1093/cercor/bhs265

Liu, S., Li, A., Zhu, M., Li, J., & Liu, B. (2019). Genetic influences on cortical myelination in the human brain. *Genes Brain Behav, 18*(4), e12537. doi:10.1111/gbb.12537

Lord, C., & Rutter, M. (2003). Social communication questionnaire (SCQ). *Torrance, CA: WPS*.

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K., & Bishop, S. L. (2012). *Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)*. Torrence, CA: Western Psychological Services.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord, 24*(5), 659-685.

Lyall, A. E., Shi, F., Geng, X., Woolson, S., Li, G., Wang, L., . . . Gilmore, J. H. (2015). Dynamic development of regional cortical thickness and surface area in early childhood. *Cereb Cortex, 25*(8), 2204-2212. doi:10.1093/cercor/bhu027

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., . . . Dietz, P. M. (2020). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. *MMWR Surveill Summ, 69*(4), 1-12. doi:10.15585/mmwr.ss6904a1

Mann, C., Bletsch, A., Andrews, D., Daly, E., Murphy, C., Murphy, D., & Ecker, C. (2018). The effect of age on vertex-based measures of the grey-white matter tissue contrast in autism spectrum disorder. *Mol Autism, 9*, 49. doi:10.1186/s13229-018-0232-6

McGee, A. W., Yang, Y., Fischer, Q. S., Daw, N. W., & Strittmatter, S. M. (2005). Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. *Science, 309*(5744), 2222-2226. doi:10.1126/science.1114362

McKinnon, C. J., Eggebrecht, A. T., Todorov, A., Wolff, J. J., Elison, J. T., Adams, C. M., . . . Pruett, J. R., Jr. (2019). Restricted and repetitive behavior and brain functional connectivity in infants at risk for developing autism spectrum disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging, 4*(1), 50-61. doi:10.1016/j.bpsc.2018.09.008

Mullen, E. M. (1995). *Mullen scales of early learning*. Circle Pines, MN: American Guidance Service.

Nordahl, C. W., Lange, N., Li, D. D., Barnett, L. A., Lee, A., Buonocore, M. H., . . . Amaral, D. G. (2011). Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America, 108*(50), 20195-20200. doi:10.1073/pnas.1107560108

Ozonoff, S., Heung, K., Byrd, R., Hansen, R., & Hertz-Picciotto, I. (2008). The onset of autism: patterns of symptom emergence in the first years of life. *Autism Res, 1*(6), 320-328. doi:10.1002/aur.53

Ozonoff, S., Young, G. S., Landa, R. J., Brian, J., Bryson, S., Charman, T., . . . Iosif, A. M. (2015). Diagnostic stability in young children at risk for autism spectrum disorder: a baby siblings research consortium study. *J Child Psychol Psychiatry, 56*(9), 988-998. doi:10.1111/jcpp.12421

Pierce, K., Carter, C., Weinfeld, M., Desmond, J., Hazin, R., Bjork, R., & Gallagher, N. (2011). Detecting, studying, and treating autism early: the one-year well-baby check-up approach. *J Pediatr, 159*(3), 458-465.e451-456. doi:10.1016/j.jpeds.2011.02.036

Pierce, K., Gazestani, V. H., Bacon, E., Barnes, C. C., Cha, D., Nalabolu, S., . . . Courchesne, E. (2019). Evaluation of the diagnostic stability of the early autism spectrum disorder phenotype in the general population starting at 12 months. *JAMA Pediatr*. doi:10.1001/jamapediatrics.2019.0624

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America, 98*(2), 676-682. doi:10.1073/pnas.98.2.676

Rosen, M. L., Amso, D., & McLaughlin, K. A. (2019). The role of the visual association cortex in scaffolding prefrontal cortex development: A novel mechanism linking socioeconomic status and executive function. *Dev Cogn Neurosci, 39*, 100699. doi:10.1016/j.dcn.2019.100699

Rowley, C. D., Sehmbi, M., Bazin, P. L., Tardif, C. L., Minuzzi, L., Frey, B. N., & Bock, N. A. (2017). Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T(1) -weighted MRI. *Hum Brain Mapp, 38*(7), 3691-3703. doi:10.1002/hbm.23624

Sacrey, L. R., Zwaigenbaum, L., Bryson, S., Brian, J., Smith, I. M., Roberts, W., . . . Garon, N. (2018). Parent and clinician agreement regarding early behavioral signs in 12- and 18-month-old infants at-risk of autism spectrum disorder. *Autism Res, 11*(3), 539-547. doi:10.1002/aur.1920

Shafee, R., Buckner, R. L., & Fischl, B. (2015). Gray matter myelination of 1555 human brains using partial volume corrected MRI images. *Neuroimage, 105*, 473-485. doi:10.1016/j.neuroimage.2014.10.054

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N., . . . Wise, S. P. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci, 28*(14), 3586-3594. doi:10.1523/jneurosci.5309-07.2008

Solso, S., Xu, R., Proudfoot, J., Hagler, D. J., Jr., Campbell, K., Venkatraman, V., . . . Courchesne, E. (2016). Diffusion tensor imaging provides evidence of possible axonal overconnectivity in frontal lobes in autism spectrum disorder toddlers. *Biol Psychiatry, 79*(8), 676-684. doi:10.1016/j.biopsych.2015.06.029

Sowell, E. R., Thompson, P. M., Leonard, C. M., Welcome, S. E., Kan, E., & Toga, A. W. (2004). Longitudinal mapping of cortical thickness and brain growth in normal children. *J Neurosci, 24*(38), 8223-8231. doi:10.1523/jneurosci.1798-04.2004

Sparrow, S. S., Cicchetti, D. V., & Balla, D. A. (2005). *Vineland Adaptive Behavior Scales, Second Edition*. Circle Pines, MN: American Guidance Service.

Spear, L. P. (2013). Adolescent neurodevelopment. *J Adolesc Health, 52*(2 Suppl 2), S7-13. doi:10.1016/j.jadohealth.2012.05.006

Sydnor, V. J., Larsen, B., Bassett, D. S., Alexander-Bloch, A., Fair, D. A., Liston, C., . . . Satterthwaite, T. D. (2021). Neurodevelopment of the association cortices: Patterns, mechanisms, and implications for psychopathology. *Neuron*. doi:10.1016/j.neuron.2021.06.016

Tomassy, G. S., Berger, D. R., Chen, H. H., Kasthuri, N., Hayworth, K. J., Vercelli, A., . . . Arlotta, P. (2014). Distinct profiles of myelin distribution along single axons of pyramidal neurons in the neocortex. *Science, 344*(6181), 319-324. doi:10.1126/science.1249766

Travers, B. G., Adluru, N., Ennis, C., Tromp do, P. M., Destiche, D., Doran, S., . . . Alexander, A. L. (2012). Diffusion tensor imaging in autism spectrum disorder: a review. *Autism Res, 5*(5), 289-313. doi:10.1002/aur.1243

Turesky, T. K., Vanderauwera, J., & Gaab, N. (2021). Imaging the rapidly developing brain: Current challenges for MRI studies in the first five years of life. *Dev Cogn Neurosci, 47*, 100893. doi:10.1016/j.dcn.2020.100893

Wang, Q., Li, H. Y., Li, Y. D., Lv, Y. T., Ma, H. B., Xiang, A. F., Jia, X. Z., & Liu, D. Q. (2021). Resting-state abnormalities in functional connectivity of the default mode

network in autism spectrum disorder: a meta-analysis. *Brain imaging and behavior*, *15*(5), 2583–2592. https://doi.org/10.1007/s11682-021-00460-5

Weitzman, C., & Wegner, L. (2015). Promoting optimal development: screening for behavioral and emotional problems. *Pediatrics, 135*(2), 384-395. doi:10.1542/peds.2014-3716

White, N., Roddey, C., Shankaranarayanan, A., Han, E., Rettmann, D., Santos, J., . . . Dale, A. (2010). PROMO: Real-time prospective motion correction in MRI using image-based tracking. *Magnetic resonance in medicine, 63*(1), 91-105. doi:10.1002/mrm.22176

Wierenga, L. M., Langen, M., Oranje, B., & Durston, S. (2014). Unique developmental trajectories of cortical thickness and surface area. *Neuroimage, 87*, 120-126. doi:10.1016/j.neuroimage.2013.11.010

Wolff, J. J., Gu, H., Gerig, G., Elison, J. T., Styner, M., Gouttard, S., . . . Piven, J. (2012). Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. *Am J Psychiatry, 169*(6), 589-600. doi:10.1176/appi.ajp.2011.11091447

Xiao, Z., Qiu, T., Ke, X., Xiao, X., Xiao, T., Liang, F., . . . Liu, Y. (2014). Autism spectrum disorder as early neurodevelopmental disorder: evidence from the brain imaging abnormalities in 2-3 years old toddlers. *J Autism Dev Disord, 44*(7), 1633-1640. doi:10.1007/s10803-014-2033-x

Yerys, B. E., Gordon, E. M., Abrams, D. N., Satterthwaite, T. D., Weinblatt, R., Jankowski, K. F., . . . Vaidya, C. J. (2015). Default mode network segregation and social deficits in autism spectrum disorder: Evidence from non-medicated children. *Neuroimage Clin, 9*, 223-232. doi:10.1016/j.nicl.2015.07.018

# CHAPTER 3: STUDY 3

The content within this section, titled “Chapter 3: Study 3,” reflects material from a paper that has been submitted for publication**.** The citation is as follows:

Chen, B., Olson, L., Rios, A., Salmina, M., Linke, A., & Fishman, I. (under review). Reduced covariation between brain morphometry and local spontaneous activity in young children with autism spectrum disorder.

## ABSTRACT

While disruptions in brain maturation in the first years of life in ASD are well documented, little is known about how brain structure and function are related in young children with ASD compared to typically developing peers. We applied a multivariate pattern analysis to examine covariation patterns between brain morphometry and local brain spontaneous activity in 38 toddlers and preschoolers with ASD and 31 typically developing children using T1-weighted structural magnetic resonance imaging (MRI) and resting-state functional MRI data acquired during natural sleep. The results revealed significantly reduced brain structure-function correlations in ASD. The resultant brain structure and function composite indices were associated with age among typically developing children but not in those with ASD, suggesting mistiming of typical brain maturational trajectories early in life in autism. Additionally, the brain function composite indices were associated with overall developmental and adaptive behavior skills in the ASD group, highlighting the neurodevelopmental significance of early local brain activity in autism.

## INTRODUCTION

While the current consensus is that autism spectrum disorder (ASD) originates prenatally affecting early fetal neurodevelopment (Courchesne, Gazestani, & Lewis, 2020), the clinical diagnosis of ASD cannot be made before behavioral symptoms fully manifest (with the median age at diagnosis in the US currently being 49 months (Maenner et al., 2023)), substantially limiting our ability for early identification. The implications of delayed detection and identification are significant given the profound impact of early interventions on the developing brain, especially in the first years of life, during the critical window of rapid brain maturation and peak experience-dependent neuroplasticity (Tau & Peterson, 2010).

Although cumulative neuroimaging evidence has shown alterations in both structural (i.e., neuroanatomy) and functional development of the brain in ASD, these studies are largely based on older children and adolescents, with only a few consistent findings emerging from neuroimaging studies in infants and toddlers at risk for, or with early diagnosis of autism. Multiple studies have reported early brain overgrowth, including enlarged brain volumes and head circumferences, accelerated surface area expansion, and increased structural connectivity across white matter tracts in the first years of life in young children with ASD, at a group level, when compared to typically developing age peers (Courchesne et al., 2001; Hazlett et al., 2005; Hazlett et al., 2011; Solso et al., 2016; Wolff et al., 2012; Xiao et al., 2014). Besides these early *structural* brain findings in ASD detected with anatomical and diffusion MRI, a small but growing number of studies have examined *functional* brain organization and connectivity in infants and toddlers with (or at risk for) ASD using functional MRI acquired during natural sleep. Earlier studies have primarily focused on brain function in putative language regions and reported reduced fMRI activation in response to speech sounds, absent or reversed hemispheric

lateralization for speech processing, and diminished inter-hemispheric connectivity between language regions in young children with ASD (Dinstein et al., 2011; Eyler, Pierce, & Courchesne, 2012; Lombardo et al., 2015). More recent studies have investigated whole brain intrinsic functional connectivity (iFC) and functional networks in infants with high familial risk for ASD (due to having an older sibling with autism) who were followed prospectively (Eggebrecht et al., 2017; Emerson et al., 2017; Marrus et al., 2018; McKinnon et al., 2019). While these prospective studies provided unique opportunities to study neurodevelopment before behavioral symptoms of ASD emerge, this sampling design is inherently biased given the exclusive focus on children from families with a significant familial risk (while most individuals with ASD do not have older siblings with the disorder (Szatmari et al., 2016)). More recent studies examining resting-state functional connectivity in toddlers diagnosed with ASD (ages 1.5 to 3.5 years) found disruptions in multi-sensory circuitry, including atypically increased iFC between visual and sensorimotor networks (B. Chen et al., 2021) and between thalamus and sensory cortices (Linke et al., 2023), which were associated with greater autism symptoms and poorer clinical outcomes, such as sleep disturbances.

