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Neurobehavioral Processes Shaping Infant Development in the First 6 Postnatal Months and Emerging Differences in Autism

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B.S., Georgia Institute of Technology, 2016

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An abstract of
A dissertation submitted to the Faculty of the
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Abstract

Neurobehavioral Processes Shaping Infant Development in the First 6 Postnatal Months and Emerging Differences in Autism

By Zeena Ammar

The first 6 months of life mark a period of immense change in infants. At birth, infants demonstrate a host of reflexive and spontaneous behaviors that allow them to engage with and learn from the world around them. These behaviors not only encourage interactions with their environment, but also with their caregiver, whose responses facilitate later development. As infants age and learn from these interactions, they undergo a shift from these reflexive behaviors to more volitional behaviors as they become more alert and take part in contingent social interactions. Accompanying these behavioral shifts are significant changes in infant brain development. During the first two years of life, the structure of the infant brain is established with increasing gray and white matter volume, cortical thickness, surface area, and synaptogenesis. As the brain is undergoing these changes, it is particularly susceptible to disruptions in development possibly resulting in neurodevelopmental conditions such as autism. Despite the evidence highlighting these first 6 months as a dynamic and critical period of development, few have longitudinally studied this period of development to explore changes in the infant brain and behavior. Understanding development during these first 6 months is vital for understanding the processes underlying typical brain and behavioral development and can provide benchmarks from which to compare atypical trajectories. This thesis aims to fill this gap in early brain and behavior research by developing measures of entrainment during infant-caregiver interactions (Study 1), identifying differences in trajectories of motor development between neurotypical infants and infants later diagnosed with autism (Study 2), and mapping longitudinal developmental trajectories of white matter lateralization (Study 3).

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INTRODUCTION

The first 6 months of life are marked by rapid growth and change in behavioral and brain development (Dubois et al., 2014; Nagy, 2011; P. R. Rochat, 2001; Shultz et al., 2018). Although newborns are often viewed as having a limited behavioral repertoire, they actually exhibit many reflex-like predispositions and spontaneous behaviors that allow them to engage with their surroundings and create opportunities for learning and social interaction (Needham et al., 2002; Rousseau et al., 2017; Rovee & Rovee, 1969; Thelen, 1981). For example, newborns exhibit reflexive grasping, which facilitates contact with and results in attention from their caregivers (Lozoff et al., 1977; Twitchell, 1965). They also display reflex-like attention to faces (M. H. Johnson et al., 1991) and spontaneous and varied facial expressions that elicit an emotional response from their caregivers (Lozoff et al., 1977; D. S. Messinger, 2002; Oster, 1978; Oster & Ekman, 1978). These predispositions can act as necessary signals to caregivers who respond to their infants in a timely and developmentally appropriate manner (Malatesta & Izard, 1984). The resulting exchanges provide infants with opportunities to learn about the effects of their actions on their environment. As they age, these learning opportunities facilitate important behavioral changes as infants go from spending the majority of their time asleep and exhibiting reflex-like spontaneous behaviors to spending more of their time alert and engaging in contingent social interactions (Lozoff et al., 1977; Nagy, 2011; P. R. Rochat, 2001; Sreenberg & Morris, 1974).

Accompanying this shift in behaviors are drastic changes in the structural and functional organization of the infant brain (Kolb & Fantie, 2009; Tau & Peterson, 2010). The newborn brain is not simply a smaller version of an adult brain. At birth, the infant's brain has significantly more synapses than the adult brain and is prepared to be remodeled by experiences that will help strengthen certain connections and prune connections that are not functional (Gilmore et al., 2018;

Ilyka et al., 2021; Kolb & Fantie, 2009; Tau & Peterson, 2010; Tierney & Nelson, 2009). Given the rapid changes in both brain and behavior in the first 6 postnatal months, infants are particularly susceptible to disruptions in development that could have cascading effects (Knudsen, 2004). These disruptions could lead infants down an atypical developmental path, possibly resulting in neurodevelopmental conditions, such as autism or ADHD (Bradshaw et al., 2022; Iverson, 2021)

Given that early postnatal life is a highly dynamic and critical period of development, it is an important focus of study for understanding both typical and atypical development. However, few studies have longitudinally mapped developmental trajectories of change in brain and behavior through the first postnatal months. This gap in the literature has left several important questions unanswered: What are the mechanisms of developmental change in brain and behavior during early infancy? How is the brain specializing during this time, and what experiences may drive these changes? When and in what domains can behavioral differences be detected between infants who are neurotypical and those with developmental delays? The answers to these questions are essential to tackle as they will provide insight into the developmental processes needed to support optimal brain and behavior development. They will also provide benchmarks from which atypical development can be compared to understand their mechanisms and possibly provide ideas for diagnostic markers and periods in which interventions may be the most helpful. This thesis is an attempt to address these big picture questions at the level of both behavior and brain by (a) developing novel measures of entrainment during infant-caregiver interaction, a critical mechanism of developmental change during early infancy (Study 1), (b) identifying early differences in trajectories of motor development between neurotypical infants (NT) and infants later diagnosed with autism (AUT) (Study 2), and (c) examining longitudinal developmental trajectories of white matter lateralization, a neural architecture that both supports and reflects the specialization of behavioral and cognitive skills throughout development (Study 3)

Mechanisms of Developmental Change in Early Infancy

The behavioral changes occurring in early infancy may be supported by at least two critical features and/or drivers of developmental mechanisms: (a) infants' ability to create their own opportunities for learning and (b) infant-caregiver interactions.

Infants Create Their Own Opportunities for Learning

One crucial feature of developmental change is that infants are active participants in creating their own opportunities for learning (Nagy, 2011; Shultz et al., 2018). Infants are born with reflexive predispositions that allow them to act on the world around them. Studies have shown that infants learn by observing the effects of their initially spontaneous behaviors on their environment (Ainsworth, 1969; Goldberg, 1977; Needham et al., 2002; Rovee & Rovee, 1969; Sargent et al., 2014; Watson, 1967). Early reflexes, such as the palmar grasp, the Moro reflex, sucking, and rooting, are all present from birth and create opportunities for infants to learn about the effects of their actions on the environment (Ingram, 1962; Lozoff et al., 1977; Prechtl, 1958; Rousseau et al., 2017). The palmar grasp allows infants to hold onto their caregivers (Futagi et al., 2012). Holding on to their caregivers can act as an attention-grabbing signal or a sign of "recognition, affection or appreciation" (Lozoff et al., 1977) from the infant. Furthermore, during feeding, it can act as an indicator of satiation, with infants decreasing the strength of their grasp on the breast the longer they have fed (Berecz et al., 2020; Buka & Lipsitt, 1991; Futagi et al., 2012; Halverson, 2012). The Moro, a startle reflex, aids infant survival as it signals to the caregiver that the infant may fall or have been startled, in which the typical caregiver response is to hold them tighter or pick them up (Futagi et al., 2012; Rousseau et al., 2017). The sucking reflex facilitates successful feeding and can act as a signal for attention during feeding (Goldberg, 1977). When infants stop sucking during feeding, their caregiver will "jiggle" them to resume infant sucking. However, work by Kaye and Brazelton (1971) found that, in reality, caregivers' "jiggling" behavior did nothing to restart sucking (Kaye & Brazelton, 1971; Kaye & Wells, 1980). Instead, the infants had learned that the cessation of their sucking resulted in attention from their caregiver by promoting social behaviors, such as looking, talking, and touching, that tended to decrease when infants were sucking during feedings (Dunn, 1977). The rooting reflex, in which an infant turns their head in response to stimulation of their mouths, helps infants find their caregiver's nipple (Prechtl, 1958), and caregivers will shift either their infant or breast to help the infant feed (Blauvelt, 1962). By acting on the world through their predispositions, infants create their own opportunities for social learning, as the meaning of their initially spontaneous behaviors is transformed by witnessing the consequences of their actions on the environment.

Infants' ability to learn by detecting the contingency between their actions and the effects on the surrounding environment was demonstrated in two studies by Needham et al. (2002) and Rovee & Rovee (1969). They used paradigms in which infants' spontaneous limb movements resulted in immediate and highly reinforcing consequences that facilitated learning. Rovee & Rovee (1969) connected an infant's leg to a mobile such that when the infant kicked, the mobile would spin. Spontaneous kicking behavior is quite common in infancy, allowing the infants to frequently observe that the mobile would spin every time they moved their leg. Eventually, infants picked up on this contingency and began to kick more intentionally to spin the mobile explicitly. Similarly, Needham et al. (2002) took advantage of spontaneous arm movement in infants. They placed Velcro mittens on infants who saw that each random touch of a Velcroed object in front of them would result in them picking up and being able to explore the object. Furthermore, the sticky mittens accelerated the acquisition of volitional reaching and grasping in those same infants because of the experiences afforded by the mittens. These studies highlight that early spontaneous or reflexive behaviors can be the precursors for later volitional, goal-directed actions by creating learning opportunities that transform initially reflexive behaviors into volitional actions. Although most existing evidence for these transitions comes from experimentally manipulated tasks (Needham et al., 2002; Rovee & Rovee, 1969), few prospective, longitudinal studies have mapped trajectories of both reflexive and volitional action in the same infants to better understand how and when these transitions occur in both typical and atypical development. Longitudinal mapping of change in reflexive and volitional actions is a primary goal of Study 2.

The Infant-Caregiver Dyad

Another crucial way infants learn about the meaning of their own initially spontaneous actions is through the reaction of their caregiver. Just as babies notice the contingencies between their leg movements and the mobile, they also notice contingencies between their behaviors and their caregiver's responses. Caregiver responses are adapted to be incredibly reinforcing, matching infants' needs and scaffolding their emerging skills (Smith & Gasser, 2005). This learning through dyadic interactions has been explored in face-to-face interactions measuring the synchrony of contingent vocalization, gaze, and affect between infants and their caregivers (Cohn & Tronick, 1987; Feldman, 2007; Northrup & Iverson, 2020). Studies have found that caregivers will match the affect of their infants, look at their eyes when infants gaze at them, and respond to their vocalizations because caregivers are so motivated and primed to respond to their babies (Lozoff et al., 1977; D. S. Messinger, 2002; Oster, 1978; Oster & Ekman, 1978).

Not only is it essential for these interactions to be mutually reinforcing, but for further learning to occur, they also need to be mutually adaptive. This means that over time, through the caregiver's support and responses to their infants, the infant's behavior will become increasingly contingent (Lozoff et al., 1977; Northrup & Iverson, 2020; Smith & Gasser, 2005). Infants' spontaneous facial expressions are a perfect example of this change. These facial expressions, which are typically non-contingent and formed in the absence of incoming stimuli, result in emotional responses from the caregiver (Lozoff et al., 1977). Caregivers will respond by imitating their infants' expressions, increasing the infants' attention to caregivers' faces. The meaning that the caregiver assigns to their infant's spontaneous behaviors and how they change their behaviors in response provides important feedback for newborns to learn about the meaning of their own initially spontaneous actions. As these dyadic interactions play a prominent role in infant learning, it is vital to understand exactly how caregivers adapt to changes in their infant's developmental stage and how they may impact infant development. Study 1 provides a critical step in this direction by developing a novel method for measuring eyeblink entrainment, an important signal of mutual engagement, and testing for its presence during interactions between young infants and their caregivers.

Early Brain Development

Equally rapid and drastic changes in infant brain shape and function in infants' first postnatal months are not only driven by genetic information but also by epigenetic processes such as early infant experiences (Kolb & Fantie, 2009; Tierney & Nelson, 2009). Brain development in humans is a highly protracted process (Tierney and Nelson 2009). It begins roughly 2 weeks post-conception and continues well into adulthood (Gilmore et al., 2018). In postnatal development, the brain changes rapidly within the first two years as the brain's basic structure is established (Gilmore et al., 2018). This period is marked by increases in gray and white matter volume, cortical thickness and surface area, and synaptogenesis, followed by the start of synaptic pruning, as well as spontaneous neuron firing and remodeling of excitatory and inhibitory synapses within cortical areas.

Structural Development

Longitudinal work in the first 3 months of life by Holland et al. (2014) found that the brain changes most rapidly within the first few days of life, increasing in volume at a rate of 1% per day (Holland et al., 2014). This rate decreases to about 0.4% increase per day by the end of the third postnatal month, with the brain having grown roughly 64%. By the end of the first year, the brain will have doubled in size (Gilmore et al., 2007; Knickmeyer et al., 2008). This volume change is driven predominantly by the drastic changes in gray matter volume (synapses and dendrites) with slower changes in white matter (Dubois et al., 2014; Gilmore et al., 2018; Knickmeyer et al., 2008). The cortical gray matter increases in volume by 108-149% in the first year of life, with the most rapid changes occurring in the first 3 months (Holland et al., 2014; Knickmeyer et al., 2008). Similarly, subcortical gray matter volume also has a significant increase of 105 % in the first year due to new synapses developing in the cortex (J. P. Bourgeois & Rakic, 1993; J.P. Bourgeois et al., 1994; Huttenlocher, 1979; Huttenlocher & Dabholkar, 1997). Studies suggest that gray matter matures more quickly in occipital, parietal, and frontal regions supporting sensory and motor functions respectively in early development (Gilmore et al., 2007). White matter myelination begins prenatally in the second semester and follows a similar pattern (Wilson et al., 2021; Kagen and Herschkowitz, 2005), such that white matter tracts in the primary sensory and motor pathways are the most mature at birth. Overall, white matter tracts tend to mature posteriorly to anteriorly, from subcortical structures to cortical structures (Gilmore et al., 2018; Knickmeyer et al., 2008). These changes are accompanied by increasing gyrification in the first two years (Li et al., 2014). Additionally, studies have shown regionally heterogeneous increases in surface area and cortical thickness that continue into late toddlerhood and childhood, respectively (Gilmore et al., 2018)

Functional Development

The structural organization of brain development lays the groundwork for understanding the maturation of neural circuits that mediate the brain's functional abilities (Gao et al., 2017). Most functional studies in infant brain development focus on resting state functional connectivity during infant sleep given that scanning infants while awake is methodologically challenging, although strides are being made to make this feasible (Yates et al., 2021). Studies in the first few months of life have found that infants demonstrate BOLD signal patterns similar to that of adults in the sensorimotor (Allievi et al., 2016; Arichi et al., 2012), visual (Karen et al., 2008) and auditory networks (Dehaene-Lambertz et al., 2002). A longitudinal study by Gao et al. (2015) found that these sensorimotor and auditory networks reached adult-like patterns of connectivity before the primary and secondary visual networks, followed by the dorsal attention and default mode networks. Similar to patterns found in structural development, the frontoparietal executive control networks remained immature throughout the first year of life (Gao et al., 2015)

These many structural and functional changes in the brain are critical for supporting new behaviors during the first postnatal months. These behaviors affect how infants act on the world around them and therefore shape their experiences that in turn shape the developing brain. Very few studies have longitudinally explored how these early experiences shape brain development and vice versa, but the few studies that do exist have shed some much-needed light on this relationship (Dai et al., 2019; Eggebrecht et al., 2017; Girault et al., 2019; O’Muircheartaigh et al., 2014). O'Muircheartaigh et al. (2014) found associations between the development of myelin volume fraction across the brain and individual cognitive abilities from 3 months to 4 years of age. Specifically, they found that white matter development in the frontal and temporal cortices was related to expressive and language abilities, and this relationship became stronger as the infants aged. On the functional neuroimaging side, Eggbreght et al. (2017) found that at 12 and 24 months of age joint attention is associated with connections between the visual and dorsal attention networks and the visual network and parietal default mode network. Although these studies have begun to fill the gap in the literature, the field is still missing longitudinal trajectories of brain development in early infancy and their associations with development in multiple behavioral domains. To begin filling this gap, Study 3 will describe a longitudinal structural neuroimaging study in NT infants that charts trajectories of white matter lateralization in nine major tracts from birth to 6 months.

Autism Spectrum Disorder

Although it is critical to better understand neurotypical behavioral and brain development, this information is also paramount to better understand early deviations from such processes in infants with developmental delays such as autism. Autism is a highly complex neurodevelopmental condition that may impact mechanisms of learning in very early infancy (Bradshaw et al., 2022; Shultz et al., 2018). Although autism can only be diagnosed in toddlerhood, it has been hypothesized that initial, even subtle, disruptions to foundational mechanisms of social adaptive action may lead to differing early experiences and thus increasingly divergent developmental trajectories, culminating in the phenotypic form of autism (Shultz et al., 2018). Therefore, identifying early deviations from typical developmental processes in the first postnatal months is an important area of focus for understanding the biological mechanisms underlying autism.

What is Autism Spectrum Disorder and How is it Diagnosed?

Autism spectrum disorder, or autism, is a developmental condition characterized by differences in social interactions, communication, focused interests, and repetitive behaviors. Even with these diagnostic criteria, autism is highly heterogenous, meaning that the traits exhibited by those on the spectrum vary substantially. This heterogeneity led to the change in diagnostic criteria in the DSM-5, combining previously separate diagnoses into an umbrella term of autism spectrum disorder (American Psychiatric Association, 2013).

Currently, 1 in every 36 children is diagnosed with autism (Maenner, 2023). As autism is highly heritable, the probability of receiving a diagnosis increases if that child has a first-degree relative with autism with the recurrence rate being 20% or 1 in 5 (Ozonoff et al., 2011). It is possible to diagnose a child with autism at 2 years, yet most children will not receive a diagnosis until around 4-5 years (Elsabbagh & Johnson, 2010). The diagnostic process involves interviewing the child's parents and putting together a detailed history using interview instruments, such as the Autism Diagnostic Interview-Revised (ADI-R) and observing the child in a controlled environment using an assessment tool such as the Autism Diagnostic Observation Schedule (ADOS).

Studying Autism in Early Life

Even though autism is diagnosed in the toddler years, it is thought to affect development from birth or even from the mid- to late-fetal period (Baron-Cohen et al., 2015; Elsabbagh &

Johnson, 2010; Shultz et al., 2018; Willsey et al., 2013). As a result, there is a significant focus in research to understand the early unfolding of autism to identify early signs and underlying mechanisms leading to a diagnosis, with the hope of facilitating the development of support and services for infants and their families. One type of study, the 'Baby-Sibling' study design, has been instrumental in revealing early differences between AUT and NT infants (Szatmari et al., 2016). These Baby-Sibling studies are prospective longitudinal studies in which a younger sibling of a child with autism is followed from birth until at least 36 months of age. This study design capitalizes on the heritability of autism and the fact that these siblings have a recurrence likelihood of 20% (Ozonoff et al., 2011). These studies have led to findings of differences in both brain (Eggebrecht et al., 2017; Emerson et al., 2017; Hazlett et al., 2005, 2011, 2017; J. Liu et al., 2019; Wolff et al., 2012, 2015) and behavior development (*social*: Chawarska et al., 2013; Elsabbagh, Fernandes, et al., 2013; Elsabbagh, Gliga, et al., 2013; Jones & Klin, 2013; Shic et al., 2014; *motor*: Bradshaw et al., 2018; Heathcock et al., 2015; Iverson et al., 2019; Kohen-Raz et al., 1992; Libertus et al., 2014; Nickel et al., 2013 l*anguage*: Gamliel et al., 2009; Hudry et al., 2014; Patten et al., 2014; Paul et al., 2013; Schoen et al., 2011) in autism.

Gaps in the Autism Field

Despite all the early differences noted between NT and AUT infants, many studies have been unsuccessful in identifying differences in early life, with some even concluding that no differences in social behavior are present in the first year of life (Ozonoff et al., 2010; Shen & Piven, 2017). On the one hand, this is surprising because, given what has been reviewed above, many of the foundational aspects of social development are in play during this period as babies are rapidly gaining new skills as their behaviors are being shaped by their caregivers. On the other hand, these studies may have failed to find differences for one of two reasons: 1) they were conducted using cross-sectional developmental ages that prevented them from uncovering longitudinal differences or 2) they were not looking for building blocks or precursors to behaviors that are present in older children with autism and therefore were looking for behaviors developmentally inappropriate in younger infants. However, there is evidence that some of these early deviations from typical behavior exist in AUT infants. For example, in the social behavioral domain, work by Jones and Klin (2011) found that AUT infants showed different patterns of eye-looking behaviors as early as two months of age. In the motor domain, a study by Bhat et al. (2012) showed that AUT infants at 3 and 6 months showed fine and gross motor delays compared to NT infants (Bhat et al., 2012). Finally, Yirmiya et al. (2006) found that at 5 months of age, AUT infants and their caregivers have less synchronous interactions and longer delays in responses to one another's behaviors. These preliminary findings, combined with the developmental principles reviewed above, suggest that the first postnatal months are in fact, a critical period to explore, particularly within the context of dyadic interactions and early reflexive and exploratory behaviors. To gain further knowledge on early markers of behavioral changes in autism and considering the limitations of earlier studies, Study 2 will describe differences in longitudinal trajectories of motor behaviors in NT and AUT infants.

To summarize, previous studies show that the first postnatal months are characterized by widespread and rapid changes across brain and behavior development. Although several behavioral systems have been well-studied in this early postnatal period, few studies have mapped longitudinal changes in brain and behavior in typical development and deviations from AUT infants. This thesis intends to shed light on gaps of knowledge in early behavioral and neural development, as well as on their derailment in AUT infants. The main hypotheses to be tested are:

Study 1, Eyeblink entrainment is measurable and present in the first 6 months of life; Study 2, AUT infants will exhibit a delayed decrease in reflexive grasping and delayed onset of volitional grasping; Study 3, White matter lateralization is present and time-varying in the first 6 months of life.

Study 1

In Study 1, I developed and applied a method to measure behavioral entrainment, an important component of dyadic interaction. Infant-caregiver entrainment occurs when infants and their caregivers coordinate and synchronize their behaviors, providing an opportunity for learning as described in this introduction. Disruptions to the interactions within a dyad are characteristic of neurodevelopmental conditions, with atypical entrainment shown in adults with autism (Nakano et al 2011). In Study 1, I specifically focused on eyeblink entrainment (the synchronization of eyeblinks between two interacting partners which can be indicative of mutual engagement (Nakano & Kitazawa, 2010)), a particularly useful longitudinal marker of entrainment given that eye blinking can be measured consistently throughout infancy unlike other markers of entrainment (mutual eye gaze, facial expression) that may undergo marked changes during this period. Blinking has the advantage of being a behavior that can easily be captured from birth (Bacher & Smotherman) and could be used to identify early differences between NT and AUT infants. As described previously, some have not been able to find differences between NT and AUT infants in the first 6 months of life. However, the behaviors they measured may not have made sense to explore in early infancy, making it difficult to find any differences. Study 1 fills this gap by providing an easily captured and quantitative measure of infant-caregiver interactions and social learning which is critical for infant development. The results from Study 1 show that infants and caregivers do entrain their blinks to one another and provide a baseline from which atypical patterns of entrainment can be compared in future studies.

