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Signature:

Arick Wang Date

Development of social-visual engagement in infant rhesus monkeys (*Macaca mulatta*)

By

Arick Wang
Doctor of Philosophy
Department of Psychology

Jocelyne Bachevalier, Ph.D.
Advisor

Harold Gouzoules, Ph.D.
Committee Member

Warren R. Jones, Ph.D.
Committee Member

Longchuan Li, Ph.D.
Committee Member

Elaine F. Walker, Ph.D.
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

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By

Arick Wang
M.A. – Emory University, 2015
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Abstract

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By Arick Wang

Autism Spectrum Disorder (ASD) consists of a range of pervasive developmental disorders, including impairments of social interaction and communication (DSM-V). Despite high degree of heterogeneity in ASD symptoms, reliable deviations in social-visual engagement occur in infancy. Yet, the neural mechanisms supporting these early developing social skills (and the lack thereof) remain unclear. Given technical and ethical limitations in performing longitudinal neuroimaging studies in human infants, a nonhuman primate (NHP) model was used to delineate the neural changes linked to the emergence of these social skills. The first study traced social-visual engagement skills in infant rhesus macaques from 2 to 24 weeks (≈ 2 to 24 months in human infants), using methodologies paralleling human infant studies. The changes in interest to facial cues in infant macaques closely parallel those reported in human infants, with a few species-specific differences due to differing ethological constraints. The second study followed the development of gaze following, a more elaborate form of social-visual engagement in the same animals. Gaze following skills emerged later in monkeys than in humans, though there were important changes in mutual eye gaze and saccade velocities, two critical behaviors associated with gaze following. Finally, the neural underpinning of these early developing social skills was assessed using longitudinal neuroimaging tool (Diffusion Tensor Imaging, DTI) on infants of Studies 1 and 2. Prolonged development of white matter tracts were found within the three cortical visual pathways supporting visual perception, motion, and attention throughout infancy. These changes in white-matter properties showed moderate to strong correlations with the behavioral changes reported above. Collectively, the NHP brain-behavior findings suggest that the period between 2-24 weeks represents a critical period for the refinement of social skills that parallels the fine-tuning of neural connections in social visual pathways. The early-emerging and highly-conserved quantitative phenotypes between the two species indicate that infant NHP provide a critical model to study genetic variations, molecular, or experimental manipulations altering the normative development of social-visual attention. Such studies will further our knowledge of the brain-behavior pathogenesis of ASD and will ultimately help validate efficacy of potential therapeutic treatments for attenuating social deficits in ASD.

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General Introduction

Deficits in social cognition are one of the main symptoms affecting many neurodevelopmental disorders; specifically, it is a hallmark symptom of Autism Spectrum Disorder (ASD), Asperger's Syndrome, and Rett's Syndrome (APA, 2013). The very first case descriptions provided by Kanner (1943) noted delayed language acquisition and atypical social responses (not responding to name). Today, one of the diagnostic criteria established by the DSM-V for ASD consists of difficulties in social communication and interaction; though due to the heterogeneity of ASD, the magnitude of social impairments may differ greatly across individuals. Additional studies have shown a disruption in a number of social behaviors in ASD, including a large reduction in shared affect in parental interaction and reciprocal smiling (Zwaigenbaum et al., 2005; Estes et al., 2015; Wetherby et al, 2004). As efforts have been made to diagnose ASD at earlier ages, many studies have begun to use high-risk populations (i.e., younger siblings of children already diagnosed with ASD) to help characterize early behavioral markers linked to the development of ASD (Zwaigenbaum et al., 2009). Thus, many studies are focused on the development of early social skills, such as social-visual engagement, mutual eye gaze and gaze following skills as measures of social cognition.

Broadly speaking, social-visual engagement involves flexibly changing one's behavior or attention in response to social information – including preferential looking towards faces over non-social scenes or objects and orienting one's attention towards the direction of a name being called. These forms of social behavior are present relatively early in development and allow for the study of social cognition in preverbal infants. Additionally, even these relatively simple forms of social-visual engagement can be disrupted in ASD. For example, children who are later diagnosed with ASD, have reduced responding to their names noticeable in the first two years of life (Zwaigenbaum et al., 2005; Nadig & Mulligan, 2017; Miller et al., 2017). Additionally, there are deficits in responding to joint attention cues (Sullivan et al., 2007; Yoder et al., 2009) as well as fewer bids for joint attention (Goldberg et al., 2005; Landa et al., 2006). Recently, the use of eye-tracking methodologies in prospective studies of high-risk infants and typically developing children have provided objective measurable differences in social-visual engagement of infants who later will be diagnosed with ASD (Frazier et al., 2017). The analysis of gaze behavior provides an intermediary between brain development and social behavior and has been explored as a possible biomarker for ASD (Pierce et al., 2016). Visual orienting is a directly observable behavior that may be indexed by neurophysiological processes. Klin and colleagues (2015) showed a disruption in the attention to biological motion in 2-year olds with ASD as compared to typically developing (TD) and developmentally delayed (DD) controls. Namely, unlike TD and DD toddlers, toddlers with ASD did not preferentially orient to point-light displays of biological motion and instead preferred non-social physically contingent point-light displays. It has been shown that the spontaneous orienting to biological motion is present in newborns (Simion et al., 2008) and it is theorized that the neural underpinnings overlap with brain regions involved face and gaze processing (Pelphrey et al., 2005). A malfunctioning of these neural structures in early infancy may not only derail the preference for biological motion but may greatly affect the development of procesing social information as well. Indeed, at 6 months, Chawarska and colleagues (2013) found a reduction of spontaneous orienting in infants that later develop ASD compared to TD infants, and Jones and Klin (2013) found reliable deviations in the longitudinal changes in attention to the eyes when comparing infants with ASD to TD and DD infants. Specifically, Jones and Klin showed short videoclips of social scenes (i.e., toddlers playing in a kindergarten setting or an adult talking to the infant from the screen) to TD, DD and high-risk

ASD (i.e., having an older sibling with an ASD diagnosis) infants. Typically developing infants have a general increase in attention to the eyes early on from 2 to 12 months that slightly decline to reach a trough around 17 months before increasing again. In contrast, infants that were later diagnosed with ASD started to attend to the eyes similarly to TD at 2 months but showed a progressive decline in attention to the eyes thereafter.

It is believed that social-visual engagement, including attention to the eyes in infancy, is supported by attentional and social visual networks, such as the amygdala, ventral striatum, medial prefrontal cortex, and cortical areas along the superior temporal sulcus – all areas known to be altered in adults with ASD (Sato et al., 2012; Kampe et al., 2001; Wang et al., 2011). Thus, the changes in developmental trajectories of social-visual attention in infancy in ASD are hypothesized to be due to a number of early neural changes in these structures, specifically the theory that reflexive subcortical visual processing becomes increasingly supported and modulated by voluntary cortical visual processing throughout childhood development (Klin et al., 2015). However, inferences to this neural network have been made through neuroimaging studies conducted in adults with ASD that only provide an approximation of neural changes that might support the changes in attention to social cues occurring in infancy. Given the difficulty to acquire densely sampled, longitudinal neuroimaging data across infant development in humans, the development of an animal model that will help better delineate the neural contributions to social-visual engagement throughout infancy is clearly warranted.

Whereas a number of studies have made use of mouse models to identify various genetic and molecular components of social behavior that may be implicated in ASD (Bey & Jiang, 2014), these attempts to link the knowledge on genetic mouse models to neural substrates have been limited (Ellegood & Crawley, 2015). Furthermore, the relatively restricted social repertoire of rodents and significant differences in their brain structures compared to humans have limited the use of mouse models to study the development of early social skills (Watson & Platt, 2012). Rhesus macaques provide a more appropriate animal model for understanding neurodevelopmental changes in early life, and in particular macaques are an ideal model for understanding the social deficit seen in childhood psychopathologies given (1) the rich and complex social structure in which they develop and navigate, (2) the similarity with humans in brain and cognitive functions development, and (3) the visual system is the primary sensory information that monkeys use to navigate the world (Machado & Bachevalier, 2003).

In this introduction, we will first review and compare behavioral studies in humans and nonhuman primates (NHPs) studies on social-visual engagement and attention. We will then provide a brief review of the neural substrates of social-visual engagement, focusing on gaps in the literature that led to the three studies described in this thesis. Generally, the goal of the three studies was to characterize the development of early social skills in the monkeys, social-visual engagement, mutual eye gaze, and gaze following, and to identify the neural networks supporting these early developing skills using noninvasive neuroimaging techniques.

Social-visual engagement in humans

Social-visual engagement is the ability to actively use visual information to change one's behavior and attention appropriately based upon social contingencies. Baron-Cohen (1992) proposed that social cognition consists of modular and progressively developing set of social-visual behavior that has been since expanded upon by Perret and Emery (1994). The components of social-visual behavior consist of:

1) An eye direction detection system that is characterized by early gaze preferences;
2) Direction of attention detector that processes attentional cues from the head and body;
3) Intentionality detector that infers communicative intent behind gaze; and
4) Shared attention mechanism.

Behaviors such as gaze following and joint attention are contingent upon several of these components. Finally, Baron-Cohen (1992) believed that this set of social-visual behaviors ultimately give rise to theory of mind. This framework of the progressive development of social cognition provides some insight into studies of the predictive nature of early eye gaze and future sociality and communication skills (Brooks & Melzoff, 2005; Brooks & Melzoff, 2008; Riby et al., 2013), highlighting the developmental importance of social-visual engagement from infancy. Indeed, deviations in infant social-visual engagement are detectable by 24 months and have been shown to be predictive of later ASD diagnosis (Klin et al., 2012; Klin & Jones, 2015).

The early eye direction detection is present almost immediately following birth and preference for directed eye gaze over averted gaze occurs as early as 2-5 days in human infants (Senju & Johnson, 2009; Farroni et al., 2003). The preference for direct eye gaze continues to develop and by 3 months of age, infants typically pay more attention to the eyes of others than any other part of the face, and pay attention the face more than any other part of the body (Haith et al., 1977). In a detailed investigation of the developmental trajectory of early eye gaze, Jones and Klin (2013) conducted a prospective longitudinal study with typically developing (TD) infants and high-risk ASD infants watching pre-recorded video scenes of actresses talking to the infants over the first 24 months. Although both groups of infants began fixating at the eyes for similar period of time during the first 3-4 months of life, ASD infants showed a steady decline in attention to the eye region thereafter, extending until 24 months. On the other hand, TD infants maintained attention to the eyes of others until 9 months of age, followed by a brief decline between 9-17 months, a further increase to reach a plateau relatively stable until 24 months.

As infants age, the eye direction detector system works in conjunction with an intentionality detector and more elaborate forms of social-visual attention arises. By 4 months of age, infants begin to utilize eye direction to direct their attention, marked by the emergence of spontaneously gaze following (Farroni et al., 2004; Senju & Johnson, 2009). This utilization of social-visual engagement to direct their attention continues to develop and becomes relatively stable at 9 months (Mundy et al., 2007). This ability to gaze follow is a crucial social-cognitive ability that facilitates social learning and language acquisition later in childhood (Brooks & Meltzoff, 2015; Morales et al., 2000; Tenenbaum et al., 2015). Furthermore, the disruption of these processes, including deficits in gaze following, is closely related to ASD (Gillespie-Lynch et al., 2013; Thorup et al., 2016).

Finally, gaze following evolves into a mechanism for establishing joint attention. By 18 months, infants reliably establish joint attention through gaze following in triadic social interactions (Brooks & Meltzoff, 2002). Specifically, joint attention refers to the ability to coordinate visual attention between two persons and an external object or event in order to share attention (Mundy et al., 2009). This utilization of social-visual information to establish joint attention represents a particularly meaningful moment in development. As with eye gaze and gaze following, diminished joint attention is a consistent feature in children with ASD (Charman, 2003; Dawson et al., 2004; Lord et al., 2000; Mundy, 2016). Although face and eye detection are the foundation for gaze following, establishing joint attention is particularly crucial for social referencing and requires an inference of intentionality (Carpenter et al., 1998). Thus, impairments in joint attention are considered one of the early steps of a cascade of behaviors leading to theory of mind and additionally considered an incredibly important observational marker for ASD diagnosis (Autism Diagnosis Observation Schedule; Lord et al., 2012; Mundy, 2017). Furthermore, deficits in joint attention are linked to reduced acquisition of social-communication skills (Murray et al., 2008) and are one of the longest-lasting symptoms of ASD. Indeed, although establishing joint attention in response to other individuals can be improved in children with ASD, they still fail to initiate joint attention well into adolescence (Charman, 2003; Mundy et al., 2009; Hobson & Hobson, 2007). To sum, attention to faces and eyes in particular as well as gaze following and joint attention have become critical early markers for children at risks of developing ASD.

Social-visual engagement in nonhuman primates

Rhesus macaques live in a highly social environment in which social-visual engagement can be crucial to surviving. It is believed that social-visual attention has been a primary selective pressure in the evolution of the primate brain and remains largely conserved from NHPs into humans (Chance & Jolly, 1970; Dunbar & Shultz, 2007). Social-visual attention is important from birth, being used to adjust and modulate aggressive, threatening, and affiliative behaviors according to the observed dominance hierarchy (Deaner, Khera, & Platt, 2005). In adulthood, eye-contact and gaze is often used to recruit allies and assert dominance before more aggressive behaviors are ever displayed (Maestripieri & Wallen, 1997; Shepherd & Platt, 2008; Emory, 2000). Thus, the evolutionary and social importance of social-visual attention in primates allows for drawing parallels between human and nonhuman primate studies.

Social-visual engagement in rhesus macaques develops in a similarly progressive manner as humans. Kuwahata and colleagues (2014) tested the visual preference for configural patterns in the first 3 months of infancy, finding that infants reliably preferred face-like schematic configurations over any other patterns by 1 month of age. Similarly, Lutz and colleagues (1998) determined that a preference for face-like drawings over distorted pen drawings of rhesus macaque faces emerged on average at 6 weeks of age.

Although this preference seems to develop after birth, further studies were conducted to better delineate the relative contributions of innate face processing and experience driven development of social cognition. Indeed, recent NHP findings suggest that there may be an innate preference that aids in the development of face processing. Specifically, it has been noted that infant monkeys that are reared without exposure to any faces for 6-24 months will quickly develop a facial preference to the first face or face-like stimuli they regularly witness (Sugita, 2008). Face scanning behavior continues to develop in the first 3 months of life. Parr and colleagues (2016) showed an initial strong preference for conspecific faces over heterospecific faces that shifted to an increase in attention to heterospecific faces around 5 weeks of age. Another study conducted with the same animals also showed that infant monkeys develop a preference for direct gaze that emerges in the first 3 months, and that viewing patterns towards direct faces versus indirect faces showed different developmental timelines (Muschinski et al., 2016). Although these studies do provide insight into the development of social-visual attention in NHPs, they all presented procedural limitations given that the stimuli used were side-by-side still images of two different face exemplars. The lack of dynamic facial expressions and features may fail to properly capture visual attention in a social domain.