Critically, a vast majority of neuroimaging studies in ASD, including those in toddler and preschool years reviewed above, have examined brain structural and functional indices separately using MRI data from a single modality (e.g., fMRI or anatomical MRI), with patterns of *covariation* between brain structural and functional development largely overlooked. With regard to neuroanatomy or morphometric brain development, both cortical thickness (CT) and surface area (SA) contribute to the cortical volume growth, albeit each following distinct, non-overlapping maturational trajectories (Brown et al., 2012; Wierenga, Langen, Oranje, & Durston, 2014) rooted in distinct neurobiological processes, including distinctive genetic underpinnings

(Panizzon et al., 2009; Strike et al., 2019). The normative developmental trajectories of CT and SA have been extensively studied, with cortical thinning and surface area expansion shown across most of the brain during toddler and preschool years (Bethlehem et al., 2022; Frangou et al., 2022; Remer et al., 2017). As noted above, the limited evidence available on the brain volumetric and morphometric development in early childhood in ASD suggests that these trajectories may be accelerated (i.e., have an earlier peak) in young children with ASD (Courchesne et al., 2001; Hazlett et al., 2005; Hazlett et al., 2011; Schumann et al., 2010; Xiao et al., 2014). The first years of life is also a period of rapid development of brain functional organization and activity, which can be estimated with blood oxygenation level-dependent (BOLD) signal using fMRI. Although resting-state fMRI data have been primarily used – whether in general population, in ASD, or in other clinical populations – to examine large-scale functional connectivity patterns based on the strength of the correlations between BOLD signal fluctuations in spatially distant brain regions (Biswal, Yetkin, Haughton, & Hyde, 1995; Fox & Raichle, 2007), resting-state fMRI data can also be used to quantify *local spontaneous brain activity* within a given brain region. For example, regional spontaneous brain activity can be characterized with fractional amplitude of low-frequency fluctuation (fALFF) metric, which measures the relative contribution of low frequency BOLD signal fluctuations to the entire frequency range detectable by BOLD-optimized MRI sequences (Zou et al., 2008; Zuo et al., 2010). Only a handful of studies have investigated local spontaneous activity in ASD, with evidence of disrupted local activity observed in school-aged children, adolescents and adults, albeit with mixed, region- and age-specific pattern of results (Di Martino et al., 2014; Guo et al., 2017; Itahashi et al., 2015; Karavallil Achuthan, Coburn, Beckerson, & Kana, 2023). However, no published study to date has investigated local spontaneous activity in the first years of life in

ASD, limiting our knowledge of the maturational aspects and early developmental trajectories of local spontaneous brain activity in ASD.

Motivated by the dearth of research leveraging *multimodal* MRI data in early childhood in ASD and aiming to improve our understanding of the multivariate relationships between brain structure and function in early neurodevelopment in ASD, this study set out to examine the covariation patterns between brain morphometry and local spontaneous activity in young children with ASD as compared to typically developing (TD) age-matched peers, using both structural MRI and resting-state functional MRI data acquired during natural sleep. We utilized canonical correlation analysis (CCA), a statistical method allowing investigation of joint multivariate relationships, to identify a set of brain morphometric and local spontaneous activity measures that are maximally correlated (indicating co-maturation) in typical development and to compare this brain structure-function covariation pattern to that observed in the ASD cohort. We hypothesized that young children with ASD would exhibit reduced brain structure-function correlations when compared to TD children.

## METHODS

### Participants

This study includes data from participants enrolled in the San Diego State University (SDSU) Toddler MRI Project, a longitudinal study of early brain markers of ASD. Children between 18 and 42 months with a diagnosis of ASD or behavioral concerns consistent with ASD symptoms were referred to the Project from multiple sources, including specialty autism clinics, state-funded early education and developmental evaluation programs, local pediatricians, community clinics, and autism service providers, and have been followed longitudinally through 5 years of age. TD children were recruited from the community via digital and social media

advertisement in and around the San Diego County. Co-occurring neurological disorders (e.g., cerebral palsy), history of perinatal CNS infection or gross CNS injury, non-febrile seizures, and contraindications for MRI served as exclusionary for children in either group. Participants with known syndromic forms of ASD (e.g., fragile X or Rett syndrome), as ascertained from parent report, were also excluded. In order to limit known risk factors associated with developmental delays among children enrolled in the TD group, TD participants were further screened and excluded for prematurity (<36 weeks of gestation), family history (in first-degree relatives) of ASD, intellectual disability, or other heritable psychiatric or neurological disorders. The research protocol was approved by the institutional review boards of SDSU, University of California San Diego (UCSD), and the County of San Diego Health and Human Services Agency. Written informed consent was obtained from the caregivers.

This study includes cross-sectional data (from one of the longitudinal study visits completed between 2016 and early 2020) from 38 young children with ASD and 31 TD children, ages 1.5 – 5.5 years, for whom both high-quality T1 (anatomical) and two runs of resting-state functional MRI data acquired during natural sleep were available. Participants with ASD and TD children were matched at the group level on age (see Table 1 for demographic and developmental characteristics of the sample).

**Diagnostic and Developmental Assessment**

Diagnoses of ASD or clinical best estimate (Ozonoff et al., 2015) in children younger than 3 years of age were established upon enrollment using standardized measures in combination with expert clinical judgement, in accordance with the current recommendations by the American Academy of Pediatrics and Society for Developmental and Behavioral Pediatrics (Lipkin & Macias, 2020; Weitzman & Wegner, 2015). Because diagnostic evaluation is repeated

at follow-up visits in the context of the larger longitudinal Project, only data from children with confirmed diagnosis, based on the DSM-5 (American Psychiatric Association, 2013) diagnostic criteria, were included in the current study. ASD diagnoses were supported by the Autism Diagnostic Observation Schedule-Second Edition (Lord et al., 2012) administered by research-reliable clinicians, the Social Communication Questionnaire (Lord & Rutter, 2003) or the Autism Diagnostic Interview-Revised (Lord, Rutter, & Le Couteur, 1994) administered to caregivers of children older than 36 months, and expert clinical judgment. Developmental skills were assessed in all (TD and ASD) participants with the Mullen Scales of Early Learning (MSEL; Mullen, 1995), a clinician-administered standardized assessment of cognitive, language, and motor development. Total developmental quotient (DQ) was calculated as an average of four developmental quotients (for each MSEL subscale: Receptive Language, Expressive Language, Fine Motor, and Visual Reception) derived by dividing the subscale age-equivalence score by the child's chronological age and multiplying by 100 (Messinger et al., 2013). The DQ metric was utilized to avoid the relatively common floor effect of the MSEL Early Learning Composite Standard Score, which was observed in 7 out of 38 children in the ASD cohort (consistent with other reports in cohorts of young children with ASD (Lord et al., 2006; Munson et al., 2008)). The Vineland Adaptive Behavior Scales, 2nd Edition, Survey Interview (Vineland-II; Sparrow, Cicchetti, & Balla, 2005), a semi-structured interview, was administered to caregivers to assess the child's adaptive development skills demonstrated at home and other settings. The Vineland-II Adaptive Behavior Composite (ABC) score was used in the analysis. For inclusion and retention in the TD group, children had below-clinical cutoff scores on the ASD screener, the SCQ (all TD scores $\leq 10$; see Table 1), and demonstrated developmental skills falling no more than 1.5 SD

below the normative mean for their age on measures of early learning and development (the MSEL subscales).

## MRI Data Acquisition

MRI data were collected during natural nocturnal sleep on a GE Discovery MR750 3T MRI scanner at the UCSD Center for Functional Magnetic Resonance Imaging, using a Nova Medical 32-channel head coil. First, a multiband multi-echo planar imaging (EPI) sequence allowing simultaneous acquisition of multiple slices was used to acquire two fMRI runs (400 volumes per each 6-minute run) with high spatial resolution and fast acquisition (TR=800ms, TE=35ms, flip angle=52°, 72 slices, multiband acceleration factor=8, 2mm isotropic voxel size, matrix=104x104, FOV=20.8cm). Two separate 20s spin-echo EPI sequences with opposing phase encoding directions were also acquired using the same matrix size, FOV and prescription to correct for susceptibility-induced distortions. High-resolution anatomical images were acquired next with a fast 3D spoiled gradient recalled (FSPGR) T1-weighted sequence (0.8mm isotropic voxel size, NEX=1, TE/TI=min full/1060ms, flip angle=8°, FOV=25.6cm, matrix=320x320, receiver bandwidth 31.25hz). Motion during anatomical scans was corrected in real-time using three navigator scans and real-time prospective motion correction (PROMO) (White et al., 2010), and images were bias corrected using the GE PURE option.

In preparation for the scan night, and to optimize MRI data acquisition, a comprehensive habituation protocol was implemented. An individualized scan night sleep strategy (e.g., time of arrival, approximating home-like sleeping arrangements, including access to a double MRI bed for co-sleeping families, rocking chair, modular playpen mounted on the MRI bed resembling a crib, ambient lighting in the MRI suite, etc.) was developed for each child, based on the typical bedtime routines assessed in advance with an in-house Sleep Habits Questionnaire. To habituate

the child to the scanning environment, the parents were instructed to practice nightly inserting soft foam child-size earplugs after the child had fallen asleep, and to play an mp3 file containing the MRI sounds of the scan sequences employed in the study at progressively louder volumes for a week. On the night of the scan, noise protection was achieved with MRI compatible headphones (MR Confon) and earplugs. Scanning commenced after approximately 30-50 minutes of sleep.

**MRI Data Preprocessing and Quality Assessment**

T1 anatomical MRI images were processed for automated cortical reconstruction using FreeSurfer version 5.3.0 recon-all (https://surfer.nmr.mgh.harvard.edu), which generated pial and white matter surfaces for each individual. All T1 anatomical images were visually inspected for motion-related and other artifacts and FreeSurfer outputs were examined slice-by-slice by two independent raters to identify any inaccuracies in surface placement. Whole-brain average gray/white contrast-to-noise ratio (CNR) was calculated for each participant's T1 image.

Functional MRI data were preprocessed with FMRIB's Software Libraries (FSL v5.0.10) (Smith et al., 2004), Matlab 2015b (Mathworks Inc., Natick, MA, USA) using SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, UK), and the CONN toolbox v17f (Whitfield-Gabrieli & Nieto-Castanon, 2012; http://www.nitrc.org/projects/conn). Preprocessing steps included correction for susceptibility-induced distortions using the two spin-echo EPI acquisitions with opposite phase encoding directions and FSL's TOPUP tools; motion correction using rigid-body realignment as implemented in SPM12; spatial smoothing using a 6mm Gaussian kernel at full-width half maximum; outlier detection using the Artifact Detection Toolbox as installed with CONN v17f (ART; https://www.nitrc.org/projects/artifact_detect) to identify outlier volumes with frame-wise displacement (FD) >0.5mm and/or changes in signal

intensity >3 standard deviations; and nuisance regression including censoring of outliers detected by the ART toolbox, regression of the 6 motion parameters and their derivatives, and the first five PCA components derived from the CSF and white matter compartments using aCompCor (Behzadi, Restom, Liau, & Liu, 2007). The structural images were co-registered to the mean functional image, segmented and normalized to the Montreal Neurological Institute (MNI) atlas space using non-linear registration and the default tissue probability maps included with SPM12. The white matter (WM) and CSF probability maps obtained from segmentation of the structural image for each subject were thresholded at 0.95 and eroded by 1 voxel. These thresholded and eroded masks were applied to functional images to extract WM and CSF time courses, which were submitted to a principal component analysis with aCompCor (Behzadi et al., 2007) to be used for nuisance regression. Functional images were directly normalized to MNI space with the same non-linear registration as used for the structural images. Mean head motion was indexed by root mean square of displacement (RMSD) across two fMRI runs, calculated from rigid-body realignment of the raw data prior to TOPUP correction.

**Brain Structural and Functional Variables and Statistical Analyses**

Two brain morphometric measures, surface area (SA) and cortical thickness (CT), were extracted for each participant from 34 regions of interest (ROIs) per hemisphere from the Desikan-Killiany atlas implemented in FreeSurfer (Desikan et al., 2006). Local spontaneous activity was indexed with the fALFF measure extracted from the fMRI data in each voxel and averaged within the same ROIs. FALFF is calculated as the power within the low frequency range (0.01 – 0.1 Hz) divided by the total power of the entire frequency spectrum, using the implementation included with the CONN toolbox:

$$fALFF = \sqrt{\frac{\sum_t (h(t) * BOLD(x,t))^2}{\sum_t BOLD(x,t)^2}}$$

The SA, CT, and fALFF variables of interest were submitted to linear regressions to exclude potential confounds; namely, CNR and total brain volume were regressed out of the two morphometric measures (SA and CT) and head motion indexed by mean RMSD across two fMRI runs was regressed out of the local spontaneous activity measure (fALFF).

In order to investigate the covariation patterns between brain structure (SA and CT) and function (local spontaneous activity, fALFF), sparse canonical correlation analysis (SCCA) was implemented using the 'PMA' package in R (Witten, Tibshirani, & Hastie, 2009). Canonical correlation analysis (CCA) is a multivariate statistical technique that identifies linear combinations of two sets of variables – such as brain morphometry and local spontaneous activity measures – with maximal correlation between them (Hardoon, Szedmak, & Shawe-Taylor, 2004). CCA is particularly suited to identifying the source of common statistical variation among data from multiple modalities (such as brain anatomical and functional variables), without assuming any directionality (Zhuang, Yang, & Cordes, 2020). To avoid model overfitting and enhance interpretability of the structure-function covariation, SCCA, a variant of CCA, was used because it identifies parsimonious sources of variation by setting a maximum number of variables with minimal contribution to interpretable linear combinations, or canonical variates, to exactly zero (thereby inducing sparsity on canonical coefficients). A pair of canonical variates (structural CV and functional CV) capturing the highest brain structure-function correlation among TD children was extracted from SCCA. In order to examine whether young children with ASD show comparable structure-function covariation pattern to that observed in neurotypical development, corresponding canonical vectors derived from the TD data were applied to the ASD data. Significance of the difference in canonical correlations (or

correlations between canonical variates generated with the SCCA) between the TD and ASD groups was determined with permutation testing. Specifically, bootstrapping was carried out by randomly splitting the whole (combined ASD and TD) sample in half, with 1000 iterations, and calculating the difference in canonical correlations by applying the canonical vectors derived from half of the sample to the other half. The group difference in canonical correlation was determined to be statistically significant at $p<0.05$ on the bootstrapping distribution.