Study 2

In Study 2, I examined the development of reflexive and volitional grasping in the first 6 months of life in NT and AUT infants. Although motor delays are not considered a core diagnostic feature of autism, many have found evidence for differences in motor behaviors and motor milestones in AUT infants (Bhat et al., 2012; Flanagan et al., 2012; Iverson et al., 2019; Libertus et al., 2014). Although these differences have been established, it is still unknown whether these differences can first be observed in early infancy and if they occur first in reflexive or volitional behaviors. To fill this gap in the literature, I prospectively and longitudinally assessed reflexive and volitional grasping in 20 AUT infants and 125 NT infants once a month during the first 6 postnatal months. Reflexive grasping was assessed using the NICU Neurobehavioral Network Scales (Lester & Tronick, 2004) and volitional grasping was tested using the fine motor subscale, specifically the block series, of the Bayley Scales of Infant and Toddler Development (Bayley, 2012). I found significant differences in developmental trajectories of volitional grasping. This difference in trajectories began around 173 days and continued through the oldest age of testing (6 months). I found no statistically significant differences in reflexive grasping behavior due to a lack of variability within the sample of AUT infants (with nearly all AUT infants showing persistence of the reflex at 6 months). However, qualitatively, although several NT infants no longer exhibit this reflexive behavior by 5 months, only one AUT infant no longer displays the reflex, suggesting that this reflexive behavior may persist longer in AUT infants.

Study 3

In Study 3, I focused on brain development in the first 6 months of life, particularly the development of lateralization in white matter tracts. Lateralization patterns are a prominent structural feature of brain white matter. They have been investigated as a neural architecture that indicates and supports the specialization of cognitive processing and observed behaviors, e.g., language skills. Multiple neurodevelopmental disorders have been associated with atypical lateralization, reinforcing the need for careful measurement and study of this structural characteristic. Unfortunately, there is little consensus on the direction and magnitude of lateralization in major white matter tracts during the first months and years of life – the period of most rapid postnatal brain growth and cognitive maturation. In addition, no studies have examined white matter lateralization in a longitudinal pediatric sample – preventing confirmation of if and how white matter lateralization changes over time. Using a densely sampled longitudinal dataset from NT infants aged 0-6 months, Study 2 aims to 1) chart trajectories of white matter lateralization in nine major tracts and 2) link variable findings from cross-sectional studies of white matter lateralization in early infancy. I show that lateralization patterns are time-varying and tract-specific and that differences in lateralization results during this period may reflect the dynamic nature of lateralization through development, which can be missed in cross-sectional studies.

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STUDY 1. Eyeblink Entrainment in Infant-Caregiver Dyads

Abstract

Entrainment, the coordination and synchronization between two systems, is a crucial aspect of interpersonal interactions, particularly in infant-caregiver dyadic interactions. Caregivers and infants coordinate their vocalizations, eye gaze, and facial expressions, providing a critical foundation for social learning and cognitive development. Disruptions to these dyadic interactions can have cascading consequence on subsequent development and have been shown to be characteristic of neurodevelopmental conditions such as autism. Therefore, it is critical to study early entrainment behaviors to understand early infant development. Recent research has shown that subtle behaviors, such as eyeblinks, are also entrained during social interactions. For example, eyeblink entrainment is present between communicative partners at attentional breakpoints, and this entrainment phenomenon may reflect mutual engagement which is a critical part of social learning (Nakano et al., 2010, Rose & Gravel, 2011). Although eyeblink entrainment has been studied in adults, its presence in infant-caregiver interactions remain unexplored, as there are currently no methods available for non-invasive automatic identification of infant eyeblinks. This study has two aims: 1) develop and test a semi-automated algorithm and graphical user interface (GUI) to detect infant and caregiver eyeblinks and 2) use this method to determine whether eyeblink entrainment is present during interactions between 3-6-month-old neurotypical infants and their caregivers. Using video recordings from 41 infant-caregiver dyads, I show that infants and caregivers entrain their blinks to one another with infants blinking 560-890 ms before their caregivers.

Introduction

Entrainment, which describes a dynamic interaction between two coordinating systems, is a ubiquitous feature of biological systems and plays a critical role in interpersonal synchrony (Clayton et al., 2005). Nowhere is this more apparent than in infant-caregiver dyadic interactions, one of the most foundational and influential relationships in a child's development (Feldman, 2007). A dyad, otherwise known as a dynamically interacting partnership, develops from the entrainment between two individuals. Behavioral entrainment occurs when an infant and caregiver within a dyad coordinate and synchronize their behaviors (Clayton et al., 2005; Feldman, 2007; Lense et al., 2022). Entrainment between infant and parent allows the dyad to co-create a shared relationship serving as the foundation for the specialization of an infant's social brain networks and contributing to a child's social and cognitive development, including capacity for empathy, self-regulation, social fitness, theory of mind, intimacy, and symbol use (Feldman, 2007; Rocha et al., 2020). Disruptions of behavioral entrainment are characteristic of neurodevelopmental conditions such as autism spectrum disorder (autism) or other conditions (i.e., maternal depression or anxiety) (Feldman, 2003, 2007; Mayo & Gordon, 2020; Weinberg & Tronick, 1998; Zlochower & Cohn, 1996). These disruptions can result in early atypical experiences in dyadic interactions that can have significant effects in later childhood, making the study of early entrainment critical for understanding infant development (Northrup & Iverson, 2015; Wan et al., 2013).

Eyeblink entrainment – the synchronization of eyeblinks between social partners – has recently been identified as a fundamental element of dyadic social interaction in both human adults and non-human species. Although blinks are traditionally thought to only play a role in protecting and lubricating the eyes (Evinger, 1995; Evinger et al., 1991), several lines of evidence suggest that the timing of eyeblinks may play an important role in engagement and entrainment within social interaction. First, patterns of eye blinking have been shown to index engagement with ongoing visual content. Studies in adults and toddlers indicate that people adjust the timing of their blinks based on what they are watching, with blink rates decreasing during an engaging movie, video clip, or while completing a visual task (Descroix et al., 2018; Nakano & Miyazaki, 2019; Ranti et al., 2020; Shin et al., 2015; Shultz et al., 2011) and increasing when individuals are completing a less engaging task. Similar effects were reported in non-human species, with birds showing blink inhibition during moments of increased vigilance (Yorzinski et al. 2016). Relatedly, both adult humans and macaques coordinate the timing of their blinks with the blinks of their social partner (Nakano et al. 2010; Ballesta et al. 2016), a phenomenon that, in humans, is disrupted in autistic adults (Nakano et al. 2011). Eyeblinks also act as a paralinguistic cue with interlocutors synchronizing their blinks at 'feedback slots' or breakpoints of speech at the end of a thought (Cummins, 2012; Hömke et al., 2017, 2018; Nakano & Kitazawa, 2010). These blinks tend to occur with nods and other physical responses during conversations and provide conversational signals (Hömke et al., 2017, 2018). More specifically, blinks are considered grounding signals or signals of understanding by the listener in response to the speaker during a conversation (Hömke et al., 2017). Thus, within the context of dyadic interaction, eyeblink entrainment may signal that the interactive partners have established coordination with one another (Shockley et al., 2009) and are mutually engaged (with both partners decreasing eyeblinks during moments when they are highly engaged and increasing eyeblinks at moments when they are less engaged).

Though blink entrainment indexes mutual engagement, a central feature of dyadic interaction, it has yet to be investigated in the context of infant-caregiver interactions. Given that mutual engagement is critical for social learning (Rose & Gravel, 2011), it would be valuable to develop a measure of blink entrainment that can be leveraged to index mutual engagement during infant-caregiver dyadic interaction. Unfortunately, there are currently no available methods for automating the identification of infant eyeblinks non-invasively. Most studies of infant blinking manually code videos to identify infant blinks (a very time-intensive and laborious process) (Bacher & Allen, 2009; Bacher & Smotherman, 2004b; Descroix et al., 2015, 2018) or have used more invasive methodologies such as EMG (Tomita et al., 1989) or electrode needles (Blank et al., 1983).

The aims of this study are twofold. First, to develop and test a new semi-automated algorithm with an accompanying guided user interface (GUI) to detect infant and caregiver eye blinking. Second, to leverage this new method to test whether eyeblink entrainment is present during interactions between 3- to 6-month-old neurotypical infants and their caregivers. Given substantial evidence of clear temporal structure within dyadic interactions of 3- to 6-month-old infants (Feldman, 2007; Northrup & Iverson, 2020; Rocha et al., 2020), I hypothesize that eyeblink entrainment will be observed.

As disruptions in mutual engagement may signal disruptions to typical learning processes (Rose & Gravel, 2011), identifying such early deviations may be critical in aiding the early identification of children at increased likelihood for neurodevelopmental conditions such as autism. This study provides an important first step in developing and testing new methodology for future longitudinal work exploring the role of mutual engagement in early learning and facilitating the discovery of early divergences in atypical development.

Materials and Methods

Participants

Participants were 41 neurotypical infants (21 female and 20 male infants with a mean age of 4.82 months) and their caregivers (37 mothers and 4 fathers) (see Table 1.1 for participant characterization details). Participants were selected from a cohort of infants participating in the NIH-funded Autism Center of Excellence (ACE) prospective longitudinal study of infants at low- and elevated-likelihood for autism at the Marcus Autism Center, Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, GA. The research protocol was approved as bearing no significant risk by the Human Investigations Committee at Emory University School of Medicine. "Neurotypicality" of infants was defined at enrollment by the absence of familial genetic likelihood for neurodevelopmental disorders and the absence of medical concerns: All infants had no history of autism in first-, second-, or third-degree relatives, no developmental delays in first-degree relatives, no pre- or perinatal complications, no history of seizures, no known medical conditions or genetic disorders, and no hearing loss or visual impairment.

Dyadic interaction data were collected when the participants were between 3 and 6 months old; given the longitudinal nature of the ACE, some participants (n=12) contributed more than one session to the analyses resulting in an average of 1.39 (±0.67 s.d.) visits per dyad and a total of 57 dyadic interaction sessions used for this study.

	Participants
Mean age at Testing, months (s.d.)	4.82 (1.14)
Infant Sex	
Male	n = 20
Female	n = 21
Race and Ethnicity %	
White	
Hispanic	12.20%
Non-Hispanic	41.46%
Black	
Hispanic	7.32%
Non-Hispanic	9.76%
Asian	2.44%
American Indian or Alaskan Native	2.44%
Mixed Race	7.32%
Other	2.44%
Unknown	14.63%
Maternal Education %	
Professional Degree	9.76%
Master's Degree	34.15%
College Degree	19.51%
Associate Degree	4.88%
Courses Toward College	7.32%
Trade or Vocational School	2.44%
Highschool or GED	4.88%
Unknown	17.07%
Yearly Household Income %	
>150,000	12.20%
80,001-150,000	19.52%
<80,000	29.28%
Unknown	21.95%

Table 1.1: Demographics of Participant Sample

Experimental Setting and Equipment

Recordings of infant-caregiver interactions were collected using a bi-directional audio-visual recording system located at Marcus Autism Center (Figure 1.1). In the recording setup, caregivers and infants were seated separately, each facing hidden cameras and matched at eye level to make the interaction feel as natural as possible. When recording began, infants and caregivers could see and hear each other in real time. Video recordings were sampled at 29.97 fps and written to DVDs.

Figure 1.1: Infant-Caregiver Live Interaction Equipment Set-up.

Procedure

Each experimental session began with the infant and caregiver entering the testing room while a children's video played on the infant's presentation screen (e.g., Baby Mozart, Bear in the Big Blue House). The experimenter buckled the infant into the bassinet, standardizing the infant's eye position relative to the teleprompter (28 inches away from the screen, subtending an approximate 24° x 32° portion of each infant's visual field). Caregivers were seated in a soundproof booth and instructed to interact with their infant as they usually would. Interactions were 70 seconds (n=42) or 90 seconds (n=15) in duration. To ensure consistency across dyads, only the first 70 seconds of each interaction were analyzed.

Prior to the infant-caregiver interaction, infants were eye-tracked while viewing video scenes of an actress looking directly into the camera and playing the role of a caregiver (e.g., singing, talking in motherese), as described in Jones and Klin (2013). These data were collected as part of the broader study and were not analyzed in the present paper.

Analysis and Measures

Exclusionary Criteria for Infant-Caregiver Interaction Videos: To ensure accurate detection of eyeblinks, recordings were excluded from analysis when there were distractors in the caregiver booth (such as a sibling being held by the caregiver), the caregiver was wearing glasses that made it difficult to see their eyes, or if there was an object that occluded the eye of the infant or caregiver for most of the video. Additionally, to account for variability in infant state, videos were excluded if the infant was crying, sleeping, moving excessively, or fussing for much of the video. Using these criteria, 33% of the total possible live recordings were excluded from the analysis. A total of 57 dyadic interaction sessions were used in analyses.

Identification of Eyeblinks

To develop a new approach for the semi-automated identification of eyeblinks we used DeepLabCut, a markerless pose estimation software that can be trained to identify points on the eye without requiring that anything be affixed to the participant. This approach avoids the use of obtrusive data collection measures (e.g. EMG sensors, etc.), thereby facilitating naturalistic interaction between infants and their caregivers. DeepLabCut-generated timeseries of eye feature positions were input to an in-house algorithm to identify eyeblinks (instances when the top eyelid occluded at least half of the pupil), which were then verified using an in-house graphical user interface. These steps are detailed below.

DeepLabCut Markerless Pose Estimation: DeepLabCut (v 2.1 and 2.2) was used to identify 9 points on the video recordings of infant and caregiver eyes (Figure 1.2). Nine videos were selected as the training set for eye identification in DeepLabCut. To ensure accurate identification of eye features across a variety of videos, the training videos were selected to include dyads from a variety of racial and ethnic backgrounds (4 White, 2 Black/African American, 1 American Indian/ Alaskan Native, 1 Other, and 1 Not Reported) and with varying data quality (i.e., from minimal to high infant or caregiver motion).

Infant and caregiver eyes were labeled on 869 frames across all 9 videos. The specific frames to be labeled were chosen using a k-means clustering method (Nath et al., 2019). The k-means clustering approach first down-samples the video and clusters all the frames using k-means, such that each frame is treated as a vector. Once clustered, frames from different clusters are selected for labeling. Selecting frames in this manner ensures that there is variability between frames selected for labeling and is the recommended method for sparse behaviors, such as infant blinking (Nath et al., 2019). Of the labeled frames, 814 frames (95% of frames) were used to train the network, and 53 frames (5% of frames) were used to test the network. The neural network was trained using the training set frames for 1,030,000 iterations. Twenty outlier frames per video, selected by DeepLabCut, were corrected and the neural network was refined using the same number of iterations. This process was repeated 3 times. The full videos were labeled for each iteration and evaluated for accuracy of labeling through visual inspection and error quantification (test error was: 2.03 pixels, train: 1.19 pixels (image size was 718 by 478). The 3rd iteration of the model was used to evaluate all 57 videos for this study.

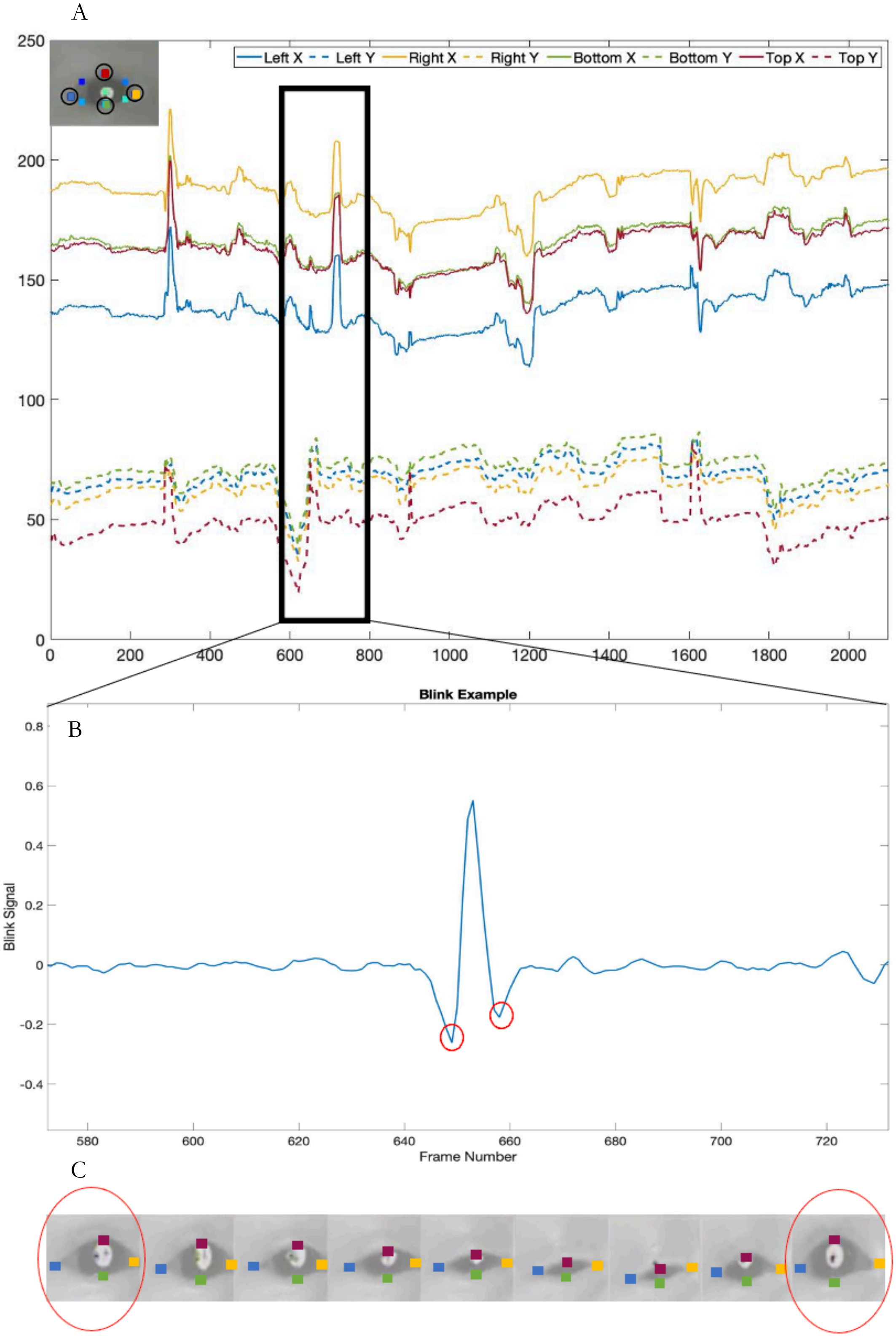
A
Left X
Left Y
Right X
Right Y
Bottom X
Bottom Y
Top X
Top Y
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
1800
2000
Blink Example
B
Blink Signal
0.8
0.6
0.4
0.2
0
-0.2
-0.4
580
600
620
640
660
680
700
720
Frame Number
C

Figure 1.2: DeepLabCut Eye Identification and Output. (A) Nine points were labeled on each eye: left and right corners, 3 points on the top and bottom lid, and a final point placed on the pupil. The left and right corners and the middle points on the top and bottom lid (circled in black) were used to formulate the final signal used to identify blinks. Time course of X- and Y- coordinates, output by DeepLabCut, for each of the points used to identify eye blinks. **(B)** An exemplar peak denoting an eyeblink. The troughs (indicated by a red circle) before and after the peak represent the onset and offset of a blink with the peak representing the moment the eye is closed. (C) A frame-by-frame example of an infant's blink. The moment when the eye is closed corresponds to the peak in the blink signal.

<u>Analysis of DeepLabCut Coordinates:</u> The X- and Y- coordinates and the likelihood values of the 9 points of infant and caregiver eyes determined by DeepLabCut were used to build a semi-automated process to detect eyeblinks in MATLAB (MATLAB 2020B). Raw X- and Y-coordinates for an exemplar infant are shown in Figure 1.2A. Points with a likelihood value of <.98 were replaced with a NaN placeholder since including those under this threshold resulted in less accurate blink identification. Next, data points were standardized (point coordinate - mean coordinate of all points) to reduce the noise created by motion. For the infants, all missing data were filled by linear interpolation using the MATLAB function *interp()*. This step allowed for continuous identification of the whole eye, even if it was partially blocked by the infant (i.e., hand in front of their face) or partially off-screen. As the video of the caregivers included their whole face and their faces were never outside the camera view, this was not a necessary step in identification of caregiver blinks. Next, the area of the eye was calculated assuming the shape of the eye was an ellipse. The Euclidian distance between the left and right (a), and top and bottom

points (b) were used to calculate pi*a*b for every frame (Figure 1.2A). The area of the eye was plotted against the frame number for the duration of the video. This signal was then smoothed using a boxcar/square wave moving average with a local and a global smoother. For the caregiver signal, any data points marked with a NaN were then replaced with the mean signal value. As caregivers only had missing data due to covering their eyes for peekaboo, this method of replacing the NaNs with the mean signal value prevented false-positive identification of the eye when it was not visible. Finally, for both infants and caregivers, the signal was centered around 0 by subtracting it from the mean signal, and the signal was flipped such that a blink was indicated by a sharp increase in signal (Figure 1.2B).

<u>Peak Detection:</u> Peaks, or moments when the eye closed, were detected using the *findpeaks()* function in MATLAB. Blink onset and offset were marked by the troughs sandwiching the peaks as they indicated moments where the eye was open at its widest (circled in red in Figure 1.2B).To determine blink length, we calculated the distance between the trough before and after the peak. Possible blink peaks were filtered using the *findpeaks()* function based on three criteria for infants and caregivers, respectively: minimum peak prominence (0.14,0.17), minimum peak width (13,4.25), and maximum peak width (1.8, 9.95). The criteria were determined by building histograms of these peak characteristics for peaks that corresponded with blinks identified by hand and for peaks that did not correspond to blinks and selecting values that included all the blinks peaks and minimized the amount of non-blink peaks. The final output of the blink detection algorithm was a table with the frame number of the peak, the onset and offset of the blink, and the blink duration.