Although, the development of eye and face detection to elaborated gaze following and joint attention in rhesus macaques is still disputed (Emery, 2000; Ferrari et al., 2000; Itakura, 2004), NHPs certainly act upon ever-changing contingencies and rely on visual information to alter their own landscape of social saliency, and gaze following has been observed in laboratory settings in adult monkeys (Mosher et al., 2011; Simpson et al., 2017). However, the developmental timeline of gaze following is not well delineated in rhesus macaques. Within semi-free ranging monkeys, gaze following has been reported to emerge at around 6 months (Tomasello et al., 2001; Teufel et al., 2010; Rosati et al., 2016). Similarly, reciprocal face-face interactions with the infant's mother becomes a significant part of the infant's behavioral repertoire at 3 months (Ferrari et al., 2009), suggesting a more elaborate use of social-visual engagement than eye detection. By 1 year, monkeys reliably use gaze as a directional cue (Senju & Johnson, 2009). Still, as for the studies on attention to facial cues, studies on gaze following have a number of methodological limitations when compared to the human literature. Studies in semi-free ranging monkeys only provide for detection of gross differences in gaze behavior (i.e., live observation notes or frame-by-frame coding of monkey behavior recorded from a distance instead of precise eye-tracking methods), and many laboratory studies using eye-tracking methodology either relied on an human experimental actor or primarily used still images, neither may be ecologically relevant for engaging infant monkeys in social-visual behaviors (Rosati et al., 2016; Simpson et al., 2010). Thus, the degree to which the current NHP studies can inform human studies is fairly limited, as the lack of ecological relevance and changes in research methodology may not capture the same behavioral milestones between species. To address this shortcoming in the literature, the first two chapters of this thesis serve to better characterize the development of social-visual attention skills in nonhuman primates. Methodology established for human infant studies (Jones & Klin, 2013) were adapted for use with NHPs and ecologically relevant videoclips of other rhesus macaques were shown. Studying the longitudinal development of eye attention (Chapter 1) and gaze following (Chapter 2) will add to our understanding of the degree to which NHP infants attend to the social environment around them in a way human children do.

Neural substrates of social-visual engagement

There are several subcortical and cortical regions of the brain that are face selective in both humans and nonhuman primates. So far, our understanding of the neural underpinning of social-visual engagement and early face processing is mainly derived from studies of adult brains. Thus, the emergence (either functionally or structurally) of face processing systems throughout infancy is not well understood, though several inferences can be made from the extensive literature in adult face processing.

Morton and Johnson (1991) first published a two-process theory of the development of face processing that suggested a mostly developed subcortical route present at birth, and a later emergence of cortical face processing areas. The two-process theory is supported by studies that have shown that the fairly basic face preference at birth (infants preferentially orient towards face-like schematic patterns over other basic patterns; Johnson et al., 1991; Goren et al., 1975) may be supported by a subcortical route, which is then further modulated by the development of cortical visual areas that will become specialized for face selectivity (Farroni et al., 1999). This theory, however, presupposes that subcortical visual systems are innately driven to detect faces or face-like configurations at birth. At its foundation, the two-process theory is based upon two well-documented processes that ensure imprinting in domestic chicks onto a mother hen. First, the optic tectum (subcortical component) contains a visual template of a hen that directs attention of the chicks to objects that match the template, then the mesopallium (cortical homologue) forms a strong representation of the object and an irreversible memory and attachment to the object the chick attends (Morton & Johnson, 1991; Horn, 2004). Thus, a similar neural organization has been proposed for the attention to faces in primates. It is believed, as shown in Figure 1, that the primate subcortical areas, including the superior colliculus, pulvinar, and amygdala, contain a template that directs attention of neonates to faces. The innate attention to faces could then either directly or indirectly guide the development of face specificity in cortical areas (Shultz, 2005).

One alternative theory for early social-visual attention is the “sensory hypothesis” first proposed by Kleiner and Banks (1987). The sensory hypothesis stipulates that all visual preferences (including face preference) in infants can be explained by the spatial frequencies of the stimuli. Throughout the last 30 years, the sensory hypothesis has suggested that innate preferences for faces are driven by visual statistics and spatial organization. Specifically, the innate preference for faces may be due to neural organization of low visual features, such as high- versus low-spatial frequencies, and square versus curved edges. In the visual cortical system, preferences for combinations of features, such as curved edges with low-spatial frequency blobs, occurs independently (Farley et al., 2007). Similarly, the binocular correlation model (BCM) suggests that neonatal biases towards faces are a result of a visual filtering resulting from limited binocular integration in newborns (Wilkinson et al., 2014). The correlation of salient areas in each eye (i.e., the eyes and mouth) serve to amplify these areas to create a representation of face-like stimuli in the visual system. This model relies highly on visual experience – specifically that patches of cortex within the ventral visual pathway (see Figure 1) are repeatedly co-activated with preferences for different low-level features while viewing the faces of others, and that this repeated co-activation leads to cortical neurons that become face selective. In testing this theory, Arcaro and colleagues (2017) attempted to train face selective cortical areas to be selective of other features (specifically hands). In infant monkeys that were not exposed to faces since birth but received repeated exposures to hands, the cortical areas usually devoted to process faces failed to develop a specificity for any other body part. However, as soon as face stimuli were introduced into the infants' visual experience, cortical face specificity began to develop in the cortical areas. Therefore, the cortical area does require experience with faces to develop, and is not a pathway that is innately tuned to face processing. The development of cortical brain regions may be biased by subcortical inputs to preferentially respond to faces and face-like stimuli.

To summarize, the first theory suggests that the subcortical pathway contains an innate visual template that attracts and guides infant attention, which, in turn, biases the development of cortical areas to become face-specific (Johnson et al., 2015). The second theory suggests that early face preferences are due to low-level visual statistics in cortical areas, and that frequent experience of faces causes category-specific cortical specialization for faces. Both these theories suggest a bottom-up driven development using basic visual information. While there is likely a subcortical template that drives neonatal attention towards faces, changes in social-visual attention throughout infancy are not due solely to the presence or absence of schematic faces in the infant environment, but depend also upon meaningful social interactions between the infants and others to reinforce the development of cortical face specificity. For instance, human infants' attention is also driven by multimodal social cues, such as infant-directed speech or "motherese"

(Fernald, 1985; Cooper & Aslin, 1990). Likewise, affective touch has been shown to enhance the learning of face-identity in 4 month-old infants (Longa et al., 2017). Taken together, a subcortical template drives initial neonatal attention towards faces, whereas further development of cortical face-specificity is likely due not only to exposure to visual features of faces but also to the emergence of infants' interest to meaningful social interactions, as recently suggested by Powell and colleagues (2018).

Jones and Klin (2013) used the proposed neural circuits summarized above to explain the highly stereotyped developmental trajectories of social-visual engagement in TD and ASD infants (see Figure 2). They proposed that a subcortical template driving infant attention towards faces and eyes may explain the initially high degree of eye-attention in both TD and ASD infants. As experience with faces increases together with enhanced socially driven interactions during the first few months, the subcortical system is slowly supplanted by the developing visual cortical areas becoming specifically tuned to faces. This shift from subcortical to cortical control of attention to faces may explain the fact that in TD infants, after a slight dip in eye-attention over the first 9 months, attention to faces rises again and plateaus for the rest of infancy as infants begin to placed more social value on faces. If this proposal is correct then the pattern of eye-attention of infants later developing ASD, which is characterized by a same drop in attention to the eyes during the first 9 months as TD infants but no recovery later on in infancy, may indicate a drop of the subcortical driven attention that is not supplanted by a cortically driven attention system as children at risk of developing ASD may not place social value on faces and eyes, resulting in abnormal development in social-visual engagement processing.

Given this socially-mediated theory for the development of the social-visual attention system, do we have evidence for a differential time course of development between the subcortical and cortical attention systems in primates? Although the data on the neural brain areas supporting the development of social visual attention are still in its infancy, the next section will review the (1) subcortical pathway that includes the pulvinar, superior colliculus, and amygdala, the (2) ventral temporal visual pathway, which contains two separate processing streams, and finally the (3) dorsal parietal visual pathway, as well as their development in primates.

Subcortical visual pathway

The initial evidence for a subcortical route for face processing came from studying patients with residual face processing abilities, such as blindsight and prosopagnosia. For instance, blindsight patients with extensive damage to the visual cortex demonstrate limited ability to detect faces and facial expressions (Morris et al., 2001). Similarly, adults with prosopagnosia due to cortical damage are still able to detect the presence of faces and some facial expressions (de Gelder et al., 2003). Functional imaging of patients with prosopagnosia have revealed limited facial processing activation in the amygdala, pulvinar, and superior colliculus (de Gelder et al., 2003). Developmentally, this subcortical face processing pathway is fairly well developed at birth (Johnson, 2005; Atkinson, 2000). Basic facial processing, such as spontaneously orienting to schematic face-like patterns, is supported by this subcortical face processing (Nelson, 2001; Cassia et al., 2001).

As visual cortical face processing pathways develop in infancy, the subcortical pathways are believed to take on a more modulatory role in social-visual attention, while biasing cortical development for face specificity. For instance, the amygdala responds to direct eye contact before cortical visual processing in electrophysiological studies (Conti et al., 2007). The standard hypothesis of subcortical visual processing would indicate that even after the development of cortical visual processing areas, the amygdala and pulvinar are important for the fast processing of emotional “fight-or-flight” situations, such as quickly being able to detect threats such as snakes. This theory is supported by several studies showing faster neural response to faces of high emotional or social valence (such as engaged direct eye contact or fearful faces) in the pulvinar and amygdala when compared to other cortical face processing areas. The subcortical pathway has been shown to provide a faster neural response for processing direct versus averted eye contact well into adulthood (de Gelder, 2003; Johnson, 2005; Johnson & Morton, 1991; LeDoux, 1996). Additionally, early activation of the amygdala, superior colliculus and pulvinar is predictive of the activation of cortical face-processing areas by several hundred milliseconds (Keightley et al., 2003; George et al., 2001; Kleinhans et al., 2008). In adulthood, it is believed that rapid detection of eye contact is still mediated by the amygdala (Sato et al., 2011). Additionally, haemodynamic measures have shown higher activity in the amygdala at an earlier time point when viewing fearful faces of strangers on a screen (Dolan & Vuilleumier, 2003; Tamietto & de Gelder, 2010).

In NHPs, similar electrophysiological evidence has shown similar changes in amygdala activity when detecting the eyes of conspecifics in social scenes (Mosher et al., 2014). Additionally, PET studies have also shown that the amygdala is highly responsive to changes in the gaze of others, and that full, adult amygdalectomy does generate noticeable decreases in gaze monitoring (Kawashima et al., 1999; Young et al., 1995).

Several studies have also implicated the amygdala in deficits of social attention and engagement as well, leading to the theory that early disruption of amygdala function can result in further atypical development in a network of areas involved in processing socially salient stimuli (Skuse et al., 2003). Postmortem and MRI studies from ASD patients have reported abnormalities in the amygdala (Bauman & Kemper, 1985; Abbell et al., 1999). Additionally, neonatal amygdala lesions in monkeys decrease mother-infant mutual gaze and disrupt social preference (Bauman et al., 2004), and result in subtle alterations in social interactions in the first months of life (Raper et al., 2014). Not only do these results highlight the importance of subcortical structures in social cognition, but it underscores the proposal that early disruptions of the amygdala may have long-lasting effects on social behavior well into adulthood. However, the underlying mechanisms of how early the subcortical visual pathway shapes social-visual engagement remains under debate.

Specifically, the extent to how independent the subcortical visual pathway is remains under heavy debate. Neuroendocrine response to visual threats has remained evolutionarily conserved across many vertebrates, including fish, suggesting an important role in thalamus-amygdala pathway for processing fearful stimuli (Carr, 2015). However, there are still contradictory studies that show no changes in behavior after complete amygdala lesions (Tsuchiya et al., 2009), suggesting that the amygdala may not be solely responsible for the processing of affectively and motivationally significant features such as fearful faces. Rather, it is more likely that affective and motional stimuli engage several brain sites, including the amygdala through the subcortical visual pathways, but also in cortical visual pathways simultaneously, rather than hierarchically (Pessoa & Adolphs, 2010).

Cortical visual processing

Research onto the neural substrate of visual functions has demonstrated that a large portion of the cortex, especially in primates, is devoted to the processing of visual signals. One earlier influential paper demonstrated that in adult monkeys visual information that reaches the visual primary area (V1) is then processed through a series of visual cortical association areas using three different cortical routes: a dorsal parietal route that extends from LIP to frontal cortical areas, critical for visuospatial attention (Ungerleider and Mishkin, 1982), a ventral temporal route, which contains two streams that extend from V1. The first projects successively within cortical areas of the ventral temporal gyrus posteriorly from V2 to V3, V4, TEO and finally area TE anteriorly, and is critical for stimulus processing, and the second extends from V1 into areas of the superior temporal sulcus (STS) that includes MT, MST, FST and AST, critical for motion (Boussaoud et al., 1990). Broadly, these three major streams of visual processing play a critical role in the processing of face stimuli as will be reviewed below.

Ventral visual pathway: Within the dorsal temporal visual pathway, faces are primarily processed through two distinct multisynaptic streams both originating from V1. The first stream runs from V1 through the IT that includes TE and TEO. In humans, this stream includes the face-selective areas of the lateral occipital face area (OFA) and the medial fusiform face area (FFA). The second stream goes from V1 through the STS and includes MT, MST and FST and other motion sensitive areas in the anterior portion of the STS (Boussaoud et al., 1990; Ungerleider & Mishkin, 1982). Between these two processing streams, Haxby and colleagues (2000) proposed that the IT stream face is important for the detection of faces and facial features, whereas the STS stream detects changeable aspects of the face, such as the perception of eye gaze and lip movement. Additionally, the STS areas have reciprocal connections with the intraparietal sulcus, responsible for directing attention, and subcortical visual processing areas. These reciprocal connections provide (1) a pathway for using preferences to facial cues processed by the subcortical system and (2) to direct attention to faces mediated by the ventral and dorsal cortical steams of visual processing.

The medial fusiform gyrus within the IT stream, an area extensively studied in face processing literature, seems to be more holistic tuned to faces than the motion pathway, responding to identity of individuals rather than the changeable features of a face, such as head or gaze direction. Several studies in humans using simultaneous and delayed matching of identical or different pictures of a stimulus individual have demonstrated identity-unique activity in an area of the fusiform gyrus known as the FFA (Haxby et al., 1999; Kanwisher et al., 1997; George et al., 1999). Recent fMRI studies have shown that, whereas the subcortical visual stream is sensitive to face-like stimuli based upon internal features, such as eyes and nose, the FFA is selective for both internal and external features of the face, such as hair, head outline, neck, and shoulders (Kamps et al., 2018). Taken together, the FFA seems to be uniquely tuned to process invariant facial aspects, such as identity, gender, and face form. While NHPs do not have a functionally defined FFA, studies have found face-selective regions in the monkey IT (Tsao et al., 2003; Pinsk et al., 2005). Both electrophysiological and functional neuroimaging studies in monkeys (Bruce & Young, 1986; Hadj-Bouziane et al., 2008; Tsao et al., 2003; Tsao & Livingstone., 2008) have demonstrated several cortical areas along IT responsive and selective to face stimuli. Specifically, the largest face-selective region within IT, located in TE, is thought to be the NHP homologue to the human FFA (Tsao et al., 2006). This theory is validated by computational transformations (Tsao et al., 2003) with the assumption that neighborhood relations between specific cortical areas are evolutionarily conserved (Van Essen et al., 2001; Tootell, Tsao, & Vanduffel, 2003; Sereno & Huang, 2006).

The second stream of processing in the ventral visual pathway contains face selective and motion sensitive areas along the STS. In humans, both fMRI and EEG studies have confirmed differential activity when a person views another in profile versus direct gaze (Hoffman & Haxby, 2000; Puce et al., 2000). Studies in adult split-brain patients have also implicated the STS as mediators for gaze following (Kingstone, 2009). The STS has further been implicated in various forms of social-visual engagement in human adulthood, differentially firing when attending to lip-reading, body posture, and even American Sign Language (Calvert et al., 1997; Howard et al., 1996; Neville et al. 1998). Interestingly, attending to the changeable aspects of a face, such as gaze or head direction or facial expressions, revealed no difference in activation of the FFA, suggesting the area is attuned to the holistic identity of the face rather than the perceptual features of a face (Hoffman & Haxby, 2000). In the experiment, Hoffman and Haxby conducted an fMRI experiment in which subjects viewed still images of strangers and were asked to provide either the direction of the gaze or indicate whether the picture was the same as the previous picture. They found a functional dissociation in which the STS provided a much stronger response when attending to the gaze, whereas the lateral fusiform gyrus provided a response only when asked if the identity matched.