Associations between age and the canonical variates capturing maximally correlated brain morphometry and local activity variables were examined with linear regressions conducted separately in the TD and ASD groups. Finally, associations between canonical variates and overall developmental and adaptive behavior skills were examined with linear regression models, with structural CV or functional CV as predictors and MSEL Total DQ or Vineland-II ABC as outcome variables, controlling for age and sex (with separate regression models in the ASD and TD groups).

## RESULTS

The results of the SCCA performed on the TD data revealed a significant, positive canonical correlation between brain morphometry and local spontaneous activity ($r = 0.81$, $p < 0.001$; see Figure 1A). Structural and functional canonical variates (CVs) contributing to this canonical correlation are presented in Figure 2, which depicts canonical coefficients illustrative of the relationship between the initial variables (i.e., SA, CT, fALFF) and the CVs, for each ROI in the left (top panel) and right (bottom panel) hemispheres. As can be seen in Figure 2, this pair of canonical variates was characterized by generally reduced SA and greater CT being associated with lower fALFF, with only one exception of higher fALFF in the right cuneus cortex. Specifically, the structural CV implicated lower SA in bilateral orbitofrontal, anterior cingulate,

and inferior frontal cortices, and greater CT in bilateral caudal middle frontal, lateral orbitofrontal, and inferior frontal cortices, and cuneus, precuneus, pericalcarine, and supramarginal cortices (see Figure 2 legend for a detailed list of ROIs). Together, lower SA and higher CT in these regions covaried with lower fALFF in left inferior frontal, caudal and rostral middle frontal, superior frontal, and supramarginal cortices and right orbitofrontal cortex, and higher fALFF in cuneus.

After applying the canonical vectors derived from the TD data to the ASD group, the structure-function canonical correlation found in the ASD group was reduced ($r = 0.25$, $p = 0.136$; see Figure 1B). To determine whether this difference between the canonical correlations observed in the TD and ASD groups was significant, permutation testing with 1000 iterations was conducted to estimate the bootstrapping distribution by randomly splitting the dataset in half and calculating the difference in structure-function canonical correlations by applying the canonical vectors derived from half of the sample to the other half. Permutation testing (see Figure 1C) determined that the structure-function correlation in the ASD group was significantly reduced ($p<0.05$).

Testing for links between the canonical variates of brain morphometry and local activity and child's age, linear regressions revealed that both structural and functional CVs were significantly, negatively correlated with age in the TD ($r$=-0.72 and -0.75, respectively), but not in the ASD group ($r$=-0.26 and -0.25, respectively; see Figure 3), with significant diagnostic group by age interactions for both structural CV ($p$=0.01) and functional CV ($p$=0.005).

Finally, linear regression models testing for relationships between canonical variates and overall developmental and adaptive behavior skills among children with ASD revealed significant associations between functional CV and overall developmental skills (Mullen Total

DQ; partial $r$=-0.43, $p$=0.009) and adaptive functioning (Vineland-II ABC; partial $r$=-0.37, $p$=0.026) after controlling for age (see Figure 4). No significant associations with behavioral indices were found for the structural CV, and there were no relationships between structural or functional CVs and developmental or adaptive skills in TD children.

## DISCUSSION

We used both structural and functional MRI data acquired during the same scanning session to examine the covariation patterns between brain morphometry and local spontaneous activity in young children with ASD compared to age-matched TD children. A multivariate statistical approach – canonical correlation analysis – was implemented to identify a pair of canonical variates or linear combinations of brain morphometric (SA, CT) and local spontaneous activity measures (fALFF) that maximally covary in typical development, indicating co-maturation. The CCA revealed a general covariation pattern of lower SA and higher CT associated with overall lower fALFF in children who are typically developing. This pattern of structure-function covariation (between brain structural metrics and local brain activity) was found to be significantly reduced in children with ASD, as determined with permutation testing. We also set out to examine whether the canonical variates capturing maximally correlated brain morphometry and local activity variables are associated with age as well as overall developmental and adaptive behavior skills in children with ASD and in TD peers. Age-related analyses revealed that while the canonical variates of brain structure and function were significantly associated with age, cross-sectionally, in TD children, these age relationships were not observed in the ASD group. Furthermore, among young children with ASD, the functional canonical variate capturing local spontaneous activity across the brain (which covaries with brain

structural metrics) was significantly associated with indices of general development and adaptive behavior skills.

*Weaker brain structure-function coupling early in life in autism*

Most notably, these results provide initial evidence of reduced brain structure-function correlation in young children with ASD relative to TD children, suggesting that the covariation or close dependence between brain morphometry and local spontaneous activity in ASD deviates from typical neurodevelopment during early childhood. Although the covariation between brain structure and function have not been previously studied in young children, with or without autism, a recent study (Qi et al., 2020) reported results of a fusion analysis between fALFF and gray matter (GM) volume in school-age children and adults with ASD. Utilizing the ABIDE datasets (Di Martino et al., 2017; Di Martino et al., 2014), the authors reported findings linking autism symptoms with patterns of covariance between greater fALFF in broadly distributed cortical regions (e.g., dorsolateral prefrontal, inferior frontal, superior/middle temporal gyrus) but reduced fALFF in subcortical regions (e.g., thalamus and caudate) and greater GM volumes in partially overlapping cortical areas such as dorsolateral prefrontal and superior/middle temporal gyrus. Also using the ABIDE dataset from school-age children, Chen and colleagues identified atypical concordance patterns between the function (measured with ALFF) in GM and white matter (WM) regions, with higher GM/WM functional covariance observed in children with ASD and linked with autism symptoms (H. Chen, Long, Yang, & He, 2021). While these results are not directly comparable to the present findings due to considerable methodological differences and disparate age range, they highlight the need for multimodal neuroimaging studies utilizing multivariate statistical methods, which allow modelling complex neurodevelopmental processes jointly and examining how they co-develop across time and individuals in ASD. Our

study also contributes to the broader literature on the development of structure-function coupling in human brain networks and how it relates to cognitive development and psychopathology (Baum et al., 2020). Overall, our finding of the weaker brain structure-function coupling in children with ASD suggests that the fundamental aspects of brain development may be uncoupled early in life in autism, likely contributing to the disrupted circuit formation, with distributed effects on brain function and connectivity across the entire lifespan.

*Atypical age-related effects: evidence of mistimed brain development trajectories in autism*

The importance of studying developmental trajectories jointly across different brain maturation indices, especially during early childhood, is further supported by the differential age-related effects (albeit observed cross-sectionally) in both brain morphometry and local spontaneous activity detected in our study. Namely, we found that the structural and functional canonical variates (underlying the brain structure-function coupling) were significantly associated with age in typical development, but such relationship was absent among children with ASD. This suggests that maturational trajectories of covariation between brain structure and function may be mistimed in early childhood in ASD. This observation is in line with other findings of atypical age-related effects observed in unimodal studies examining maturation of functional network connectivity and cortical myelination across early childhood in ASD (B. Chen et al., 2021; B. Chen et al., 2022). These findings extend the notion of atypical neurodevelopment and mistimed brain maturational trajectories in autism to early childhood. Given the profound brain maturational changes, peak neuroplasticity, and remarkable advances in cognitive, behavioral, and socio-emotional development characterizing the first years of life (Bornstein, 2014; Tau & Peterson, 2010), it is critical to examine brain maturation and its timing in autism during this developmental period, rather than making inferences from neuroimaging

studies in older children and adolescents (cf. (He et al., 2020; Uddin, Supekar, & Menon, 2013)). It is possible that the variable (distinct from neurotypical) brain maturational trajectories, including atypical brain structure-function coupling, in young children with autism contribute to variable treatment response among children with autism (Vivanti, Prior, Williams, & Dissanayake, 2014) despite the robust evidence of efficacy of early interventions (Landa, 2018). Critically, the links between canonical variate capturing brain local spontaneous activity and overall developmental and adaptive behavior skills detected in the ASD group suggest that brain function, specifically local spontaneous activity, may be a meaningful neurobiological feature that is related to developmental and behavioral outcomes in ASD.

*Potential limitations*

While this study is the first known investigation of the multivariate relationship between brain morphometry and local spontaneous activity in the first years of life in ASD, interpretation of its results is somewhat limited by the moderate sample size due to known challenges of acquiring high quality multimodal MRI data in young children, and in particular in children with neurodevelopmental disorders (Hendrix & Thomason, 2022; Nordahl et al., 2016; Turesky, Vanderauwera, & Gaab, 2021). As such, we applied a parsimonious multivariate model (SCCA) to extract composite indices that capture maximally correlated brain morphometry and local activity variables. This data-driven approach allowed for examination of the overall structure-function covarying patterns with simultaneous data reduction, which is most appropriate for high-dimensional data with a moderate sample size. However, CCA also comes with some limitations; for instance, the relationship between the two modalities (sets of variables) is assumed to be linear and the directionality of the linear relationship (or canonical correlations) identified with CCA are indeterminate (Zhuang et al., 2020). Additionally, this approach is not

suitable for identifying region-specific abnormalities in ASD. Future larger-scale and longitudinal studies are needed to examine the age-related trajectories of brain structure-function covariation patterns longitudinally. Finally, as with any correlational approach, the identified function-structure covarying patterns do not infer causation. Hence, we cannot discern if the reduced brain structure-function correlation in ASD originates from atypical brain morphometry, local spontaneous activity, or other neurodevelopmental processes not directly examined in this study. However, the observed links with developmental and adaptive behavior skills suggest that brain function (local spontaneous activity) may be particularly clinically relevant at this age.

*Conclusions*

To our knowledge, this study is the first to characterize the brain structure-function covariation, using multimodal MRI measures acquired during the same scanning session and a multivariate pattern analysis, in the first years of life in ASD. The overall brain structure-function correlation was significantly reduced in young children with ASD compared to typically developing children, and the neurotypical age-related relationship in the structural and functional indices capturing maximally correlated brain morphometry and local activity measures was absent in the ASD group, suggesting mistimed developmental trajectory of the brain structure-function coupling. Furthermore, the identified association between the index of local spontaneous activity and overall developmental and adaptive behavior skills in the ASD cohort highlights the importance of local brain activity in early developmental outcomes.

**Acknowledgements**

This research was supported by the National Institutes of Health (R01 MH107802 to I. Fishman). The first author (Bosi Chen) is supported by the Autism Speaks Royal Arch Mason Predoctoral Fellowship and the Society of Clinical Child and Adolescent Psychology/American Psychological Association (APA) Division 53 Donald Routh Dissertation Grant Award. The funding sources had no role in study design, writing of the report, or the decision to submit the article for publication. The authors are grateful to Mikaela Kinnear, Ph.D., Chris Fong, M.A., and Lisa Mash, Ph.D., of San Diego State University, and Tiffany Wang, M.S., of University of California San Diego, for invaluable assistance with data collection. The authors' strongest gratitude goes to the children and families who so generously dedicated their time and effort to this research.

Chapter 3, in full, is currently under review for publication. Chen, B., Olson, L., Rio, A., Salmina, M., Linke, A., and Fishman, I. The dissertation author was the primary investigator and author of this paper.

**Table 3.1 Participant Characteristics**

| | ASD (n = 38) | TD (n = 31) | ASD vs. TD | |
|---|---|---|---|---|
| | Mean ± SD (Min - Max) | Mean ± SD (Min - Max) | $t/X^2$ | *p* value |
| Age at scan (months) | 44.6 ± 14.8 (18 - 69) | 44.2 ± 13.7 (18 - 65) | $t(67) = 0.14$ | 0.89 |
| Gender (M/F) | 30/8 | 16/15 | $\chi^2(1) = 5.74$ | 0.02 |
| Ethnicity (Hispanic/Non-Hispanic)[a] | 16/18 | 9/22 | $\chi^2(1) = 2.23$ | 0.14 |
| Race (White/More-than-one/Black/Asian)[b] | 21/8/0/2 | 22/4/3/0 | -- | -- |
| Gestational age (weeks)[c] | 38.6 ± 2.4 (31 - 43) | 39.6 ± 1.1 (37 - 42) | $t(59) = -2.00$ | 0.05 |
| Birth weight (grams)[d] | 3230.0 ± 571.8 (2041 - 4394) | 3511.2 ± 366.7 (2863 - 4082) | $t(59) = -2.26$ | 0.03 |
| Delivery method (Vaginal/C-section)[e] | 24/11 | 21/9 | $\chi^2(1) = 0.02$ | 0.90 |
| Maternal education level (%)[f] | | | | |
| High school or some college credit, but <1 year | 34% | 6% | -- | -- |
| Associate degree/vocational school | 11% | 6% | -- | -- |
| Bachelor's degree | 11% | 35% | -- | -- |
| Master's degree | 24% | 42% | -- | -- |
| Professional degree (MD, PhD, JD) | 11% | 10% | -- | -- |
| MSEL Total Developmental Quotient[g] | 74.8 ± 23.0 (14 - 107) | 103.5 ± 13.2 (71 - 134) | $t(66) = -6.15$ | <0.001 |
| Vineland-II Adaptive Behavior Composite | 75.8 ± 10.6 (55 - 100) | 106.1 ± 12.7 (80 - 127) | $t(66) = -10.75$ | <0.001 |
| SCQ Total Score[h] | 16.3 ± 7.9 (3 - 35) | 4.0 ± 2.9 (0 - 10) | $t(58) = 7.79$ | <0.001 |
| ADOS-2 Calibrated Severity Score | 6.3 ± 2.1 (2 - 10) | -- | -- | -- |
| RMSD (mm) | 0.13 ± 0.04 (0.05 - 0.21) | 0.10 ± 0.03 (0.05 - 0.18) | $t(67) = 3.28$ | 0.002 |
| Total Brain Volume ($cm^3$) | 1095.4 ± 98.5 (787.9 - 1298.9) | 1067.2 ± 105.2 (813.9 - 1293.5) | $t(67) = 1.15$ | 0.26 |
| Gray/White CNR | 2.1 ± 0.2 (1.7 - 2.5) | 2.0 ± 0.2 (1.3 - 2.3) | $t(67) = 1.86$ | 0.07 |

Note: M = male; F = female; MSEL = Mullen Scales of Early Learning; Vineland-II = Vineland Adaptive Behavior Scales, 2nd Edition; SCQ = Social Communication Questionnaire; ADOS-2 = Autism Diagnostic Observation Schedule, 2nd Edition; RMSD = root mean square displacement; CNR = contrast to noise ratio.