Semi-Automated Blink Identification with Guided User Interface (GUI): Given the variability in caregiver and infant blinking (e.g. blink length, degree of eyelid closure, percentage of blinks accompanied by saccades, etc), the peak detection algorithm alone resulted in many false positives (75.78% for infants and 25.62% for caregivers). To reduce false positives, a GUI was created to allow a trained experimenter to confirm the presence of a blink and adjust the onset and/or offset of the blink.

The GUI allows the user to assign one of three possible events for the possible blinks they are presented with. The frames could be coded as a blink (the frame would be assigned a 1), as lost data (the frame would be assigned 'NaN'), or as neither a blink nor lost data (the frame would be assigned a 0). Blinks were identified as instances when the eyelid occluded at least half of the pupil, with blink onset defined as the frame in which the top lid began to move towards the bottom lid (Figure 1.2C). Moments where the infant closed their eyes due to fussing, crying, or yawning, were not included as blinks for this study (Bacher & Allen, 2009; Bacher & Smotherman, 2004b), however, blinks accompanied by saccades were included (Rottach et al., 1998). Lost data were defined as frames in which no part of the infant or caregivers' right eye was visible.

Verifying Algorithm and GUI accuracy: Accuracy of our semi-automated method for blink detection was assessed by comparing blinks identified by the algorithm and GUI to blinks hand coded by a trained experimenter in a subset of 20 videos. Using the manually coded videos as the ground truth, the algorithm and GUI were 98.83% and 98.20% accurate at identifying eyeblinks and 97.04% and 98.79% accurate at identifying lost frames for infants and caregivers, respectively.

GUI Coding Reliability: Two coders were trained to code the blinks using the GUI. The coders passed the training requirements with 99.33 % and 99.40 % accuracy for infant blinks and 98.90% and 98.65% for caregiver blinks compared to the trainer before being allowed to code videos on their own using the GUI.

Peristimulus Time Histograms

To examine how the timing of infant eye blinking varied with respect to caregiver eye blinking, I used peristimulus time histograms (PSTHs). PSTHs were created by computing counts of an infant's blinks occurring in 30-ms bins in an 800-ms window surrounding each caregiver eyeblink. The bin counts were calculated for each infant across all caregiver blinks and then averaged across all infants to obtain group means.

To test whether the observed changes in infant blink rate differed from those expected by chance, I computed a second set of PSTHs from permuted infant blink data. As before, each infant's blink sequences permuted by circular shifting of individual data 5,000 times. PSTHs were then computed on each of those permuted datasets. The mean instantaneous blink rate, during each bin, across all 5,000 PSTHs from permuted data quantified the blink rate one would expect if infant eyeblinks were random with respect to caregiver eyeblinks. If, on the other hand, infant eyeblinks were time-locked to caregiver eyeblinks and not random, one would expect to see significant deviations from the permuted data distribution. The 2.5th and 97.5th percentiles of instantaneous blink rate across all PSTHs from permuted data acted as a $p=0.05$ confidence level against which to compare blink rates in the actual data (two-tailed comparisons).

Results

Infant and Caregiver Blink Rate

Average blink rates of both infants and caregivers (mean = 4.3 blinks per minute and mean = 15.1 blinks per minute, respectively) were consistent with those reported in the literature for their respective age groups (Bacher & Allen, 2009; Bacher & Smotherman, 2004a, 2004b; Nakano & Kitazawa, 2010) (see Figure 1.3). Infant blink rate ranged from 0 – 15.4 blinks per minute and 0.9 to 43.7 blinks per minute for caregivers. Outliers were retained in all analyses given previous reports of large inter-individual variability in blink rates that span the ranges reported here (Bacher & Allen, 2009; Bacher & Smotherman, 2004a, 2004b; Nakano & Kitazawa, 2010).

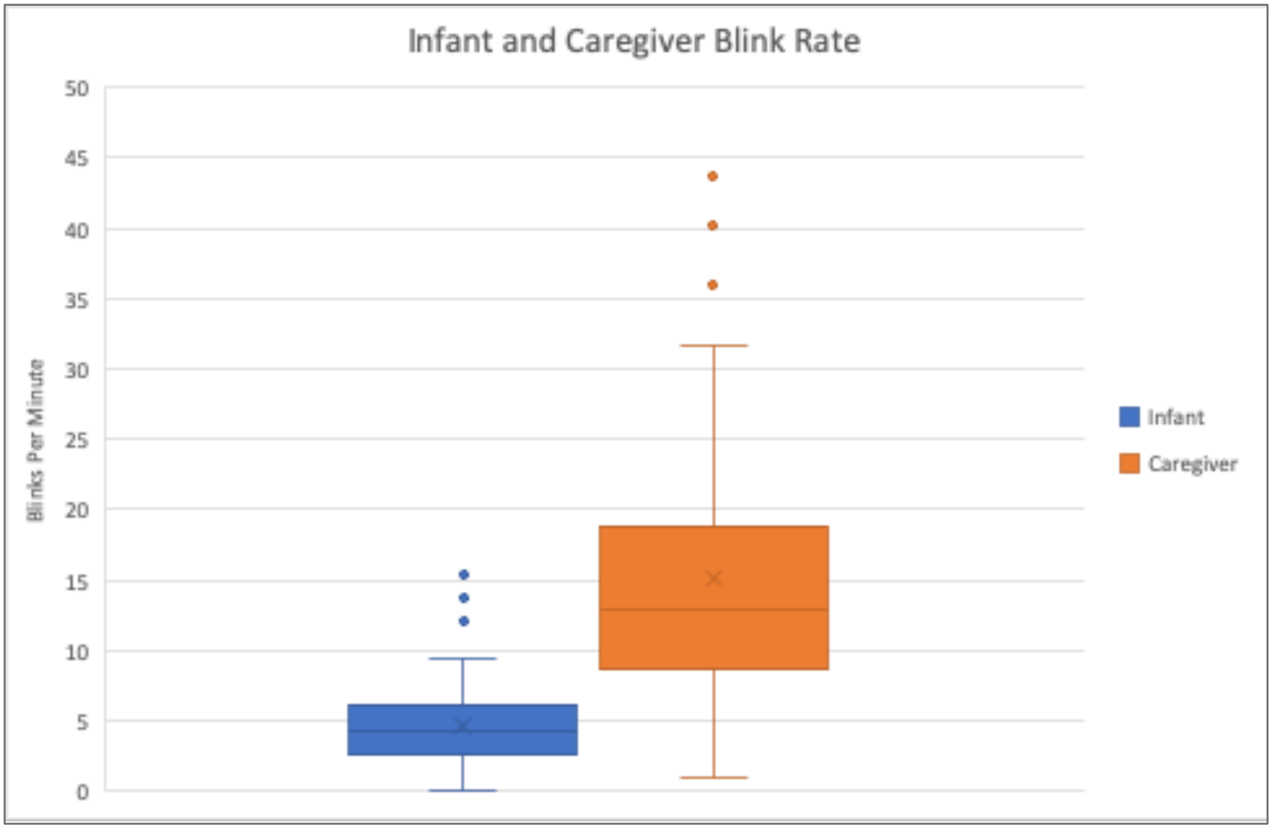

Figure 1.3: Infant and Caregiver Blink Rates. Box and whisker plot showing the minimum, maximum, mean, interquartile range, and outliers of blinks per minute for infants and their caregivers.

Infant Blinking Relative to Caregiver Blinking

As shown in Figure 1.4A, the PSTH for infants reveals a 4.31% increase in infant blink rate, reaching its maximum approximately 726 ms before caregiver blinks. This indicates a statistically significant increase in infant blink rate ($p < 0.05$), occurring just prior to moments when caregivers blinked. Furthermore, a 3.2% decrease in infant blink rate was observed, reaching its minimum 200 ms after the caregivers' blinks ($p < 0.05$). This indicates a statistically significant decrease in infant blink rate ($p < 0.05$), occurring just after moments when caregivers blinked.

Caregiver Blinking Relative to Infant Blinking

To determine whether caregiver eye blinking was also time-aligned to infant eye blinking, a second PSTH was created using the infant blinks as the reference event and the caregiver blinks as the target event. As shown in Figure 1.4B, a 4.16% increase in caregiver blink rate was observed, reaching its maximum 690 ms after infant eyeblinks. This indicates a statistically significant increase in caregiver blink rate ($p < 0.05$), occurring just after moments when infants blinked. Furthermore, a 3.30% decrease in caregiver blink rate was observed, reaching its minimum 200 ms before the infants' blinks ($p < 0.05$). This indicates a statistically significant decrease in caregiver blink rate ($p < 0.05$) occurring just before the infants blinked.

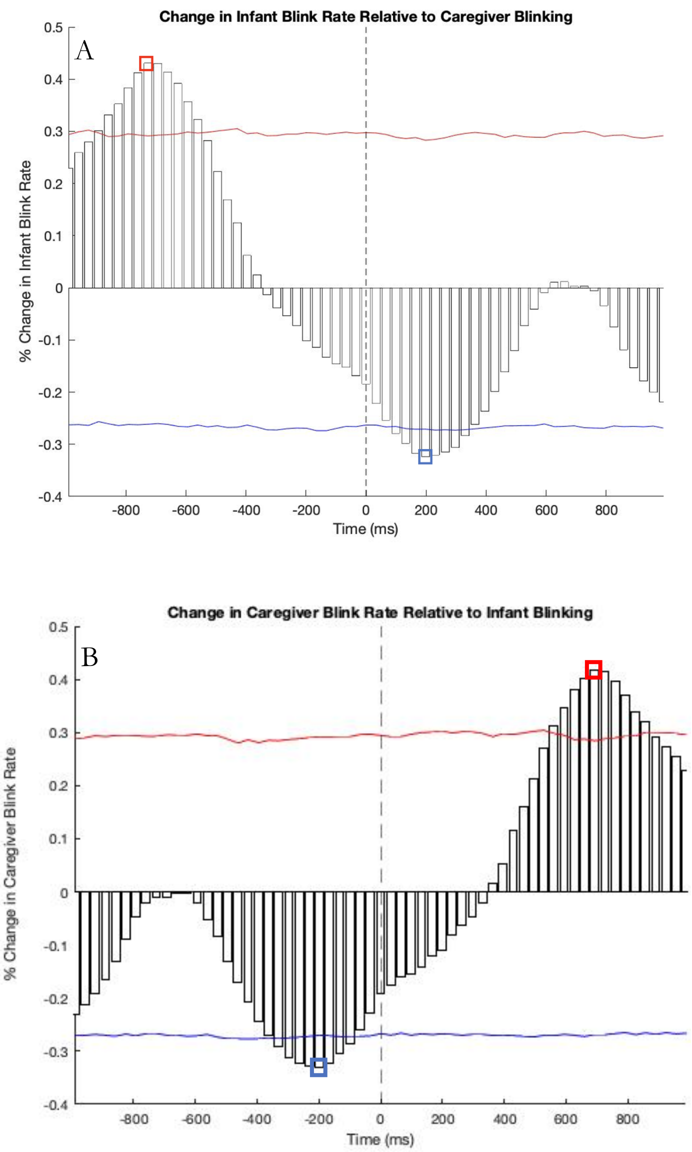

Figure 1.4: Time-Locked Infant and Caregiver Eyeblinks During Dyadic Interaction. PSTHs show the percent change in blinks per minute from the mean of permuted blink data. (A) PSTH shows the percent change in infant blink rate relative to moments when their caregiver blinked. (B) PSTH shows the percent change in caregiver blink rate relative to moments when their infant blinked. The blue and red lines mark the .025 and .975 confidence intervals respectively. Percent change in blinks per minute beyond these levels indicates a change in blinks per minute that is greater than expected by chance (two-tailed, $p < 0.05$). The maximum increase and decrease in

blinks per minute are indicated by the red and blue squares, respectively.

Discussion

This study aimed to develop and validate a semi-automated, non-invasive method to identify infant blinks during naturalistic interactions and determine if blink entrainment is present amongst 3- to 6-month-old infants and their caregivers. I successfully developed and applied a semi-automated method for identifying blinks. The algorithm and GUI together were 98.83% and 98.20% accurate at identifying frames with eyeblinks for infants and caregivers, respectively. This method is accurate, takes half the time required to identify the blinks by hand, and is noninvasive compared to other currently available methods.

Using this semi-automated blink detection method, I found that entrainment is present in infant-caregiver dyadic interactions, with infants showing a significant increase in blink rate approximately 560-890 milliseconds *before* their caregiver blinks, and caregivers showing a significance increase in blink rate approximately 560-860 milliseconds *after* their infant blinks. This lag in eyeblink entrainment is consistent with that observed in previous studies showing that adults watching a pre-recorded video blinked 250-500 milliseconds *after* the actor in the video blinked (Nakano et al. 2010).

One possible interpretation of the time lag observed in our study is that caregivers are entraining their blinks to their infants' blinks (rather than vice versa). This infant-leading model would suggest that caregivers are following their infants' cues by maintaining their engagement and inhibiting their eyeblinks until their infant blinks. This interpretation is consistent with reports that caregivers follow their infant's lead in other domains. For example, caregivers tend to vocalize approximately 1.75 seconds *after* the vocalizations of their 3- to 9-month-old infants (Northrup and Iverson, 2020). One possible explanation for this infant-leading model is that caregivers are extremely sensitive to and strive to match their infant's signals and level of engagement during their interactions. A study by Cohn and Tronick (1987) demonstrated that, when an infant displays positive affect, the mother will remain positive until the infant either becomes neutral or disengages from the interaction. Only then will the mother break her positive affect. Similarly, in our study caregivers may be inhibiting their blinks to maintain engagement until the infant blinks and disengages from the interaction, ensuring that mutual engagement is maintained for as long as the infant is willing or able to do so.

Studies examining the synchronization of infant and caregiver behaviors further suggest that the lead-lag structure of the interactions may change as infants age (Feldman, 2007; Feldman et al., 1996, 1999; Lavelli & Fogel, 2005; Lester et al., 1985). Lester et al. (1985) studied the rhythmic structure of infant-caregiver interactions measured using monadic phase coding. They found that infants showed 'dominance' or infant leading by an average of 4.8 seconds during the interaction at 3 months. However, at 5 months, the degree of synchrony in behaviors increased, meaning that the lag time decreased between infant behavior and maternal responses. Similarly, Feldman et al. (1996 and 1999) found evidence that 3-month-old infants' affective states preceded affective changes in their caregivers by approximately 1.5 to 2 seconds. However, by 9 months, they found a decreased time lag between infants and their caregivers, suggesting a shift in the lead-lag structure of interaction. Given evidence of changes in the structure of dyadic interactions in the first year of life, future studies should examine whether temporal synchronization of infant and caregiver eyeblinks increases as infants age. Establishing longitudinal trajectories of entrainment could build a baseline from which atypical dyadic interactions can be identified.

In addition to evidence of eyeblink entrainment, I found that infants inhibit their blinks before their caregiver blinks, and that caregivers inhibit their blinks before their infant blinks. I wish to propose two possible explanations for these unexpected findings. For infants, blink inhibition prior to caregiver blinking may be explained by a reduced physiological need to blink: directly after an infant has blinked, there is a decreased physiological need to blink directly as the tear film has already been restored (Bacher & Smotherman, 2004a). Thus, infants increased blinking ~560-890 ms prior to their caregiver's blink may drive the brief period of blink inhibition that follows. For caregivers, blink inhibition prior to infant blinking may reflect increased engagement with their infant's cues. If infant eye blinking indexes a 'breakpoint' in a bout of mutual engagement (Nakano, 2010), caregivers may be sensitive to signs that their infant is beginning to disengage (e.g. fussiness, distress, shift in attentional focus) and may inhibit their blinks as they attend to their infant's change in behavior. Future work should examine infant and caregiver behaviors before and after they blink to investigate these possible explanations.

Relatedly, although the present study did not examine patterns of infant and caregiver blinking relative to vocalizations, future work could examine the potential role of eyeblinks as a paralinguistic cue in infant-caregiver interaction by characterizing the timing of caregiver and infant blinks relative to their speech and vocalization patterns (such as in Nakano et al 2010 and 2011). If future studies do find evidence that eyeblinks function as a paralinguistic cue in early development, further work could examine whether eyeblink entrainment plays a role in infant expressive or receptive language development. Given the associations between early vocal coordination and later infant development, it would not be surprising that the two are linked (Iverson, 2022).

In conclusion, this study provides evidence of eyeblink entrainment between 3-to 6-month-old infants and their caregivers. Additionally, I have developed and validated a novel, semi-automated approach to coding blinks that can facilitate future studies in this area. Future work should focus on mapping the development of eyeblink entrainment from birth to determine how caregiver-infant dyadic interactions may change over time. Eyeblink entrainment has the advantage of being a marker that can be measured at all stages of infancy, unlike other behaviors often coded in studies of infant-caregiver dyads. Those behaviors, like smiling or vocalizations, emerge at different developmental stages and may not be valuable indicators of synchrony across all ages. Caregivers may alter their behavior in studies if they know they are being observed; eyeblink entrainment can provide an unconscious, implicit measure in evaluating the dyad. As early dyadic interactions provide the foundational moments for social learning in infancy, disruptions of entrainment early in life may have downstream effects on later development. The significant effects of atypical dyadic interactions on infant development make longitudinal measures of entrainment exceptionally valuable for understanding both typical and atypical development.

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STUDY 2. Differences in Developmental Trajectories of Reflexive and Volitional Grasping in Neurotypical Infants and Infants Later Diagnosed with Autism

Abstract

Autism is characterized by differences in social interactions, communication, focused interests, and repetitive behaviors. Although motor deficits are not considered one of these core features, significant motor differences between neurotypical (NT) and autistic (AUT) individuals have been reported by several studies. These differences begin in infancy and include a variety of motor behaviors: sitting, standing, walking, head lag, and grasping. Despite the large body of work in this field, there are two gaps. First, the age at which these motor differences first emerge is unknown, resulting from the few studies that have prospectively and longitudinally studied infant motor development in the first 6 months of life, a period marked by rapid change in early development. Second, while many have studied the development of voluntary motor behaviors, few studies have mapped the development of reflexive behaviors in the first 6 months of life in AUT infants. As many early motor behaviors in infants are spontaneous and reflexive, it is important to know if differences between NT and AUT infants arise in these early reflexive behaviors. This study aims to address these gaps by prospectively mapping longitudinal trajectories of reflexive and volitional grasping in 125 NT and 20 AUT infants from birth to 6 months and determining (1) when differences between NT and AUT infants first arise and (2) whether these differences are found in reflexive and/or volitional grasping. The results show that group differences emerge in volitional grasping as early as 163 days, whereas no significant group differences were found in trajectories of reflexive grasping.

Introduction

Autism spectrum disorder, or autism, is a developmental condition characterized by differences in social interactions, communication, focused interests, and repetitive behaviors. Currently, 1 in every 36 children is diagnosed with autism (Maenner, 2023). Although autism can only be reliably diagnosed by the second year of life, it impacts neurodevelopment by as early as the mid- to late-fetal period (Willsey et al., 2013), motivating a focus on understanding the unfolding of autism in early infancy.

Although motor deficits are not considered a core feature of autism, a number of studies have reported significant differences in motor behaviors between NT and AUT individuals (Bhat et al., 2012; Bradshaw et al., 2018, 2018; Flanagan et al., 2012; Heathcock et al., 2015; Iverson et al., 2019; Libertus et al., 2014). Findings from parent reports and retrospective home videos have provided initial evidence that AUT infants have delayed attainment of gross motor milestones, such as sitting, standing and walking (Arabameri & Sotoodeh, 2015; Reindal et al., 2020; Teitelbaum et al., 1998) and fine motor skills (Bolton et al., 2012; Gernsbacher et al., 2008), with some differences observed by as early as 6 months (Sacrey et al., 2015). These findings have been supported by differences in motor behaviors found in prospective studies and studies conducting direct testing (Bhat et al., 2012; Flanagan et al., 2012; Iverson et al., 2019). For example, Iverson et al. (2019) found that at 6-months AUT infants had increased failure rates on the fine motor assessment items of the Mullen Scales of Early Learning compared with infants with a low-likelihood of developing autism (Iverson et al., 2019). Additionally, Libertus et al. (2014) found that 6-months-old infants with a high likelihood for developing autism showed reduced grasping and object manipulation skills compared to infants with a low-likelihood of developing autism (Libertus et al., 2014).

There are two major gaps within this field. The first is that few studies have prospectively and longitudinally studied infant motor development in the first 6 months of life with no studies densely sampling infant motor behavior beginning at birth. Consequently, the age at which motor differences first emerge is unknown. The second is that few studies have explored the development of reflexive behaviors in those with autism and even fewer of those have explored these reflexes in the first 6 months of life. Early motor behaviors during the newborn period are often thought of as being as spontaneous or reflexive (Futagi et al., 2012; Ingram, 1962; Nagy, 2011; Rousseau et al., 2017). These early reflexes are subcortically mediated and are largely thought to ‘disappear’ by the end of first year of life as the infant’s cortex matures and begins to inhibit these subcortical regions (Futagi & Suzuki, 2010; M. H. Johnson, 1990; Melillo et al., 2022; Teitelbaum et al., 1998; Thelen et al., 1984; Zafeiriou, 2004). Although most studies of motor differences in young infants at elevated-likelihood for autism have investigated voluntary, goal-directed movements, evidence from older infants and children suggest that motor reflexes may be impacted as well (Chinello et al., 2018; Healy et al., 2022; Melillo et al., 2022; Teitelbaum et al., 2003). For instance, persistent presence of reflexes, specifically sucking, rooting, and grasping, was observed in 12- to 17-month-old (Chinello et al., 2018). Additionally, snout and visually rooting reflexes were found in 4- to 6-year-old children with autism compared to their NT peers (Healy et al., 2022). However, the evidence for differences in reflexive behaviors remains scarce particularly in infancy.

Filling these two gaps will have important implications for determining when motor differences first arise and if these differences either occur in reflexes, or goal-directed, voluntary motor behaviors or both. This distinction has important implications for understanding brain systems implicated in early motor disruptions and for understanding precursors to later motor differences. For instance, motor action in early infancy is characterized by reflexive and spontaneous actions that are thought to be subserved by subcortical neural mechanisms (Futagi et al., 2012; Futagi & Suzuki, 2010). On the other hand, volitional, goal-directed movements that arise later in infancy are thought to be subserved by cortical neural mechanisms (Johnson, 1990; Melillo et al., 2022; Teitelbaum et al., 1998; Thelen et al., 1984; Zafeiriou, 2004). Understanding when and how motor systems are impacted by autism (earlier reflexive phases vs later-emerging motor skills) could direct future studies in autism to establish specific neural circuitry differences. Furthermore, knowing when differences first emerge could help identify time periods when early intervention would have the greatest influence on later development.