The STS stream is also evolutionarily conserved between humans and NHP, demonstrating selectivity for changeable aspects of the face such as gaze. STS cortical areas in macaques are sensitive to both eye contact and gaze direction: some cells would differentially fire for direct gaze and establishing eye contact, whereas others would fire selectively for profile views of other monkeys (Perret et al., 1985; Perret & Mistlin, 1990; Perret et al., 1992). The STS is also specifically responsive for conspecific gaze and, when damaged, gaze following is negatively impacted (Kamphuis et al., 2009, Roy et al., 2014). Portions of the anterior STS have been reported to be selective for biological motion as well (Perret et al., 1985).

Interactions between the STS and the amygdala (Amaral et al., 1992) have been shown to be critically important for evaluating face identity and facial expressions (Gothard et al., 2007; Hoffman et al., 2007; Mosher et al., 2010). ERP studies conducted in both monkeys and humans suggest that feedback connections from the amygdala to STS may serve to help modulate amygdala responses, and feedforward connections from STS to the amygdala may serve to increase attention during emotional, social, or threatening situations (Mehta et al., 2000; Puce et al., 1999). Histological and functional MRI studies have also shown reciprocal anatomical and functional connections between STS and the dorsal attention network, specifically with the intraparietal sulcus responsible for directing attention and focus (Harries & Perret, 1991; Callejas, Shulman, & Corbetta, 2014).

In sum, the STS is responsive to the perceptual features and changeable aspects of a face such as gaze direction, whereas the IT is responsive to the holistic processing of face identity. Indeed, the predominant theory in the literature holds that face processing occurs in parallel between the two streams –the FFA (or primate TE) for the identification of identity and gender, and the STS for the identification of expression and gaze (Haxby et al., 2000; Gobbini & Haxby, 2007; O’Toole, 2002; Bernstein et al., 2018).

Dorsal visual pathway: The dorsal visual pathway stretches from the occipital lobe forward and dorsally into the parietal lobe. Although this pathway is more implicated in spatial relation than social-visual engagement, areas of the dorsal visual pathway do process important social information and are involved in attentional processes. For example, cortical areas within the lateral intraparietal sulcus (LIP) are critical for shifting attention to salient stimuli. The LIP has shown activation when subjects are monitoring the gaze of other subjects (Okada et al., 2008; Akiyama et al., 2007) or when individuals perceived and remembered faces (Lee & Kuhl, 2018). Additionally, the LIP has functional connectivity with the ventral visual pathways, specifically through the STS (Callejas, Shulman, & Corbetta, 2014). Finally, cortical areas within LIP then project to the Frontal Eye Field (FEF) in the dorsolateral portion of the prefrontal cortex and responsible for saccadic eye movements for the purpose of visual field perception and awareness, as well as for voluntary eye movement. It then projects back to the LIP as well as posterior cortical areas of the two ventral pathways, such as TEO and MT. In this way the FEF can influence the activation of neurons in the extrastriate visual cortex and is a source of top-down control on visual processing, including the processing of social signals (Schall, 2009).

Advances in the anatomical and functional organization of the ventral and dorsal visual pathways have provided strong evidence for the critical role of these cortical areas in social-visual engagement, but the development of these areas in early infancy and how they support infant social-visual engagement are not as well studied.

Development of the ventral and dorsal cortical pathways

Few human infant neuroimaging studies have followed the development of visual cortical areas supporting early social skills. For example, in human infants, studies using both PET and fMRI have reported that (1) maturation of primary visual areas (V1/V2/V3) occurs early in life

(Chugani and Phelps, 1986; Chugani et al., 1987; Gao et al., 2015), (2) unique resting state networks, including primary visual cortex, sensorimotor areas, and lateral parietal cortex exist in the infant brain (Fransson et al., 2007) and (3) FC between primary visual cortex and other areas within the visual system is present in infants -neonates to 2 years (Lin et al., 2008). In a more recent fMRI experiment by Deen and colleagues (2017), 4- to 6-month old human infants viewed dynamic videos of other faces and dynamic videos of environments. Even at this early age, cortical areas showed selective activation while infants viewed dynamic faces over scenes, with distinct regions responding preferentially to human faces versus natural scenes. The spatial structure of these responses is very similar to that observed in adults, and extends throughout cortex, including occipital, temporal, and parietal regions. Thus, although the anatomical maturation of human cortex is slow and asynchronous, basic aspects of functional organization are present across cortex from a very early age. Yet, the strength of signals in these areas is lesser in the infants than in the adults, suggesting subsequent refinement and more specificity in responses between the different areas. The authors suggested that the early-developing category preferences in cortex provides basic functional structures that will subsequently lead to the strong category-selective regions observed in adults. The relatively low selectivity at birth and emergence of face selectivity suggest that cortical face selectivity may begin relatively early on, but has a slower, protracted development that lasts throughout infancy. Using EEG in longitudinal studies in human infants have likewise shown a later development of the parietal attentional areas that is correlated with increases in bids to establish joint attention (Mundy et al., 2003; Mundy et al., 2009).

Such protracted development of the visual cortical pathways has been demonstrated in monkeys as well. Histological findings indicate that ventral face processing areas have a more prolonged development throughout infancy and are not fully developed at birth (Webster et al., 1991; Webster et al., 1994; Rodman & Consuelos, 1994). Metabolic studies in rhesus macaques have shown that areas along the ventral visual streams are immature at birth, but reach adult-like metabolic levels by 3 months of age (Distler et al., 1996). MRI and electrophysiological studies have likewise shown a later development of face-selective patches within the ventral visual pathways in rhesus macaques (Livingstone et al., 2017; Arcaro and Livingstone, 2017; Rodman et al., 1991; Rodman et al., 1993). Additionally, there is a later development of the connection between IT and parietal zones responsible for visual attention (Rodman & Consuelos, 1994) and metabolic studies in monkeys have also shown an abrupt maturational step in both the STS and the ventral area of the LIP at around 3 months of age (Distler et al., 1996).

Unfortunately, the relative contribution of cortical and subcortical visual pathways during infant development is only beginning to be mapped and remains poorly understood. This particular limitation in our knowledge of neural substrates is particularly glaring because the first 2 years of life represent the most substantial neural growth (Johnson, 2001). As previously discussed, it is believed that although the subcortical visual pathway is responsible for early face and eye detection, as the cortical visual pathway begins to mature, there is an increased salience for socially relevant signals. This shift from subcortical to cortical modulation may be disrupted in infants that later develop ASD, which results in a cascade of social deficits seen in ASD. This would suggest that there is a susceptible time period during which extensive changes in the strength of connections between the subcortical and cortical visual pathways may occur. To characterize the changes within the networks supporting early developing social visual engagement, the third aim of this thesis will use diffusion tensor imaging (DTI) to follow the development of anatomical connectivity of the subcortical and cortical networks, but only the white matter development in the cortical networks has been analyzed.

Several investigators have proposed advantages for the use of DTI in studying white-matter maturation (Barkovich, 2000; Geng et al., 2012). Additionally, the measurements extracted from DTI have been shown to be more stable than standard structural MRI intensity measures of white matter (Zhang et al., 2005). Tractography methods for DTI can assess microstructure properties of white matter bundles, as well as more accurately identify white matter tracts and changes in fiber shape or microstructure (McKinstry et al., 2002; Mori & van Zijl, 2002). Chapter 3 of this thesis will assess the longitudinal development of white matter tracts within the subcortical visual processing areas and the ventral and dorsal visual pathways in the same infant rhesus macaques for which we obtained the behavioral data in Chapters 1 and 2.

Summary

Endophenotypes are a quantifiable component of a neurodevelopmental or psychiatric condition that capture a core feature of the condition, but not the symptoms themselves (Gould & Gottesman, 2006). Endophenotypes believed to be greatly important in understanding complex neurodevelopmental and psychiatric disorders because they are more likely to mark the derailment of normative functions, allowing us to achieve a better understanding of potential causal mechanisms (Dawson et al., 2002). Social-visual engagement is an important behavior that develops in infancy and deviations in the development of social-visual engagement can lead to a cascade of effects, causing more severe deficits in social cognition and thus providing a measurable endophenotype that can be adapted to an animal model. The similarities in brain structure, parallels in behavioral repertoire, and the progressive nature in which many social behaviors are acquired in infancy make rhesus macaques an ideal animal model for studying social-visual engagement (see *Table 1*). Previous studies in social-visual engagement in infant rhesus macaques have several methodological limitations from either being (1) purely observational, or (2) do not consider the ecological relevance of species-related stimuli.

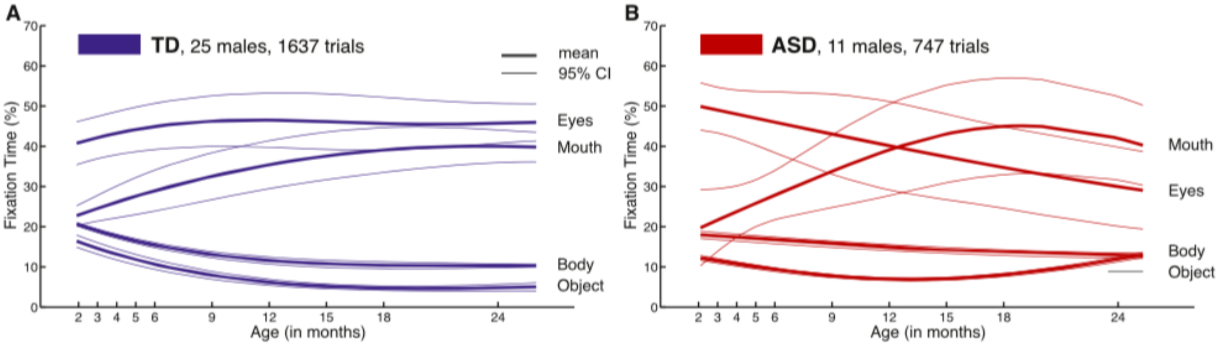
In further studying the endophenotype of social-visual engagement in rhesus macaques, species-relevant social stimuli, translational methods that can be used in both humans and infants, and longitudinal behavioral and neuroimaging studies performed on the same animals must be used to characterize the infant development of eye attention and gaze following. This project adapts methodologies used in human infant studies of social-visual engagement to infant rhesus macaques, studying attentional changes in rhesus macaque viewing patterns of social stimuli through early infancy. Leveraging the benefits of an animal model, this project also collects high-quality neuroimaging data while the infant is under anesthesia. In Chapter 1, we predict that nonhuman primates will display a trajectory of eye attention in infancy similar to that of typically developing human infants at corresponding ages and using the same methodology. For Chapter 2, we will examine the development of gaze following. We hypothesize that gaze following will not be present at birth, but will become apparent in the second month of life. In Chapter 3, we will utilize longitudinal DTI from a subset of the subjects of Chapters 1 and 2. We hypothesize that there will be correlations between important behavioral developmental shifts obtained from the first two chapters and maturational changes in the development of anatomical white matter tracts, such as increases in streamline counts (an estimation of the number of axons connecting two brain areas of interest), and increases in measures of axonal microstructure, such as fractional anisotropy and mean diffusivity. Specifically, we expect to see changes within the ventral perceptual pathway connectivity (between V4, TEO, TE, and amygdala) associated with changes in face preference and mutual eye gaze, within STS connectivity (between MT, MST, and FST) associated changes in social face perception and gaze following, and within the dorsal visual pathway connectivity (between MT, LIP, and FEF), associated with emergence of volitional spatial attention.

Figures and Tables

Figure 1: Summary of visual pathways

Caption for Figure 1: Summary of the visual pathways implicated in social-visual engagement. Those studied in this dissertation include the ventral motion pathway (in green) along the superior temporal sulcus (STS) which includes areas MT, MST and FST responsible for the processing of the changeable aspects of the face, and the perception of eye gaze; the ventral object pathway (in red) along the inferior temporal (IT) cortex including areas TEO, TE and the amygdala, implicated in early face detection and perceptual of basic facial features; and dorsal attention pathway including LIP and FEF that is responsible for visuo-spatial perception and involved in volitional attention. Included in this figure, not studied in this dissertation is a subcortical visual pathway (in blue) that includes the superior colliculus, pulvinar, and the amygdala.

Figure 2: Development of social-visual attention in TD and ASD human infants



Caption for Figure 2: Reproduced from Klin, Shultz & Jones (2015) summarizing the developmental trajectories of social-visual attention typically developing (TD) infants and those later diagnosed with ASD from birth until 24 months. **(A)** TD infants' attention to the eyes was greater than the mouth and body at all timepoints and showed a slight rise, reaching a peak at around 9-12 months, next declines reaching a trough at 19-20 months, then rising again. Attention the mouth region slowly increased throughout development. In contrast, **(B)** ASD diagnosed infants had a gradual decline in the attention to the eyes and a drastic increase in attention to the mouth, with attention to the mouth being greater than that of the eyes following 12 months of age.

Table 1: Early milestones in infant social behaviors in both humans and monkeys

Human Behavior	**Age**		**Monkey Behavior**
Reciprocal smiling	*1-2 months*	*1-2 weeks*	Reciprocal lipsmacking
Alertness when in the presence of strangers	*4 months*	*4 weeks*	Increase in observant behavior while on mom
Express emotions to external events	*4-5 months*	*5 weeks*	Use of fear-grimace/lipsmack in social exploration
Play peek-a-boo	*>6 months*	*8-12 weeks*	First play behavior emerges

Table 1: A summary of some important milestones in infant social behavior in humans compared to similar time points in monkey infants, keeping in mind that 1 month in human infants approximately corresponds to 1 week in infant monkeys. Human infant behaviors transcribed from a published textbook on infant behavior (Gassel et al., 1934). Monkey data are from published findings (Kaburu et al., 2016; Ferrari et al., 2009; Hinde & Spencer-Booth, 1967).

References:

Abell, F., Krams, M., Ashburner, J., Passingham, R., Friston, K., Frackowiak, R., … Frith, U. (1999). The neuroanatomy of autism: a voxel-based whole brain analysis of structural scans. *Neuroreport*, *10*(8), 1647–1651.

Akiyama, T., Kato, M., Muramatsu, T., Umeda, S., Saito, F., & Kashima, H. (2007). Unilateral amygdala lesions hamper attentional orienting triggered by gaze direction. *Cerebral Cortex (New York, N.Y. : 1991)*, *17*(11), 2593–2600. https://doi.org/10.1093/cercor/bhl166

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

Amaral, D. G., Price, J. L., Pitkänen, A., & Carmichael, T. (1992). The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. In J. Aggleton, J. Aggleton (Ed.) (pp. 1–66). New York, NY: Wiley-Liss.

Arcaro, M. J., & Livingstone, M. S. (2017). A hierarchical, retinotopic proto-organization of the primate visual system at birth. *eLife*, *6*. https://doi.org/10.7554/eLife.26196

Arcaro, M. J., Schade, P. F., Vincent, J. L., Ponce, C. R., & Livingstone, M. S. (2017). Seeing faces is necessary for face-domain formation. *Nature Neuroscience*, *20*(10), 1404–1412. https://doi.org/10.1038/nn.4635

Atkinson, J. (2000). *The Developing Visual Brain*. OUP Oxford. Retrieved from https://www.ebook.de/de/product/3255762/janette_atkinson_the_developing_visual_brain.html

Barkovich, A. J. (2000). Concepts of myelin and myelination in neuroradiology. *AJNR. American Journal of Neuroradiology*, *21*(6), 1099–1109.