[a]Ethnicity data are missing for 4 ASD participants; [b]Race data are missing for 5 ASD and 1 TD participants; [c]Gestational age data are missing for 4 ASD and 1 TD participants; [d]Birth weight data are missing for 2 ASD participants; [e]Delivery method data are missing for 3 ASD and 1 TD participants; [f]Maternal education data are missing for 3 ASD participants; [g]MSEL Total Developmental Quotient data is missing for 1 ASD participant; [h]SCQ data are missing for 6 ASD and 3 TD participants

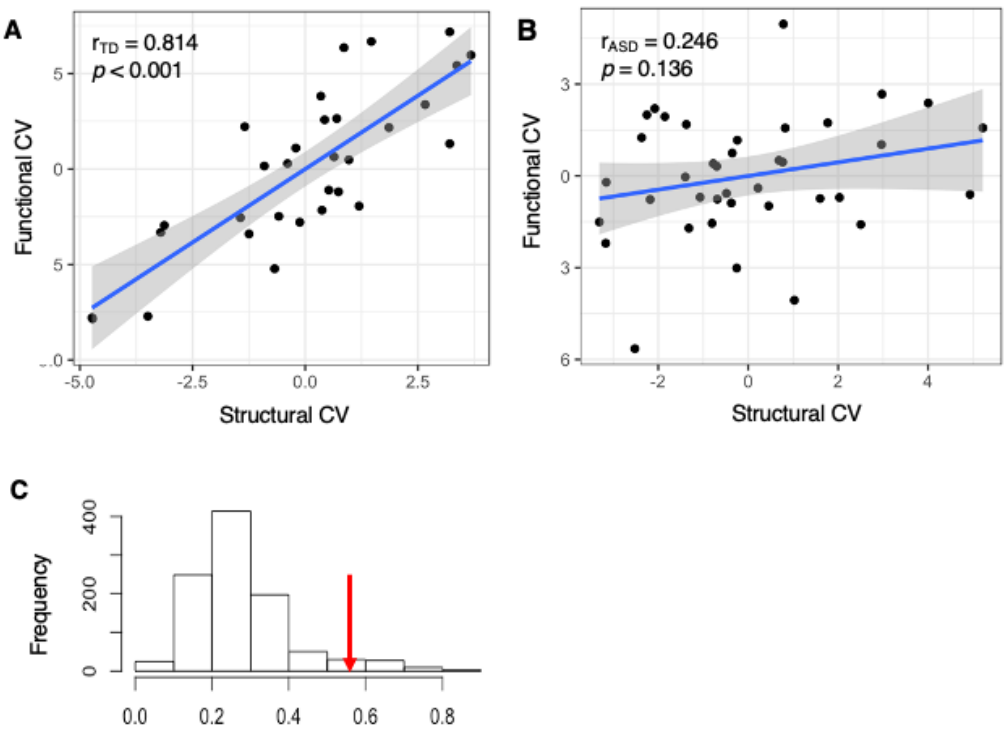


**Figure 3.1 Canonical correlations between brain structural and functional indices**
Scatterplots representing canonical correlations between structural and functional CVs (canonical variates) in the TD (A) and ASD (B) groups. (C) Bootstrapping distribution of the difference in canonical correlations after randomly splitting the whole sample in half and applying the canonical vectors derived from one half of the sample to the other half (1000 iterations); red arrow indicates the difference in canonical correlations depicted in panels A and B ($r_{TD} - r_{ASD} = 0.57$).

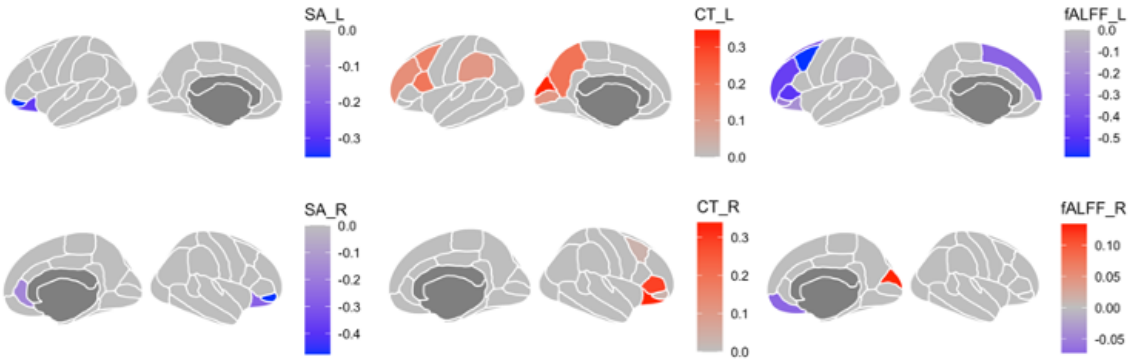


**Figure 3.2 ROIs of highest brain structure-function correlation in TD children**
Canonical vectors of surface area (SA), cortical thickness (CT), and fALFF from bilateral regions of interest (ROIs) with maximized structure-function correlation in typically developing (TD) children, derived from sparse canonical correlation analysis (SCCA). Top and bottom panels depict ROIs from left and right hemisphere, respectively. ROIs contributing to each structural and functional canonical vectors are: for SA, L pars orbitalis, L lateral orbitofrontal, R pars orbitalis, R lateral orbitofrontal, R rostral anterior cingulate; for CT, L cuneus, L precuneus, L pars opercularis, L caudal middle frontal, L rostral middle frontal, L pericalcarine, L supramarginal, R lateral orbitofrontal, R pars triangularis, R caudal middle frontal, R pars orbitalis; for fALFF, L caudal middle frontal, L pars triangularis, L rostral middle frontal, L superior frontal, L pars orbitalis, L lateral orbitofrontal, L supramarginal, R cuneus, R medial orbitofrontal. L=left; R=right.

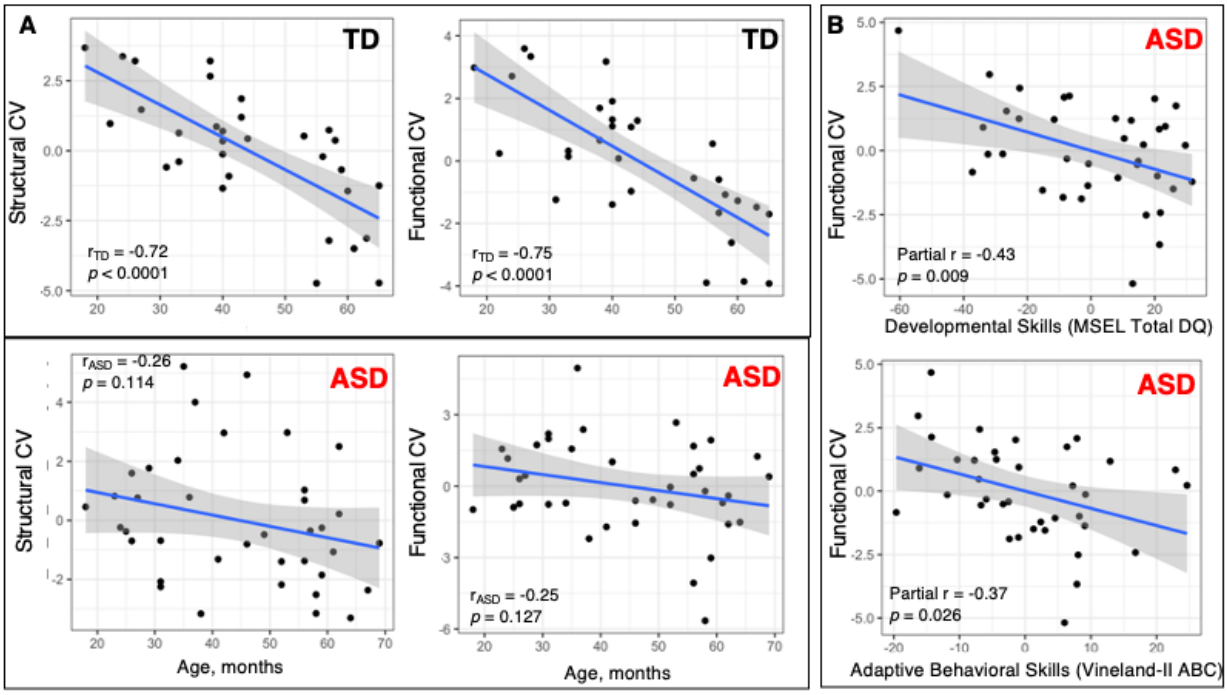


**Figure 3.3 Correlations between brain indices and age and developmental skills**
**A:** Correlations between structural and functional canonical variates (CVs) and age, plotted separately in the TD (**top**) and ASD (**bottom panel**) groups. **B:** Partial correlations* between functional CV and MSEL Total DQ **(top)** and Vineland-II ABC **(bottom)** in children with ASD (*controlling for age; values on the X and Y axes represent residuals).

## References


APA. (2013). Diagnostic and statistical manual of mental disorders (DSM-5®): American Psychiatric Pub.

Baum, G. L., Cui, Z., Roalf, D. R., Ciric, R., Betzel, R. F., Larsen, B., et al. (2020). Development of structure-function coupling in human brain networks during youth. Proc Natl Acad Sci U S A, 117(1), 771-778.

Behzadi, Y., Restom, K., Liau, J., & Liu, T. T. (2007). A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. Neuroimage, 37(1), 90-101.

Bethlehem, R. A. I., Seidlitz, J., White, S. R., Vogel, J. W., Anderson, K. M., Adamson, C., et al. (2022). Brain charts for the human lifespan. Nature, 604(7906), 525-533.

Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med, 34(4), 537-541.

Bornstein, M. H. (2014). Human infancy…and the rest of the lifespan. Annu Rev Psychol, 65, 121-158.

Brown, T. T., Kuperman, J. M., Chung, Y., Erhart, M., McCabe, C., Hagler, D. J., Jr., et al. (2012). Neuroanatomical assessment of biological maturity. Curr Biol, 22(18), 1693-1698.

Chen, B., Linke, A., Olson, L., Ibarra, C., Reynolds, S., Müller, R. A., et al. (2021). Greater functional connectivity between sensory networks is related to symptom severity in toddlers with autism spectrum disorder. J Child Psychol Psychiatry, 62(2), 160-170.

Chen, B., Linke, A., Olson, L., Kohli, J., Kinnear, M., Sereno, M., et al. (2022). Cortical myelination in toddlers and preschoolers with autism spectrum disorder. Dev Neurobiol, 82(3), 261-274.

Chen, H., Long, J., Yang, S., & He, B. (2021). Atypical Functional Covariance Connectivity Between Gray and White Matter in Children With Autism Spectrum Disorder. Autism Res, 14(3), 464-472.

Courchesne, E., Gazestani, V. H., & Lewis, N. E. (2020). Prenatal Origins of ASD: The When, What, and How of ASD Development. Trends Neurosci, 43(5), 326-342.

Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., et al. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology, 57(2), 245-254.

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage, 31(3), 968-980.

Di Martino, A., O'Connor, D., Chen, B., Alaerts, K., Anderson, J. S., Assaf, M., et al. (2017). Enhancing studies of the connectome in autism using the autism brain imaging data exchange II. Sci Data, 4, 170010.

Di Martino, A., Yan, C. G., Li, Q., Denio, E., Castellanos, F. X., Alaerts, K., et al. (2014). The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. Mol Psychiatry, 19(6), 659-667.

Dinstein, I., Pierce, K., Eyler, L., Solso, S., Malach, R., Behrmann, M., et al. (2011). Disrupted neural synchronization in toddlers with autism. Neuron, 70(6), 1218-1225.

Eggebrecht, A. T., Elison, J. T., Feczko, E., Todorov, A., Wolff, J. J., Kandala, S., et al. (2017). Joint Attention and Brain Functional Connectivity in Infants and Toddlers. Cereb Cortex, 27(3), 1709-1720.

Emerson, R. W., Adams, C., Nishino, T., Hazlett, H. C., Wolff, J. J., Zwaigenbaum, L., et al. (2017). Functional neuroimaging of high-risk 6-month-old infants predicts a diagnosis of autism at 24 months of age. Science translational medicine, 9(393), eaag2882.