To address these gaps, we prospectively mapped longitudinal trajectories of both reflexive grasping and volitional grasping in infants with and without autism, from birth to 6 months. Grasping is a crucial exploratory behavior that infants use to learn about and interact with the world around them and many studies have found associations between infant grasping behavior and motor, cognitive, language, and social development (Gerson & Woodward, 2014; Libertus & Needham, 2011; P. Rochat, 1989; Skerry et al., 2013). For example, grasping provides opportunities for infants and toddlers to initiate moments of joint attention with their caregiver that facilitates future social learning (K. M. Johnson & Woods, 2016). Developmental changes in grasping in NT infants has been well documented. In the newborn period, grasping is reflexive and is typically elicited by placing one's finger onto the ulnar side of an infant's hand and applying pressure to their palm resulting in the closure of their fingers (Futagi et al., 2012). Reflexive grasping can be elicited from birth, begins to wane around 5 months, and should no longer be present by the end of the first year of life (Futagi et al., 2012; Twitchell, 1965; Zafeiriou et al., 1995). By contrast, volitional grasping (when an infant reaches for and grasps an object) first emerges at around 4 months of age (Berthier & Keen, 2006).

Overall, this study will extend previous reports of deficits in volitional grasping in autism at 6 months of age by examining: (1) when these motor differences first arise and (2) whether differences are observed in both reflexive and volitional behaviors. Given that early disruptions to primary reflexives are typically associated with major neurological impairments (Hobo et al., 2014; Pavão et al., 2013), I expected to observe no initial differences in reflexive grasping between in NT and AUT infants. However, given previous reports of persistent reflex-like behaviors in autism (Chinello et al., 2018; Healy et al., 2022; W. Jones & Klin, 2013; Shultz et al., 2018), I hypothesized that trajectories of reflexive grasping will decrease earlier in NT infants compared to AUT. Finally, I hypothesized that there would be a delayed onset and decreased observations of volitional grasping in AUT infants compared to NT infants.

Materials and Methods

Participants

Participants (145 infants; 125 NT infants and 20 AUT infants) were selected from a cohort of infants participating in the NIH-funded Autism Center of Excellence prospective longitudinal study of infants at low- and elevated-likelihood for autism at the Marcus Autism Center, Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, GA. The research protocol was approved as bearing no significant risk by the Human Investigations Committee at Emory University School of Medicine. All infants were enrolled at birth and followed until 2 or 3 years of age.

Infants were considered to be neurotypical on the basis of having no familial genetic likelihood for neurodevelopmental disorders and no medical concerns: all NT infants had no history of autism in first-, second-, or third-degree relatives, no developmental delays in first-degree relatives, no pre- or perinatal complications, no history of seizures, no known medical conditions or genetic disorders, and no hearing loss or visual impairment. Infants in the autism group were assigned a diagnosis of autism by an expert clinician at 2 or 3 years of age (see Diagnostic Procedures for details).

	AUT (n=20)	NT (n=125)
Gestational Age, mean (SD), weeks	38.85 (1.57)	39.02 (1.79)
Sex		
Male	70%	55.20%
Female	30%	44.80%
Race		
White	60%	74.40%
Black	20.00%	12.80%
Asian	10.00%	0.80%
American Indian or Native Alaskan	0%	0.80%
Mixed Race	5.00%	4.00%
Other	5.00%	0.80%
Unknown	0%	6.40%
Ethnicity		
Hispanic, Latinx	0%	12.00%
Non-Hispanic, Latinx	100%	88.80%
Maternal Education		
Professional Degree	5%	15.20%
Master's Degree	20%	35.20%
College Degree	40%	28.80%
Associates Degree	15%	3.20%
Courses Toward College	10%	5.60%
Trade or Vocational School	5%	2.40%
Unknown	0%	8.00%
Yearly Household Income		
>150,000	15%	28.00%
80,001-150,000	35%	39.20%
<80,000	30%	20.80%
Unknown	20%	12.00%
ADOS CSS, Mean (SD)	6.05(2.58)*	3.09(2.33)*
Unknown	0%	40%
Mullen, 24 months, Mean (SD)		
Visual reception AE	23.8(4.8)* [4]	27.2 (6.1)*[1]
Receptive AE	18.8(8.0)* [4]	26.2 (5.8)*[2]
Expressive AE	19.8(8.4)* [4]	24.6(7.1)*[2]
Gross Motor AE	21.9(3.9) [4]	22.4(3.5)[3]
Fine motor AE	22.4(3.3)* [4]	24.7 (4.1)*[1]

Table 2.1: Demographics of Participant Sample. * Indicates $p<0.05$ using a two-tailed t-test[1] Indicates 56% unknown scores. [2] Indicates 56.8% unknown scores. [3] Indicates 76% unknown scores. [4] Indicates 10% unknown scores.

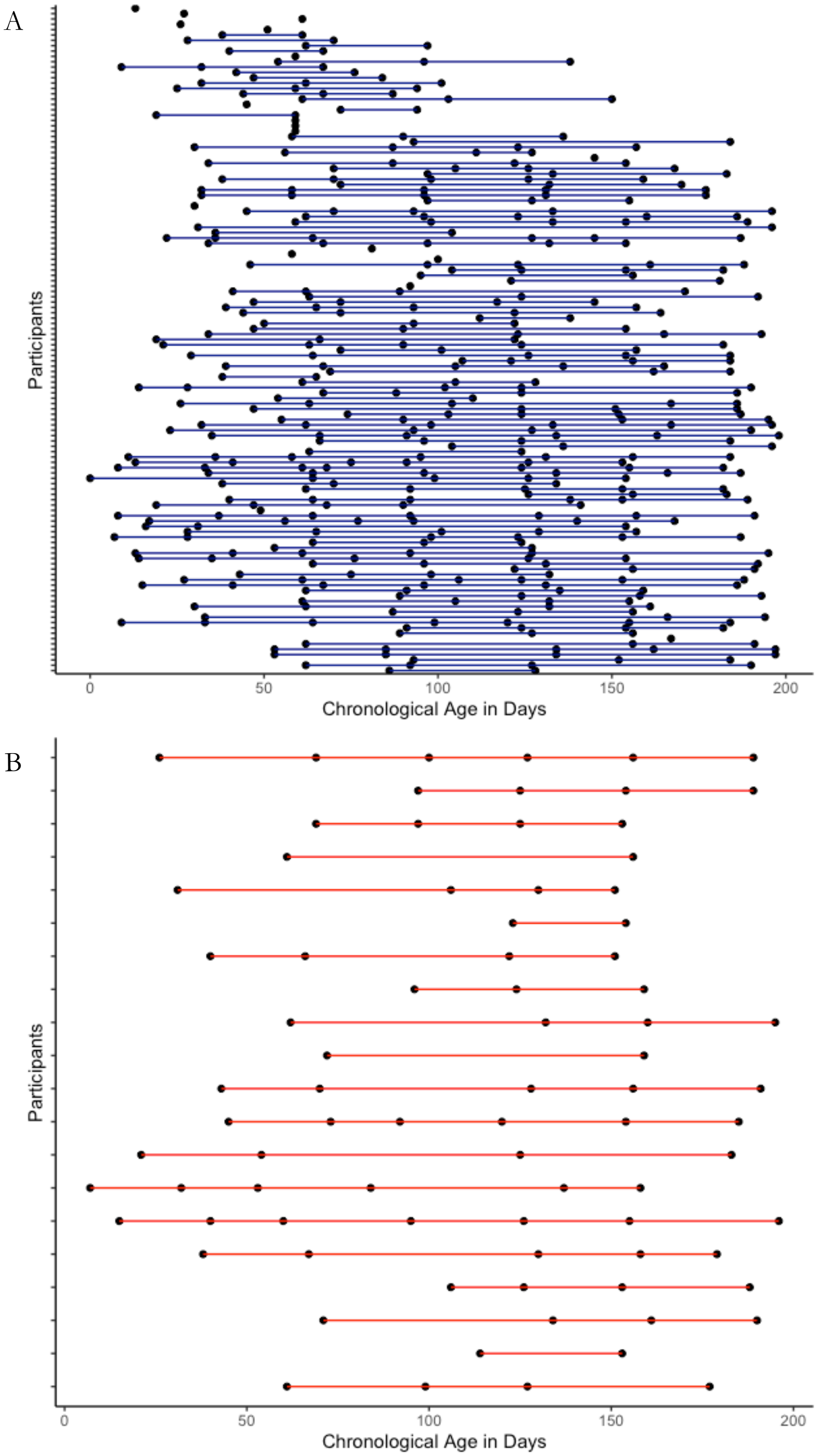

Figure 2.1 Sampling Distribution and Age of Testing of NT (A) and AUT (B) Infants. Each participant's visits are denoted with a solid black circle and connected with a line.

Motor Assessments

Infants were tested by a licensed clinical psychologist, speech language pathologist, or doctoral student at up to 7 timepoints from birth to 6 months (Figure 2.1). NT infants had on average 3.5 ±1.6 visits and AUT infants had on average 4.2 ± 1.4 visits. Reflexive and volitional grasping was assessed at each visit using the NICU Network Neurobehavioral Scales (NNNS) and the Bayley Scales of Infant and Toddler Development (Bayley, 2012; Lester & Tronick, 2004), respectively.

Reflexive Grasping: The NNNS measures neonatal neurological reflexes, neurobehavioral organization and motor development and can be used to evaluate neonates who are born at term, preterm, medically fragile or typically developing. Item number 23 in the NNNS was used to measure infant reflexive grasping. Grasping was elicited by the experimenter using their finger to place slight pressure on the infant's palm and the score was determined by gauging the strength of the resulting grasp around the finger. This item is scored from a 1 - 4 on each hand: 1 – No grasp response, 2- Short, weak flexion, 3- Strong and sustained grasp for several seconds that relaxes, and 4 – Prolonged, excessive grasp; tips of infant's fingers turn white, maybe long latency to relax or no relaxation at all.

For this study the left and the right-hand measures were averaged, and this average was binarized such that if the infant received a score of 2 or 3 on the NNNS, the reflex was considered to be present, and the infant was assigned a binary score of 1. If the infant scored a 1 on the NNNS, they were assigned a binary score of 0 indicating the absence of the reflex. Sessions where infants scored a 4 on either hand were excluded (n=2 for AUT infants, n=6 for NT infants) as this score is indicative of atypical grasping behavior (more consistent with neurological issues) that was not under investigation in this study.

Volitional Grasping: The Bayley is a standardized assessment for children from birth to 42 months. Item number 15 in the Bayley Fine Motor subscale was used to measure infant volitional reaching and grasping. The infant was presented with a red block and received a 0 if they could not grasp the block with their whole hand and a 1 if they were able to grasp the block. The decision to binarize NNNs and Bayley scores (to reflect presence/absence of grasping) was made because this feature of the data most directly addresses our study aims.

Diagnostic Procedures

Infants received a clinical evaluation at 2 years of age, conducted by expert clinicians who were blind to the infant's likelihood for autism. Assessments included the *Autism Diagnostic Observation Schedule* (ADOS; typically ADOS 1 (Lord et al., 1999), or the Toddler Module (Lord et al., 2008) or ADOS 2 (Lord et al., 1999)), the *Mullen Scales of Early Learning* (Mullen, 1995), and the *Vineland Adaptive Behavior Scales II* (Sparrow et al., 2012). These assessments evaluated social disability, developmental level across visual receptive and expressive language, and fine and gross motor domains, and adaptive function in the child's daily life, respectively. The participants had to meet the criteria for autism spectrum disorder as defined by the DSM-5 (American Psychiatric Association & American Psychiatric Association, 2013) to receive an autism diagnosis.

To confirm the diagnosis, an additional diagnostic review procedure was conducted. Two clinicians, blind to the participant's likelihood for autism, reviewed all available assessment materials for each child. Two clinicians, blind to the participant's likelihood for autism, independently reviewed all available assessment materials and recordings for each child and provided a clinician's best estimate diagnosis. If the diagnosis assigned by the two clinicians to a child differed, a broader group of expert clinicians discussed and reviewed that child's information and achieved consensus.

Data Analysis

Logistic Regression Models: Data were first fitted using a generalized additive model in R (version 4.2.3) using a binomial link function. The *gam()* function from the mgcv package in R adopts a method of curve fitting which penalizes curvature using a smoothing parameter and finds the optimal smooth parameter value using restricted maximum likelihood. A feature of this fitting procedure is to suggest a simple linear fit should it align best with the data. The model fit a linear trend to the data allowing the developmental changes of volitional grasping to be mapped using a logistic regression model (*glm()* function) on a log odds scale which assumes that the relationship between the NNNS and the Bayley scores and age is linear on the log scale. A slightly different approach to the logistic regression model needed to be taken for the reflexive data. As can be seen in Figure 2.2B, there is little variability in the AUT sample with only 1 participant receiving a score of 0 on the reflexive grasping assessment. This distribution of the data results in a quasi-complete separation that can occur when binary data contain observations predominantly take on a single value across and within participants. To correct for this in the modeling approach, the logistic regression (using *logitsf()*) was run with a bias reduction of maximum likelihood estimates. For the final model, the log odds were converted to probabilities to map the probability of infants displaying reflexive or volitional grasping from birth to 6 months of age that allowed for the identification for any subtle changes in trajectory shape over time. To identify differences in reflexive and volitional behaviors between AUT and NT infants an interaction effect between age in days and diagnosis was used in the model.

Results

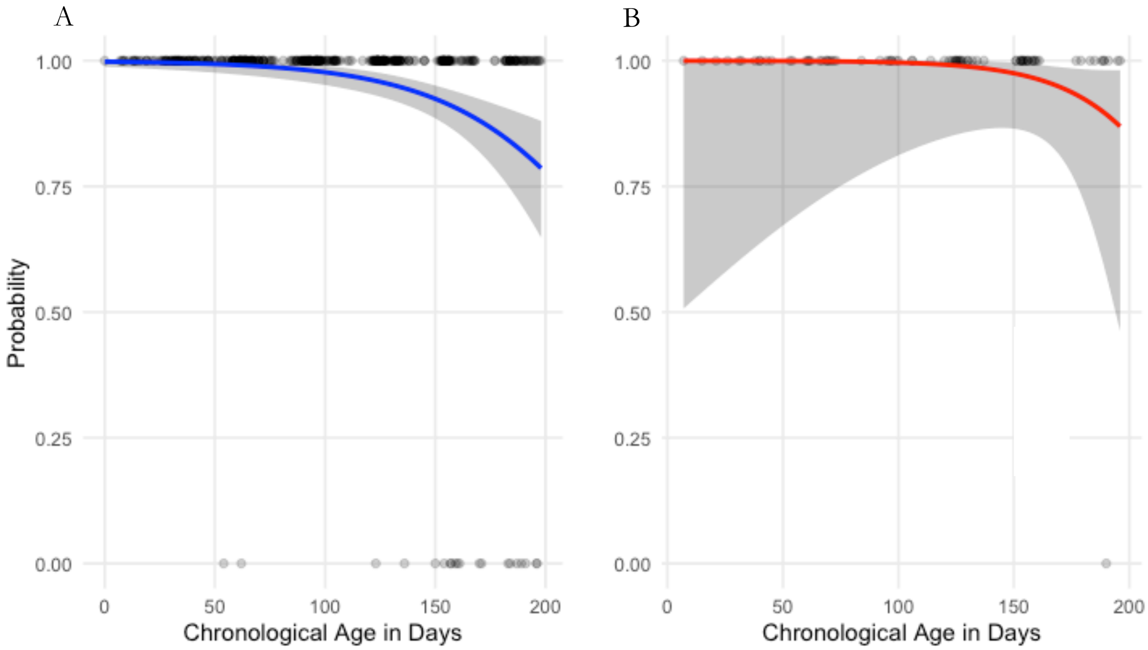

Figure 2.2: Developmental Trajectories and Raw Data of Reflexive Grasping in the First 6 Months of Life in NT (A) and AUT (B) Infants. Solid lines represent trajectories mapped with a logistic regression model using a log odds scale. Light gray shading represents the 95% confidence band with darker gray circles representing the raw scores for each participant sessions. The large confidence band for the autism group is expected given the lack of variability of the scores for AUT infants.

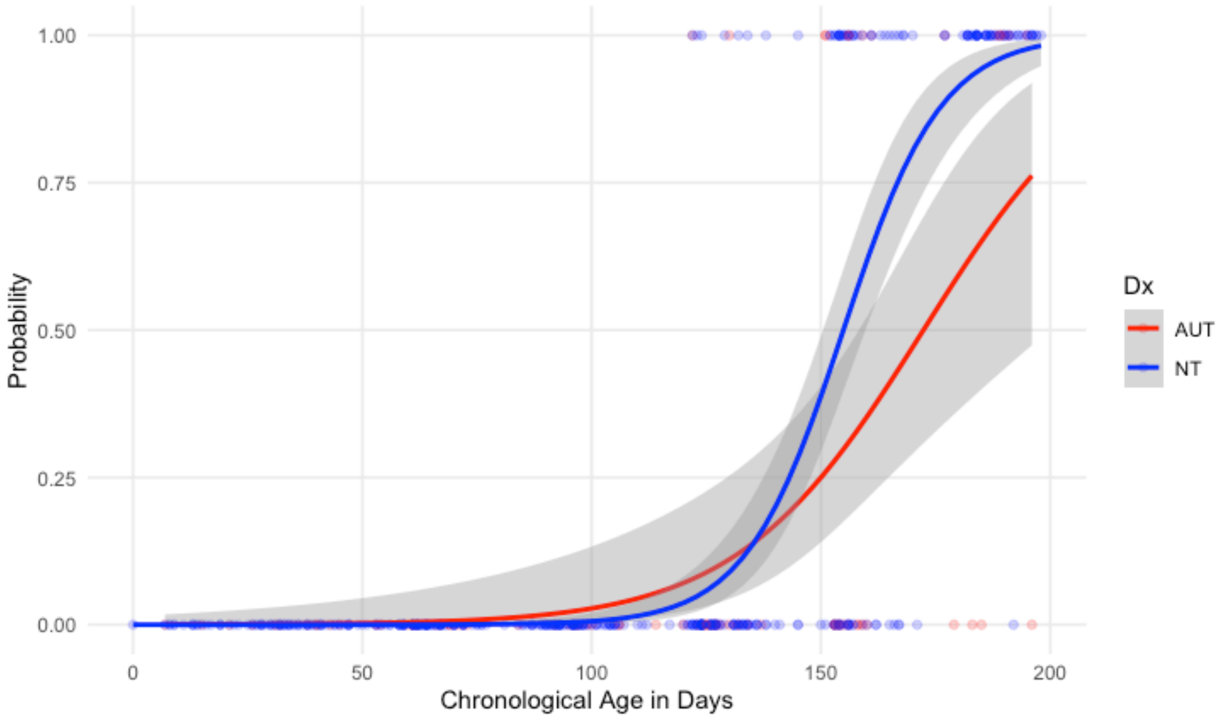

Figure 2.3: Developmental Trajectories of Volitional Grasping in NT and AUT and infants. Solid lines represent trajectories mapped with a logistic regression model using a log odds scale. Light gray shading represents the 95% confidence band with blue and red circles representing the raw scores for each participant sessions for NT and ASD infants respectively. Significant differences in trajectories are denoted by moments in which there is not overlap of the confidence intervals of each trajectory.

Table 2.2: Logistic Regression Model Output for Reflexive Grasping

Variable	**Coefficient**	**Standard Error**	**P-value**
Intercept	9.433	4.856	0.00019
Age in Days	-0.038	0.028	0.18921
Diagnosis	-3.191	4.945	0.62519
Age in Days x Diagnosis	0.014	0.029	0.75315

Table 2.3: Logistic Regression Model Output for Volitional Grasping

Variable	Coefficient	Standard Error	P-value
Intercept	-8.481	2.190	0.00011
Age in Days	0.049	0.014	0.00360
Diagnosis	-5.998	2.814	0.03303
Age in Days x Diagnosis	0.044	0.018	0.01380

Logistic Model

Two logistic regressions were used for this study to determine if there were differences between AUT and NT infants in their reflexive and volitional grasping behaviors. The first analyzed the relationship between grasping reflex and diagnosis, the interaction between diagnosis and age, and age. No significant effects were found for this model. This is likely due to the lack of variability in NNNS scores among the autism group, as visualized in Figure 2.2B (with the majority of infants continuing to show the reflex through 6 months of age). Only one subject showed an absence of the reflex during the expected time frame, yielding extremely wide confidence intervals as discussed in the Figure 2.2B legend.

The second model analyzed the relationship between volitional grasping and diagnosis, the interaction between diagnosis an age, and age. Age and diagnosis showed a significant effect on Bayley score and there was a significant interaction effect in the model output (Age in days x Diagnosis) meaning that the difference between the trajectories for AUT and NT infants increases over time. For ease of interpretation, log odds were converted to probabilities to visualize the trajectories of these behaviors. To determine moments when a significant difference between the AUT and NT infants emerged, I searched for time when the confidence intervals were non-overlapping. The difference first emerges at 163 days and the largest difference between the two trajectories was found at 173 days where there was a .34 difference in mean probability of displaying voluntary grasping.

Discussion

This study examined whether motor differences, measured by grasping behavior, arise before 6 months of age in autism and if they are found in reflexive or volitional behaviors. Here, I mapped the development of reflexive and volitional grasping, an essential motor behavior that affords opportunities for exploring and learning about one's environment in early infancy, in a sample of AUT and NT infants. No differences in the developmental trajectories of reflexive grasping were found between the AUT and NT infants, but significant differences were found in the volitional grasping behavior, beginning at 163 days and peaking at around 173 days.

The similarity in developmental trajectories of reflexive grasping between both groups of infants suggest that there may not be a fundamental disruption in early reflexes. As reflexive behaviors are thought to be subserved by subcortical neural networks, this may suggest that subcortical circuity is not impacted by autism in the first 6 months of life. However, these findings do not preclude the possibility that differences in the grasping reflex could arise later in development, particularly as the grasping reflex does not begin to decline until 5 months and disappears at the end of first year of life. Findings from the NT group reflexive grasping follow the trends observed in the literature as a slight decline is observed around 5 months of age, capturing the initial decline of the reflex in 19 out of 125 (15.2%) infants. By contrast, in the autism group, only 1 out of 20 (5%) infants displayed no reflex at 190 days. Although the lack of variability makes it impossible to statistically compare the two groups due to the large confidence bands of the AUT group, the pattern in the autism group is consistent with previous suggestions of persistent reflexes in autism. For example, Jones and Klin (2013) found that early eye-looking, a behavior hypothesized to be reflex-like in the newborn period, is present at greater levels and for a more extended period in AUT infants. Additionally, persistent reflexes have been found in toddlers (12-17 months) (Chinello et al., 2018) and older children (4-6 years) (Healy et al., 2022) indicating a possibly protracted developmental trajectory. Future work should extend this study by measuring reflexive grasping through the first year of life, as reflexive grasping is not expected to decline more fully until 6-12 months (Twitchell, 1965).