Baron-Cohen, S, (1992). The theory of mind hypothesis of autism: history and prospects of the idea. *The Psychologist,* 5, 9-12.

Bauman, M., & Kemper, T. L. (1985). Histoanatomic observations of the brain in early infantile autism. *Neurology*, *35*(6), 866–874. https://doi.org/10.1212/wnl.35.6.866

Bauman, M. D., Lavenex, P., Mason, W. A., Capitanio, J. P., & Amaral, D. G. (2004). The development of social behavior following neonatal amygdala lesions in rhesus monkeys. *Journal of Cognitive Neuroscience*, *16*(8), 1388–1411. https://doi.org/10.1162/0898929042304741

Bernstein, M., Erez, Y., Blank, I., & Yovel, G. (2018). An Integrated Neural Framework for Dynamic and Static Face Processing. *Scientific Reports*, *8*(1), 7036. https://doi.org/10.1038/s41598-018-25405-9

Bey, A. L., & Jiang, Y. (2014). Overview of mouse models of autism spectrum disorders. *Current Protocols in Pharmacology*, *66*, 5. https://doi.org/10.1002/0471141755.ph0566s66

Boussaoud, D., Ungerleider, L. G., & Desimone, R. (1990). Pathways for motion analysis: cortical connections of the medial superior temporal and fundus of the superior temporal visual areas in the macaque. *The Journal of Comparative Neurology*, *296*(3), 462–495. https://doi.org/10.1002/cne.902960311

Brooks, R., & Meltzoff, A. N. (2005). The development of gaze following and its relation to language. *Developmental Science*, *8*(6), 535–543. https://doi.org/10.1111/j.1467-7687.2005.00445.x

Brooks, R., & Meltzoff, A. N. (2008). Infant gaze following and pointing predict accelerated vocabulary growth through two years of age: a longitudinal, growth curve modeling study. *Journal of Child Language*, *35*(1), 207–220.

Brooks, R., & Meltzoff, A. N. (2015). Connecting the dots from infancy to childhood: A longitudinal study connecting gaze following, language, and explicit theory of mind. *Journal of Experimental Child Psychology*, *130*, 67–78. https://doi.org/10.1016/j.jecp.2014.09.010

Bruce, V., & Young, A. (1986). Understanding face recognition. *British Journal of Psychology*, *77*(3), 305–327. https://doi.org/10.1111/j.2044-8295.1986.tb02199.x

Callejas, A., Shulman, G. L., & Corbetta, M. (2014). Dorsal and ventral attention systems underlie social and symbolic cueing. *Journal of Cognitive Neuroscience*, *26*(1), 63–80. https://doi.org/10.1162/jocn_a_00461

Carpenter, M., Nagell, K., & Tomasello, M. (1998). Social cognition, joint attention, and communicative competence from 9 to 15 months of age. *Monographs of the Society for Research in Child Development*, *63*(4), i–vi,1-143.

Calvert, G. A., Bullmore, E. T., Brammer, M. J., Campbell, R., Williams, S. C., McGuire, P. K., … David, A. S. (1997). Activation of auditory cortex during silent lipreading. *Science (New York, N.Y.)*, *276*(5312), 593–596.

Carr, J. A. (2015). I'll take the low road: the evolutionary underpinnings of visually triggered fear. *Frontiers in Neuroscience*, *9*, 414. https://doi.org/10.3389/fnins.2015.00414

Cassia, V. M., Simion, F., & Umiltaa, C. (2001). Face preference at birth: the role of an orienting mechanism. *Developmental Science*, *4*(1), 101–108. https://doi.org/10.1111/1467-7687.00154

Chance, M. R. A., & Jolly, C. J. (1970). *Social groups of monkeys, apes and men.* London, U.K: Cape.

Charman, T. (2003). Why is joint attention a pivotal skill in autism? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *358*(1430), 315–324. https://doi.org/10.1098/rstb.2002.1199

Chawarska, K., Macari, S., & Shic, F. (2013). Decreased spontaneous attention to social scenes in 6-month-old infants later diagnosed with autism spectrum disorders. *Biological Psychiatry*, *74*(3), 195–203. https://doi.org/10.1016/j.biopsych.2012.11.022

Chugani, H. T., & Phelps, M. E. (1986). Maturational changes in cerebral function in infants determined by 18FDG positron emission tomography. *Science (New York, N.Y.)*, *231*(4740), 840–843.

Chugani, H. T., Phelps, M. E., & Mazziotta, J. C. (1987). Positron emission tomography study of human brain functional development. *Annals of Neurology*, *22*(4), 487–497. https://doi.org/10.1002/ana.410220408

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

Cooper, R. P., & Aslin, R. N. (1990). Preference for infant-directed speech in the first month after birth. *Child Development*, *61*(5), 1584–1595.

Dawson, G., Webb, S. J., Carver, L., Panagiotides, H., & McPartland, J. (2004). Young children with autism show atypical brain responses to fearful versus neutral facial expressions of emotion. *Developmental Science*, *7*(3), 340–359.

Dawson, G., Webb, S., Schellenberg, G. D., Dager, S., Friedman, S., Aylward, E., & Richards, T. (2002). Defining the broader phenotype of autism: genetic, brain, and behavioral perspectives. *Development and Psychopathology*, *14*(3), 581–611.

Deaner, R. O., Khera, A. V., & Platt, M. L. (2005). Monkeys Pay Per View: Adaptive Valuation of Social Images by Rhesus Macaques. *Current Biology*, *15*(6), 543–548. https://doi.org/10.1016/j.cub.2005.01.044

Deen, B., Richardson, H., Dilks, D. D., Takahashi, A., Keil, B., Wald, L. L., … Saxe, R. (2017). Organization of high-level visual cortex in human infants. *Nature Communications*, *8*, 13995. https://doi.org/10.1038/ncomms13995

de Gelder, B., Frissen, I., Barton, J., & Hadjikhani, N. (2003). A modulatory role for facial expressions in prosopagnosia. *Proceedings of the National Academy of Sciences*, *100*(22), 13105–13110. https://doi.org/10.1073/pnas.1735530100

Distler, C., Bachevalier, J., Kennedy, C., Mishkin, M., & Ungerleider, L. (1996). Functional development of the corticocortical pathway for motion analysis in the macaque monkey: A 14C-2-deoxyglucose study. *Cerebral Cortex*, *6*(2), 184,195.

Dolan, R. J., & Vuilleumier, P. (2003). Amygdala automaticity in emotional processing. *Annals of the New York Academy of Sciences*, *985*, 348–355.

Dunbar, R. I. M., & Shultz, S. (2007). Evolution in the Social Brain. *Science*, *317*(5843), 1344–1347. https://doi.org/10.1126/science.1145463

Ellegood, J., & Crawley, J. N. (2015). Behavioral and Neuroanatomical Phenotypes in Mouse Models of Autism. *Neurotherapeutics*, *12*(3), 521–533. https://doi.org/10.1007/s13311-015-0360-z

Emery, N. J. (2000). The eyes have it: the neuroethology, function and evolution of social gaze. *Neuroscience and Biobehavioral Reviews*, *24*(6), 581–604.

Estes, A., Zwaigenbaum, L., Gu, H., John, T. S., Paterson, S., Elison, J. T., … Piven, J. (2015). Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life. *Journal of Neurodevelopmental Disorders*, *7*(1). https://doi.org/10.1186/s11689-015-9117-6

Farley, B. J., Yu, H., Jin, D. Z., & Sur, M. (2007). Alteration of Visual Input Results in a Coordinated Reorganization of Multiple Visual Cortex Maps. *Journal of Neuroscience, 27*(38), 10299–10310. https://doi.org/10.1523/jneurosci.2257-07.2007

Farroni, T., Mansfield, E. M., Lai, C., & Johnson, M. H. (2003). Infants perceiving and acting on the eyes: Tests of an evolutionary hypothesis. *Journal of Experimental Child Psychology, 85*(3), 199–212. https://doi.org/10.1016/s0022-0965(03)00022-5

Farroni, T., Massaccesi, S., Pividori, D., & Johnson, M. H. (2004). Gaze Following in Newborns. *Infancy*, *5*(1), 39–60. https://doi.org/10.1207/s15327078in0501_2

Farroni, T., Simion, F., Umilta, C., & Barba, B. D. (1999). The gap effect in newborns. *Developmental Science*, *2*(2), 174–186. https://doi.org/10.1111/1467-7687.00066

Fernald, A. (1985). Four-month-old infants prefer to listen to motherese. *Infant Behavior and Development*, *8*(2), 181–195. https://doi.org/10.1016/s0163-6383(85)80005-9

Ferrari, P. F., Kohler, E., Fogassi, L., & Gallese, V. (2000). The ability to follow eye gaze and its emergence during development in macaque monkeys. *Proceedings of the National Academy of Sciences*, *97*(25), 13997–14002. https://doi.org/10.1073/pnas.250241197

Ferrari, P. F., Paukner, A., Ionica, C., & Suomi, S. J. (2009). Reciprocal Face-to-Face Communication between Rhesus Macaque Mothers and Their Newborn Infants. *Current Biology*, *19*(20), 1768–1772. https://doi.org/10.1016/j.cub.2009.08.055

Fransson, P., Skiöld, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., & Aden, U. (2007). Resting-state networks in the infant brain. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(39), 15531–15536. https://doi.org/10.1073/pnas.0704380104

Frazier, T. W., Strauss, M., Klingemier, E. W., Zetzer, E. E., Hardan, A. Y., Eng, C., & Youngstrom, E. A. (2017). A Meta-Analysis of Gaze Differences to Social and Nonsocial Information Between Individuals With and Without Autism. *Journal of the American Academy of Child & Adolescent Psychiatry*, *56*(7), 546–555. https://doi.org/10.1016/j.jaac.2017.05.005

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

Geng, X., Gouttard, S., Sharma, A., Gu, H., Styner, M., Lin, W., … Gilmore, J. H. (2012). Quantitative tract-based white matter development from birth to age 2years. *NeuroImage, 61*(3), 542–557. https://doi.org/10.1016/j.neuroimage.2012.03.057

George, N., Dolan, R. J., Fink, G. R., Baylis, G. C., Russell, C., & Driver, J. (1999). Contrast polarity and face recognition in the human fusiform gyrus. *Nature Neuroscience*, *2*(6), 574–580. https://doi.org/10.1038/9230

George, N., Driver, J., & Dolan, R. J. (2001). Seen Gaze-Direction Modulates Fusiform Activity and Its Coupling with Other Brain Areas during Face Processing. *NeuroImage*, *13*(6), 1102–1112. https://doi.org/10.1006/nimg.2001.0769

Gillespie-Lynch, K., Elias, R., Escudero, P., Hutman, T., & Johnson, S. P. (2013). Atypical Gaze Following in Autism: A Comparison of Three Potential Mechanisms. *Journal of Autism and Developmental Disorders*, *43*(12), 2779–2792. https://doi.org/10.1007/s10803-013-1818-7

Gobbini, M. I., & Haxby, J. V. (2007). Neural systems for recognition of familiar faces. *Neuropsychologia*, *45*(1), 32–41. https://doi.org/10.1016/j.neuropsychologia.2006.04.015

Goldberg, W. A., Jarvis, K. L., Osann, K., Laulhere, T. M., Straub, C., Thomas, E., … Spence, M. A. (2005). Brief Report: Early Social Communication Behaviors in the Younger Siblings of Children with Autism. *Journal of Autism and Developmental Disorders*, *35*(5), 657–664. https://doi.org/10.1007/s10803-005-0009-6

Gould, T. D., & Gottesman, I. I. (2006). Psychiatric endophenotypes and the development of valid animal models. *Genes, Brain, and Behavior*, *5*(2), 113–119. https://doi.org/10.1111/j.1601-183X.2005.00186.x

Goren, C. C., Sarty, M., & Wu, P. Y. (1975). Visual following and pattern discrimination of face-like stimuli by newborn infants. *Pediatrics*, *56*(4), 544–549.

Gothard, K. M., Battaglia, F. P., Erickson, C. A., Spitler, K. M., & Amaral, D. G. (2007). Neural responses to facial expression and face identity in the monkey amygdala. *Journal of Neurophysiology*, *97*(2), 1671–1683. https://doi.org/10.1152/jn.00714.2006

Hadj-Bouziane, F., Bell, A. H., Knusten, T. A., Ungerleider, L. G., & Tootell, R. B. H. (2008). Perception of emotional expressions is independent of face selectivity in monkey inferior temporal cortex. *Proceedings of the National Academy of Sciences*, *105*(14), 5591–5596. https://doi.org/10.1073/pnas.0800489105

Haith, M. M., Bergman, T., & Moore, M. J. (1977). Eye contact and face scanning in early infancy. *Science (New York, N.Y.)*, *198*(4319), 853–855.

Harries, M. H., & Perrett, D. I. (1991). Visual processing of faces in temporal cortex: physiological evidence for a modular organization and possible anatomical correlates. *Journal of Cognitive Neuroscience*, *3*(1), 9–24. https://doi.org/10.1162/jocn.1991.3.1.9

Haxby, Hoffman, & Gobbini. (2000). The distributed human neural system for face perception. *Trends in Cognitive Sciences*, *4*(6), 223–233.

Haxby, J. V., Ungerleider, L. G., Clark, V. P., Schouten, J. L., Hoffman, E. A., & Martin, A. (1999). The effect of face inversion on activity in human neural systems for face and object perception. *Neuron*, *22*(1), 189–199.

Hobson, J. A., & Hobson, R. P. (2007). Identification: The missing link between joint attention and imitation? *Development and Psychopathology*, *19*(2). https://doi.org/10.1017/s0954579407070204

Hoffman, K. L., Gothard, K. M., Schmid, M. C., & Logothetis, N. K. (2007). Facial-expression and gaze-selective responses in the monkey amygdala. *Current Biology : CB*, *17*(9), 766–772. https://doi.org/10.1016/j.cub.2007.03.040

Hoffman, E. A., & Haxby, J. V. (2000). Distinct representations of eye gaze and identity in the distributed human neural system for face perception. *Nature Neuroscience*, *3*(1), 80–84. https://doi.org/10.1038/71152

Horn, G. (2004). Pathways of the past: the imprint of memory. *Nature Reviews Neuroscience*, *5*(2), 108–120. https://doi.org/10.1038/nrn1324

Howard, R. J., Brammer, M., Wright, I., Woodruff, P. W., Bullmore, E. T., & Zeki, S. (1996). A direct demonstration of functional specialization within motion-related visual and auditory cortex of the human brain. *Current Biology : CB*, *6*(8), 1015–1019.

Itakura, S. (2004). Gaze-following and joint visual attention in nonhuman animals. *Japanese Psychological Research*, *46*(3), 216–226. https://doi.org/10.1111/j.1468-5584.2004.00253.x

Johnson, M. H. (2001). Functional brain development in humans. *Nature Reviews. Neuroscience*, *2*(7), 475–483. https://doi.org/10.1038/35081509

Johnson, M. H. (2005). Subcortical face processing. *Nature Reviews. Neuroscience*, *6*(10), 766–774. https://doi.org/10.1038/nrn1766

Johnson, M. H., Dziurawiec, S., Ellis, H., & Morton, J. (1991). Newborns' preferential tracking of face-like stimuli and its subsequent decline. *Cognition*, *40*(1–2), 1–19.

Johnson, M. H., Senju, A., & Tomalski, P. (2015). The two-process theory of face processing: Modifications based on two decades of data from infants and adults. *Neuroscience & Biobehavioral Reviews*, *50*, 169–179. https://doi.org/10.1016/j.neubiorev.2014.10.009

Kanner, L. (1943). Autstic disturbances of affective contact. *Nervous Child*, *2*, 217,250.