Eyler, L. T., Pierce, K., & Courchesne, E. (2012). A failure of left temporal cortex to specialize for language is an early emerging and fundamental property of autism. Brain, 135(Pt 3), 949-960.

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci, 8(9), 700-711.

Frangou, S., Modabbernia, A., Williams, S. C. R., Papachristou, E., Doucet, G. E., Agartz, I., et al. (2022). Cortical thickness across the lifespan: Data from 17,075 healthy individuals aged 3-90 years. Hum Brain Mapp, 43(1), 431-451.

Guo, X., Chen, H., Long, Z., Duan, X., Zhang, Y., & Chen, H. (2017). Atypical developmental trajectory of local spontaneous brain activity in autism spectrum disorder. Sci Rep, 7, 39822.

Hardoon, D. R., Szedmak, S., & Shawe-Taylor, J. (2004). Canonical Correlation Analysis: An Overview with Application to Learning Methods. Neural Computation, 16(12), 2639-2664.

Hazlett, H. C., Poe, M., Gerig, G., Smith, R. G., Provenzale, J., Ross, A., et al. (2005). Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. Arch Gen Psychiatry, 62(12), 1366-1376.

Hazlett, H. C., Poe, M. D., Gerig, G., Styner, M., Chappell, C., Smith, R. G., et al. (2011). Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. Archives of general psychiatry, 68(5), 467-476.

He, C., Chen, H., Uddin, L. Q., Erramuzpe, A., Bonifazi, P., Guo, X., et al. (2020). Structure-Function Connectomics Reveals Aberrant Developmental Trajectory Occurring at Preadolescence in the Autistic Brain. Cereb Cortex, 30(9), 5028-5037.

Hendrix, C. L., & Thomason, M. E. (2022). A survey of protocols from 54 infant and toddler neuroimaging research labs. Dev Cogn Neurosci, 54, 101060.

Itahashi, T., Yamada, T., Watanabe, H., Nakamura, M., Ohta, H., Kanai, C., et al. (2015). Alterations of local spontaneous brain activity and connectivity in adults with high-functioning autism spectrum disorder. Mol Autism, 6, 30.

Karavallil Achuthan, S., Coburn, K. L., Beckerson, M. E., & Kana, R. K. (2023). Amplitude of low frequency fluctuations during resting state fMRI in autistic children. Autism Res, 16(1), 84-98.

Landa, R. J. (2018). Efficacy of early interventions for infants and young children with, and at risk for, autism spectrum disorders. Int Rev Psychiatry, 30(1), 25-39.

Linke, A. C., Chen, B., Olson, L., Ibarra, C., Fong, C., Reynolds, S., et al. (2023). Sleep Problems in Preschoolers With Autism Spectrum Disorder Are Associated With Sensory Sensitivities and Thalamocortical Overconnectivity. Biol Psychiatry Cogn Neurosci Neuroimaging, 8(1), 21-31.

Lipkin, P. H., & Macias, M. M. (2020). Promoting Optimal Development: Identifying Infants and Young Children With Developmental Disorders Through Developmental Surveillance and Screening. Pediatrics, 145(1).

Lombardo, M. V., Pierce, K., Eyler, L. T., Carter Barnes, C., Ahrens-Barbeau, C., Solso, S., et al. (2015). Different functional neural substrates for good and poor language outcome in autism. Neuron, 86(2), 567-577.

Lord, C., Risi, S., DiLavore, P. S., Shulman, C., Thurm, A., & Pickles, A. (2006). Autism from 2 to 9 years of age. Arch Gen Psychiatry, 63(6), 694-701.

Lord, C., & Rutter, M. (2003). Social communication questionnaire (SCQ). Torrance, CA: WPS.

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K., & Bishop, S. L. (2012). Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). Torrence, CA: Western Psychological Services.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord, 24(5), 659-685.

Maenner, M. J., Warren, Z., Williams, A. R., Amoakohene, E., Bakian, A. V., Bilder, D. A., et al. (2023). Prevalence and characteristics of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020. MMWR Surveillance Summaries, 72(2), 1.

Marrus, N., Eggebrecht, A. T., Todorov, A., Elison, J. T., Wolff, J. J., Cole, L., et al. (2018). Walking, Gross Motor Development, and Brain Functional Connectivity in Infants and Toddlers. Cereb Cortex, 28(2), 750-763.

McKinnon, C. J., Eggebrecht, A. T., Todorov, A., Wolff, J. J., Elison, J. T., Adams, C. M., et al. (2019). Restricted and Repetitive Behavior and Brain Functional Connectivity in Infants at Risk for Developing Autism Spectrum Disorder. Biol Psychiatry Cogn Neurosci Neuroimaging, 4(1), 50-61.

Messinger, D., Young, G. S., Ozonoff, S., Dobkins, K., Carter, A., Zwaigenbaum, L., et al. (2013). Beyond autism: a baby siblings research consortium study of high-risk children at three years of age. J Am Acad Child Adolesc Psychiatry, 52(3), 300-308.e301.

Mullen, E. M. (1995). Mullen scales of early learning. Circle Pines, MN: American Guidance Service.

Munson, J., Dawson, G., Sterling, L., Beauchaine, T., Zhou, A., Elizabeth, K., et al. (2008). Evidence for latent classes of IQ in young children with autism spectrum disorder. Am J Ment Retard, 113(6), 439-452.

Nordahl, C. W., Mello, M., Shen, A. M., Shen, M. D., Vismara, L. A., Li, D., et al. (2016). Methods for acquiring MRI data in children with autism spectrum disorder and intellectual impairment without the use of sedation. Journal of neurodevelopmental disorders, 8, 20-20.

Ozonoff, S., Young, G. S., Landa, R. J., Brian, J., Bryson, S., Charman, T., et al. (2015). Diagnostic stability in young children at risk for autism spectrum disorder: a baby siblings research consortium study. J Child Psychol Psychiatry, 56(9), 988-998.

Panizzon, M. S., Fennema-Notestine, C., Eyler, L. T., Jernigan, T. L., Prom-Wormley, E., Neale, M., et al. (2009). Distinct genetic influences on cortical surface area and cortical thickness. Cereb Cortex, 19(11), 2728-2735.

Qi, S., Morris, R., Turner, J. A., Fu, Z., Jiang, R., Deramus, T. P., et al. (2020). Common and unique multimodal covarying patterns in autism spectrum disorder subtypes. Mol Autism, 11(1), 90.

Remer, J., Croteau-Chonka, E., Dean, D. C., 3rd, D'Arpino, S., Dirks, H., Whiley, D., et al. (2017). Quantifying cortical development in typically developing toddlers and young children, 1-6 years of age. Neuroimage, 153, 246-261.

Schumann, C. M., Bloss, C. S., Barnes, C. C., Wideman, G. M., Carper, R. A., Akshoomoff, N., et al. (2010). Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. J Neurosci, 30(12), 4419-4427.

Solso, S., Xu, R., Proudfoot, J., Hagler, D. J., Jr., Campbell, K., Venkatraman, V., et al. (2016). Diffusion Tensor Imaging Provides Evidence of Possible Axonal Overconnectivity in Frontal Lobes in Autism Spectrum Disorder Toddlers. Biol Psychiatry, 79(8), 676-684.

Sparrow, S. S., Cicchetti, D. V., & Balla, D. A. (2005). Vineland Adaptive Behavior Scales, Second Edition. Circle Pines, MN: American Guidance Service.

Strike, L. T., Hansell, N. K., Couvy-Duchesne, B., Thompson, P. M., de Zubicaray, G. I., McMahon, K. L., et al. (2019). Genetic Complexity of Cortical Structure: Differences in Genetic and Environmental Factors Influencing Cortical Surface Area and Thickness. Cereb Cortex, 29(3), 952-962.

Szatmari, P., Chawarska, K., Dawson, G., Georgiades, S., Landa, R., Lord, C., et al. (2016). Prospective Longitudinal Studies of Infant Siblings of Children With Autism: Lessons Learned and Future Directions. J Am Acad Child Adolesc Psychiatry, 55(3), 179-187.

Tau, G. Z., & Peterson, B. S. (2010). Normal development of brain circuits. Neuropsychopharmacology, 35(1), 147-168.

Turesky, T. K., Vanderauwera, J., & Gaab, N. (2021). Imaging the rapidly developing brain: Current challenges for MRI studies in the first five years of life. Dev Cogn Neurosci, 47, 100893.

Uddin, L. Q., Supekar, K., & Menon, V. (2013). Reconceptualizing functional brain connectivity in autism from a developmental perspective. Front Hum Neurosci, 7, 458.

Vivanti, G., Prior, M., Williams, K., & Dissanayake, C. (2014). Predictors of outcomes in autism early intervention: why don't we know more? Front Pediatr, 2, 58.

Weitzman, C., & Wegner, L. (2015). Promoting optimal development: screening for behavioral and emotional problems. Pediatrics, 135(2), 384-395.

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect, 2(3), 125-141.

Wierenga, L. M., Langen, M., Oranje, B., & Durston, S. (2014). Unique developmental trajectories of cortical thickness and surface area. Neuroimage, 87, 120-126.

Witten, D. M., Tibshirani, R., & Hastie, T. (2009). A penalized matrix decomposition, with applications to sparse principal components and canonical correlation analysis. Biostatistics, 10(3), 515-534.

Wolff, J. J., Gu, H., Gerig, G., Elison, J. T., Styner, M., Gouttard, S., et al. (2012). Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. Am J Psychiatry, 169(6), 589-600.

Xiao, Z., Qiu, T., Ke, X., Xiao, X., Xiao, T., Liang, F., et al. (2014). Autism spectrum disorder as early neurodevelopmental disorder: evidence from the brain imaging abnormalities in 2-3 years old toddlers. J Autism Dev Disord, 44(7), 1633-1640.

Zhuang, X., Yang, Z., & Cordes, D. (2020). A technical review of canonical correlation analysis for neuroscience applications. Hum Brain Mapp, 41(13), 3807-3833.

Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J., et al. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. J Neurosci Methods, 172(1), 137-141.

Zuo, X. N., Di Martino, A., Kelly, C., Shehzad, Z. E., Gee, D. G., Klein, D. F., et al. (2010). The oscillating brain: complex and reliable. Neuroimage, 49(2), 1432-1445

# INTEGRATED SUMMARY

Although behavioral signs of ASD emerge early in life and can be reliably identified during the second year of life, relatively little is known about the developmental trajectories of brain structure and function in ASD during this critical neurodevelopmental period. This is in great part due to the known challenges associated with conducting neuroimaging studies in early childhood especially with young children with developmental disabilities. The three studies described above aimed to enhance our understanding of early brain development in ASD by examining multiple aspects of neurodevelopment which are distinct and complementary to each other. Each study provided new insights into how critical neurodevelopmental processes in ASD deviate from typical developmental trajectories in the first years of life. Specifically, this dissertation examined functional network organization and connectivity, intracortical myelination, and covariation between brain morphometry and local spontaneous activity using multimodal MRI data (i.e., structural and functional MRI) acquired during natural sleep from young children with and without ASD. Study 1 found atypically increased functional connectivity between visual and sensorimotor networks in young children with ASD and this overconnectivity was linked with greater autism symptoms. Study 2 reported differential age-related trajectories in an MRI-proxy of intracortical myelin between children with ASD and TD children, although no group differences in intracortical myelin content were identified. Finally, Study 3 showed reduced brain structure-function correlation between brain morphometric measures (surface area and cortical thickness) and local spontaneous activity in our ASD cohort using a multivariate pattern analysis, with the resultant local spontaneous activity index associated with general developmental and adaptive behavior skills in children with ASD.

Altogether, these three studies highlight the importance of integrating multimodal data and examining distinct but complementary anatomical and functional brain measures to elucidate the trajectories of postnatal brain development during the first years of life when ASD behavioral symptoms first manifest. While the three studies apply distinct methodological and analytical approaches, some convergent themes in the results have emerged. One somewhat unexpected finding is that little or no group differences were identified in functional network connectivity and intracortical myelin content between the ASD and TD groups in Studies 1 and 2. At the same time, differential age-related trajectories were consistently found in young children with ASD as compared to maturational trajectories observed in the TD cohort. This pattern of results suggests that fundamental neurodevelopmental processes may be mistimed in ASD, with potentially greater inter-individual variability of brain maturational trajectories found among children with ASD. Similarly, Study 3 identified absence of neurotypical age-related effects in covarying brain morphometric and local spontaneous activity indices, which may have contributed to the overall reduced brain structure-function correlation observed in the ASD cohort. The atypical trajectories of brain development in ASD identified across all three studies are generally consistent with the current understanding that the ASD phenotype is the result of cascading neurodevelopmental effects that commence very early in development (Constantino, Charman, & Jones, 2021; Courchesne et al., 2019; Wolff, Jacob, & Elison, 2018). The results of the three studies also provide substantial evidence that several early building blocks of brain structural and functional development are implicated in the first years of life in ASD, highlighting the notion that there is not a single neurobiological process, but rather a confluence of multiple neurodevelopmental factors that contribute to the ASD phenotype. Hence, our understanding of how these processes interact over time and how they differentially relate to later developmental

and clinical outcomes are important questions to answer. Finally, several of the indices identified as having atypical neurodevelopmental trajectories in children with ASD (e.g., multisensory functional connectivity in Study 1 or local spontaneous activity correlated with brain morphometry in Study 3) were associated with clinical and developmental indices (including autism symptom severity and overall developmental skills), suggesting that the neurobiology underlying these measurable brain indices contributes to the ASD phenotype.