Group differences in volitional grasping were found by as early as 163 days, with AUT infants exhibiting less volitional grasping behavior than NT infants. These findings are consistent with other studies showing decreased grasping activity in 6 month old infants with a high-likelihood for developing autism (Libertus et al., 2014). Additionally, studies have described delays across many motor skills in AUT infants in relation to their neurotypical peers (Arabameri & Sotoodeh, 2015; Bhat et al., 2012; Bradshaw et al., 2018; Flanagan et al., 2012; Heathcock et al., 2015; Iverson et al., 2019; Reindal et al., 2020; Teitelbaum et al., 1998). This study adds to the existing literature by showing when these differences unfold as previous work has not explored differences in grasping behavior from birth or in a relatively dense, longitudinal sample.

Achieving a better understanding of the processes underlying early motor deficits in autism has important intervention implications. One possibility is that our findings may reflect deficits at the neurological or musculature level. If this is the case, then future work should focus on exploring the neural mechanisms underlying fine motor skills and their role in the development of autism. This explanation would lead to further research on interventions for autism that would focus on physical therapy and supporting motor milestone development, which has been shown to benefit social development later in life (Bhat et al., 2012; Iverson, 2021, 2022; Libertus & Needham, 2011). Alternatively, the observed differences in volitional grasping could be due to differences in the way in which initially reflexive or spontaneous behaviors are shaped and transformed into volitional ones. In the first few months of life infants learn by observing the impact of their initially spontaneous and/or reflexive actions on their environment (K. M. Johnson & Woods, 2016; Needham et al., 2002; Rovee & Rovee, 1969; Sargent et al., 2014). Work by Sargent et al (2014) demonstrated this mechanism of learning in early infancy. They tied an infant's leg to a mobile and leveraged the spontaneous kicking behavior of infants to spin the mobile. Infants eventually detected the contingency between their leg kicks and the mobile spinning and began to intentionally kick with the goal of spinning the mobile. A similar phenomenon is observed in dyadic interactions between infants and their caregivers (K. M. Johnson & Woods, 2016; Shultz et al., 2018). Caregivers provide highly reinforcing feedback by providing meaning and responding their infant's spontaneous behaviors (Cohn & Tronick, 1987; Iverson, 2021; Kaye & Wells, 1980; Northrup & Iverson, 2020). Infants are able to, once again, detect the relationship between their actions and caregiver responses resulting in infants intentionally eliciting further interactions with their caregiver. In AUT infants, there is a possibility of a disruption in the infants' ability to process the effects of reflexive grasping on their environment or caregiver. If that is the case, this could result a disruption to the process through which initially spontaneous grasping is transformed to volitional grasping. These dyadic interactions are critical for social development and are disrupted in autism. If findings from this future work establish that the developmental trajectories of these behaviors are influenced by early dyadic interactions, this would lead to a very different pathway for intervention research than the first account (see above). In this case, interventions would focus on parent mediated interventions to support these interactions to enhance future motor development.

In conclusion, this study provides insights into motor development differences between AUT and NT infants in the first 6 months of life, specifically in reflexive and volitional grasping behaviors. The findings highlight the need to study motor development in early infancy, suggesting that motor differences may emerge as early as 4 months of age in autism. Future research should continue to investigate motor developmental trajectories, extending through the first year of life, and specifically explore the role of infant-caregiver interactions in shaping motor development in autism, which could have implications for early interventions targeting motor delays.

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STUDY 3. Lateralization of major white matter tracts during infancy is time-varying and tract-specific

The material in this chapter has been submitted for publication at Cerebral Cortex. Aiden L. Ford and I contributed equally to this work.

Abstract

Lateralization patterns are a major structural feature of brain white matter and have been investigated as a neural architecture that indicates and supports the specialization of cognitive processing and observed behaviors, e.g., language skills. Many neurodevelopmental disorders have been associated with atypical lateralization reinforcing the need for careful measurement and study of this structural characteristic. Unfortunately, there is little consensus on the direction and magnitude of lateralization in major white matter tracts during the first months and years of life – the period of most rapid postnatal brain growth and cognitive maturation. In addition, no studies have examined white matter lateralization in a longitudinal pediatric sample – preventing confirmation of if and how white matter lateralization changes over time. Using a densely sampled longitudinal dataset from neurotypical infants aged 0-6 months, we aim to 1) chart trajectories of white matter lateralization in nine major tracts and 2) link variable findings from cross-sectional studies of white matter lateralization in early infancy. We show that patterns of lateralization are time-varying and tract-specific and that differences in lateralization results during this period may reflect the dynamic nature of lateralization through development, that can be missed in cross-sectional studies.

Introduction

The lateralization of brain regions and networks is a core feature of structural and functional neural organization. Growing evidence indicates that hemispheric biases in specific structures are associated with the emergence of adaptive behaviors as early as the first year of life (Adibpour et al., 2018; Bortfeld et al., 2009; Dehaene-Lambertz et al., 2002; Elison et al., 2013), motivating the theory that lateralization is a neural architecture that supports and reflects the specialization of behavioral and cognitive skills throughout development. Two notable examples include language acquisition and sensorimotor perception, which, in adulthood, are lateralized to the left and contralateral hemispheres, respectively (Friederici, 2011; Friederici & Alter, 2004; Hugdahl, 2000; Ocklenburg et al., 2014).

Many neurodevelopmental disabilities associated with social and cognitive differences are marked by atypical lateralization patterns (Moncrieff, 2010). Atypical asymmetries in the structural regions and functional networks associated with motor skills, language ability, and social processing have been consistently reported in autistic children and adults (Floris et al., 2016, 2021; Keehn et al., 2015; Kleinhans et al., 2008; Lindell & Hudry, 2013; J. Liu et al., 2019; Travers et al., 2012). These atypical asymmetries are also observed in lateralized behaviors, such as increased variability in handedness in autistic children (Lindell & Hudry, 2013; Markou et al., 2017). Both neurological and behavioral differences in lateralization emerge from the first months of life and persist well into toddlerhood (Keehn et al., 2015; J. Liu et al., 2019). Lateralization differences in brain structure and function have also been observed in ADHD (Hale et al., 2014), dyslexia (Langer et al., 2017), and schizophrenia and bipolar disorder (Ho et al., 2017).

Given the relevance of neural lateralization for both basic and clinical science, it would be broadly useful to have a clear understanding of when brain lateralization emerges during development. Moreover, evidence from infancy studies shows the lateralization of early behaviors can shift direction, motivating additional investigation into whether neural lateralization also changes over time. For example, in a review of the handedness literature, Morange and Bloch (1996) explain that infants favor their left hand during the 'pre-reaching' phase, before four months of age (Morange & Bloch, 1996). This preference shifts between four and six months of age and infants begin to prefer their right hand for reaching and grasping behaviors. Instabilities in behavioral lateralization are also found in facial expressions. Best and Queen (1989) found that while adult smiles are more animated on the left side of the face, indicating that motor control of emotional expression is specialized in the right hemisphere, infants show emotion more strongly on the right side of their face, indicating a left hemisphere specialization (Best & Queen, 1989). These results suggest that lateralization is early emerging and may change over time at both the level of brain and behavior during infancy.

Despite evidence demonstrating that the lateralization of infant behaviors is time-varying, few studies have tested the hypothesis that lateralization patterns in the developing brain may also be time-varying. In this study, we focus specifically on the lateralization of major white matter tracts connecting subcortical and cortical regions of the brain. White matter fibers are a key structural element of the brain that organize and maximize brain-wide communication. Moreover, lateralization in white matter may support lateralization in other brain tissues and features, including gray matter and functional connectivity (Casey et al., 2005; Ocklenburg et al., 2016; Passingham et al., 2002).

Most of our current understanding of the lateralization of major white matter tracts is informed by cross-sectional studies conducted in infants, toddlers, and adults (findings from fractional anisotropy (FA) analyses summarized in Figure 3.1). These studies show that tract asymmetry (typically measured through differences in microstructure metrics derived from diffusion tensor imaging (DTI)) is present in infancy and early childhood (Bisiacchi & Cainelli, 2022; O'Muircheartaigh et al., 2013), however results are inconsistent from study to study. Although these inconsistencies may, in part, be due to variability in participant samples, and MRI acquisition and analytic strategy, many in the field have attributed them to the absence of longitudinal work (Bisiacchi & Cainelli, 2022; Cohen et al., 2016; Dubois et al., 2009; O'Muircheartaigh et al., 2013; Saadani-Makki et al., 2019). For example, lateralization of the uncinate fasciculus (UF) appears to differ depending on the age of the participants within the study sample. In samples of infants, average age 4.8 months researchers found no lateralization in the UF (Dubois et al., 2016). However, Johnson et al. (2014), in a study of older toddlers aged two to four years, reported rightward lateralization of the UF (Johnson et al., 2014). To determine if these changes in lateralization are the result of differences in participant ages from cross-sectional studies, rather than differences in acquisition or analysis, it is critical to employ a longitudinal design to map the lateralization of white matter tracts over time in the same individuals. This approach becomes all the more important when mapping asymmetries during the first months of life, which, despite being the focus of few studies, is the period of most rapid postnatal brain growth (see Figure 3.1) (Holland et al., 2014).

Ultimately, potential evidence of time-varying change in brain lateralization from cross-sectional studies, alongside well-characterized instabilities in behavioral lateralization in infancy motivate the need for longitudinal studies exploring the development of brain lateralization during the first months of life (Bisiacchi & Cainelli, 2022). Our work fills this gap in the literature and focuses on the first six months of life. This developmental window marks the period of greatest postnatal maturation in brain development, particularly in white matter structures, and shows remarkable change in infant behavior as they acquire and exhibit new skills (Holland et al., 2014; Ilyka et al., 2021). Using a densely sampled, longitudinal dataset where participants completed up to three DTI sessions from birth to six months, we aim to identify periods where the lateralization of major white matter tracts changes over time as well as link variable findings from cross-sectional studies of white matter lateralization in early infancy (Figure 3.1). Paired with functional principal components analysis, a nonparametric statistical technique ideal for mapping the non-linear shape of development, this experimental design allows us to study the dynamics of early white matter lateralization.

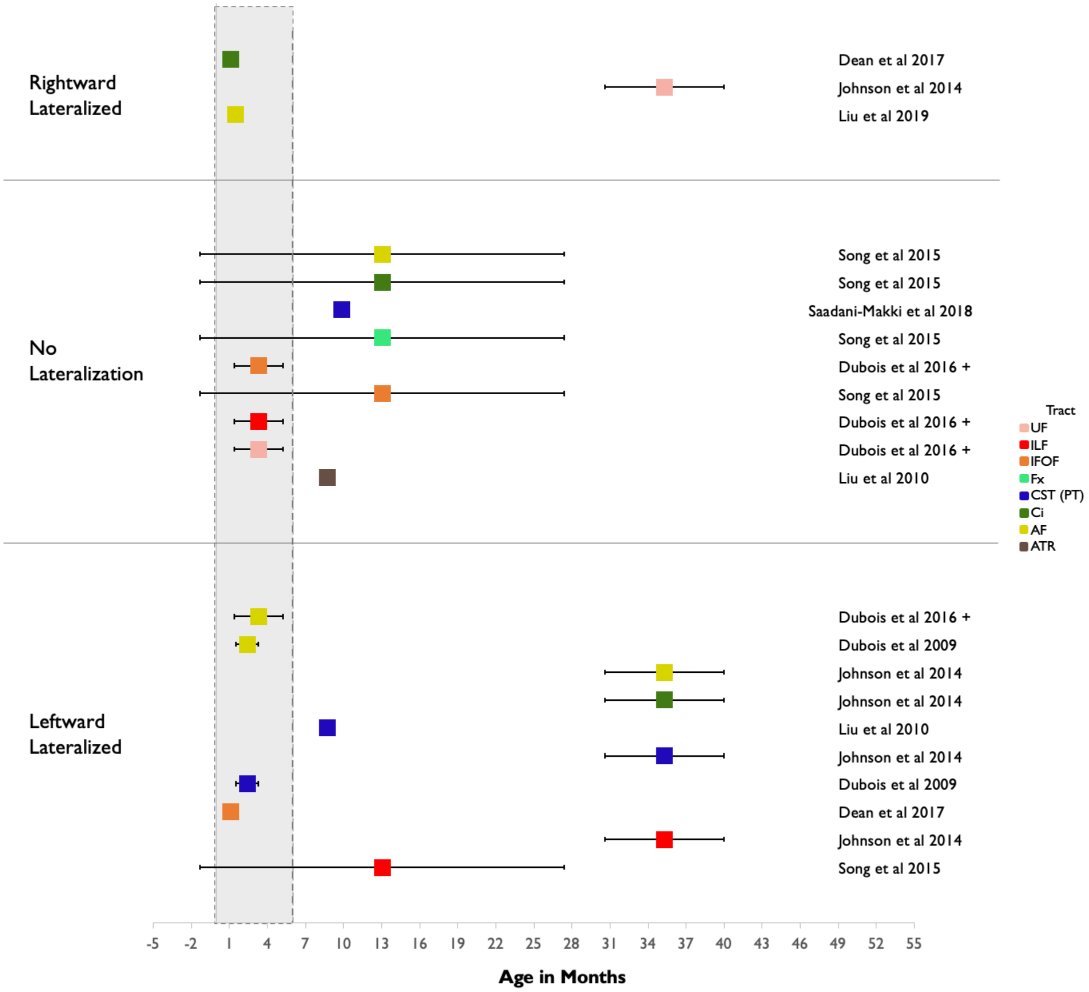

Figure 3.1: Lateralization in FA of Major White Matter Tracts Measured Across Infant Neuroimaging Studies. Studies were included if they met the following criteria: 1) neurotypical population, 2) participants under 36 months of age, 3) analyzed FA measures in tracts of interest (Dubois et al., 2009; Liu et al., 2010; Johnson et al., 2014; Song et al., 2015; Dubois et al., 2016; Dean et al., 2017; Liu et al., 2019; Saadani-Makki et al., 2019). Tracts are categorized as either right, left, or not lateralized. Colored squares denote the mean age of the participant sample and length of black bars indicates approximate spread of included ages. The shaded box highlights the studies that included infants within the newborn to 6-month age range. The '+' symbol denotes papers whose mean age was not reported, as a result the midpoint and age range provided is indicated here.

Materials and Methods

Participants: All participants were neurotypical infants enrolled in prospective longitudinal studies of infants at high- and low-likelihood for autism at the Marcus Autism Center in Atlanta, GA, USA. Infants in this sample had a mean gestational age of 38.6 weeks (SD=1.98 weeks) and were considered neurotypical if they had no autistic first-, second-, or third-degree relatives, no developmental delays in first degree relatives, no pre- or perinatal complications, no history of seizures, no known medical conditions or genetic disorders, and no hearing loss or visual impairment. Infants with contraindications for MRI were not allowed to participate. Infants were not excluded based on handedness as it cannot be confidently determined at this age range. The Emory University Institutional Review board approved the research protocol for this study.

Unlike many longitudinal sampling designs where data were collected at regular, fixed intervals, scans were scheduled for each infant at three pseudorandom time points between birth and six months (Holland et al., 2014). This non-uniform longitudinal sampling design provides practical and methodological advantages for mapping developmental trajectories in that 1) without rigid sampling windows, families have increased flexibility for scheduling and completing infant visits and 2) collected data cover the entire 0–6-month period of interest, reducing the risk of over-interpolating trajectory shape during curve-fitting (Figure 3.2) (Yao et al., 2005).

A total of 135 scans were initially acquired from 81 infants. Six scans were excluded from this total during pre-processing because less than the minimum number of six volumes of the image were collected (Basser et al., 1994) (four scans) or distortion correction could not be performed (two scans). An additional three scans were excluded during post-processing because the age at scanning led to edge effects during curve-fitting (Yao et al., 2005). Our final data set included 126 scans collected from 78 infants, with 53.8%, 30.8%, and 15.4% of infants contributing one, two and three longitudinal scans, respectively. The distribution of participant age at each scan is displayed in Figure 3.2.

	(N = 78)
Infant Sex	31f, 47m
Gestational Age at birth, mean (SD)	38.6wks (2)
Race (N=68)	
Black	8.8%
Native	1.5%
White	86.8%
More than one race	2.9%
Maternal Education (N=66)	
High School	1.5%
College Courses	6.0%
Associate's Degree	1.5%
College Degree	30.0%
Graduate Degree	60.0%
Household Income (N=65)	
< $40000	7.8%
$40000-$80000	17.2%
$80001-$100000	18.8%
$100001-$150000	25.0%
> $150001	31.2%

Table 3.1: Demographics of Participant Sample. Nine participants did not complete the Family Demographic Form, and additional participants declined to answer specific questions about Race, Maternal Education, and Household Income. For information categories with a participant number less than the total sample, the N is specified next to the category title.

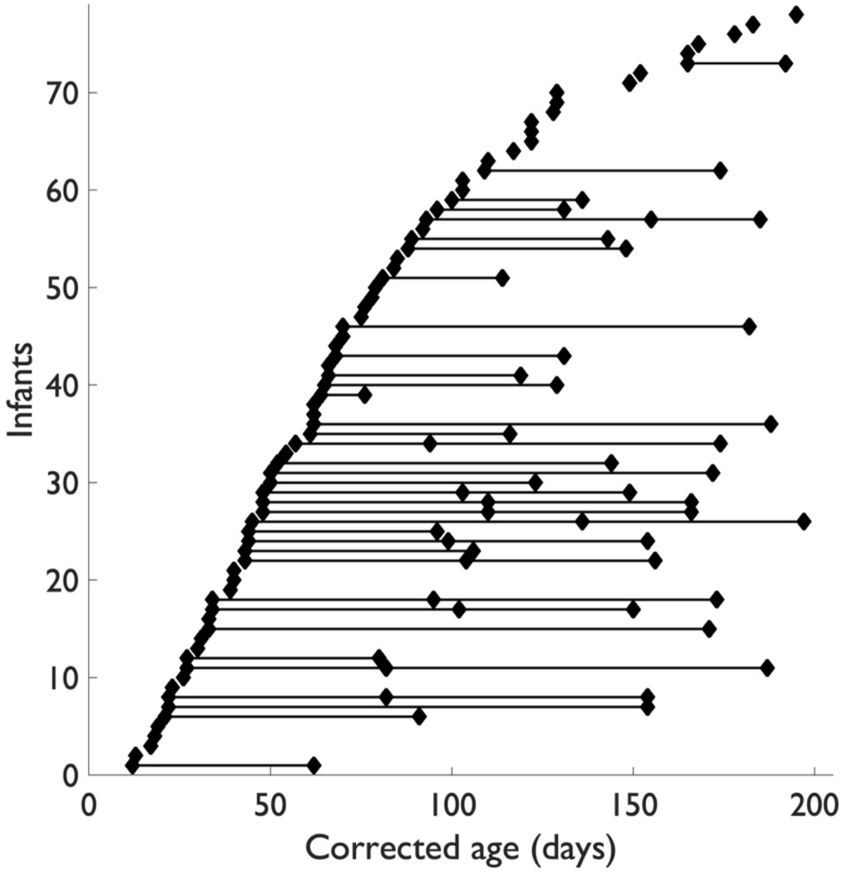

Figure 3.2: Included Scans for All Infant Subjects. Infants completed up to three scans between birth and 6 months, using a non-uniform, longitudinal sampling design.

MRI data acquisition: Infant scans were acquired at Emory University's Center for Systems Imaging Core on a 3T Siemens Tim Trio (n=26) or a 3T Siemens Prisma (n=52) scanner, using a 32-channel head coil. All infants were scanned during natural sleep, using the following procedure. First, infants were swaddled, rocked, and/or fed to encourage natural sleep. Once asleep, the infant was placed on a pediatric scanner bed. Scanner noise was reduced below 80 dBA by using: 1) sound attenuating pediatric headphones, equipped with MR-safe optical microphones to enable real-time monitoring of in-ear sound levels throughout the scan session; and 2) a custom-built acoustic hood, inserted into the MRI bore (Valente et al., 2014). To mask the onset of scanner noise, white noise—gradually increasing in volume—was played through the headphones prior to the first sequence. An MRI-compatible camera (MRC Systems) was mounted on the head coil to enable monitoring of the infant throughout the scan. A trained experimenter remained in the scanner room and the procedure was stopped if the infant awoke or if an increase in sound level was observed.

Diffusion MRI data from the Tim Trio scanner were acquired using a multiband sequence (Feinberg et al., 2010; Moeller et al., 2010) with the following parameters: repetition time (TR) of 6200 ms, echo time (TE) of 74 ms, a multiband factor of 2 combined with parallel imaging (GRAPPA) with an acceleration factor of 2, a field-of-view (FOV) of 184×184, image matrix of 92×92, b value of 0/700 s/mm^2, spatial resolution of 2 mm isotropic, and 61 diffusion directions, 56 slices covering the whole brain. An extra 6 averages of b0s were collected to improve the signal-to-noise ratio (SNR) of the baseline diffusion MRI signal. The total scan time for the diffusion MRI sequence was 7 minutes 26 seconds.

Diffusion MRI data from the Prisma scanner were acquired using a multiband sequence with the following parameters: repetition time (TR) of 2330 ms, echo time (TE) of 86.6 ms, a multiband factor of 4 without parallel imaging acceleration, a field-of-view (FOV) of 184×184, image matrix of 106×106, b value of 0/700 s/mm^2, spatial resolution of 1.75mm isotropic, and 89 diffusion directions, 68 slices covering the whole brain. An extra 6 averages of b0s were collected to improve the signal-to-noise ratio (SNR) of the baseline diffusion MRI signal. The total scan time for the diffusion MRI sequence was 3 minutes 58 seconds.

For diffusion imaging protocols on both scanners, b0 images were acquired in the opposite phase encoding direction (posterior-to-anterior) for removing susceptibility-related distortion in diffusion (Andersson et al., 2003).