Kanwisher, N., McDermott, J., & Chun, M. M. (1997). The fusiform face area: a module in human extrastriate cortex specialized for face perception. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(11), 4302–4311.

Kampe, K. K. W., Frith, C. D., Dolan, R. J., & Frith, U. (2001). Reward value of attractiveness and gaze. *Nature*, *413*(6856), 589. https://doi.org/10.1038/35098149

Kamps, F. S., Morris, E. J., & Dilks, D. D. (2019). A face is more than just the eyes, nose, and mouth: fMRI evidence that face-selective cortex represents external features. *NeuroImage*, *184*, 90–100. https://doi.org/10.1016/j.neuroimage.2018.09.027

Keightley, M. L., Winocur, G., Graham, S. J., Mayberg, H. S., Hevenor, S. J., & Grady, C. L. (2003). An fMRI study investigating cognitive modulation of brain regions associated with emotional processing of visual stimuli. *Neuropsychologia*, *41*(5), 585–596. https://doi.org/10.1016/s0028-3932(02)00199-9

Kingstone, A. (2009). Taking a real look at social attention. *Current Opinion in Neurobiology*, *19*(1), 52–56. https://doi.org/10.1016/j.conb.2009.05.004

Kleiner, K. A., & Banks, M. S. (1987). Stimulus energy does not account for 2-month-olds’ face preferences. *Journal of Experimental Psychology. Human Perception and Performance*, *13*(4), 594–600.

Kleinhans, N. M., Richards, T., Sterling, L., Stegbauer, K. C., Mahurin, R., Johnson, L. C., … Aylward, E. (2008). Abnormal functional connectivity in autism spectrum disorders during face processing. *Brain*, *131*(4), 1000–1012. https://doi.org/10.1093/brain/awm334

Klin, A., & Jones, W. (2008). Altered face scanning and impaired recognition of biological motion in a 15-month-old infant with autism. *Developmental Science*, *11*(1), 40,46. https://doi.org/10.1111/j.1467-7687.2007.00608.x

Klin, A., Shultz, S., & Jones, W. (2015). Social visual engagement in infants and toddlers with autism: Early developmental transitions and a model of pathogenesis. *Neuroscience & Biobehavioral Reviews*, *50*, 189–203. https://doi.org/10.1016/j.neubiorev.2014.10.006

Kawashima, R., Sugiura, M., Kato, T., Nakamura, A., Hatano, K., Ito, K., … Nakamura, K. (1999). The human amygdala plays an important role in gaze monitoring. A PET study. *Brain : A Journal of Neurology*, *122 (Pt 4)*, 779–783. https://doi.org/10.1093/brain/122.4.779

Kuwahata, H., Adachi, I., Fujita, K., Tomonaga, M., & Matsuzawa, T. (2004). Development of schematic face preference in macaque monkeys. *Behavioural Processes*, *66*(1), 17–21. https://doi.org/10.1016/j.beproc.2003.11.002

Landa, R., & Garrett-Mayer, E. (2006). Development in infants with autism spectrum disorders: a prospective study. *Journal of Child Psychology and Psychiatry*, *47*(6), 629–638. https://doi.org/10.1111/j.1469-7610.2006.01531.x

LeDoux, J. E. (1996). *The Emotional Brain*. New York, NY: Simon and Schuster.

Lee, H., & Kuhl, B. A. (2016). Reconstructing Perceived and Retrieved Faces from Activity Patterns in Lateral Parietal Cortex. *The Journal of Neuroscience*, *36*(22), 6069–6082. https://doi.org/10.1523/jneurosci.4286-15.2016

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

Livingstone, M. S., Vincent, J. L., Arcaro, M. J., Srihasam, K., Schade, P. F., & Savage, T. (2017). Development of the macaque face-patch system. *Nature Communications*, *8*, 14897. https://doi.org/10.1038/ncomms14897

Longa, L. D., Gliga, T., & Farroni, T. (2019). Tune to touch: Affective touch enhances learning of face identity in 4-month-old infants. *Developmental Cognitive Neuroscience*, *35*, 42–46. https://doi.org/10.1016/j.dcn.2017.11.002

Lord, C., Luyster, R., Guthrie, W., & Pickles, A. (2012). Patterns of developmental trajectories in toddlers with autism spectrum disorder. *Journal of Consulting and Clinical Psychology*, *80*(3), 477–489. https://doi.org/10.1037/a0027214

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Leventhal, B. L., DiLavore, P. C., … Rutter, M. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*, *30*(3), 205–223.

Lutz, C. K., Lockard, J. S., Gunderson, V. M., & Grant, K. S. (1998). Infant monkeys' visual responses to drawings of normal and distorted faces. *American Journal of Primatology*, *44*(2), 169–174. https://doi.org/10.1002/(sici)1098-2345(1998)44:2<169::aid-ajp7>3.0.co;2-u

Machado, C. J., & Bachevalier, J. (2003). Non-human primate models of childhood psychopathology: the promise and the limitations. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *44*(1), 64–87.

Maestripieri, D., & Wallen, K. (1997). Affiliative and submissive communication in rhesus macaques. *Primates*, *38*(2), 127–138. https://doi.org/10.1007/bf02382003

McKinstry, R. C., Miller, J. H., Snyder, A. Z., Mathur, A., Schefft, G. L., Almli, C. R., … Neil, J. J. (2002). A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology*, *59*(6), 824–833. https://doi.org/10.1212/wnl.59.6.824

Mehta, A. D., Ulbert, I., & Schroeder, C. E. (2000). Intermodal selective attention in monkeys. II: physiological mechanisms of modulation. *Cerebral Cortex (New York, N.Y. : 1991)*, *10*(4), 359–370. https://doi.org/10.1093/cercor/10.4.359

Morales, M., Mundy, P., Delgado, C. E. ., Yale, M., Neal, R., & Schwartz, H. K. (2000). Gaze following, temperament, and language development in 6-month-olds: A replication and extension. *Infant Behavior and Development*, *23*(2), 231–236. https://doi.org/10.1016/s0163-6383(01)00038-8

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2011). Videos of conspecifics elicit interactive looking patterns and facial expressions in monkeys. *Behavioral Neuroscience*, *125*(4), 639–652. https://doi.org/10.1037/a0024264

Miller, M., Iosif, A.-M., Hill, M., Young, G. S., Schwichtenberg, A. J., & Ozonoff, S. (2017). Response to Name in Infants Developing Autism Spectrum Disorder: A Prospective Study. *The Journal of Pediatrics*, *183*, 141–146. https://doi.org/10.1016/j.jpeds.2016.12.071

Mori, S., & van Zijl, P. C. M. (2002). Fiber tracking: principles and strategies - a technical review. *NMR in Biomedicine*, *15*(7–8), 468–480. https://doi.org/10.1002/nbm.781

Morris, J. S., DeGelder, B., Weiskrantz, L., & Dolan, R. J. (2001). Differential extrageniculostriate and amygdala responses to presentation of emotional faces in a cortically blind field. *Brain : A Journal of Neurology*, *124*(Pt 6), 1241–1252. https://doi.org/10.1093/brain/124.6.1241

Morton, J., & Johnson, M. H. (1991). CONSPEC and CONLERN: a two-process theory of infant face recognition. *Psychological Review*, *98*(2), 164–181.

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2010). Response characteristics of basolateral and centromedial neurons in the primate amygdala. *The Journal of*

Neuroscience : The Official Journal of the Society for Neuroscience, *30*(48), 16197–16207. https://doi.org/10.1523/JNEUROSCI.3225-10.2010

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2011). Videos of conspecifics elicit interactive looking patterns and facial expressions in monkeys. *Behavioral Neuroscience*, *125*(4), 639–652. https://doi.org/10.1037/a0024264

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2014). Neurons in the monkey amygdala detect eye contact during naturalistic social interactions. *Current Biology : CB*, *24*(20), 2459–2464. https://doi.org/10.1016/j.cub.2014.08.063

Mundy, P. C. (2016). *Autism and Joint Attention*. Guilford Publications. Retrieved from https://www.ebook.de/de/product/24980021/peter_c_mundy_autism_and_joint_attention.html

Mundy, P. (2017). A review of joint attention and social-cognitive brain systems in typical development and autism spectrum disorder. *European Journal of Neuroscience*, *47*(6), 497–514. https://doi.org/10.1111/ejn.13720

Mundy, P., Fox, N., & Card, J. (2003). EEG coherence, joint attention and language development in the second year. *Developmental Science*, *6*(1), 48–54. https://doi.org/10.1111/1467-7687.00253

Mundy, P., Sullivan, L., & Mastergeorge, A. M. (2009). A parallel and distributed-processing model of joint attention, social cognition and autism. *Autism Research*, *2*(1), 2–21. https://doi.org/10.1002/aur.61

Murray, D. S., Creaghead, N. A., Manning-Courtney, P., Shear, P. K., Bean, J., & Prendeville, J.-A. (2008). The Relationship Between Joint Attention and Language in Children With Autism Spectrum Disorders. *Focus on Autism and Other Developmental Disabilities*, *23*(1), 5–14. https://doi.org/10.1177/1088357607311443

Muschinski, J., Feczko, E., Brooks, J. M., Collantes, M., Heitz, T. R., & Parr, L. A. (2016). The development of visual preferences for direct versus averted gaze faces in infant macaques (Macaca mulatta). *Developmental Psychobiology*, *58*(8), 926–936. https://doi.org/10.1002/dev.21421

Nadig, A., & Mulligan, A. (2017). Intact non-word repetition and similar error patterns in language-matched children with autism spectrum disorders: A pilot study. *Journal of Communication Disorders*, *66*, 13–21. https://doi.org/10.1016/j.jcomdis.2017.03.003

Nelson, C. A. (2001). The development and neural bases of face recognition. *Infant and Child Development*, *10*(1–2), 3–18. https://doi.org/10.1002/icd.239

Neville, H. J., Bavelier, D., Corina, D., Rauschecker, J., Karni, A., Lalwani, A., … Turner, R. (1998). Cerebral organization for language in deaf and hearing subjects: Biological constraints and effects of experience. *Proceedings of the National Academy of Sciences*, *95*(3), 922–929. https://doi.org/10.1073/pnas.95.3.922

Okada, T., Sato, W., Kubota, Y., Usui, K., Inoue, Y., Murai, T., … Toichi, M. (2008). Involvement of medial temporal structures in reflexive attentional shift by gaze. *Social Cognitive and Affective Neuroscience*, *3*(1), 80–88. https://doi.org/10.1093/scan/nsm027

O’Toole, A. J., Roark, D. A., & Abdi, H. (2002). Recognizing moving faces: a psychological and neural synthesis. *Trends in Cognitive Sciences*, *6*(6), 261–266. https://doi.org/10.1016/s1364-6613(02)01908-3

Parr, L. A., Murphy, L., Feczko, E., Brooks, J., Collantes, M., & Heitz, T. R. (2016). Experience-dependent changes in the development of face preferences in infant rhesus monkeys. *Developmental Psychobiology*, *58*(8), 1002–1018. https://doi.org/10.1002/dev.21434

Pelphrey, K. A., Morris, J. P., Michelich, C. R., Allison, T., & McCarthy, G. (2005). Functional anatomy of biological motion perception in posterior temporal cortex: an FMRI study of eye, mouth and hand movements. *Cerebral Cortex (New York, N.Y. : 1991)*, *15*(12), 1866–1876. https://doi.org/10.1093/cercor/bhi064

Perret, D. I., & Emery, N. J. (1994). Understanding the intentions of others from visual signals: Neurophysiological evidence. *Cahiers de Psychologie Cognitive/Current Psychology of Cognition*, *13*(5), 683–694.

Perrett, D. I., Hietanen, J. K., Oram, M. W., & Benson, P. J. (1992). Organization and functions of cells responsive to faces in the temporal cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *335*(1273), 23–30. https://doi.org/10.1098/rstb.1992.0003

Perrett, D. I., & Mistlin, A. J. (1990). Wiley series in neuroscience, Vol. 2. Comparative perception, Vol. 2. Complex signals. In W. C. Stebbins & M. A. Berkley, W. C. Stebbins & M. A. Berkley (Eds.) (pp. 187–215). Oxford, England: John Wiley and Sons.

Perrett, D. I., Smith, P. A., Potter, D. D., Mistlin, A. J., Head, A. S., Milner, A. D., & Jeeves, M. A. (1985). Visual cells in the temporal cortex sensitive to face view and gaze direction. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *223*(1232), 293–317. https://doi.org/10.1098/rspb.1985.0003

Pessoa, L., & Adolphs, R. (2010). Emotion processing and the amygdala: from a ’low road’ to ’many roads’ of evaluating biological significance. *Nature Reviews. Neuroscience*, *11*(11), 773–783. https://doi.org/10.1038/nrn2920

Pierce, K., Marinero, S., Hazin, R., McKenna, B., Barnes, C. C., & Malige, A. (2016). Eye Tracking Reveals Abnormal Visual Preference for Geometric Images as an Early Biomarker of an Autism Spectrum Disorder Subtype Associated With Increased Symptom Severity. *Biological Psychiatry*, *79*(8), 657–666. https://doi.org/10.1016/j.biopsych.2015.03.032

Pinsk, M. A., DeSimone, K., Moore, T., Gross, C. G., & Kastner, S. (2005). Representations of faces and body parts in macaque temporal cortex: a functional MRI study. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(19), 6996–7001. https://doi.org/10.1073/pnas.0502605102

Powell, L. J., Kosakowski, H. L., & Saxe, R. (2018). Social Origins of Cortical Face Areas. *Trends in Cognitive Sciences*, *22*(9), 752–763. https://doi.org/10.1016/j.tics.2018.06.009

Puce, A., Allison, T., & McCarthy, G. (1999). Electrophysiological studies of human face perception. III: Effects of top-down processing on face-specific potentials. *Cerebral Cortex (New York, N.Y. : 1991)*, *9*(5), 445–458. https://doi.org/10.1093/cercor/9.5.445

Puce, A., Smith, A., & Allison, T. (2000). ERPS evoked by viewing facial movements. *Cognitive Neuropsychology*, *17*(1–3), 221–239. https://doi.org/10.1080/026432900380580

Raper, J., Wallen, K., Sanchez, M. M., Stephens, S. B. Z., Henry, A., Villareal, T., & Bachevalier, J. (2013). Sex-dependent role of the amygdala in the development of emotional and neuroendocrine reactivity to threatening stimuli in infant and juvenile rhesus monkeys. *Hormones and Behavior*, *63*(4), 646–658. https://doi.org/10.1016/j.yhbeh.2013.01.010

Riby, D. M., Hancock, P. J., Jones, N., & Hanley, M. (2013). Spontaneous and cued gaze-following in autism and Williams syndrome. *Journal of Neurodevelopmental Disorders*, *5*(1), 13. https://doi.org/10.1186/1866-1955-5-13

Rodman, H. R., & Consuelos, M. J. (1994). Cortical projections to anterior inferior temporal cortex in infant macaque monkeys. *Visual Neuroscience*, *11*(1), 119–133.