**Limitations and Future Directions**

While atypical age-related trajectories were identified using cross-sectional data, future studies are needed to confirm these relationships longitudinally. Our results also need to be replicated in other independent samples of young children with and without ASD. The sample sizes across the three studies, while somewhat variable, were relatively moderate limiting our ability to meaningfully examine heterogeneity and sex differences in brain development in young children with ASD. Nevertheless, this ASD cohort exhibited a full range of developmental skills and autism symptom severity, largely due to the concerted effort to recruit a representative sample as well as due to implementation of sleep MRI protocol. One of the biggest criticisms in autism neuroimaging research is that the majority of past studies have reported somewhat biased results because they primarily included children and adolescents with ASD with relatively high level of functioning, given the need to cooperate with scanning requirements (i.e., being able to stay still inside the MRI scanner for 30-60 minutes without sedation). This essentially precluded participation from children younger than 5 years of age and those representing the entire range of abilities on the autism spectrum, including children with severe autism symptoms. Scanning young children with ASD during natural sleep has shown promise in studying the developing brain across the entire autism spectrum. We anticipate that more neuroimaging studies with

young children will adopt this approach in the future with efforts to share best practices in MRI acquisition strategies, habituation procedures, scanning protocols, and analytical approaches in the infant and toddler neuroimaging community (Chen, Linke, Olson, Ibarra, Kinnear, et al., 2021; Gilmore et al., 2018; Hendrix & Thomason, 2022). Lastly, the three studies largely relied on group-level comparisons between ASD and TD due to lack of power to detect individual differences or potential subgroups or subtypes of ASD. Moving beyond group-level analysis, studies examining neural correlates of individual variability and developmental trajectories are necessary to parse the behavioral heterogeneity of ASD (Lord, Bishop, & Anderson, 2015), which can be particularly useful for developing and refining existing targeted intervention strategies, and measuring the impact of interventions on brain structural and functional maturation.

**Conclusions**

Despite these limitations, to our knowledge, this is the first series of studies to comprehensively characterize multiple neurodevelopmental processes in toddlers and preschoolers with ASD. This dissertation demonstrates the potential of multimodal neuroimaging methods to reveal the maturational pathways of critical brain structural and functional changes that characterize the first years of life in ASD. These three studies provide initial evidence that the age-related trajectories of functional network connectivity, intracortical myelin, brain morphometry, and local spontaneous activity differ from neurotypical trajectories, and are related to autism symptoms and overall developmental skills. However, many important questions remain about the mechanistic interactions among these processes, individual variability in the neurodevelopmental trajectories, and how these early brain abnormalities relate to later outcomes. Future multimodal longitudinal studies aimed at clarifying the relationships between

multiple neurodevelopmental processes and parsing the neural and behavioral heterogeneity of ASD are essential in moving the research forward. Ultimately, knowledge gained from these studies may aid with the search for reliable biomarkers, which could be potentially identified prior to the emergence of behavioral symptoms. This is particularly meaningful for early detection and intervention and can contribute to the development of more targeted prevention and intervention programs.

**Reflections on Autism Research**

As the field of autism research moves forward, the research community has come to realize the importance of engaging key stakeholders such as individuals with ASD and their families in the design, execution, and interpretation of results for research studies. The SDSU Toddler MRI Project has made concerted efforts in engaging families from a wide range of communities in Southern California and accommodating their needs throughout their study participation. For instance, research visits, including behavioral evaluations and scanning sessions, were offered on weekends, after hours, and school holidays or breaks, to expand accessibility of research participation. This is particularly important for the families with limited socioeconomic resources (e.g., access to childcare, paid time off). The research team members offered to provide childcare for siblings of participants as needed during research visits, to help remove financial constraints associated with participation. Furthermore, as part of the research protocol, brief summaries of the evaluations including testing results, diagnostic impressions, and recommendations were provided to participants' parents or guardians who often find the reports useful for initiating interventional services or accommodations at schools. Providing this benefit to the families at no cost has allowed the Project to target under-served populations who may not seek diagnostic evaluations due to cost, or experience additional delays in accessing

resources. The implementation of these measures has allowed us to promote more equitable participation in research by families from under-resourced and traditionally under-represented communities and to maintain long-term cooperative relationship with these families.

Overall, upon reflection, besides understanding the etiology of ASD, it is also critical to consider the translational value of our work and how neuroimaging research can contribute to improving the quality of life of people with ASD and supporting their families. Although this dissertation only includes cross-sectional data collected from one time point or study visit, ongoing longitudinal data collection is under way in the context of the SDSU Toddler MRI Project, following up these children when they turn 5 years of age. With the knowledge gained from this dissertation in early brain development in ASD, I hope to continue to explore how deviations in early neurodevelopmental processes relate to later developmental outcomes including ASD symptoms, cognitive and adaptive functioning, and other co-occurring conditions (such as ADHD) assessed at school-entry age. Broadly, in addition to developing pharmacological and behavioral intervention programs for individuals with ASD whose health and overall wellbeing are significantly impacted by the core symptomatology or related challenges associated with ASD, we need to also consider how to influence policy making and public perception and attitudes towards neurodiverse populations (Lord et al., 2022) in order to create a more inclusive and equitable society that allows full participation and contribution by neurodiverse as well as neurotypical individuals.

REFERENCES


Abrahám, H., Vincze, A., Jewgenow, I., Veszprémi, B., Kravják, A., Gömöri, E., & Seress, L. (2010). Myelination in the human hippocampal formation from midgestation to adulthood. *Int J Dev Neurosci, 28*(5), 401-410. doi:10.1016/j.ijdevneu.2010.03.004

Almli, C. R., Rivkin, M. J., & McKinstry, R. C. (2007). The NIH MRI study of normal brain development (Objective-2): newborns, infants, toddlers, and preschoolers. *Neuroimage, 35*(1), 308-325. doi:10.1016/j.neuroimage.2006.08.058

Andrews, D. S., Avino, T. A., Gudbrandsen, M., Daly, E., Marquand, A., Murphy, C. M., . . . Ecker, C. (2017). In Vivo Evidence of Reduced Integrity of the Gray-White Matter Boundary in Autism Spectrum Disorder. *Cereb Cortex, 27*(2), 877-887. doi:10.1093/cercor/bhw404

APA. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5®)*: American Psychiatric Pub.

Arnold, S. E., & Trojanowski, J. Q. (1996). Human fetal hippocampal development: I. Cytoarchitecture, myeloarchitecture, and neuronal morphologic features. *J Comp Neurol, 367*(2), 274-292. doi:10.1002/(sici)1096-9861(19960401)367:2<274::Aid-cne9>3.0.Co;2-2

Avino, T. A., & Hutsler, J. J. (2010). Abnormal cell patterning at the cortical gray-white matter boundary in autism spectrum disorders. *Brain Res, 1360*, 138-146. doi:10.1016/j.brainres.2010.08.091

Barbas, H. (2015). General cortical and special prefrontal connections: principles from structure to function. *Annu Rev Neurosci, 38*, 269-289. doi:10.1146/annurev-neuro-071714-033936

Beckmann, C. F. (2012). Modelling with independent components. *Neuroimage, 62*(2), 891-901. doi:10.1016/j.neuroimage.2012.02.020

Bethlehem, R. A. I., Seidlitz, J., White, S. R., Vogel, J. W., Anderson, K. M., Adamson, C., . . . Alexander-Bloch, A. F. (2022). Brain charts for the human lifespan. *Nature, 604*(7906), 525-533. doi:10.1038/s41586-022-04554-y

Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med, 34*(4), 537-541. doi:10.1002/mrm.1910340409

Bornstein, M. H. (2014). Human infancy…and the rest of the lifespan. *Annu Rev Psychol, 65*, 121-158. doi:10.1146/annurev-psych-120710-100359

Brown, T. T., & Jernigan, T. L. (2012). Brain development during the preschool years. *Neuropsychol Rev, 22*(4), 313-333. doi:10.1007/s11065-012-9214-1

Brown, T. T., Kuperman, J. M., Chung, Y., Erhart, M., McCabe, C., Hagler, D. J., Jr., . . . Dale, A. M. (2012). Neuroanatomical assessment of biological maturity. *Curr Biol, 22*(18), 1693-1698. doi:10.1016/j.cub.2012.07.002

Casanova, M. F., Buxhoeveden, D. P., Switala, A. E., & Roy, E. (2002). Neuronal density and architecture (Gray Level Index) in the brains of autistic patients. *J Child Neurol, 17*(7), 515-521. doi:10.1177/088307380201700708

Chapman, T. W., & Hill, R. A. (2020). Myelin plasticity in adulthood and aging. *Neurosci Lett, 715*, 134645. doi:10.1016/j.neulet.2019.134645

Chen, B., Linke, A., Olson, L., Ibarra, C., Kinnear, M., & Fishman, I. (2021). Resting state functional networks in 1-to-3-year-old typically developing children. *Dev Cogn Neurosci, 51*, 100991. doi:10.1016/j.dcn.2021.100991

Chen, B., Linke, A., Olson, L., Ibarra, C., Reynolds, S., Müller, R. A., . . . Fishman, I. (2021). Greater functional connectivity between sensory networks is related to symptom severity in toddlers with autism spectrum disorder. *J Child Psychol Psychiatry, 62*(2), 160-170. doi:10.1111/jcpp.13268

Chen, B., Linke, A., Olson, L., Kohli, J., Kinnear, M., Sereno, M., . . . Fishman, I. (2022). Cortical myelination in toddlers and preschoolers with autism spectrum disorder. *Dev Neurobiol, 82*(3), 261-274. doi:10.1002/dneu.22874

Constantino, J. N., Charman, T., & Jones, E. J. H. (2021). Clinical and Translational Implications of an Emerging Developmental Substructure for Autism. *Annu Rev Clin Psychol, 17*, 365-389. doi:10.1146/annurev-clinpsy-081219-110503

Conti, E., Mitra, J., Calderoni, S., Pannek, K., Shen, K. K., Pagnozzi, A., . . . Guzzetta, A. (2017). Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: A diffusion MRI study. *Hum Brain Mapp, 38*(5), 2333-2344. doi:10.1002/hbm.23520

Corsello, C. M., Akshoomoff, N., & Stahmer, A. C. (2013). Diagnosis of autism spectrum disorders in 2-year-olds: a study of community practice. *J Child Psychol Psychiatry, 54*(2), 178-185. doi:10.1111/j.1469-7610.2012.02607.x

Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., . . . Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology, 57*(2), 245-254.

Courchesne, E., Pramparo, T., Gazestani, V. H., Lombardo, M. V., Pierce, K., & Lewis, N. E. (2019). The ASD Living Biology: from cell proliferation to clinical phenotype. *Mol Psychiatry, 24*(1), 88-107. doi:10.1038/s41380-018-0056-y

Damoiseaux, J. S., Rombouts, S. A., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., & Beckmann, C. F. (2006). Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences of the United States of America, 103*(37), 13848-13853. doi:10.1073/pnas.0601417103

Dawson, G., Jones, E. J., Merkle, K., Venema, K., Lowy, R., Faja, S., . . . Webb, S. J. (2012). Early behavioral intervention is associated with normalized brain activity in young children with autism. *J Am Acad Child Adolesc Psychiatry, 51*(11), 1150-1159. doi:10.1016/j.jaac.2012.08.018

de Bie, H. M., Boersma, M., Wattjes, M. P., Adriaanse, S., Vermeulen, R. J., Oostrom, K. J., . . . Delemarre-Van de Waal, H. A. (2010). Preparing children with a mock scanner training protocol results in high quality structural and functional MRI scans. *Eur J Pediatr, 169*(9), 1079-1085. doi:10.1007/s00431-010-1181-z

Dean, D. C., 3rd, Dirks, H., O'Muircheartaigh, J., Walker, L., Jerskey, B. A., Lehman, K., . . . Deoni, S. C. (2014). Pediatric neuroimaging using magnetic resonance imaging during non-sedated sleep. *Pediatr Radiol, 44*(1), 64-72. doi:10.1007/s00247-013-2752-8

Deoni, S. C., Dean, D. C., 3rd, Remer, J., Dirks, H., & O'Muircheartaigh, J. (2015). Cortical maturation and myelination in healthy toddlers and young children. *Neuroimage, 115*, 147-161. doi:10.1016/j.neuroimage.2015.04.058

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., . . . Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage, 31*(3), 968-980. doi:10.1016/j.neuroimage.2006.01.021

Di Martino, A., O'Connor, D., Chen, B., Alaerts, K., Anderson, J. S., Assaf, M., . . . Milham, M. P. (2017). Enhancing studies of the connectome in autism using the autism brain imaging data exchange II. *Sci Data, 4*, 170010. doi:10.1038/sdata.2017.10

Di Martino, A., Yan, C. G., Li, Q., Denio, E., Castellanos, F. X., Alaerts, K., . . . Milham, M. P. (2014). The autism brain imaging data exchange: towards a large-scale evaluation of the intrinsic brain architecture in autism. *Mol Psychiatry, 19*(6), 659-667. doi:10.1038/mp.2013.78

Dinstein, I., Pierce, K., Eyler, L., Solso, S., Malach, R., Behrmann, M., & Courchesne, E. (2011). Disrupted neural synchronization in toddlers with autism. *Neuron, 70*(6), 1218-1225. doi:10.1016/j.neuron.2011.04.018

Dosenbach, N. U., Nardos, B., Cohen, A. L., Fair, D. A., Power, J. D., Church, J. A., . . . Schlaggar, B. L. (2010). Prediction of individual brain maturity using fMRI. *Science, 329*(5997), 1358-1361. doi:10.1126/science.1194144