Data preprocessing: Infant data were preprocessed using FSL (6.0.03) and in-house MATLAB code (R2016b, MathWorks Inc. 2016). Preprocessing steps included correcting inter-volume motion artifacts, removing susceptibility distortion using the "topup" function in FSL and eddy-current distortion and motion correction using FSL's "eddy" tool (Andersson & Sotiropoulos, 2016). Diffusion MRI parameters were estimated using weighted least squares estimators (Koay et al., 2006; Veraart et al., 2013).

Image Registration: Tensor-based registration was used to align infant brain images to a common space (Zhang et al., 2006, 2007). Unlike T1- and T2-weighted images that are isointense by approximately 6 months of age and contrast flip after 6 months (Paus et al., 2001), tensor maps maintain relatively consistent orientation information about white and gray matter microstructure, thereby allowing for more accurate and detailed mapping of corresponding features between individuals (Pecheva et al., 2017; Zhang et al., 2006, 2007). Infant brain images were aligned to a sample-specific template using multilevel registration (Guimond et al., 2000; Zhang et al., 2006). First, each infant's tensor map was aligned to the tensor map of a randomly chosen infant participant using 6-degree of freedom (df) rigid body transformations. Second, the aligned images from all participants were averaged to create the initial target template with 6-df. Third, individual tensor maps were aligned to the initial target template using 12-df affine transformations and then averaged to form the intermediate 12-df target template. Fourth, individual tensor maps were registered to the 12-df intermediate target template using diffeomorphic registration and then averaged to create the sample-specific diffusion tensor template (Guimond et al., 2000). After the sample-specific diffeomorphic template was built, individual tensor maps were aligned to the template space using rigid (i.e., 6-df), then affine (i.e., 12-df), and then diffeomorphic registration, as described above (Zhang et al., 2006) for group analysis. This approach would not bias the registration results due to the initial target template and avoid large deformations in any single step.

Fiber Tractography

Infant white matter pathways: Whole-brain tractography, seeded from the whole-brain white matter mask (mean fractional anisotropy, FA >0.15), was performed using FACT (Cook et al., 2006; Mori et al., 1999) in Camino (http://camino.cs.ucl.ac.uk/). After all possible streamlines representing white matter connectivity in the infant brain were constructed, white matter for the whole brain (WB) and nine major pathways was delineated using TrackVis (http://www.trackvis.org), using methods described in previous literature (see Supplementary Figure 3.1 for details). Tracts of interest included the arcuate fasciculus (AF), anterior thalamic radiation (ATR), cingulum (Ci), fornix (Fx), inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), uncinate fasciculus (UF), and corticofugal tracts originating in the pre- and post-central gyrus (M1 and S1). The WB mask was created by combining all tracts and white matter of the corpus callosum and binarizing them to form a single mask. The corpus callosum was not analyzed in this present work as commissural tracts cross between the hemispheres. Two-dimensional views of the tracts are shown in Figures 3.3 and 3.4 and three-dimensional views are shown in Supplementary Figure 3.2.

DTI-derived metrics for quantifying white matter development: Fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and trace diffusivity (Tr) were used to quantify tract-specific white matter development (Basser et al., 1994; Basser & Pierpaoli, 2011; Dubois et al., 2014). Mean FA, AD, RD, and Tr values were calculated within each tract-of-interest and averaged within hemispheres.

Harmonization of data collected on Siemens Trio and Prisma scanners using ComBat: As part of a standard processing pipeline for multiple analyses using this dataset, ComBat—a regression-based harmonization technique that models additive and multiplicative site/scanner effects in each voxel—was applied to harmonize infant data collected on Siemens Trio and Prisma 3T scanners (W. E. Johnson et al., 2007). An FA mask (FA>0.05) was applied to each infant's tensor map in template space and all voxels within the FA mask were input into the ComBat model. White matter tract masks were then multiplied with the FA mask to ensure that only harmonized voxels were included in regression analyses. Data harmonization was performed for each DTI metric separately using ComBat software (https://github.com/Jfortin1/ComBatHarmonization) in Matlab (R2016b, The MathWorks Inc. 2016).

Note however, that applying ComBat (and by extension, other harmonization techniques), does not impact lateralization values or trajectory shape because computing the lateralization index controls for scanner dependent differences in FA values. For example, say using one scanner consistently results in higher FA values. When the difference in FA values between the left and right hemisphere is calculated, the scanner-dependent magnitude difference is removed from the result.

Statistical Analyses

Lateralization index: The extent of white matter lateralization was calculated between the left and right hemispheres using: (left value – right value)/(left value + right value), from (Dubois et al., 2009). This index was separately calculated for each tract of interest across each DTI metric at each timepoint. Group trajectories of lateralization indices for each tract across each metric were constructed using Functional Principal Components Analysis (Yao et al., 2005).

Gestational age: Infant age at each scan was corrected for length of gestation to control for the effects of gestational age when mapping lateralization over time. For example, an infant scanned at 4 weeks after birth with a gestational age of 39 weeks had a corrected age of 3 weeks.

Functional Principal Components Analysis: Given the possibility that lateralization processes may be time-varying from 0-6 months, we implemented a nonparametric statistical approach that places few assumptions on the shape of the group trajectory.

Functional Principal Components Analysis (FPCA) is an extension of Functional Data Analysis designed to accommodate sparsely sampled longitudinal data (Yao et al., 2005). Using Principal Components Analysis by Conditional Expectation, a data-driven, nonparametric curve-fitting algorithm, FPCA constructs non-linear trajectories at the group and individual level. Mean growth curves of white matter lateralization were built for nine major white matter tracts and whole brain white matter using the PACE package, version 2.17, in MATLAB R2019b. Simultaneous 95% confidence bands for both the mean curves and rate of change curves were computed using bootstrapped data, resampled with replacement at the individual level over 1,000 iterations. This approach is robust to noise in individual data as it models variance within the sample across the entire trajectory. For this reason, simultaneous confidence bands are the gold standard for constructing confidence bands because, when compared to pointwise confidence intervals, which evaluate variability for each unit of time (in this case, days), they are more conservative and accurate. Areas of the trajectory where the confidence bands do not include 0 denoted time periods of significant lateralization; leftward where the value was positive and rightward where the value was negative. The rate of change of lateralization was determined by taking the first derivative of the group trajectory, and periods of significant lateralization velocity were again denoted by regions where bootstrapped simultaneous confidence intervals did not include 0.

Infant Sex: To assess the potential effect of infant sex on patterns of white matter lateralization we took two approaches. First, we split the sample into males and females and constructed sex-specific trajectories. Sex differences were measured as time periods when the confidence bands for male and female trajectories for each tract did not overlap.

Considering that halving our sample size, from categorizing infants by sex, can make confidence bands less reliable since coverage decreases across the experimental window, we used a second, complementary approach to test for sex effects. Variance in individual trajectory shapes relative to the group mean can be assessed using principal component (PC) functions and corresponding PC scores (Yao et al., 2005). Thus, we compared the PC scores of males and females for the components derived from the trajectory of each tract using two-tailed Student's T-tests.

Results

Across the four DTI metrics examined, lateralization of major white matter structures was most prominently observed in fractional anisotropy (FA). Given that FA is a second order metric derived from AD and RD we reported the FA results in detail below and include the AD, RD, and Tr results in Supplementary Figures 3.4-3.6.

The tracts were grouped into categories representing broad developmental trends: time-varying lateralization, constant lateralization, and no lateralization. Time-varying lateralization described tracts that begin with some lateralization status (right-biased, no bias, left-biased) that ultimately changed to a different status; constant lateralization described tracts that measured a significant left- or right-bias from 0-6 months; no lateralization described tracts that measured no significant bias from 0-6 months.

The AF, ATR, Fx, and UF all showed time-varying lateralization of FA values (Figure 3.3C). At birth, the AF showed no hemispheric bias that became a transient left-bias by 35 days (about 1 month). Leftward lateralization continued through 107 days (3.5 months) and then returned to no significant bias. The ATR also began unlateralized, and then showed a right-bias after 98 days (3 months). The UF showed a right-bias at birth that was no longer significant by 47 days (1.5 months). Finally, the Fx initially showed a right-bias that was gone by 34 days (1 month) and then became a left-bias after 158 days (5 months).

FA in the IFOF, ILF, M1, and S1 showed constant lateralization (Figure 3.3D). S1 and ILF had a right-bias from birth to 6 months, and M1 and IFOF had a left-bias from birth to 6 months. FA in whole brain white matter also showed constant rightward lateralization (Figure 3.3A). In the nine tracts examined in this study, only the Ci remained unlateralized from 0-6 months (Figure 3.3B).

No significant effect of infant sex was observed when 1) comparing lateralization trajectories between male and female infants (Supplemental Figure 3.3) and 2) testing for differences in principal component scores between male and female infants (Supplemental Table 3.1), indicating that male and female infants in our sample did not show differences in the lateralization of major white matter tracts from 0-6 months.

Next, change rates (i.e., the first derivatives) of lateralization trajectories for whole brain white matter and the tracts were derived to provide additional insight into the temporal dynamics of lateralization (Figure 3.4). For tracts with time-varying lateralization, we expected lateralization change rates to be nonzero, however computing the trajectory of the rate of change allowed us to identify exactly when in the first 6 months the lateralization of these tracts began to shift direction. For tracts with constant lateralization, the trajectory of the rate of change showed whether the extent of that lateralization, even when the direction remained constant, changed from 0-6 months, i.e., did a rightward lateralized tract become more rightward lateralized over time? Positive rates of change in lateralization indicated that the tract became more leftward lateralized from its baseline and negative rates of lateralization indicated that the tract became more rightward lateralized from its baseline.

The rate of change curve for FA in the ATR showed lateralization in the ATR significantly shifted towards the right from 79 to 119 days (2.5 to 4 months), directly preceding the period when the tract was significantly rightward lateralized (Figure 3.4C). The direction of FA lateralization rate in the UF was significantly rightward from 37 to 90 days (1.25 to 3 months) (Figure 3.4C). This shift towards rightward lateralization mapped on to the period when the UF transitioned from being leftward lateralized to unlateralized. Finally, lateralization in the Fx transitioned to the left from 56 days (about 2 months) (Figure 3.4C). FA in the Fx began rightward lateralized, so this change in lateralization rate resulted in significant changes in tract lateralization, from rightward lateralized, to unlateralized, to ultimately leftward lateralized by 6 months of age. Whole brain white matter showed a significant shift towards rightward lateralization from 40-80 days (1.3 to 2.6 months), leading to an overall greater rightward lateralization bias (Figure 3.4A). Finally, the rate of lateralization in tracts with constant lateralization – IFOF, ILF, M1, S1 – and no lateralization – Ci –, was not significantly different from 0 during the first 6 months of life (Figure 3.4D, 3.4B).

In summary, the lateralization of major cortical and subcortical white matter structures during the first 6 postnatal months, as measured through tract FA, unfolded in a tract-specific pattern and for four of nine tracts, the direction of lateralization changed over time.

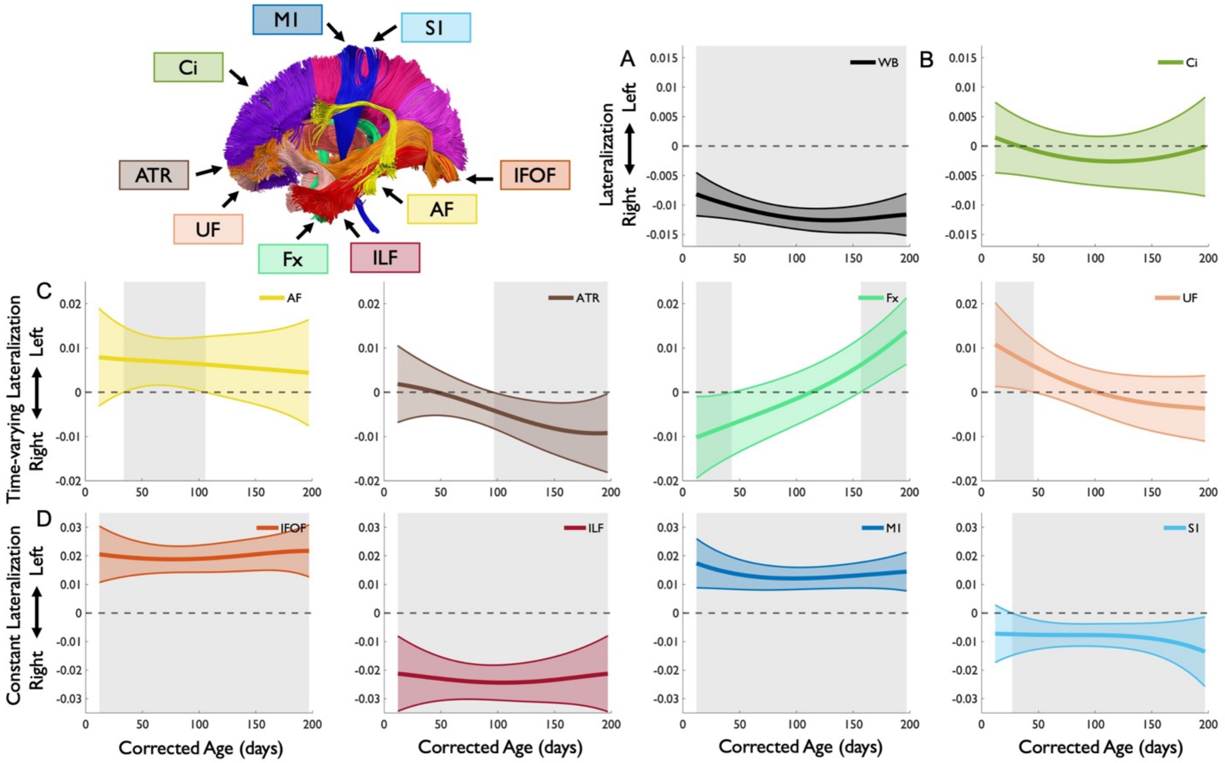

Figure 3.3: Patterns of Time-Varying, Constant, and No Lateralization in FA in Major White Matter Tracts. Lateralization in WB white matter (A) and the Ci, which shows no lateralization (B), are in the top row. Remaining tracts are grouped into those showing time-varying lateralization (C) – i.e., when the direction of significant lateralization changes from 0-6 months – and constant lateralization (D). Time periods where lateralization values significantly differ from zero are denoted by gray shading. Abbreviations: whole brain (WB), arcuate fasciculus (AF), anterior thalamic radiation (ATR), cingulum (Ci), fornix (Fx), inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), corticofugal tracts originating in motor (M1) and sensorimotor cortices (S1), and uncinate fasciculus (UF).

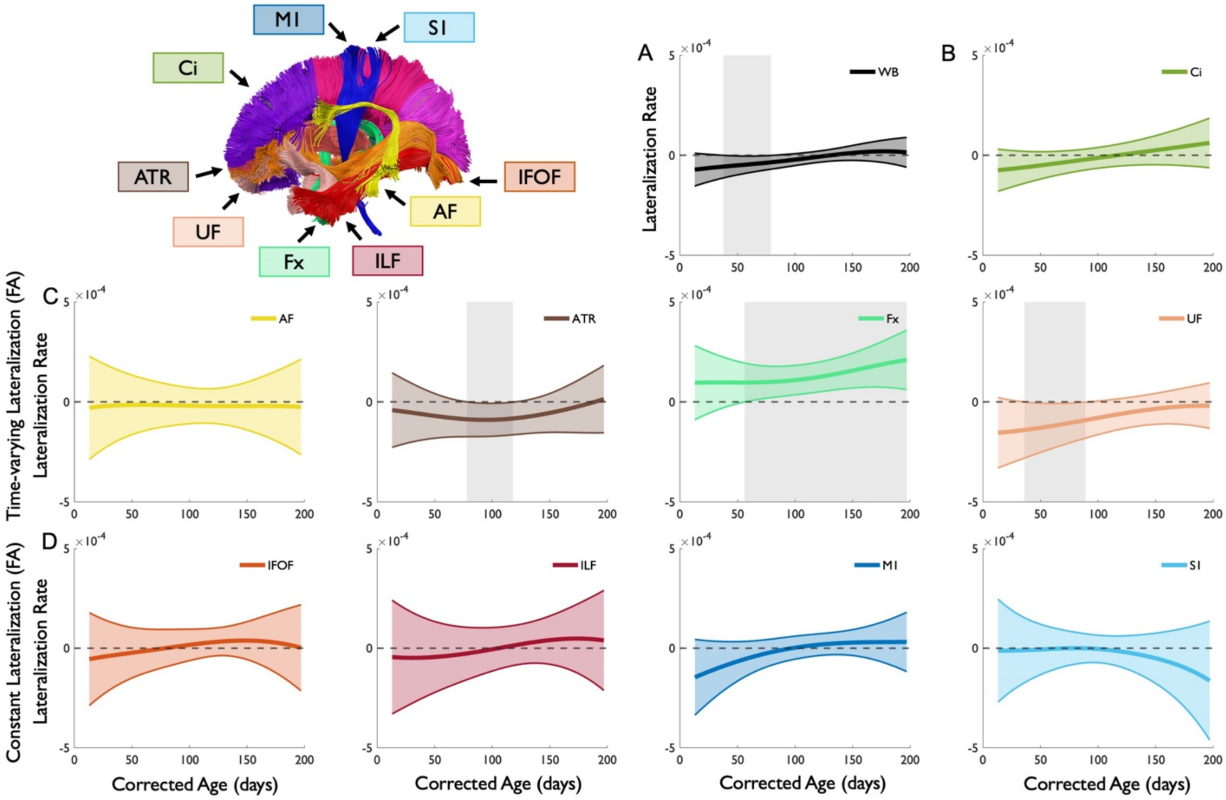

Figure 3.4: Tracts with Time-Varying Lateralization Show Significant Change in Rate of Lateralization in FA. Plot organization corresponds to the schema established from FA values in Figure 3.3: A) whole brain, B) no lateralization, C) time-varying lateralization, and D) constant lateralization. Time periods where lateralization rate significantly differs from zero are denoted by gray shading. Abbreviations: whole brain (WB), arcuate fasciculus (AF), anterior thalamic radiation (ATR), cingulum (Ci), fornix (Fx), inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), corticofugal tracts originating in motor (M1) and sensorimotor cortices (S1), and uncinate fasciculus (UF).

Discussion

This study is the first to use a longitudinal design to characterize brain lateralization during early infancy – the period of most rapid postnatal brain growth. We used a longitudinal dataset to map the trajectories of hemispheric asymmetries in white matter tract FA to answer the important question: Do lateralization patterns change during infant development? We found that major white matter structures, including whole brain white matter and cortical and subcortical tracts, are predominantly lateralized in the first six postnatal months, and that lateralization changes direction in a subset of tracts. Of the ten structures studied here, the AF, ATR, UF, and Fx showed time-varying lateralization, the M1, S1, ILF, IFOF, and whole brain white matter showed constant lateralization, and the Ci showed no lateralization.

As our results present the first trajectories of white matter lateralization in early infancy, we briefly highlight convergent findings with the small group of cross-sectional studies in this age range. Our results for the AF, IFOF, UF, and M1 subdivision of the corticofugal tracts are consistent with those of Dubois et al. (2009), Dubois et al. (2016), and Dean et al. (2017), from samples with respective average ages of 3.33, 4, and 1 months old (Dean et al., 2017; Dubois et al., 2009, 2016). To note, the lateral corticospinal tracts (CST) primarily originate in the M1 subdivision of the corticofugal tracts (Rea, 2015), so we compared our M1 results with published CST results. Our results differed for two tracts: the Ci reported in Dean et al. (2017) and ILF as reported in Dubois et al. (2016). We found no significant lateralization in the Ci, whereas Dean et al. (2017) reported rightward lateralization in their sample of one-month-old neonates. However, our results move towards a rightward lateralization during the age range matching their sample. This difference in results may be caused by our more conservative approach of using simultaneous confidence bands to estimate trajectory shape. We found constant rightward lateralization in the ILF, whereas Dubois et al. (2016) found no lateralization. This difference may stem from our larger sample size (78 versus 21) that would allow us to detect hemispheric asymmetries more readily. No other studies have examined lateralization in the ATR, Fx, or S1 subdivision of the corticofugal tracts from 0-6 months.

Confirmation from our trajectories that white matter lateralization can change direction, even within a month (Figure 3.3C), provides a compelling explanation for the variable results present in the developmental neuroimaging literature (summarized in Figure 3.1) – that differences in the ages of participants, even as small as a few months, may change lateralization results. As such, densely-sampled longitudinal studies of brain lateralization are necessary to link results between cross-sectional studies conducted with different age groups, particularly during early infancy because of the speed of neurodevelopment. For example, two studies highlighted in Figure 3.1 measured lateralization in FA of the UF. In a sample of infants around four months old, Dubois et al. (2016) found no lateralization in the UF, and then in a sample of older toddlers, Johnson et al. (2014) found FA in the UF to be rightward lateralized. Our longitudinal data provide support and additional insights into these cross-sectional differences, demonstrating that the UF is left lateralized at birth, unlateralized by four months of age (consistent with Dubois et al. 2016), and then begins to shift towards rightward lateralization after 6 months (consistent with Johnson et al., 2014). The use of longitudinal designs can also clarify results from cross-sectional studies that collapse across wider age ranges. A study of lateralization in FA of the fornix included participants from early infancy through later toddlerhood and reported no lateralization (Song et al., 2015). However, our data show that the fornix is lateralized before six months of age - rightward lateralized in the first 30 days, not lateralized from 30 to 160 days, and leftward lateralized after 160 days – a time-varying pattern of lateralization that may be masked if results from participants across this age range were analyzed together.

Given that FA is a measure of the degree of anisotropic water diffusivity in brain tissue and provides a proxy measure of white matter tract integrity (Coelho et al., 2021; Lynch et al., 2020; Wolff, et al., 2012; Young et al., 2020), the lateralization of FA in major white matter structures may indicate that neuroanatomical processes related to white matter maturation are occurring more efficiently in one hemisphere compared to the other. Specific processes tied to changes in FA are those known to change the extra-axonal space, most notably axon myelination and increases in axon density (Friedrich et al., 2020; Uddin et al., 2019). Haynes et al. (2005) used immunostaining to show that markers of axon growth and elongation, as well as adult-like myelination, are present after five months gestation and are highest in the first postnatal months (Haynes et al., 2005), reinforcing that neuroanatomical processes related to white matter lateralization are prominently shaping fetal and infant brain development.