Rodman, H. R., Scalaidhe, S. P., & Gross, C. G. (1993). Response properties of neurons in temporal cortical visual areas of infant monkeys. *Journal of Neurophysiology*, *70*(3), 1115–1136. https://doi.org/10.1152/jn.1993.70.3.1115

Rodman, H. R., Skelly, J. P., & Gross, C. G. (1991). Stimulus selectivity and state dependence of activity in inferior temporal cortex of infant monkeys. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(17), 7572–7575. https://doi.org/10.1073/pnas.88.17.7572

Rosati, A. G., Arre, A. M., Platt, M. L., & Santos, L. R. (2016). Rhesus monkeys show human-like changes in gaze following across the lifespan. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1830), 20160376. https://doi.org/10.1098/rspb.2016.0376

Roy, A., Shepherd, S. V., & Platt, M. L. (2012). Reversible inactivation of pSTS suppresses social gaze following in the macaque (Macaca mulatta). *Social Cognitive and Affective Neuroscience*, *9*(2), 209–217. https://doi.org/10.1093/scan/nss123

Sato, W., Kochiyama, T., Uono, S., Matsuda, K., Usui, K., Inoue, Y., & Toichi, M. (2011). Rapid amygdala gamma oscillations in response to fearful facial expressions. *Neuropsychologia*, *49*(4), 612–617. https://doi.org/10.1016/j.neuropsychologia.2010.12.025

Sato, W., Toichi, M., Uono, S., & Kochiyama, T. (2012). Impaired social brain network for processing dynamic facial expressions in autism spectrum disorders. *BMC Neuroscience*, *13*(1). https://doi.org/10.1186/1471-2202-13-99

Senju, A., & Johnson, M. H. (2009). The eye contact effect: mechanisms and development. *Trends in Cognitive Sciences*, *13*(3), 127–134. https://doi.org/10.1016/j.tics.2008.11.009

Sereno, M. I., & Huang, R.-S. (2006). A human parietal face area contains aligned head-centered visual and tactile maps. *Nature Neuroscience*, *9*(10), 1337–1343. https://doi.org/10.1038/nn1777

Schall, J. D. (2009). Frontal Eye Fields. In *Encyclopedia of Neuroscience* (pp. 1635–1638). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-29678-2_1861

Shepherd, S. V., & Platt, M. L. (2007). Spontaneous social orienting and gaze following in ringtailed lemurs (Lemur catta). *Animal Cognition*, *11*(1), 13–20. https://doi.org/10.1007/s10071-007-0083-6

Schultz, R. T. (2005). Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. *International Journal of Developmental Neuroscience*, *23*(2–3), 125–141. https://doi.org/10.1016/j.ijdevneu.2004.12.012

Simion, F., Regolin, L., & Bulf, H. (2008). A predisposition for biological motion in the newborn baby. *Proceedings of the National Academy of Sciences*, *105*(2), 809–813. https://doi.org/10.1073/pnas.0707021105

Simpson, E. A., Paukner, A., Sclafani, V., Kaburu, S. S. K., Suomi, S. J., & Ferrari, P. F. (2016). Acute oxytocin improves memory and gaze following in male but not female nursery-reared infant macaques. *Psychopharmacology*, *234*(3), 497–506. https://doi.org/10.1007/s00213-016-4480-x

Simpson, E. A., Varga, K., Frick, J. E., & Fragaszy, D. (2010). Infants Experience Perceptual Narrowing for Nonprimate Faces. *Infancy*, *16*(3), 318–328. https://doi.org/10.1111/j.1532-7078.2010.00052.x

Skuse, D., Morris, J., & Lawrence, K. (2003). The amygdala and development of the social brain. *Annals of the New York Academy of Sciences*, *1008*, 91–101.

Sugita, Y. (2008). Face perception in monkeys reared with no exposure to faces. *Proceedings of the National Academy of Sciences*, *105*(1), 394–398. https://doi.org/10.1073/pnas.0706079105

Sullivan, M., Finelli, J., Marvin, A., Garrett-Mayer, E., Bauman, M., & Landa, R. (2007). Response to Joint Attention in Toddlers at Risk for Autism Spectrum Disorder: A Prospective Study. *Journal of Autism and Developmental Disorders*, *37*(1), 37–48. https://doi.org/10.1007/s10803-006-0335-3

Tamietto, M., & de Gelder, B. (2010). Neural bases of the non-conscious perception of emotional signals. *Nature Reviews. Neuroscience*, *11*(10), 697–709. https://doi.org/10.1038/nrn2889

Tenenbaum, E. J., Sobel, D. M., Sheinkopf, S. J., Malle, B. F., & Morgan, J. L. (2014). Attention to the mouth and gaze following in infancy predict language development. *Journal of Child Language*, *42*(6), 1173–1190. https://doi.org/10.1017/s0305000914000725

Teufel, C., Gutmann, A., Pirow, R., & Fischer, J. (2010). Facial expressions modulate the ontogenetic trajectory of gaze-following among monkeys. *Developmental Science*, *13*(6), 913–922. https://doi.org/10.1111/j.1467-7687.2010.00956.x

Thorup, E., Nyström, P., Gredebäck, G., Bölte, S., & Falck-Ytter, T. (2016). Altered gaze following during live interaction in infants at risk for autism: an eye tracking study. *Molecular Autism*, *7*(1). https://doi.org/10.1186/s13229-016-0069-9

Tootell, R. B. H., Tsao, D., & Vanduffel, W. (2003). Neuroimaging Weighs In: Humans Meet Macaques in Primate Visual Cortex. *The Journal of Neuroscience*, *23*(10), 3981–3989. https://doi.org/10.1523/jneurosci.23-10-03981.2003

Tsao, D. Y., Freiwald, W. A., Knutsen, T. A., Mandeville, J. B., & Tootell, R. B. H. (2003). Faces and objects in macaque cerebral cortex. *Nature Neuroscience*, *6*(9), 989–995. https://doi.org/10.1038/nn1111

Tsao, D. Y., Freiwald, W. A., Tootell, R. B. H., & Livingstone, M. S. (2006). A cortical region consisting entirely of face-selective cells. *Science (New York, N.Y.)*, *311*(5761), 670–674. https://doi.org/10.1126/science.1119983

Tsao, D. Y., & Livingstone, M. S. (2008). Mechanisms of face perception. *Annual Review of Neuroscience*, *31*, 411–437. https://doi.org/10.1146/annurev.neuro.30.051606.094238

Tsuchiya, N., Moradi, F., Felsen, C., Yamazaki, M., & Adolphs, R. (2009). Intact rapid detection of fearful faces in the absence of the amygdala. *Nature Neuroscience*, *12*(10), 1224–1225. https://doi.org/10.1038/nn.2380

Tomasello, M., Hare, B., & Fogleman, T. (2001). The ontogeny of gaze following in chimpanzees, Pan troglodytes, and rhesus macaques, Macaca mulatta. *Animal Behaviour*, *61*(2), 335–343. https://doi.org/10.1006/anbe.2000.1598

Ungerleider, L. G., & Mishkin, M. (1982). Analysis of Visual Behavior. In D. J. Ingle, M. A. Goodale, & R. J. . Mansfield, D. J. Ingle, M. A. Goodale, & R. J. . Mansfield (Eds.) (pp. 549–586). Cambridge, MA: MIT press.

Van Essen, D. C., Lewis, J. W., Drury, H. A., Hadjikhani, N., Tootell, R. B. ., Bakircioglu, M., & Miller, M. I. (2001). Mapping visual cortex in monkeys and humans using surface-based atlases. *Vision Research*, *41*(10–11), 1359–1378. https://doi.org/10.1016/s0042-6989(01)00045-1

Wang, Y., Ramsey, R., & de C. Hamilton, A. F. (2011). The Control of Mimicry by Eye Contact Is Mediated by Medial Prefrontal Cortex. *Journal of Neuroscience*, *31*(33), 12001–12010. https://doi.org/10.1523/jneurosci.0845-11.2011

Watson, K. K., & Platt, M. L. (2012). Social Signals in Primate Orbitofrontal Cortex. *Current Biology*, *22*(23), 2268–2273. https://doi.org/10.1016/j.cub.2012.10.016

Webster, M. A., & Mollon, J. D. (1991). Changes in colour appearance following post-receptoral adaptation. *Nature*, *349*(6306), 235–238. https://doi.org/10.1038/349235a0

Webster, M. A., & Mollon, J. D. (1994). The influence of contrast adaptation on color appearance. *Vision Research*, *34*(15), 1993–2020. https://doi.org/10.1016/0042-6989(94)90028-0

Wetherby, A. M., Woods, J., Allen, L., Cleary, J., Dickinson, H., & Lord, C. (2004). Early indicators of autism spectrum disorders in the second year of life. *Journal of Autism and Developmental Disorders*, *34*(5), 473–493.

Wilkinson, N., Paikan, A., Gredebäck, G., Rea, F., & Metta, G. (2014). Staring us in the face? An embodied theory of innate face preference. *Developmental Science*, *17*(6), 809–825. https://doi.org/10.1111/desc.12159

Yoder, P., Stone, W. L., Walden, T., & Malesa, E. (2009). Predicting Social Impairment and ASD Diagnosis in Younger Siblings of Children with Autism Spectrum Disorder.

Journal of Autism and Developmental Disorders, *39*(10), 1381–1391. https://doi.org/10.1007/s10803-009-0753-0

Young, A. W., Aggleton, J. P., Hellawell, D. J., Johnson, M., Broks, P., & Hanley, J. R. (1995). Face processing impairments after amygdalotomy. *Brain : A Journal of Neurology*, *118 (Pt 1)*, 15–24. https://doi.org/10.1093/brain/118.1.15

Zhang, J., Miller, M. I., Plachez, C., Richards, L. J., Yarowsky, P., van Zijl, P., & Mori, S. (2005). Mapping postnatal mouse brain development with diffusion tensor microimaging. *NeuroImage*, *26*(4), 1042–1051. https://doi.org/10.1016/j.neuroimage.2005.03.009

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., & Szatmari, P. (2005). Behavioral manifestations of autism in the first year of life. *International Journal of Developmental Neuroscience*, *23*(2–3), 143–152. https://doi.org/10.1016/j.ijdevneu.2004.05.001

Zwaigenbaum, L., Bryson, S., Lord, C., Rogers, S., Carter, A., Carver, L., … Yirmiya, N. (2009). Clinical Assessment and Management of Toddlers With Suspected Autism Spectrum Disorder: Insights From Studies of High-Risk Infants. *PEDIATRICS*, *123*(5), 1383–1391. https://doi.org/10.1542/peds.2008-1606

EARLY DEVELOPMENTAL CHANGES IN VISUAL SOCIAL ENGAGEMENT IN INFANT RHESUS MONKEYS

***ARICK WANG**[1,2], CHRISTA PAYNE[1], SHANNON MOSS[1], WARREN JONES[3],
JOCELYNE BACHEVALIER[1,2]

[1]Yerkes National. Primate Research Ctr., Emory Univ., Atlanta, GA, 30329;

[2]Dept. of Psychology, Emory Univ., Atlanta, GA, 30322;

[3]Dept. of Pediatrics, Emory Univ., Atlanta, GA, 30322;

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Abstract

Although impairments in social interaction in Autism Spectrum Disorder (ASD) differ greatly across individuals and vary throughout an individual's lifetime, an important marker of ASD in infancy is deviations in social-visual engagement such as the reliably detectable early deviations in attention to the eyes or to biological movement by (Klin, Shultz, and Jones, 2015). Given the critical nature of these early developmental periods, understanding its neurobehavioral underpinnings by means of a nonhuman primate model will be instrumental to understanding the pathophysiology of ASD. Like humans, rhesus macaques 1) develop in rich and complex social behaviors, 2) progressively develop social skills throughout infancy, and 3) have high similarities with humans in brain anatomy and cognitive functions (Machado and Bachevalier, 2003). In this study, infant rhesus macaques living with their mothers in complex social groups were eye-tracked longitudinally from birth to 6 months while viewing full-faced videos of unfamiliar rhesus monkeys. The results indicated a critical period for the refinement of social skills around 4-8 weeks of age in rhesus macaques. Specifically, infant monkeys' fixation on eyes shows an inflection in developmental trajectory, increasing from birth to 8 weeks, decreasing slowly to a trough between 14-18 weeks, before increasing again. These results parallel the developmental trajectory published in human infants (Jones & Klin, 2013) and suggest the presence of a switch in the critical networks supporting these early developing social skills that is highly conserved between rhesus macaque and human infant development.

Keywords

Macaque, Infant Development, Social Visual Engagement

Social-visual attention, defined as directing visual attention to socially relevant information, such as looking at other people’s faces, in particular the eyes, and attention to gestures, body posture, or action, is a fundamental ability of all primate species and considered the basis for social cognition (Baron-Cohen et al., 1997; Emery, 2000). As a visually orienting ability in socially gregarious species, social-visual attention is considered a primary selective pressure in the evolution of the primate brain (Chance & Jolly, 1970; Dunbar & Shultz, 2007). On a very fundamental level, social-visual attention is critical for understanding and interpreting social contingencies and intention of others, and it allows for the flexible modification of behavior based on that information. As an example, rhesus macaques evaluate information regarding dominance through the observation of social interactions and then adjust their own aggressive and affiliative behavior accordingly (Deaner, Khera & Platt, 2005), which is essential for survival. In humans, social information includes using a variety of nonverbal cues, such as eye contact or gestures that are often significant to establish joint attention in children and adults and for functioning in society (Emery, 2000). Using the gaze of another to alter one’s own landscape of saliency is a basic tenet for understanding more complex social contingencies that may emerge later in ontogeny or phylogeny, such as theory of mind (Baron-Cohen, 1992). In studies with human adults, social-visual attention is known to engage extensive areas of the human social and attentional networks including the amygdala, lateral intraparietal cortex, medial prefrontal cortex, and superior temporal sulcus (Sato et al., 2011; Kampe et al., 2001; Wang et al., 2011; Akiyama et al., 2007; Okada et al., 2008). Electrophysiology, inactivation, and fMRI studies in adult rhesus macaques have also implicated the amygdala, superior temporal sulcus, and lateral intraparietal cortex when monkeys monitor the gaze of another individuals demonstrating a conservation in the neural substrates of social-visual attention (Mosher et al., 2011; Kamphuis et al., 2009; Roy et al., 2014). Unfortunately, the neural basis of social-visual engagement in infancy remains unexplored. Due to the difficulty of acquiring frequent neuroimaging data in human infants over a short period of time, we must rely on the use of animal models to better characterize the development of social-visual engagement and its neural underpinning. Such information may be invaluable to better understand the neural origins of developmental disorders associated with profound inability to develop normal social skills, such as Autism Spectrum Disorders.

Rhesus macaques are an ideal model for studying the development of early social-visual behavior given (1) the rich and complex social structure in which they develop and navigate, (2) the similarity with humans in brain and cognitive functions development, and (3) the visual system is the primary method by which monkeys navigate the world (Machado & Bachevalier, 2003). Kuwahata and colleagues (2004) found that infants reliably preferred face-like schematic configurations over any other pattern by 1 month of age. Similarly, Lutz and colleagues (1998) determined that a preference for face-like drawings over distorted pen drawings of rhesus macaque faces emerged at 6 weeks of age. Although this preference seems to develop after birth, there is evidence to suggest an innate mechanism for face detection and preference. Specifically, it has been noted that infant monkeys that are reared without exposure to any faces for 6-24 months will quickly develop a facial preference to the first face or face-like stimuli they regularly witness (Sugita, 2008). Face scanning behavior continues to develop in the first 3 months of life. Parr and colleagues (2016) showed an initial strong preference for conspecific faces over heterospecific, whereas attention to heterospecific faces would significantly increase at around 5 weeks of age. Another study conducted with the same animals also indicated that infants develop a preference for direct gaze that emerges in the first 3 months, and that viewing patterns towards direct faces versus indirect faces showed different developmental timelines. That is, infant monkeys’ fixations to the eyes were first equivalent in duration for both gaze types in the first 2 months, but thereafter remained longer for the averted gaze faces (Muschinski et al., 2016).