Eggebrecht, A. T., Elison, J. T., Feczko, E., Todorov, A., Wolff, J. J., Kandala, S., . . . Pruett, J. R., Jr. (2017). Joint Attention and Brain Functional Connectivity in Infants and Toddlers. *Cereb Cortex, 27*(3), 1709-1720. doi:10.1093/cercor/bhw403

Emerson, R. W., Adams, C., Nishino, T., Hazlett, H. C., Wolff, J. J., Zwaigenbaum, L., . . . Piven, J. (2017). Functional neuroimaging of high-risk 6-month-old infants predicts a diagnosis of autism at 24 months of age. *Science translational medicine, 9*(393), eaag2882. doi:10.1126/scitranslmed.aag2882

Estes, A., Munson, J., Rogers, S. J., Greenson, J., Winter, J., & Dawson, G. (2015). Long-Term Outcomes of Early Intervention in 6-Year-Old Children With Autism Spectrum Disorder. *J Am Acad Child Adolesc Psychiatry, 54*(7), 580-587. doi:10.1016/j.jaac.2015.04.005

Eyler, L. T., Pierce, K., & Courchesne, E. (2012). A failure of left temporal cortex to specialize for language is an early emerging and fundamental property of autism. *Brain, 135*(Pt 3), 949-960. doi:10.1093/brain/awr364

Fair, D. A., Cohen, A. L., Dosenbach, N. U., Church, J. A., Miezin, F. M., Barch, D. M., . . . Schlaggar, B. L. (2008). The maturing architecture of the brain's default network. *Proceedings of the National Academy of Sciences of the United States of America, 105*(10), 4028-4032. doi:10.1073/pnas.0800376105

Fields, R. D. (2014). Neuroscience. Myelin--more than insulation. *Science, 344*(6181), 264-266. doi:10.1126/science.1253851

Fishman, I., Datko, M., Cabrera, Y., Carper, R. A., & Müller, R. A. (2015). Reduced integration and differentiation of the imitation network in autism: A combined functional connectivity magnetic resonance imaging and diffusion-weighted imaging study. *Ann Neurol, 78*(6), 958-969. doi:10.1002/ana.24533

Fishman, I., Keown, C. L., Lincoln, A. J., Pineda, J. A., & Müller, R. A. (2014). Atypical cross talk between mentalizing and mirror neuron networks in autism spectrum disorder. *JAMA Psychiatry, 71*(7), 751-760. doi:10.1001/jamapsychiatry.2014.83

Fishman, I., Linke, A. C., Hau, J., Carper, R. A., & Müller, R. A. (2018). Atypical Functional Connectivity of Amygdala Related to Reduced Symptom Severity in Children With Autism. *J Am Acad Child Adolesc Psychiatry, 57*(10), 764-774.e763. doi:10.1016/j.jaac.2018.06.015

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci, 8*(9), 700-711. doi:10.1038/nrn2201

Frangou, S., Modabbernia, A., Williams, S. C. R., Papachristou, E., Doucet, G. E., Agartz, I., . . . Dima, D. (2022). Cortical thickness across the lifespan: Data from 17,075 healthy individuals aged 3-90 years. *Hum Brain Mapp, 43*(1), 431-451. doi:10.1002/hbm.25364

Fransson, P., Skiöld, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., & Åden, U. (2007). Resting-state networks in the infant brain. *Proceedings of the National Academy of Sciences, 104*(39), 15531-15536.

Gao, W., Alcauter, S., Elton, A., Hernandez-Castillo, C. R., Smith, J. K., Ramirez, J., & Lin, W. (2015). Functional Network Development During the First Year: Relative Sequence and Socioeconomic Correlations. *Cereb Cortex, 25*(9), 2919-2928. doi:10.1093/cercor/bhu088

Gao, W., Alcauter, S., Smith, J. K., Gilmore, J. H., & Lin, W. (2015). Development of human brain cortical network architecture during infancy. *Brain Struct Funct, 220*(2), 1173-1186. doi:10.1007/s00429-014-0710-3

Gilmore, J. H., Knickmeyer, R. C., & Gao, W. (2018). Imaging structural and functional brain development in early childhood. *Nat Rev Neurosci, 19*(3), 123-137. doi:10.1038/nrn.2018.1

Gilmore, J. H., Shi, F., Woolson, S. L., Knickmeyer, R. C., Short, S. J., Lin, W., . . . Shen, D. (2012). Longitudinal development of cortical and subcortical gray matter from birth to 2 years. *Cereb Cortex, 22*(11), 2478-2485. doi:10.1093/cercor/bhr327

Gordon, A., & Geschwind, D. H. (2020). Human in vitro models for understanding mechanisms of autism spectrum disorder. *Mol Autism, 11*(1), 26. doi:10.1186/s13229-020-00332-7

Griffanti, L., Douaud, G., Bijsterbosch, J., Evangelisti, S., Alfaro-Almagro, F., Glasser, M. F., . . . Smith, S. M. (2017). Hand classification of fMRI ICA noise components. *Neuroimage, 154*, 188-205. doi:10.1016/j.neuroimage.2016.12.036

Guo, X., Chen, H., Long, Z., Duan, X., Zhang, Y., & Chen, H. (2017). Atypical developmental trajectory of local spontaneous brain activity in autism spectrum disorder. *Scientific reports, 7*, 39822. doi:10.1038/srep39822

Hardoon, D. R., Szedmak, S., & Shawe-Taylor, J. (2004). Canonical Correlation Analysis: An Overview with Application to Learning Methods. *Neural Computation, 16*(12), 2639-2664. doi:10.1162/0899766042321814

Haroutunian, V., Katsel, P., Roussos, P., Davis, K. L., Altshuler, L. L., & Bartzokis, G. (2014). Myelination, oligodendrocytes, and serious mental illness. *Glia, 62*(11), 1856-1877. doi:10.1002/glia.22716

Haynes, L., Ip, A., Cho, I. Y. K., Dimond, D., Rohr, C. S., Bagshawe, M., . . . Bray, S. (2020). Grey and white matter volumes in early childhood: A comparison of voxel-based morphometry pipelines. *Dev Cogn Neurosci, 46*, 100875. doi:10.1016/j.dcn.2020.100875

Hazlett, H. C., Gu, H., Munsell, B. C., Kim, S. H., Styner, M., Wolff, J. J., . . . Piven, J. (2017). Early brain development in infants at high risk for autism spectrum disorder. *Nature, 542*(7641), 348-351. doi:10.1038/nature21369

Hazlett, H. C., Poe, M., Gerig, G., Smith, R. G., Provenzale, J., Ross, A., . . . Piven, J. (2005). Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch Gen Psychiatry, 62*(12), 1366-1376. doi:10.1001/archpsyc.62.12.1366

Hazlett, H. C., Poe, M. D., Gerig, G., Styner, M., Chappell, C., Smith, R. G., . . . Piven, J. (2011). Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Arch Gen Psychiatry, 68*(5), 467-476. doi:10.1001/archgenpsychiatry.2011.39

Hendrix, C. L., & Thomason, M. E. (2022). A survey of protocols from 54 infant and toddler neuroimaging research labs. *Dev Cogn Neurosci, 54*, 101060. doi:10.1016/j.dcn.2022.101060

Hoff, G. E., Van den Heuvel, M. P., Benders, M. J., Kersbergen, K. J., & De Vries, L. S. (2013). On development of functional brain connectivity in the young brain. *Front Hum Neurosci, 7*, 650. doi:10.3389/fnhum.2013.00650

Hull, J. V., Dokovna, L. B., Jacokes, Z. J., Torgerson, C. M., Irimia, A., & Van Horn, J. D. (2016). Resting-State Functional Connectivity in Autism Spectrum Disorders: A Review. *Front Psychiatry, 7*, 205. doi:10.3389/fpsyt.2016.00205

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol, 387*(2), 167-178.

Itahashi, T., Yamada, T., Watanabe, H., Nakamura, M., Ohta, H., Kanai, C., . . . Hashimoto, R. (2015). Alterations of local spontaneous brain activity and connectivity in adults with high-functioning autism spectrum disorder. *Mol Autism, 6*, 30. doi:10.1186/s13229-015-0026-z

Johnson, M. H. (2001). Functional brain development in humans. *Nat Rev Neurosci, 2*(7), 475-483. doi:10.1038/35081509

Johnson, M. H., Gliga, T., Jones, E., & Charman, T. (2015). Annual research review: Infant development, autism, and ADHD--early pathways to emerging disorders. *J Child Psychol Psychiatry, 56*(3), 228-247. doi:10.1111/jcpp.12328

Johnson, M. H., Grossmann, T., & Farroni, T. (2008). The social cognitive neuroscience of infancy: illuminating the early development of social brain functions. *Adv Child Dev Behav, 36*, 331-372. doi:10.1016/s0065-2407(08)00008-6

Jones, E. J., Gliga, T., Bedford, R., Charman, T., & Johnson, M. H. (2014). Developmental pathways to autism: a review of prospective studies of infants at risk. *Neurosci Biobehav Rev, 39*(100), 1-33. doi:10.1016/j.neubiorev.2013.12.001

Knickmeyer, R. C., Gouttard, S., Kang, C., Evans, D., Wilber, K., Smith, J. K., . . . Gilmore, J. H. (2008). A structural MRI study of human brain development from birth to 2 years. *J Neurosci, 28*(47), 12176-12182. doi:10.1523/jneurosci.3479-08.2008

Kolb, B., & Gibb, R. (2011). Brain plasticity and behaviour in the developing brain. *J Can Acad Child Adolesc Psychiatry, 20*(4), 265-276.

Landa, R. J. (2018). Efficacy of early interventions for infants and young children with, and at risk for, autism spectrum disorders. *Int Rev Psychiatry, 30*(1), 25-39. doi:10.1080/09540261.2018.1432574

Li, G., Nie, J., Wang, L., Shi, F., Lin, W., Gilmore, J. H., & Shen, D. (2013). Mapping region-specific longitudinal cortical surface expansion from birth to 2 years of age. *Cereb Cortex, 23*(11), 2724-2733. doi:10.1093/cercor/bhs265

Li, G., Wang, L., Shi, F., Lyall, A. E., Lin, W., Gilmore, J. H., & Shen, D. (2014). Mapping longitudinal development of local cortical gyrification in infants from birth to 2 years of age. *J Neurosci, 34*(12), 4228-4238. doi:10.1523/jneurosci.3976-13.2014

Lin, W., Zhu, Q., Gao, W., Chen, Y., Toh, C. H., Styner, M., . . . Gilmore, J. H. (2008). Functional connectivity MR imaging reveals cortical functional connectivity in the developing brain. *AJNR Am J Neuroradiol, 29*(10), 1883-1889. doi:10.3174/ajnr.A1256

Liu, S., Li, A., Zhu, M., Li, J., & Liu, B. (2019). Genetic influences on cortical myelination in the human brain. *Genes Brain Behav, 18*(4), e12537. doi:10.1111/gbb.12537

Lombardo, M. V., Pierce, K., Eyler, L. T., Carter Barnes, C., Ahrens-Barbeau, C., Solso, S., . . . Courchesne, E. (2015). Different functional neural substrates for good and poor language outcome in autism. *Neuron, 86*(2), 567-577. doi:10.1016/j.neuron.2015.03.023

Lord, C., Bishop, S., & Anderson, D. (2015). Developmental trajectories as autism phenotypes. *Am J Med Genet C Semin Med Genet, 169*(2), 198-208. doi:10.1002/ajmg.c.31440

Lord, C., Charman, T., Havdahl, A., Carbone, P., Anagnostou, E., Boyd, B., . . . McCauley, J. B. (2022). The Lancet Commission on the future of care and clinical research in autism. *Lancet, 399*(10321), 271-334. doi:10.1016/s0140-6736(21)01541-5

Lord, C., Elsabbagh, M., Baird, G., & Veenstra-Vanderweele, J. (2018). Autism spectrum disorder. *Lancet, 392*(10146), 508-520. doi:10.1016/s0140-6736(18)31129-2

Lord, C., Risi, S., DiLavore, P. S., Shulman, C., Thurm, A., & Pickles, A. (2006). Autism from 2 to 9 years of age. *Arch Gen Psychiatry, 63*(6), 694-701. doi:10.1001/archpsyc.63.6.694

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K., & Bishop, S. L. (2012). *Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)*. Torrence, CA: Western Psychological Services.