For this reason, constant lateralization, versus time-varying lateralization, may reflect a maturational stage wherein the neuroanatomical infrastructure underlying increases in FA – myelination and axon growth – has established a consistent ratio across hemispheres that is minimally influenced by postnatal experience. We observed this hypothesized relationship between tract maturation and lateralization in our own data as the tracts with constant lateralization were those known to begin myelination first and support early-emerging behavioral and cognitive abilities. For example, the M1 and S1 subdivisions of the corticofugal tracts were significantly and consistently lateralized during the first six months of life. Projection fibers, which include the corticofugal tracts, are among the first tracts to begin myelination in utero (Buyanova & Arsalidou, 2021; Dubois et al., 2014). The corticofugal tracts specifically, which can be further divided into corticospinal and corticobulbar tracts, integrate distal and proximal signaling across the central nervous system, and mediate infant sensorimotor reflexes, such as the grasping reflex, present at birth (Sarnat, 1989, 2003).

We observed a similar pattern for limbic and association fibers. These tracts typically have a protracted development, with myelination peaking in early adulthood (Lebel et al., 2012). Of this type of tract, the ILF and IFOF are known to develop the fastest (Lebel et al., 2012) and our results showed that the ILF and IFOF demonstrated constant lateralization from 0-6 months. Given the role that these tracts play in early face processing and visual cognition, which are present at birth, it would make sense that they may already be showing significant levels of lateralization (Herbet et al., 2018). Moreover, the idea that more mature tracts exhibit more consistent lateralization aligns with a theory of cognitive development that posits that neural lateralization may increase ones' cognitive capacity as it would allow the brain to work efficiently through parallel processing in both hemispheres (Rogers, 2021). Thus, constant lateralization would again be expected in tracts connected to behaviors specialized early in development.

Growing evidence also indicates activity-dependent, specifically learning-dependent, changes in myelination are a key mechanism to promote brain plasticity – both in early development and later life (Fields, 2015). Thus, tracts with time-varying changes in lateralization could be reflective of activity- or experience-dependent changes in myelination, potentially as the result of experiential learning. This theory connects white matter lateralization as a potential mechanism for lateralized changes in behavior that occur during infancy. Studies in older children and adults have shown that periods of significant change in white matter tract development coincide with behavioral changes (Buyanova & Arsalidou, 2021; Lebel et al., 2012). The tracts in our study that showed significant changes in rates of lateralization (ATR, Fx, and UF) all changed at or around 50 days (6-8 weeks).

Interestingly, this age marks the beginning of a transformative period in infant behavior as infants begin to transition from exhibiting predominantly reflex-like movements – i.e., uncoordinated and spontaneous behaviors – to becoming more alert and able to use volitional movements (Shultz et al., 2018) . Infant smiling is an excellent example. From birth infants exhibit endogenous smiles, or spontaneous smiles occurring in the absence of external stimuli, typically during sleep (D. Messinger & Fogel, 2007). Then, by two-and-half-months, endogenous smiling declines and infants begin to voluntarily smile in response to the smiles and behavior of others, especially those of their caregivers (D. Messinger & Fogel, 2007). In fact, many of these well-documented behavioral transitions involve lateralized infant actions (Best & Queen, 1989; Melekian, 1981; Morange & Bloch, 1996). Future studies would be well positioned to directly investigate associations between development of lateralized behaviors and development of lateralization of white matter tracts.

In conclusion, we show that lateralization is more than a core feature of structural and functional neural organization; it is a core feature of brain development – changing by tract and with time. Therefore, the implementation of longitudinal methods, from study design to statistical analysis, offers novel insight into the developmental and functional significance of brain lateralization. With the temporal specificity offered by these methods, we can investigate the fine-grained timing of lateralization, which is key during periods of rapid and large-scale brain change (like early infancy). Moreover, we can begin to examine processes of neural and behavioral specialization – which has valuable implications for broadly characterizing brain development and identifying when divergences may arise in neurodevelopmental disorders.

Limitations: This study is limited by methodological considerations and the demographics of our recruited sample. First, the tracts included in our study are large projection and association fibers and so we acknowledge that lateralization patterns may be more variable were FA to be measured at multiple segments along each tract (Dubois et al., 2009; Goodlett et al., 2009; R. T.

Johnson et al., 2014; Lynch et al., 2020). However, as our goal was to build on the existing literature and compare results from a longitudinal approach with results from past cross-sectional approaches, we opted to keep the tracts consistent with those in the literature. Future work aiming to provide more detailed or fine-grained trajectories of time-varying lateralization should consider dividing tracts into smaller subsections and map changes overtime.

Additional methodological limitations include 1) DTI metrics (FA and other diffusivity measures), can be less robust and reliable in curved structures with crossing fibers, like the AF and Fx (D. K. Jones et al., 2013; D. K. Jones & Cercignani, 2010) and 2) our study also does not include additional measures of white matter microstructure from Neurite Orientation Dispersion and Density Imaging (NODDI (Zhang et al., 2012)) or Myelin Water Imaging (MWI (Alonso-Ortiz et al., 2015)), which can map more specific features of white matter microstructure by disentangling the different microstructural contributions to DTI measures during development (Dean et al., 2017; Lynch et al., 2020). Finally, our sample of infant participants is predominantly White and from high socio-economic status backgrounds and as such, is not representative of the racial and socioeconomic demographics of the city of Atlanta, Georgia, USA (where this study was based).

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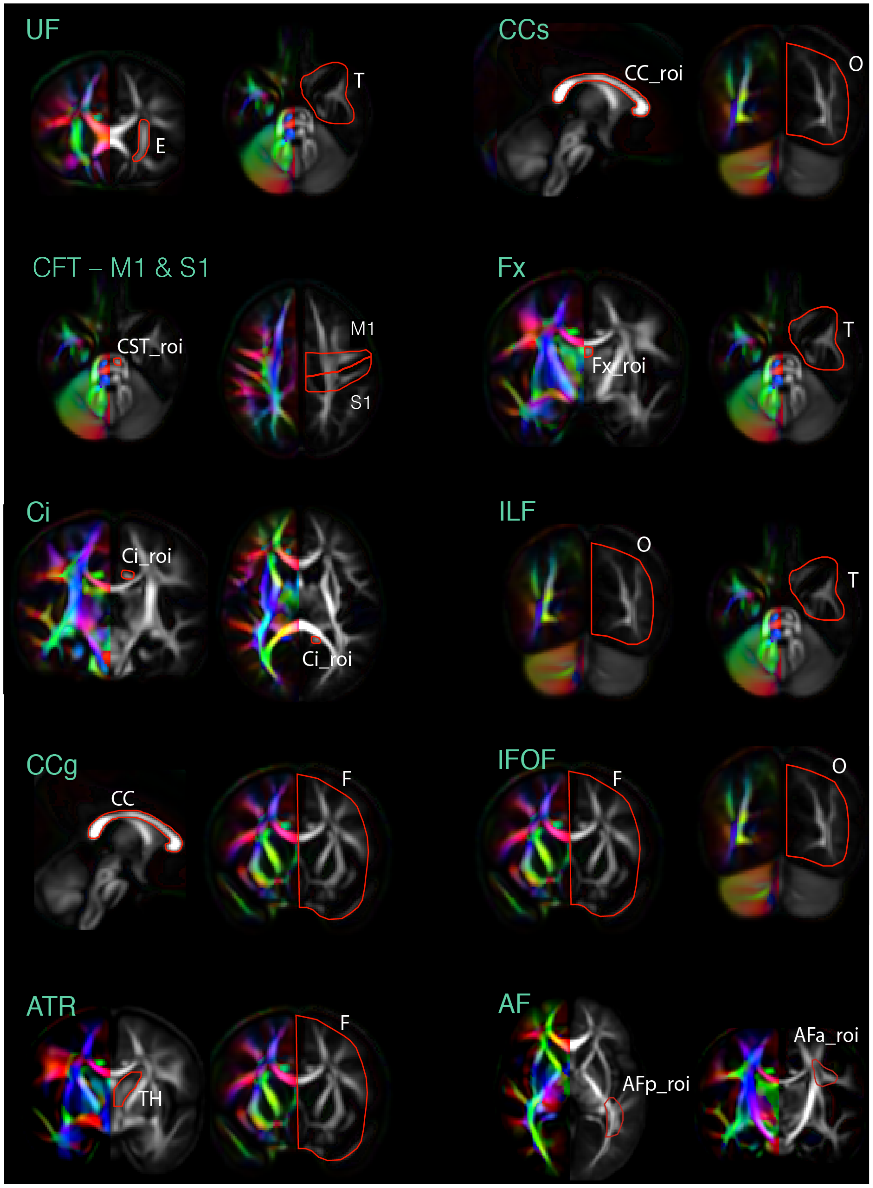
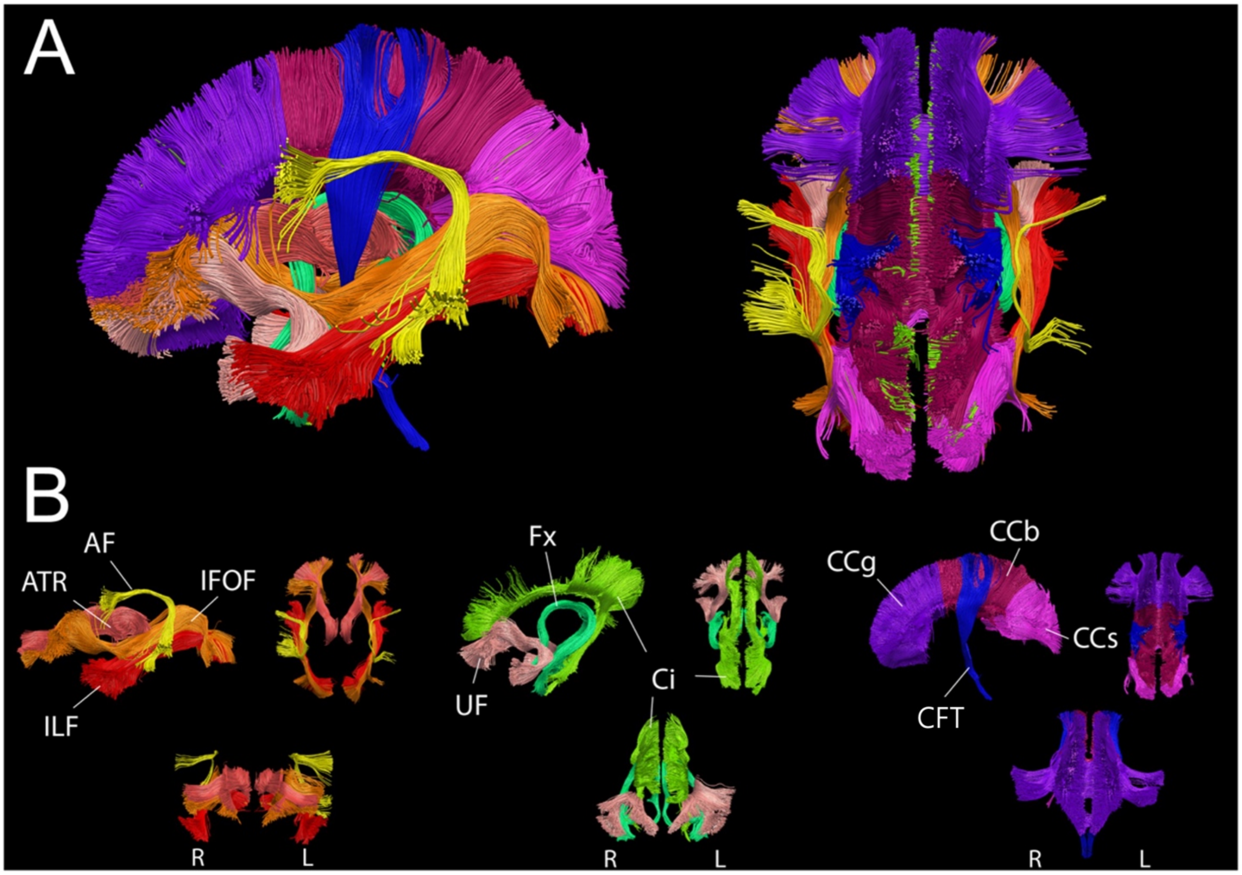
Supplemental Materials

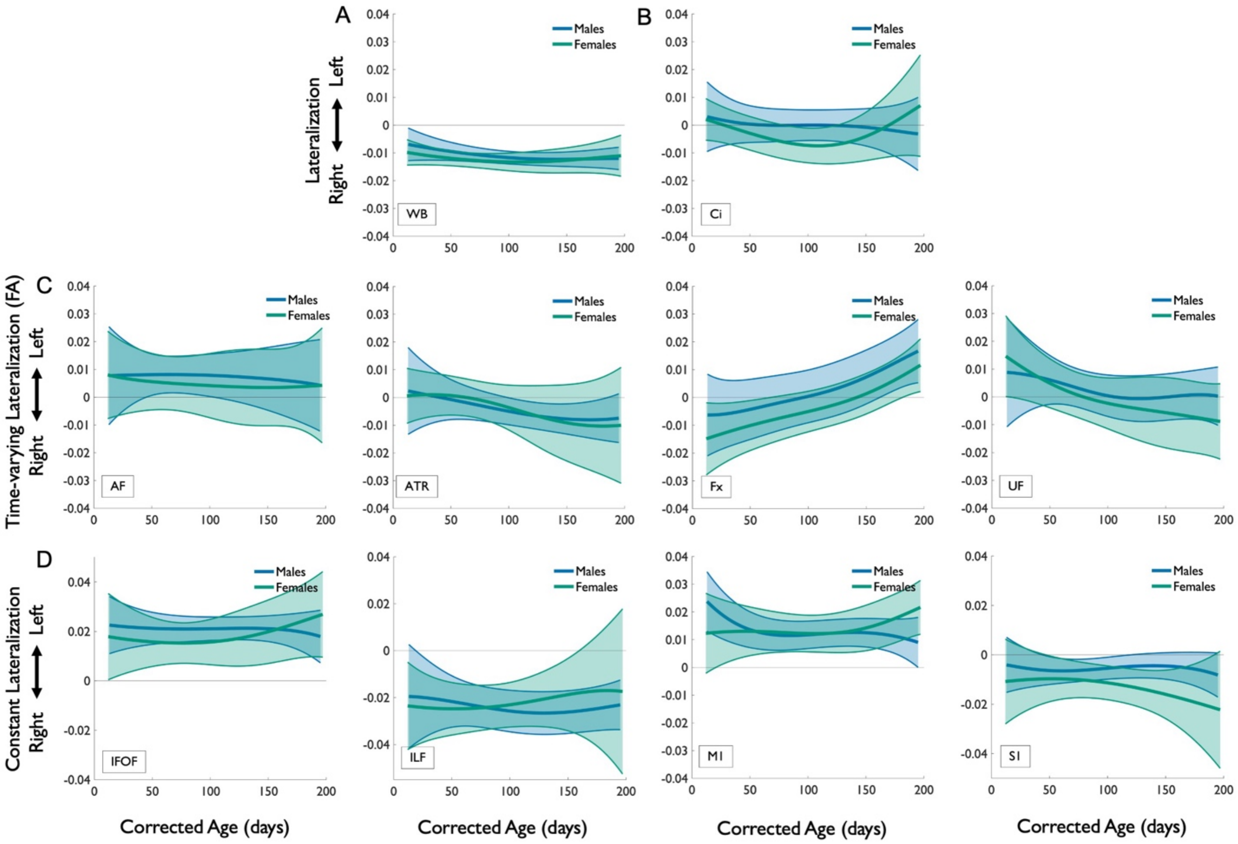


Supplementary Figure 3.1: Defining Regions of Interest (ROIs) for Delineating Major White Matter Pathways in Infant Brains. The method for delineating these tracts largely follow those outlined by Catani et al. (2008) (Catani & Thiebaut de Schotten, 2008) and others (Dubois et al., 2006; Y. Liu et al., 2010; Mori et al., 2002; Perani et al., 2011; Wolff et al., 2012). Names of delineated white-matter tracts are annotated in green. The ROIs used to delineate these tracts are annotated in white. For delineated tracts, AF: arcuate fasciculus; ATR: anterior thalamic radiation; CCg: genu of corpus callosum; CCs: splenium of corpus callosum; CFT: corticofugal tracts; Ci:

cingulum; Fx: fornix; IFOF: inferior fronto-occipital fasciculus; ILF: inferior longitudinal fasciculus; UF: uncinate fasiculus. For the ROIs used to delineate tracts, E: external capsule ROI; T: temporal ROI; CC_roi: corpus callosum ROI; O: occipital ROI; CST_roi: corticospinal tract ROI; M1: pre-central gyrus ROI; S1: post-central gyrus ROI; Fx_roi: fornix ROI; Ci_roi: cingulum ROI; F: frontal ROI; TH: thalamus ROI; AFp_roi: posterior arcuate faciculus ROI; AFa_roi: anterior arcuate fasciculus ROI.



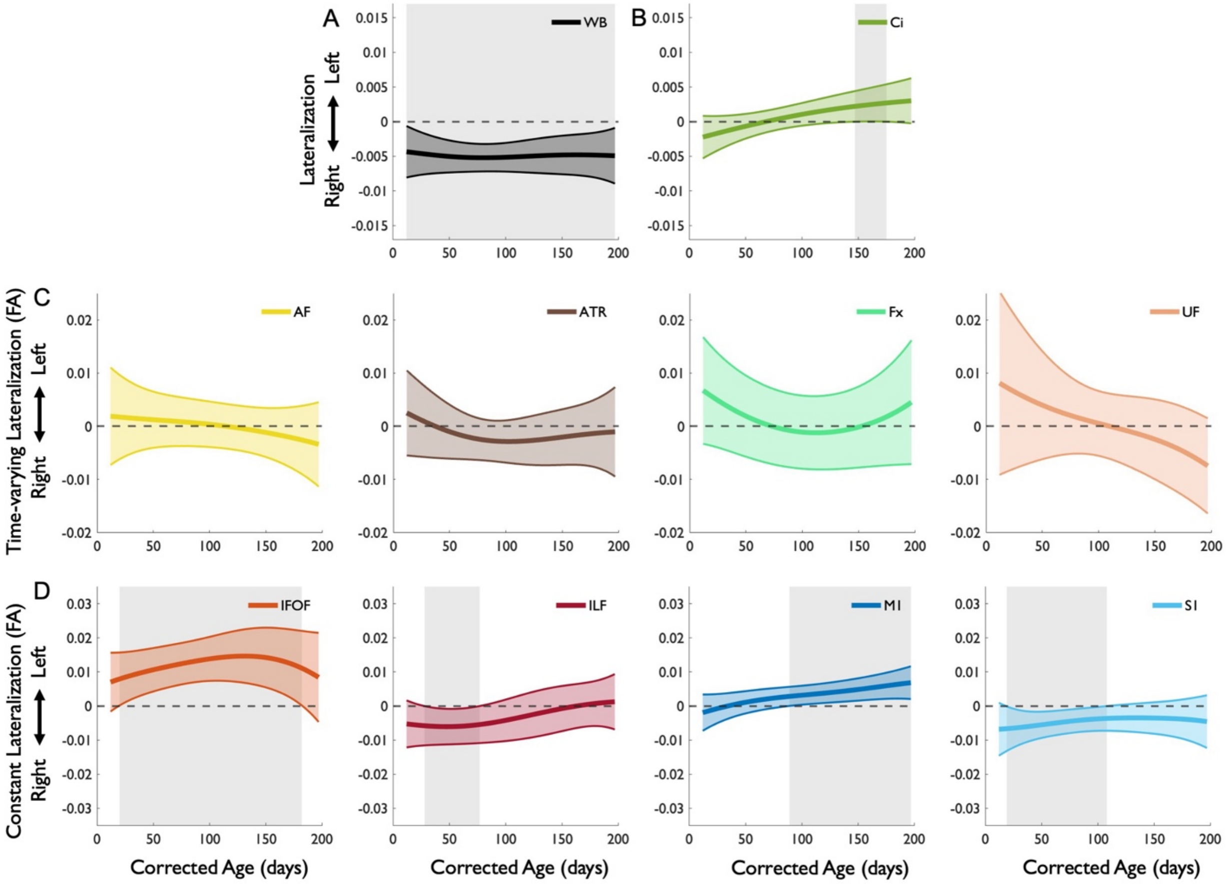
Supplementary Figure 3.2: Delineation of 9 Major White Matter Tracts. AF: arcuate fasciculus; **ATR**: anterior thalamic radiation; **CFT**: corticofugal tracts; **Ci**: cingulum; **Fx**: fornix; **IFOF**: inferior fronto-occipital fasciculus; **ILF**: inferior longitudinal fasciculus; **UF**: uncinate fasciculus. Subregions of the corpus callosum were delineated for analyses not included in this study (**CCb**: body of corpus callosum; **CCg**: genu of corpus callosum; **CCs**: splenium of corpus callosum).