In adulthood, gaze is crucial for survival within large social groups and is used to recruit allies or assert dominance (Maestripieri & Wallen, 1997; Emory, 2000). Currently, there are few studies that have explored the behavioral development of eye-looking in infancy for rhesus macaques and those that had significant limitations. Some studies used still drawing representations of faces (Lutz et al., 1998) rather than dynamic conspecific videos, which can contain more nuanced social signals to elicit looking behavior. Others were limited by poor temporal resolution, such that only 3 time points were studied in the first year (Mendelson, Haith, & Goldman-Rakic, 1982), which may not accurately capture development during a time with rapid brain development. Given the importance of interpreting gaze in both humans and nonhuman primates and the progressive development of social skills required for normal social interactions, eye-looking represents an important foundation of social cognition. Here, we precisely characterized the progressive development of social visual attention in infant rhesus macaques while viewing videos of other unfamiliar monkeys across the first 6 months of life. The study was carefully designed to resolve several limitations of previous developmental studies in monkeys. First, a large sample size of 36 infant monkeys living with their mothers in large social groups served as subjects. Second, fine grain developmental measures of social-visual attention were taken at 14 time points between postnatal Week 2 to Week 24 (6 months). Third, social-visual attention to faces was measured with a paradigm that was originally intended to mimic an eye-tracking procedure used to trace the development of social-visual attention in human infants (Jones & Klin, 2013). The similarities in procedural design were intended to assess whether social-visual attention is a phylogenetically conserved mechanisms across primate species and to develop a rhesus macaque model that will allow us to investigate the neural bases of social-visual attention in primates.

Methods

Subjects

Two cohorts of infant rhesus monkeys were tested during the 2015 and 2016 birthing seasons. A total of 36 newborn male monkeys (*Macaca mulatta*), all living with their mothers in large, socially complex groups at Yerkes National Primate Research Center (YNPRC) Field Station (Lawrenceville, GA) at Emory were assigned to the study. Both mother and infant lived in large social groups containing over 100 individuals to preserve critical social aspects of native rhesus monkey groups while allowing experimental control typical of less complex social conditions (Berard, 1989; Lovejoy & Wallen, 1988; Wallen, 2005). All infant-mother pairs remained socially housed within their original social groups for the duration of the study and infants were estimated full-term (>450g) offspring of mid-ranking multiparous mothers. While the goal of the study was to begin testing as soon after birth as possible, we also wanted to assure that bonding between mothers and infants was not impacted by the procedures, so each mother-infant pair had at least 3 days after birth prior to the first testing session. Infants were always tested with their mothers and both were immediately returned to their habitats after testing to continue bonding. Infants did not participate in the study if (1) health complications necessitated regular veterinary care and removal from their social group, (2) required to be separated from their mother that did not begin naturally lactating, or (3) were rejected by their mother shortly after birth and did not receive proper maternal care. The greatest drop-off in subjects (N = 9) occurred in the first month of life, and a total of 23 infants were tested at all time points of the study. For a summary of testing age distribution and averaged number of videos viewed at each age, see Table 1.

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University and followed the American Psychological Association standards for the ethical treatment of animals.

Eye-Tracking Procedures

Animals were tested at weeks 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 with previously established procedures that allow direct access to animals from their social groups for short periods of time (Herman et al., 2000; Maestripieri et al., 2006; McCormack et al, 2009). The mother-infant pairs were transferred to an onsite behavioral testing facility where the mother was first anesthetized (3-5 mg/kg^{-1} telazol, i.m.). The pair was then carried to a dedicated testing chamber containing a reclining seat on which the mother could rest comfortably on her back while the infant was placed on her front, ventrum-ventrum. Attached to one wall of the testing chamber was a 19” (62.6 cm, 45.27° diagonal) computer monitor (1024 x 768 pixels) where experimental stimuli were presented. Underneath the monitor was an infrared eye-tracking camera (http://www.iscan.com/ 60 Hz) mounted on a motorized gimbal, which allowed an experimenter to track the location of the infant’s eye. Following a 5-point calibration procedure, infants continued to watch videos of social rhesus macaques while freely nursing and maintaining contact with the mother (*see details in* Parr et al., 2016; Muschinski et al., 2016). In between videoclips, a centering stimulus was presented to measure any changes in calibration accuracy that may have occurred due to movement shifts in infant or mother position. If any shifts occurred, the 5 calibration points were repeated to ensure reliable and accurate calibration throughout the testing session.

Testing sessions were restricted to 30 min to limit the mother's time under anesthesia. After testing, the mother-infant pair was placed in holding cage in an adjacent room to allow for recovery of the mother from anesthesia. When the mother was fully alert, the pair was returned to their social group. Infants remained unconstrained and were free to move around, though infant was placed back onto the mother's ventrum when beginning to leave the mother and to explore the box enclosure.

Stimuli

High quality digital videos depicting unfamiliar conspecifics filmed on the rhesus monkey breeding colony maintained by the Caribbean Primate Research Center (CPRC) in Cayo Santiago, Puerto Rico were used. The videos were cropped to 10 seconds in length and played with accompanying sound. Close-up videos of both female and male monkeys from a range of ages were shown (see Figure 1a). Videos showed scenes of a single monkey, with approximately equal representation of female and male, and juvenile and adult monkeys. Juvenile monkeys showed the animal's full body; in size, each animal covered approximately 40% of space the screen video surface. Adult monkeys showed the animal's torso, arms, and head, and covered approximately 50% of on screen video surface. Videos were selected to present neutral emotional facial expressions to avoid emotional reactions from the infant and maintain a stable recording of the infant eyes; a total of 38 unique stimuli were created in this process. In between each video a 2 second inter-trial interval with a centering stimulus (circular, chiming target on an otherwise blank screen) was presented to maintain infant attention. For each testing session, a pseudorandom combination of repeated and novel stimuli was presented with an approximate 4:1 ratio, totaling 12 stimuli per session. If the infant was not attending to the screen during a stimulus, it would be marked and repeated after the end of all stimuli had been presented. This was repeated until the end of the testing session (for summary of videos average viewed see Table 1). A subset of stimuli (7 out of 38) were inverted to serve as comparison controls for changes in low-level perceptual effects and in perception, as inverted faces have been established to have different behavioral and neural responses when compared to upright faces in both humans and monkeys (Parr et al., 1999; Yin, 1969; Yovel & Kanwisher, 2005).

Data Analysis

Analysis of eye movements and coding of fixation data were performed with software written in MATLAB (MathWorks). The first phase of analysis was an automated identification of non-fixation data, comprising blinks, saccades and fixations directed away from the presented screen. Saccades were identified by eye velocity using threshold of 30°/s. Eye movements identified as fixations were coded into four regions of interest that were defined within each frame of all video stimuli: eyes, mouth, head, and body (see Fig. 1b). The regions of interest were hand traced for all frames of the video and were stored as binary bitmaps (through software written in MATLAB, see Figure 1b). Automated coding of fixation time to each region of interest then consisted of a numerical comparison of each infant's coordinate fixation data with the bitmapped regions of interest (Jones & Klin, 2013). A fixation percentage for each region of interest was calculated by comparing the fixations for the particular region to the total number of recorded fixations for the entire clip. An example of scan paths is illustrated on Figure 1C.

Statistical analyses were performed with R software, version 3.5.0 (Free Software Foundation, Inc., Boston, MA, U.S.A.). Clips that had 0 fixations recorded were excluded. Locally weighted polynomial regression was conducted using a LOESS model (Cleveland, 1979; Cleveland & Devlin, 1988) to evaluate the relationship between age and fixation percentage to each region of interest. From the LOESS model, several polynomial regressions of varying degrees were then overlaid and goodness-of-fit (GOF) was evaluated with residual standard error, the polynomial regression that produced the best GOF was used as the developmental trajectory. To determine that any polynomial regression was different from chance, a model based moving block bootstrap was conducted (Politis & Romano, 1994), repeated 5000 times using 6 blocks per run, and a pseudorandomized length per block. A polynomial model regression of the previously determined degree (i.e., degree=3 for eyes) was generated and from the regression was returned from each run. A histogram of these p-values was created and showed a relatively flat distribution with a tail at $p<0.05$ (see Figure 2). Therefore, we are confident in our regressions that resulted in $p < 0.01$.

Inverted videos were binned into three 2-month periods and a within-subject ANOVA was performed between age group and clip type to determine any differences between viewing upright and inverted faces.

Results

Attention to faces: A LOESS model between percentage of fixation to the eye-region and the age in weeks of the monkey was first conducted to estimate the shape of the data trajectory. From the regression, several degrees of polynomial regressions were fit, the third-degree polynomial provided the strongest GOF out of all models tested [$t(691)=0.18$, $F(3, 691)=4.203$, $p=0.005$]. As shown in Figure 3a, the developmental trajectory for attention to the eye-region shows an increase from 2 weeks until approximately 6 weeks. The attention then decreases to reach a trough at about 15 weeks when it begins to shift again and rise until 22 weeks. Additionally, analyses were performed on each annual cohort separately (2015, n = 12, and 2016, n = 21) and showed no differences between cohorts, indicating a high degree of overlap in their trajectories and providing proof of internal reliability and reproducibility (see Figure 4). Finally, analysis of the eye-region between inverted and upright faces showed a significantly less attention to the eyes viewing inverted faces (F(1, 1146)=4.854, p=0.028), verifying that the differences we see are due specifically to changes in face processing and not lower level perceptual features.

In contrast to the eye region, the trajectory of fixation percentage to both the mouth and body regions started with the highest points in the first few weeks and gradually decreased over time. The LOESS models more closely resembled a second-degree polynomial and fitting several polynomial regressions of varying degrees showed a second-degree polynomial provided the strongest GOF for both mouth and body regions. For the mouth region (see Fig. 3b), the polynomial provided a strong GOF [t(530)=0.03, F(2, 530)=7.318, p=0.0007], with an inflection point of around 15 weeks when attention to the mouth region begins to increase slightly. Additionally, analysis of inverted clips showed significantly increased viewing to the mouths of inverted faces (F(1,1144)=16.079, p<0.0001); this could be due to the movement present in the mouth region and when face processing is disrupted through the inversion, movement becomes the most salient area of the video.

The body region polynomial fit also provided a strong GOF [t(842)=0.02, F(2, 842)=8.897, p=0.0002], with an inflection point of close to 17 weeks when attention begins to increase slightly again (Fig. 3c). The difference in trajectory between the eyes to the body and mouth regions suggests that the third-degree polynomial is unique to the eye-region and not an overall shift in attentional patterns. For a summary of polynomial fitting for each region, see Table 2.

Discussion

This study assessed the developmental trajectory of visual attention to social stimuli in monkeys during the first 24 weeks of life using eye-tracking procedures and stimuli similar to those previously reported in human infants. The results indicate 1) important and specific shifts in attentional looking patterns to the eyes, mouth and body; 2) strong preference for the eyes in upright versus inverted faces in the first 8-12 weeks of age; and 3) these patterns of visual attention were similar to those reported in human infants for the eyes but not for the mouth. These results will be discussed in turn.

Attention to the eyes:

The findings indicated a sharp increase in attention to the eyes that reached a peak around 5-6 weeks of age, followed by a decline that reached a trough around 16 weeks before a continuous rise thereafter until the last age point assessed (i.e. 24 weeks). This developmental trajectory is in line with prior research indicating that infant macaques become sensitive to the direction of other faces at around 3-4 weeks, suggesting a time period when more elaborate forms of face processing begin to come online during the first few weeks of life (Mendelson et al., 1982). By 5-6 weeks, the infants are showing an increased preference for direct-gaze faces over averted-gaze faces (Muschinski et al., 2016). Additionally, this time period coincides with a shift from strong preferential orientation to conspecifics’ faces to heterospecifics’ faces~~.~~ (Parr et al., 2016).

Field studies in rhesus macaques have also shown that reciprocal face-face interactions between the infant and mother begin to dramatically increase and peak at close to 1 month (Ferrari et al., 2009), overlapping with our first inflection point in attention to the eyes. Thus, the development of critical mother-infant bonds that are being formed from birth to 5-6 weeks of age may also be based on the ability to focus attention to the eye-region of conspecifics and be dependent upon the prior maturation of more elaborate perceptual processing of face areas (see further discussion below). One possible explanation to the increased attention to the eyes could also be related to perceptual development, as visual acuity significantly increased in the first weeks in infant monkeys and does not reach adult-like levels until 6 weeks (Boothe et al., 1980). However, this explanation seems unlikely given that, despite weaker visual acuity than adults, infant visual abilities are proficient enough as they are able to clearly discriminate between conspecific and heterospecific faces at birth (Parr et al., 2016).

Following this peak, attention to the eyes progressively declines to reach a trough around 15-16 weeks. This decrease parallels a similar decrease in mother-infant mutual gaze as well as in face-direction preference observed by others (Ferrari et al., 2009; Muschinski et al., 2016). It also appears to follow the emergence of fear modulation with regard to different contexts during the same period (Kalin et al., 1991). Given that direct eye-contact can be an emotionally aversive stimuli for rhesus macaques, this reduction in attention to the eye-region may be due to the development and maturation of emotional-affective processes during infancy.

From 16 to 24 weeks, attention to the eyes begin to increase again. Field studies have indicated that this time period is characterized by an increase in infant exploration as they begin to seek social interactions with family members (siblings and aunts) and leave the proximity of their mothers (Hinde & Spencer-Booth, 1967; Rowell et al., 1964). Thus, following the maturation of emotional regulation, infants may begin to reestablish attention to the eye region at this time in order to identify familiarity and determine if it is safe or not to approach the other monkey while exploring.

It is also possible that changes in attention could be driven by low-level perceptual features, such as face-like configurations. Studies in both humans and monkeys have shown newborn innate attraction to face-like configurations of dots over other patterns (Goren et al., 1975; Valenza et al., 1996; Kuwahata et al., 2004). However, attention to the eye-region of inverted faces were significantly different from upright faces. Yet, since inverted faces maintain the same low-level perceptual information of the videos but disrupts face processing (Parr et al., 1999; Yin, 1969; Yovel & Kanwisher, 2005), we believe that the effects observed are specific to the social-attention to the eye region and not to low-level perceptual features or perceptual improvements over time.

Attention to the mouth and body:

In comparison to attention to the eyes, attention to the mouth starts out relatively high at around 17 percent fixation to the area compared to the rest of the scene, but this attention to the mouth begins to decline to reach a trough around 15-16 weeks. The first couple of weeks in life is characterized by the emergence of reciprocal lipsmacking, a behavior that is correlated with later-life stress and emotional regulation (Kaburu et al., 2016). The early attention to the mouth region and resulting emergence of reciprocal lipsmacking may be an ethologically relevant form of social attention, but is later overshadowed by social cues of increasing valence later in life – for example, paying attention to the eyes while establishing mother-infant mutual gaze begins to overshadow reciprocal lipsmacking. The data also indicate a later inflection point at around 17 weeks when attention to the mouth appears to slightly increase again. This increase coincides with a time in development associated with a peak in fear-grimace display and willingness to leave the mother to explore (Suomi, 1984; Hinde & Spencer-Booth, 1967). The mouth of other rhesus macaques is a socially significant area of the face and macaque lipsmacking is believed to share a homologous developmental mechanism as human speech (Morrill et al., 2012).

Additionally, lipsmacking in infancy is positively correlated with later life interest in social interaction (Paukner et al., 2013), and facial expressions are important in determining and establishing social hierarchies (de Waal & Luttrell, 1985). Attention to the mouth region is likely an important factor as infant monkeys begin to leave their mother and explores and interacts with others in the social group. However, it is possible that our stimuli did not capture the shifts in social-visual attention to the mouth given that they lacked any species-specific vocalizations emitted by the video monkey and facial expressions, and consisted of entirely unfamiliar monkeys. The use of familiar monkeys from within the infants' social groups, along with dynamic facial expressions (i.e., lipsmacking) and vocalizations from the video monkey are all factors likely to play a crucial role in directing attention to the mouth over other regions of the face and body. Similar to the eye-region, attention to the mouth region could be driven by low-level perceptual effects, especially given the movement of the mouth. Again, the significant difference in attention to the mouth region with respect to the inverted faces that maintain low-level perceptual effects while disrupting face processing provides confidence that the developmental changes described are due to changes in social-attention and not general attention or perceptual improvements over time.