Lyall, A. E., Shi, F., Geng, X., Woolson, S., Li, G., Wang, L., . . . Gilmore, J. H. (2015). Dynamic Development of Regional Cortical Thickness and Surface Area in Early Childhood. *Cereb Cortex, 25*(8), 2204-2212. doi:10.1093/cercor/bhu027

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., . . . Dietz, P. M. (2020). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. *MMWR Surveill Summ, 69*(4), 1-12. doi:10.15585/mmwr.ss6904a1

Makropoulos, A., Counsell, S. J., & Rueckert, D. (2018). A review on automatic fetal and neonatal brain MRI segmentation. *Neuroimage, 170*, 231-248. doi:10.1016/j.neuroimage.2017.06.074

Mann, C., Bletsch, A., Andrews, D., Daly, E., Murphy, C., Murphy, D., & Ecker, C. (2018). The effect of age on vertex-based measures of the grey-white matter tissue contrast in autism spectrum disorder. *Mol Autism, 9*, 49. doi:10.1186/s13229-018-0232-6

Marrus, N., Eggebrecht, A. T., Todorov, A., Elison, J. T., Wolff, J. J., Cole, L., . . . Pruett, J. R., Jr. (2018). Walking, Gross Motor Development, and Brain Functional Connectivity in Infants and Toddlers. *Cereb Cortex, 28*(2), 750-763. doi:10.1093/cercor/bhx313

McCauley, J. B., Elias, R., & Lord, C. (2020). Trajectories of co-occurring psychopathology symptoms in autism from late childhood to adulthood. *Dev Psychopathol, 32*(4), 1287-1302. doi:10.1017/s0954579420000826

McKinnon, C. J., Eggebrecht, A. T., Todorov, A., Wolff, J. J., Elison, J. T., Adams, C. M., . . . Pruett, J. R., Jr. (2019). Restricted and Repetitive Behavior and Brain Functional Connectivity in Infants at Risk for Developing Autism Spectrum Disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging, 4*(1), 50-61. doi:10.1016/j.bpsc.2018.09.008

Merz, E. C., Wiltshire, C. A., & Noble, K. G. (2019). Socioeconomic inequality and the developing brain: Spotlight on language and executive function. *Child Development Perspectives, 13*(1), 15-20. doi:10.1111/cdep.12305

Messinger, D., Young, G. S., Ozonoff, S., Dobkins, K., Carter, A., Zwaigenbaum, L., . . . Sigman, M. (2013). Beyond autism: a baby siblings research consortium study of high-risk children at three years of age. *J Am Acad Child Adolesc Psychiatry, 52*(3), 300-308.e301. doi:10.1016/j.jaac.2012.12.011

Munson, J., Dawson, G., Sterling, L., Beauchaine, T., Zhou, A., Elizabeth, K., . . . Abbott, R. (2008). Evidence for latent classes of IQ in young children with autism spectrum disorder. *Am J Ment Retard, 113*(6), 439-452. doi:10.1352/2008.113:439-452

Nordahl, C. W., Lange, N., Li, D. D., Barnett, L. A., Lee, A., Buonocore, M. H., . . . Amaral, D. G. (2011). Brain enlargement is associated with regression in preschool-age boys with autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America, 108*(50), 20195-20200. doi:10.1073/pnas.1107560108

Nordahl, C. W., Mello, M., Shen, A. M., Shen, M. D., Vismara, L. A., Li, D., . . . Amaral, D. G. (2016). Methods for acquiring MRI data in children with autism spectrum disorder and intellectual impairment without the use of sedation. *Journal of neurodevelopmental disorders, 8*, 20-20. doi:10.1186/s11689-016-9154-9

Olafson, E., Bedford, S. A., Devenyi, G. A., Patel, R., Tullo, S., Park, M. T. M., . . . Chakravarty, M. M. (2021). Examining the Boundary Sharpness Coefficient as an Index of Cortical Microstructure in Autism Spectrum Disorder. *Cereb Cortex*. doi:10.1093/cercor/bhab015

Ozonoff, S., Young, G. S., Landa, R. J., Brian, J., Bryson, S., Charman, T., . . . Iosif, A. M. (2015). Diagnostic stability in young children at risk for autism spectrum disorder: a baby siblings research consortium study. *J Child Psychol Psychiatry, 56*(9), 988-998. doi:10.1111/jcpp.12421

Palomo, R., Belinchon, M., & Ozonoff, S. (2006). Autism and family home movies: a comprehensive review. *J Dev Behav Pediatr, 27*(2 Suppl), S59-68.

Panizzon, M. S., Fennema-Notestine, C., Eyler, L. T., Jernigan, T. L., Prom-Wormley, E., Neale, M., . . . Kremen, W. S. (2009). Distinct genetic influences on cortical surface area and cortical thickness. *Cereb Cortex, 19*(11), 2728-2735. doi:10.1093/cercor/bhp026

Petersen, S. E., & Sporns, O. (2015). Brain Networks and Cognitive Architectures. *Neuron, 88*(1), 207-219. doi:10.1016/j.neuron.2015.09.027

Pfefferbaum, A., Mathalon, D. H., Sullivan, E. V., Rawles, J. M., Zipursky, R. B., & Lim, K. O. (1994). A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. *Arch Neurol, 51*(9), 874-887. doi:10.1001/archneur.1994.00540210046012

Pierce, K., Carter, C., Weinfeld, M., Desmond, J., Hazin, R., Bjork, R., & Gallagher, N. (2011). Detecting, studying, and treating autism early: the one-year well-baby check-up approach. *J Pediatr, 159*(3), 458-465.e451-456. doi:10.1016/j.jpeds.2011.02.036

Pierce, K., Gazestani, V. H., Bacon, E., Barnes, C. C., Cha, D., Nalabolu, S., . . . Courchesne, E. (2019). Evaluation of the Diagnostic Stability of the Early Autism Spectrum Disorder Phenotype in the General Population Starting at 12 Months. *JAMA Pediatr*. doi:10.1001/jamapediatrics.2019.0624

Power, J. D., Fair, D. A., Schlaggar, B. L., & Petersen, S. E. (2010). The development of human functional brain networks. *Neuron, 67*(5), 735-748. doi:10.1016/j.neuron.2010.08.017

Qi, S., Morris, R., Turner, J. A., Fu, Z., Jiang, R., Deramus, T. P., . . . Sui, J. (2020). Common and unique multimodal covarying patterns in autism spectrum disorder subtypes. *Mol Autism, 11*(1), 90. doi:10.1186/s13229-020-00397-4

Raichle, M. E. (2010). Two views of brain function. *Trends Cogn Sci, 14*(4), 180-190. doi:10.1016/j.tics.2010.01.008

Raschle, N., Zuk, J., Ortiz-Mantilla, S., Sliva, D. D., Franceschi, A., Grant, P. E., . . . Gaab, N. (2012). Pediatric neuroimaging in early childhood and infancy: challenges and practical guidelines. *Ann N Y Acad Sci, 1252*, 43-50. doi:10.1111/j.1749-6632.2012.06457.x

Raznahan, A., Shaw, P., Lalonde, F., Stockman, M., Wallace, G. L., Greenstein, D., . . . Giedd, J. N. (2011). How does your cortex grow? *J Neurosci, 31*(19), 7174-7177. doi:10.1523/jneurosci.0054-11.2011

Redcay, E., & Courchesne, E. (2008). Deviant functional magnetic resonance imaging patterns of brain activity to speech in 2-3-year-old children with autism spectrum disorder. *Biol Psychiatry, 64*(7), 589-598. doi:10.1016/j.biopsych.2008.05.020

Remer, J., Croteau-Chonka, E., Dean, D. C., 3rd, D'Arpino, S., Dirks, H., Whiley, D., & Deoni, S. C. L. (2017). Quantifying cortical development in typically developing toddlers and young children, 1-6 years of age. *Neuroimage, 153*, 246-261. doi:10.1016/j.neuroimage.2017.04.010

Reynolds, J. E., Long, X., Paniukov, D., Bagshawe, M., & Lebel, C. (2020). Calgary Preschool magnetic resonance imaging (MRI) dataset. *Data Brief, 29*, 105224. doi:10.1016/j.dib.2020.105224

Rowley, C. D., Sehmbi, M., Bazin, P. L., Tardif, C. L., Minuzzi, L., Frey, B. N., & Bock, N. A. (2017). Age-related mapping of intracortical myelin from late adolescence to middle adulthood using T(1) -weighted MRI. *Hum Brain Mapp, 38*(7), 3691-3703. doi:10.1002/hbm.23624

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Hultman, C., Larsson, H., & Reichenberg, A. (2017). The Heritability of Autism Spectrum Disorder. *Jama, 318*(12), 1182-1184. doi:10.1001/jama.2017.12141

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N., . . . Wise, S. P. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci, 28*(14), 3586-3594. doi:10.1523/jneurosci.5309-07.2008

Shen, M. D., Li, D. D., Keown, C. L., Lee, A., Johnson, R. T., Angkustsiri, K., . . . Nordahl, C. W. (2016). Functional Connectivity of the Amygdala Is Disrupted in Preschool-Aged Children With Autism Spectrum Disorder. *J Am Acad Child Adolesc Psychiatry, 55*(9), 817-824. doi:10.1016/j.jaac.2016.05.020

Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., . . . Beckmann, C. F. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proceedings of the National Academy of Sciences of the United States of America, 106*(31), 13040-13045. doi:10.1073/pnas.0905267106

Solso, S., Xu, R., Proudfoot, J., Hagler, D. J., Jr., Campbell, K., Venkatraman, V., . . . Courchesne, E. (2016). Diffusion Tensor Imaging Provides Evidence of Possible Axonal Overconnectivity in Frontal Lobes in Autism Spectrum Disorder Toddlers. *Biol Psychiatry, 79*(8), 676-684. doi:10.1016/j.biopsych.2015.06.029

Sowell, E. R., Thompson, P. M., Leonard, C. M., Welcome, S. E., Kan, E., & Toga, A. W. (2004). Longitudinal mapping of cortical thickness and brain growth in normal children. *J Neurosci, 24*(38), 8223-8231. doi:10.1523/jneurosci.1798-04.2004

Spear, L. P. (2013). Adolescent neurodevelopment. *J Adolesc Health, 52*(2 Suppl 2), S7-13. doi:10.1016/j.jadohealth.2012.05.006

Szatmari, P., Chawarska, K., Dawson, G., Georgiades, S., Landa, R., Lord, C., . . . Halladay, A. (2016). Prospective Longitudinal Studies of Infant Siblings of Children With Autism: Lessons Learned and Future Directions. *J Am Acad Child Adolesc Psychiatry, 55*(3), 179-187. doi:10.1016/j.jaac.2015.12.014

Tau, G. Z., & Peterson, B. S. (2010). Normal development of brain circuits. *Neuropsychopharmacology, 35*(1), 147-168. doi:10.1038/npp.2009.115

Thomason, M. E., Dassanayake, M. T., Shen, S., Katkuri, Y., Alexis, M., Anderson, A. L., . . . Romero, R. (2013). Cross-hemispheric functional connectivity in the human fetal brain. *Science translational medicine, 5*(173), 173ra124. doi:10.1126/scitranslmed.3004978

Tierney, A. L., & Nelson, C. A., 3rd. (2009). Brain Development and the Role of Experience in the Early Years. *Zero Three, 30*(2), 9-13.

Travers, B. G., Adluru, N., Ennis, C., Tromp do, P. M., Destiche, D., Doran, S., . . . Alexander, A. L. (2012). Diffusion tensor imaging in autism spectrum disorder: a review. *Autism Res, 5*(5), 289-313. doi:10.1002/aur.1243

Turesky, T. K., Vanderauwera, J., & Gaab, N. (2021). Imaging the rapidly developing brain: Current challenges for MRI studies in the first five years of life. *Dev Cogn Neurosci, 47*, 100893. doi:10.1016/j.dcn.2020.100893

van den Heuvel, M. P., & Hulshoff Pol, H. E. (2010). Specific somatotopic organization of functional connections of the primary motor network during resting state. *Hum Brain Mapp, 31*(4), 631-644. doi:10.1002/hbm.20893

Walhovd, K. B., Fjell, A. M., Giedd, J., Dale, A. M., & Brown, T. T. (2017). Through Thick and Thin: a Need to Reconcile Contradictory Results on Trajectories in Human Cortical Development. *Cereb Cortex, 27*(2), 1472-1481. doi:10.1093/cercor/bhv301

Weitzman, C., & Wegner, L. (2015). Promoting optimal development: screening for behavioral and emotional problems. *Pediatrics, 135*(2), 384-395. doi:10.1542/peds.2014-3716

Wierenga, L. M., Langen, M., Oranje, B., & Durston, S. (2014). Unique developmental trajectories of cortical thickness and surface area. *Neuroimage, 87*, 120-126. doi:10.1016/j.neuroimage.2013.11.010

Witten, D. M., Tibshirani, R., & Hastie, T. (2009). A penalized matrix decomposition, with applications to sparse principal components and canonical correlation analysis. *Biostatistics, 10*(3), 515-534. doi:10.1093/biostatistics/kxp008

Wolff, J. J., Gu, H., Gerig, G., Elison, J. T., Styner, M., Gouttard, S., . . . Piven, J. (2012). Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. *Am J Psychiatry, 169*(6), 589-600. doi:10.1176/appi.ajp.2011.11091447

Wolff, J. J., Jacob, S., & Elison, J. T. (2018). The journey to autism: Insights from neuroimaging studies of infants and toddlers. *Dev Psychopathol, 30*(2), 479-495. doi:10.1017/s0954579417000980

Xiao, Z., Qiu, T., Ke, X., Xiao, X., Xiao, T., Liang, F., . . . Liu, Y. (2014). Autism spectrum disorder as early neurodevelopmental disorder: evidence from the brain imaging abnormalities in 2-3 years old toddlers. *J Autism Dev Disord, 44*(7), 1633-1640. doi:10.1007/s10803-014-2033-x

Zhuang, X., Yang, Z., & Cordes, D. (2020). A technical review of canonical correlation analysis for neuroscience applications. *Hum Brain Mapp, 41*(13), 3807-3833. doi:10.1002/hbm.25090

Zöllei, L., Iglesias, J. E., Ou, Y., Grant, P. E., & Fischl, B. (2020). Infant FreeSurfer: An automated segmentation and surface extraction pipeline for T1-weighted neuroimaging data of infants 0-2 years. *Neuroimage, 218*, 116946. doi:10.1016/j.neuroimage.2020.116946

Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J., . . . Zang, Y. F. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *J Neurosci Methods, 172*(1), 137-141. doi:10.1016/j.jneumeth.2008.04.012

Zuo, X. N., Di Martino, A., Kelly, C., Shehzad, Z. E., Gee, D. G., Klein, D. F., . . . Milham, M. P. (2010). The oscillating brain: complex and reliable. *Neuroimage, 49*(2), 1432-1445. doi:10.1016/j.neuroimage.2009.09.037