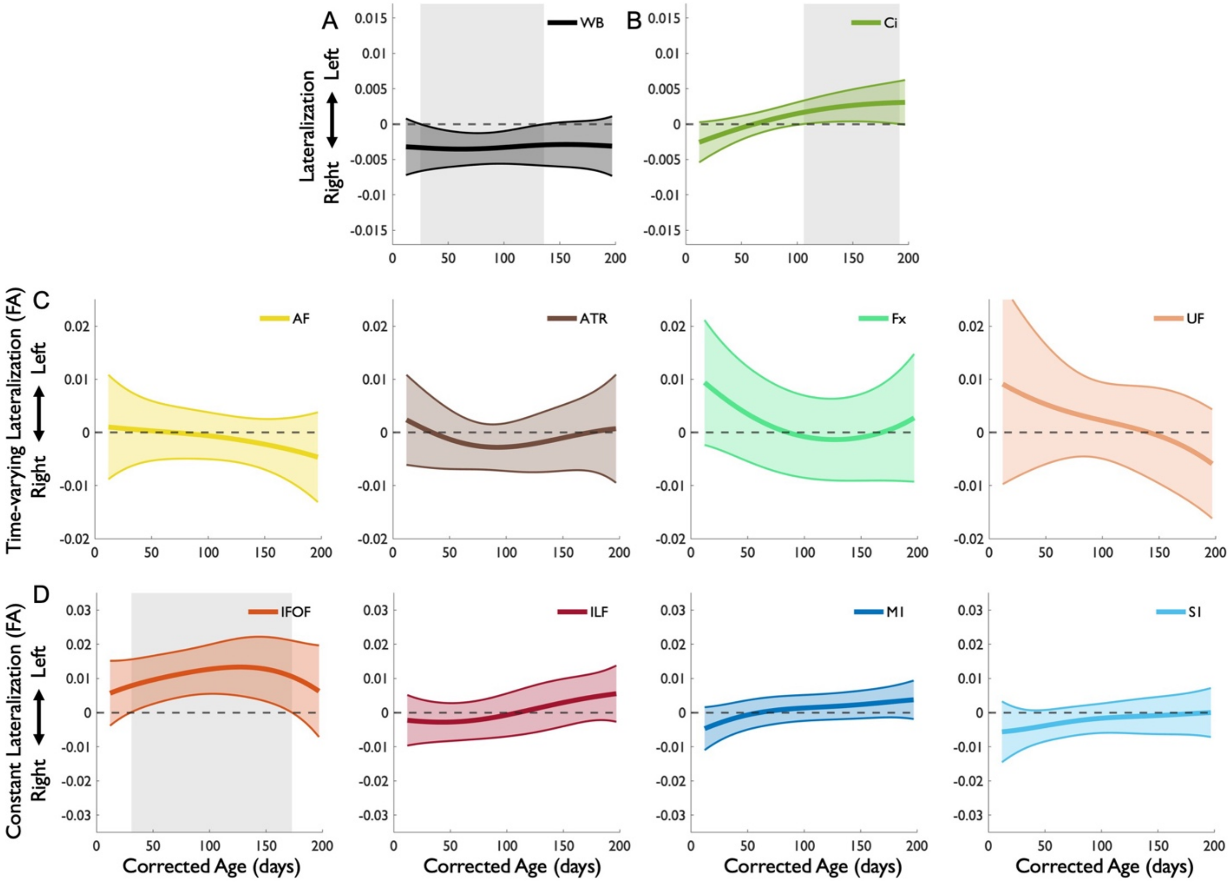
Supplementary Figure 3.3: Trajectories of FA Lateralization in Major White Matter Structures by Infant Sex. There were no periods of time where the trajectories for male and female infants did not overlap. Colored shading around the main curve indicates 95% simultaneous confidence bands. Plot organization corresponds to the schema established from FA values in Figure 3.3: A) whole brain, B) no lateralization, C) time-varying lateralization, and D) constant lateralization. Abbreviations: whole brain (WB), arcuate fasciculus (AF), anterior thalamic radiation (ATR), cingulum (Ci), fornix (Fx), inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), corticofugal tracts originating in motor (M1) and sensorimotor cortices (S1), and uncinate fasciculus (UF).

	PC1 Scores		**PC2 Scores**	
	t-value	***p-value***	***t-value***	***p-value***
WB	-1.23	0.22	-0.66	0.51
AF	-1.13	0.26	-0.67	0.51
ATR	0.07	0.94	-0.9	0.37
Ci	-1.66	0.1	-1.88	0.06
Fx	-1.6	0.11	0.27	0.79
IFOF	-1.42	0.16	-0.91	0.37
ILF	1.01	0.32	0.64	0.52
M1	-0.13	0.89	1.55	0.12
S1	1.96	0.05	-2.19	0.03
UF	-1.02	0.31	-0.77	0.44

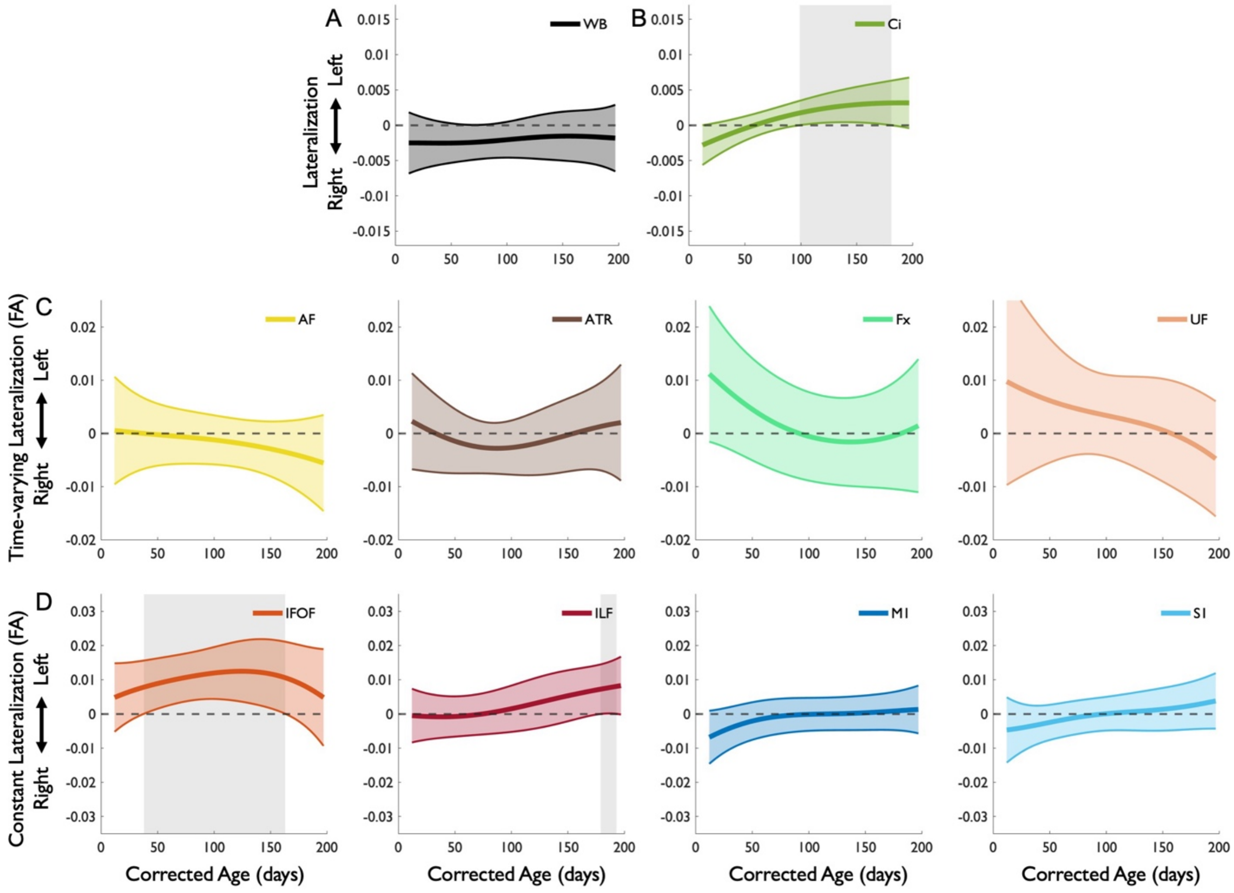
Supplementary Table 3.1: Difference in PC Scores for PC1 and PC2 Between Male and Female Infants. Difference in PC scores tested using independent two-tailed Student's t-test. For all reported tests, the df value was 76. The adjusted p-value for significance, to correct for multiple comparisons using Bonferroni correction, was 0.005.



Supplementary Figure 3.4: Lateralization of Axial Diffusion Measures in Tracts of Interest. Plot organization corresponds to the schema established from FA values in Figure 3.3: A) whole brain, B) no lateralization, C) time-varying lateralization, and D) constant lateralization. Time periods where lateralization values significantly differ from zero are denoted by gray shading. Abbreviations: whole brain (WB), arcuate fasciculus (AF), anterior thalamic radiation (ATR), cingulum (Ci), fornix (Fx), inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), corticofugal tracts originating in motor (M1) and sensorimotor cortices (S1), and uncinate fasciculus (UF).



Supplemental Figure 3.5: Lateralization of Radial Diffusion Measures in Tracts of Interest. Plot organization corresponds to the schema established from FA values in Figure 3.3: A) whole brain, B) no lateralization, C) time-varying lateralization, and D) constant lateralization. Time periods where lateralization values significantly differ from zero are denoted by gray shading. Abbreviations: whole brain (WB), arcuate fasciculus (AF), anterior thalamic radiation (ATR), cingulum (Ci), fornix (Fx), inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), corticofugal tracts originating in motor (M1) and sensorimotor cortices (S1), and uncinate fasciculus (UF).



Supplemental Figure 3.6: Lateralization of Trace Diffusion Measures in Tracts of Interest. Plot organization corresponds to the schema established from FA values in Figure 3.3: A) whole brain, B) no lateralization, C) time-varying lateralization, and D) constant lateralization. Time periods where lateralization values significantly differ from zero are denoted by gray shading. Abbreviations: whole brain (WB), arcuate fasciculus (AF), anterior thalamic radiation (ATR), cingulum (Ci), fornix (Fx), inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF), corticofugal tracts originating in motor (M1) and sensorimotor cortices (S1), and uncinate fasciculus (UF).

DISCUSSION

Summary of Research Findings

Early infancy, particularly the first 6 months of life, is a period of rapid change in brain and behavioral development (Dubois et al., 2014; Knickmeyer et al., 2008; Nagy, 2011; Shultz et al., 2018). Drastic brain growth is accompanied by a shift in infant behaviors from reflexive predispositions to more contingent and volitional actions (Gentry & Aldrich, 1948; Johnson, 1990, 2005; Johnson et al., 1991; Morton & Johnson, 1991; Shultz et al., 2018). The literature covering this age range is lacking in longitudinal studies exploring the changes occurring during this period of rapid developmental change. This gap left us asking the following questions: How does infant experience shape brain development and vice-versa? When and how are these processes disrupted in AUT infants? This thesis aimed to answer these questions and in doing so provide the field with a deeper understanding of these influential early months of life. Here, I will summarize the findings of each study and then discuss their implications and future directions.

Study 1

Study 1 focuses on early infant-caregiver interactions, specifically examining whether eyeblink entrainment is present during dyadic interactions between caregivers and 3- to 6-month-old NT infants. Entrainment is a sign of mutual adaptation and engagement between infants and their caregivers that could be an important mechanism of learning (Rose & Gravel, 2011). To conduct this analysis, I first developed and validated a semi-automated method for identifying blinks in both infants and caregivers. This method allowed me to accurately identify eyeblinks in half the time it would have taken using frame-by-frame coding methods. I found that infants and their caregivers do entrain their blinks to one another, with infants blinking between 540-840 ms before their caregivers. This finding was consistent with the infant leading hypothesis of dyadic interactions observed in other studies of this age range (Cohn & Tronick, 1987; Feldman et al., 1996, 1999; Lester et al., 1985; Northrup & Iverson, 2020). Relative to other measures of entrainment during infant-caregiver interaction (such as mutual eye gaze and facial expression) that change over development, blink entrainment has the advantage of being a measure of infant-caregiver dyadic interactions that can be easily captured at any age, facilitating future longitudinal studies of mutual engagement.

Study 2

In Study 2, I investigated the development of reflexive and volitional grasping during the first 6 months of life in NT and AUT infants. Despite previous reports of motor deficits in autism, few studies have examined when motor differences first emerge, and even fewer have examined whether early differences in motor reflexes are present (Bhat et al., 2012; Flanagan et al., 2012; Harris, 2017; Iverson et al., 2019). To address these gaps, I measured reflexive and volitional grasping prospectively and longitudinally in 20 AUT infants and 125 NT infants during the first 6 months of life. Reflexive grasping was evaluated using the NICU Neurobehavioral Network Scales (Lester & Tronick, 2004), whereas volitional grasping was examined using the fine motor subscale, specifically the block series, of the Bayley Scales of Infant and Toddler Development (Bayley, 2012). I found that there were significant group differences in the developmental trajectories of volitional grasping with AUT infants demonstrating later emergence of grasping and lower grasping scores. The trajectories between the NT and AUT infants significantly diverged at 163 days and persisted through the latest time point of testing (6 months). No statistically significant differences in reflexive grasping behavior were observed between the AUT and NT infants. However, this lack of difference may be due to the limited variability within the sample as all but 1 AUT infant continued to display the reflex at 6 months. These findings provide one of the earliest demonstrations of motor differences in autism and have implications for the development of early intervention.

Study 3

In Study 3 I used a densely sampled, longitudinal design to provide the first direct evidence that white matter lateralization can change direction, even within the short span of 3 months. From a sample of 78 NT infants, each with up to three scans from 0-6 months of age, I constructed trajectories of lateralization in nine major cortical and subcortical white matter tracts and whole brain white matter. In four of these nine tracts (AF, ATR, UF, and Fx), I found that lateralization was time-varying and changed direction in patterns specific to each tract. In four others (ILF, IFOF, M1 and S1), and whole brain white matter, lateralization remained constant, and only one tract (Ci) was not lateralized at all. These findings fill a gap in the literature as the few studies that have investigated the lateralization of major white matter tracts during early infancy, the period of greatest postnatal brain growth, and by extension vulnerability for neurodevelopmental divergence, present variable results. Many in the field have attributed these inconsistencies to the absence of longitudinal work. Overall, our findings of time-varying lateralization link published, but previously discrepant, results. Our findings suggest that white matter lateralization could be a potential mechanism for lateralized changes in behavior that occur during infancy via activity dependent myelination (Fields, 2015, Nature Neuroscience) and future studies should aim to directly test for these associations.

Implications & Future Directions

Mechanisms of Developmental Change

Two non-mutually exclusive drivers of developmental change are studied in this thesis: mutual engagement (as measured by eyeblink entrainment), a key ingredient for learning during infant-caregiver interactions (Study 1) and the fact that infants create opportunities for learning through their own initially reflexive and spontaneous actions on the world (Study 2). Future work should longitudinally explore developmental change within the context of infant-caregiver interactions and how these interactions are directly associated with learning across all domains in the first 6 months of life.

Mapping Longitudinal Change of Blink Entrainment

Study 1 provides evidence for the presence of blink entrainment, and index of mutual engagement, between 3- to 6-month-old NT infants and their caregivers. Given that mutual engagement during dyadic interactions is a key ingredient for learning, it is important to know when and how blink entrainment within a dyad emerges (Rose & Gravel, 2011). Future studies should measure eyeblink entrainment from birth to determine when it arises within dyadic interactions. I hypothesize that entrainment would emerge around 2 months of age when infants are more active and intentional participants in contingent reciprocal social interactions (Cohn & Tronick, 1987; Feldman et al., 1996, 1999; Lavelli & Fogel, 2002; Northrup & Iverson, 2020; P. R. Rochat, 2001; Zlochower & Cohn, 1996). Findings from this longitudinal work would provide an important benchmark against which to identify possible disruptions to mutual engagement in AUT infants. Although deficits in reciprocal social interaction are defining feature of the condition (American Psychiatric Association & American Psychiatric Association, 2013), when these differences first emerge – and their impact on subsequent social learning—is unknown. Future work could explore when these differences emerge by comparing trajectories of eyeblink entrainment in AUT and NT infants. As described by Shultz et al (2018) disruptions in these early interactions may contribute to many of the characteristics observed in autism making it critical to understand when they arise (Shultz et al., 2018).

Studying Associations Between Dyadic Entrainment and Learning

Mutually-adaptive infant-caregiver interactions are important for driving early learning. Research has suggested that caregivers play a critical role in shaping infants' behaviors by providing feedback that supports and reinforces the development of increasingly sophisticated infant skills and abilities (Iverson, 2021; P. R. Rochat, 2001; Shultz et al., 2018). Furthermore, infants who are provided with supplemental opportunities to experience the effects of their actions on the environment may be more likely to develop skills earlier than those who are not, showing how critical feedback is for infant learning (Needham et al., 2002; Rovee & Rovee, 1969).

Results from Study 2 showed that infant reflexive grasping begins to show signs of declining by around 6 months, with volitional reaching and grasping emerging by approximately 4 months. However, the precise developmental mechanisms facilitating this shift from reflexive to volitional behavior, are unknown. One possible candidate, that no one has yet explored within the context of motor development, is how dyadic interactions with a caregiver could scaffold and shape the development of motor skills. For instance, caregiver responses to initially reflexive infant grasping may serve to reinforce the behavior (e.g. smiling or leaning in towards their infant when she grasps the caregiver's finger) or give meaning to the infants' initially spontaneous behaviors ('oh you grabbed the rattle, do you want it?'). Future work could involve detailed observations of caregiver and environmental responses to reflexive behaviors and how those might scaffold the transition from reflexive to volitional behaviors. Uncovering this possible relationship between dyadic interactions and infant motor development would result in showing what kinds of responses from one's caregiver help to shape reaching behavior. This is particularly important as motor behavior is typically thought to shape social development (Bhat et al., 2012; Bradshaw et al., 2018; Iverson, 2021), but results from this study could show that social interactions with one's caregivers can also shape and scaffold motor development .

If this hypothesis—that caregiver reactions to initially reflexive actions scaffold motor development—were confirmed, it could have important implications for understanding motor deficits in autism. Motor delays in autism are commonly reported, yet still remain excluded from official diagnostic criteria of the condition despite the fact that it is one of the first observations by parents (Bhat et al., 2012; Bolton et al., 2012; Bradshaw et al., 2018; Flanagan et al., 2012; Gernsbacher et al., 2008; Harris, 2017; Iverson et al., 2019; Kohen-Raz et al., 1992; Libertus et al., 2014). Sensorimotor deficits are often interpreted as exacerbating or even causing social deficits (Bhat et al., 2012; Bradshaw et al., 2018, 2022; Iverson, 2021), but in fact they could very well be fueled by disruptions in social development. If this is the case, then what is specifically disrupted in autism? Are AUT infants not sensitive to the contingencies between their actions and their caregiver's responses or feedback from their environment? If this is confirmed, these findings would turn on its head the theory that autism is caused by basic sensory motor disruptions that lead to social differences (Hannant et al., 2016; Iverson, 2021; Whyatt & Craig, 2013) and is first and foremost a condition derived from atypical social processing from birth.

Early Brain and Behavior Development

The brain changes quickly in the first 6 months as demonstrated by findings described in Study 3 (Dubois et al., 2014; Gao et al., 2015; Gilmore et al., 2018; Knickmeyer et al., 2008; Kolb & Fantie, 2009). These rapid changes in the brain support equally drastic changes in behaviors during this same period of development (Kolb & Fantie, 2009; Tierney & Nelson, 2009). In turn, infant experiences, such as interactions with their caregivers, shape the developing brain (Atzil et al., 2018; Hanford et al., 2018; Ilyka et al., 2021). Although each chapter in this thesis focuses solely on brain or behavior development, the next step should be to examine reciprocal interplay between brain and behavior development.

Neural correlates of entrainment and the effects of entrainment on brain development

Findings from this thesis have led me to ask several questions: How does the state of being entrained change brain activation? How does the level of entrainment/contingency in infant-caregiver interaction shape brain development? Is entrainment a key ingredient in driving specialization of the brain? Studies have shown that early dyadic interactions influence infant brain development both concurrently and in the long term (Atzil et al., 2018; Hanford et al., 2018; Ilyka et al., 2021). However, these studies are cross-sectional with even fewer studying infants younger than 6 months of age.

To begin to tackle these questions I would propose two avenues of study. The first would begin to uncover the neural correlates of entrainment and determine if entrainment does facilitate learning. One could use EEG and functional near-infrared spectroscopy (fNIRS), a functional neuroimaging method, to determine how patterns of brain activations, and in what areas of the brain, are associated with moments of engagement or entrainment within a dyad which could be indexed using the measure of eyeblink entrainment developed in Study 1. The second would be to determine if contingency and entrainment is necessary for typical brain specialization. To begin answering this question I propose a longitudinal study to map out how levels of entrainment longitudinally predict optimal brain outcomes, for example, typical specialization of social brain networks. Findings from this study would provide an understanding of the extent to which typical trajectories of entrainment relates to typical brain specialization. These studies would be of particular interest in the field of autism research as reduced engagement with the social world Is thought to be a defining feature of autism (Chawarska et al., 2013; W. Jones & Klin, 2013; Shultz et al., 2011). If there are disruptions in the neural mechanisms associated with behavioral entrainment, then this would be valuable information for understanding a key neurobiological underpinning of autism.

Neural correlates underlying a shift from reflexive to volitional grasping

Infants undergo a period of rapid behavioral transition within the first few months of life (W. Jones & Klin, 2013; Nagy, 2011; Shultz et al., 2018). As previously discussed, newborns are equipped with spontaneous, reflex-like behaviors that facilitate early learning during this period (Needham et al., 2002; Rovee & Rovee, 1969; Sargent et al., 2014). Around 2 months of age, infants begin to display more active behaviors such as spending more time awake, responding contingently to their social partners, or actively exploring the world around them (Lozoff et al., 1977; Nagy, 2011; P. R. Rochat, 2001; Sreenberg & Morris, 1974). It is hypothesized that this shift from reflexive to volitional behaviors is accompanied by a shift from subcortical to cortical control (Chinello et al., 2018; Futagi & Suzuki, 2010; Healy et al., 2022; M. H. Johnson, 1990; Torres et al., 2013; Zafeiriou, 2004). Specifically, as the cortex matures it will inhibit signals from the spinal cord and brain stem resulting in the disappearance or suppression of the reflexive behaviors (Melillo et al., 2022; Thelen et al., 1984). Although this is a longstanding and influential hypothesis, no prospective longitudinal infant studies to date have directly examined associations between the transition from reflexive to volitional behavior and transitions from subcortical to cortical control in the brain.

The findings from Study 2 could provide an avenue for exploring these associations. I show that grasping behavior begins to transition from reflexive to volitional during the first 6 months of life. Future work can study infants beginning from birth but extending their visits through the first year as our results show that this transition may not be completed until the second half of the first year. In addition to these behavioral measures, neuroimaging data should be collected for each infant prospectively and longitudinally during the same timeframe. With trajectories of both brain and behavior development available, statistical methods such as functional linear regression (Dai et al., 2019) could be used to identify associations between trajectories. Future studies such as these will illuminate the neural mechanisms underlying critical changes in behavior. Furthermore, this work would provide an important benchmark for identifying atypical developmental trajectories in autism as findings from Jones and Klin (2013) suggest that there may be disruptions in the transition from subcortically mediated reflexive behavior to cortically mediated volitional behaviors.

Conclusion

In conclusion, the findings from this thesis shed light on the critical period of early infancy, particularly the first 6 months of life, and its impact on brain and behavioral development. The studies conducted in this thesis provide evidence for the presence of mutual engagement, as measured by eyeblink entrainment, between infants and caregivers, highlight the time-varying nature of white matter lateralization, and demonstrate early motor differences between NT and AUT infants. Future directions should focus on uncovering associations between the development of infant brain and behavior, exploring the role of dyadic interactions in guiding infant learning, and exploring early differences between NT and AUT infants. Overall, these findings contribute to our understanding of early infancy and establish normative benchmarks that can help to identify deviations early in development in autism which is critical for developing new interventions to support development before overt signs of autism emerge. insights for future research in the field of infant brain and behavioral development.

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