Attention to the body starts with the highest fixation percentage at around 30 percent then steadily declines throughout development, though continues to be a region of high fixation after the eyes. Given the neutral emotional valence of the videos as well as the close-up view that most videos had of the focal monkey, there were very few instances of the video monkey manipulating objects with hands that may have attracted attention of the infants. Therefore, the high fixation percentage to the body region may be due to the relative size of the region in comparison to those of the eye and mouth regions – there is simply more area of the video coded as the body. It is important to note, however, that by the end of our observed timeframe (24 weeks), the percent fixation towards the eye region remained higher than that of the body, further strengthening the social significance of attention to the eye region at 6 months of age.

Relationship to attention to face cues in human infants:

To obtain additional information on the phylogenetic conservation of developmental changes in social visual engagement between primate species, we also compared the changes in attention to visual cues in infant monkeys presented here with those obtained from typically developing human infants. As mentioned in the introduction, to facilitate this comparison, the infant monkeys were behaviorally tested and the data analyzed using exactly the same procedures as those used in human infants and were observed at ages (1 week to 24 weeks) that corresponded to the 2 months to 24 months age range used with the human infants (Jones & Klin, 2013).

The developmental trajectory of visual attention to the eyes of rhesus macaques closely parallels that shown in typically developing human infants. Inspection of Figures 5a and 5c demonstrate that monkeys have lower fixation percentage than human infants across this early developmental period. This species difference parallels previous cross-species data showing that, when exploring faces, monkeys make less fixations than humans, and spend more time in saccading or scanning (Dahl et al., 2009; Hu et al., 2013). Despite this difference, the developmental trajectory of attention to the eyes in early infancy is remarkably similar for the two species with a first rise in fixation percent towards the eyes for the first 6-8 weeks in monkeys and 6-8 months in humans, followed by a slow decline until 16 weeks for monkeys and 18 months in humans, and then a second rise until 24 weeks for monkeys and 24 months in humans. Thus, the cross-species shifts in attention to the eyes in early infancy is well conserved between monkeys and humans. Such similarity suggests that the eyes may provide crucial social information for face processing in both monkeys and humans (Nakato et al., 2018).

With regard to the attention to the mouth, however, the infant rhesus macaque pattern of viewing differs from that observed in human infants (see Figures 5b and 5c). Although attention to the mouth for monkeys slightly decreases during the first 2 months with a slight rise beginning at 20 weeks of age, attention to the mouth in humans increases sharply and continuously until 14 months. This species difference highlights interesting dissimilarity in the ethological relevance of the mouth regions between the two species. The overall increase seen in human infants is likely associated with the beginning of language acquisition during this early period (Klin et al., 2009; Paul et al., 2011; Wyk et al., 2010), whereas mouth movements in rhesus macaque infants are known to be used as social cues to display and indicate dominant behaviors such as threat gestures or submissive behaviors such as lipsmack cues that are mainly used during social interactions and could begin to develop at an age (4-6 months) when infant monkeys begin to leave their mother and enter in interactions with peers and other members of the social group.

Neural basis of attention to facial cues:

The specific neural mechanisms that underlie the shifts in visual attention to face cues, particularly fixation to the eyes, in early infancy are still poorly understood and remain speculative. Earlier developmental neuroanatomical and neurophysiological studies in monkeys together with more recent neuroimaging studies in both human infants and infant monkeys have indicated that visual subcortical and cortical regions responding preferentially to faces are present and spatially organized early in infancy, although fully selective face areas emerge much later (Deen et al., 2017; Livingstone et al., 2017; Arcaro et al., 2017; Arcaro & Livingstone 2017). As reviewed by Johnson (2005) and more recently by (Powell et al., 2018), several converging lines of evidence suggest that a subcortical face-processing route, including the superior colliculi, putamen and amygdala, is present at birth and is responsible for the patterns of face-related stimulus preference that are seen at that age. This subcortical route operates both more rapidly than cortical visual pathways and on low spatial visual information (LeDoux, 1996) and may be functionally more developed for newborn face preference because of the still functionally immature cortical visual areas (Johnson, 2005).

Functional neuroimaging studies in infant rhesus macaques viewing faces have shown a robust early activation of the lateral geniculate nucleus as early as 1 week of age, suggesting an early reliance on subcortical visual processing (Livingstone et al., 2017; Arcano & Livingstone, 2017, Arcano et al., 2017). In addition, sharp increase in amygdala volume, most likely resulting from increased interconnectivity of the amygdala with neocortical areas, occurs within the first six weeks of age in monkeys (Payne et al., 2010; Chareyon et al., 2012) and may help tag perceptual face processing with emotional content of a face. In contrast, the ventral temporal visual pathway involved in the perception of facial features and identity, the visual cortical areas located within the superior temporal sulcus important for the detection of facial movements and facial expressions, and the dorsal visual stream in the parietal cortex involved in spatial attentional processes are not fully developed at birth and have a more prolonged development throughout infancy (Webster et al., 1991; Webster et al., 1994; Rodman & Consuelos, 1994; Livingstone et al., 2017; Rodman et al., 1991; Rodman et al., 1993; Distler et al., 1996). Additionally, a recent functional MRI study on a subset of infants participating in the current study showed stronger functional connectivity between the most posterior cortical areas of the three visual cortical streams than in the more anterior cortical areas, and functional connectivity between the anterior portion of ventral visual stream and the amygdala increased progressively in the first 12 weeks of age (Kovatz-Balint et al., 2018). Thus, as shown in Figure 4, we speculate that at birth attention to the eyes may be supported by the early developing subcortical system. The decrease in attention to the eyes from about 6-16 weeks of age followed by the subsequent increase from 16 to 24 weeks may coincide with a decline of more reflexive subcortical processes together with a progressive rise of cortical processing as neonatal looking to faces may become more volitional when infants may choose to look at faces in order to engage contingent social interactions with positively valence. By 24 weeks of age, responses to faces in specific regions of extrastriate cortex may be further potentiated by the medial prefrontal cortex regions that respond to prosocial interactions (Johnson, 2005; Powell et al., 2018).

Summary:

Our main findings indicate that infant development of social-visual engagement is conserved in primates. The developmental trajectory for the attention to the eyes in infant rhesus macaques is comparable to that reported in human infants (Jones and Klin, 2013). Both begin with an increase in attention to the eyes that peaks relatively early in development, followed by a prolonged decrease and finally a progressive rise. The similarities in this longitudinal development implies conserved neural processing between nonhuman primates and humans. Yet, there were also important species differences that are likely guided by ethological constraint. Fixation to the mouth for example has different developmental trajectories for infant monkeys and humans, reflecting the information provided by mouth movements that are related to language acquisition in human infants at this early age but not in infant monkeys. Despite these limitations, rhesus macaques may provide a critically needed nonhuman primate model to further examine the developmental changes in neural systems engaged in social-visual attention and to assess how genetic variations as well as molecular and/or experimental manipulations of social neural networks alter social development. As recent discoveries in humans point to the importance of early-emerging and highly-conserved social phenotypes, nonhuman primate model could advance understanding of the brain-behavior pathogenesis of Autism Spectrum Disorders (ASD) as well as help validate efficacy of potential therapeutic treatments for attenuating social deficits in ASD.

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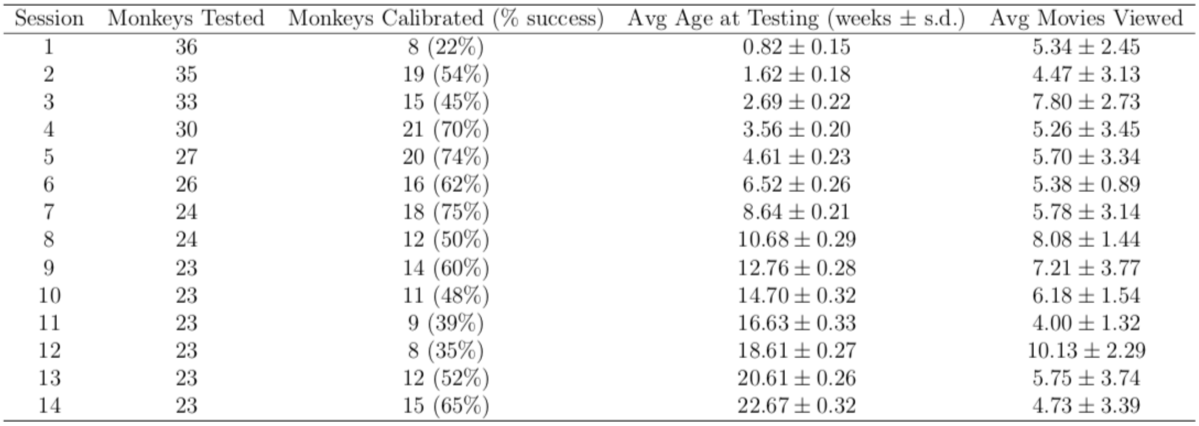
Figures and Tables

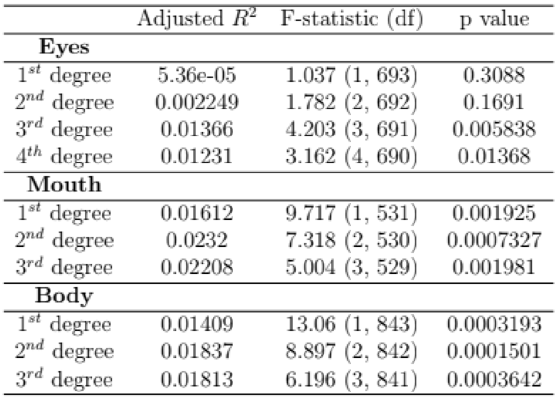
Table 1: Summary of data collection



Session	Monkeys Tested	Monkeys Calibrated (% success)	Avg Age at Testing (weeks ± s.d.)	Avg Movies Viewed
1	36	8 (22%)	0.82 ± 0.15	5.34 ± 2.45
2	35	19 (54%)	1.62 ± 0.18	4.47 ± 3.13
3	33	15 (45%)	2.69 ± 0.22	7.80 ± 2.73
4	30	21 (70%)	3.56 ± 0.20	5.26 ± 3.45
5	27	20 (74%)	4.61 ± 0.23	5.70 ± 3.34
6	26	16 (62%)	6.52 ± 0.26	5.38 ± 0.89
7	24	18 (75%)	8.64 ± 0.21	5.78 ± 3.14
8	24	12 (50%)	10.68 ± 0.29	8.08 ± 1.44
9	23	14 (60%)	12.76 ± 0.28	7.21 ± 3.77
10	23	11 (48%)	14.70 ± 0.32	6.18 ± 1.54
11	23	9 (39%)	16.63 ± 0.33	4.00 ± 1.32
12	23	8 (35%)	18.61 ± 0.27	10.13 ± 2.29
13	23	12 (52%)	20.61 ± 0.26	5.75 ± 3.74
14	23	15 (65%)	22.67 ± 0.32	4.73 ± 3.39

Caption for Table 1: A summary of the amount of data collected from infants at each testing session. There is a large degree of variation in the number of clips infants payed attention to at each session, as seen in the large standard deviation of seen by each monkey. Overall, there were far fewer inverted videos shown which accounts for the sparse number of inverted videos seen per monkey at each testing session.

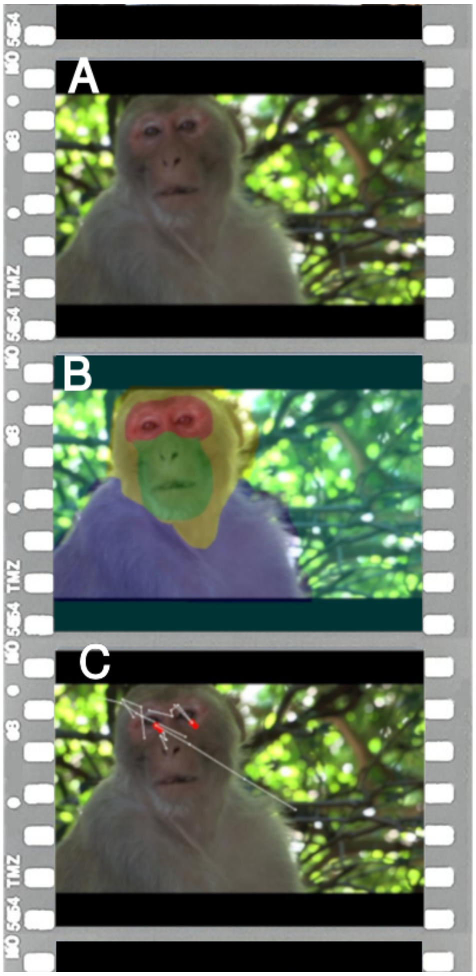
Table 2: Summary of polynomial fitting



	Adjusted R^2	F-statistic (df)	p value
Eyes			
1^{st} degree	5.36e-05	1.037 (1, 693)	0.3088
2^{nd} degree	0.002249	1.782 (2, 692)	0.1691
3^{rd} degree	0.01366	4.203 (3, 691)	0.005838
4^{th} degree	0.01231	3.162 (4, 690)	0.01368
Mouth			
1^{st} degree	0.01612	9.717 (1, 531)	0.001925
2^{nd} degree	0.0232	7.318 (2, 530)	0.0007327
3^{rd} degree	0.02208	5.004 (3, 529)	0.001981
Body			
1^{st} degree	0.01409	13.06 (1, 843)	0.0003193
2^{nd} degree	0.01837	8.897 (2, 842)	0.0001501
3^{rd} degree	0.01813	6.196 (3, 841)	0.0003642

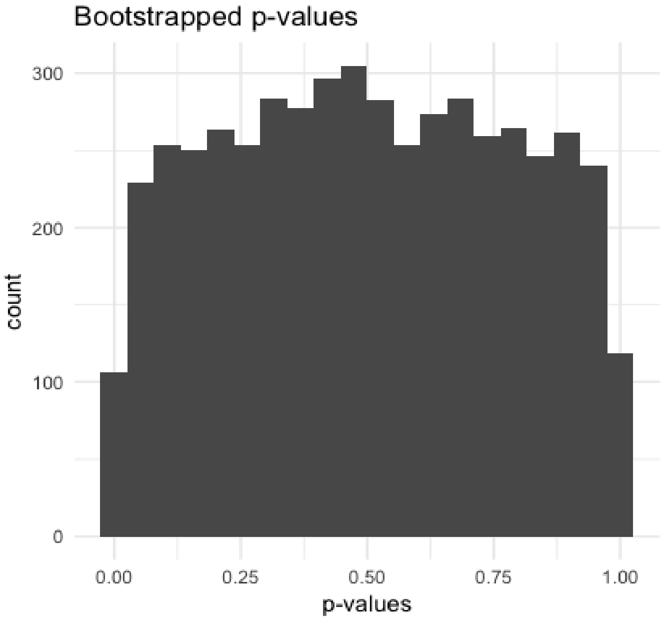
Caption for Table 2: A summary of the amount of polynomial fitting used to describe the attention the Eyes, Mouth, and Body. Initial models were estimated using a LOESS model to determine the shape of the polynomial regression. The goodness-of-fit (GOF) was evaluated for the polynomial regression: in each case the degree of polynomial chosen yielded the highest F-statistic and adjusted R^2 values, with both the polynomial of one greater and one less degree having yielding a loss in GOF.

Figure 1: Example of experimental stimuli



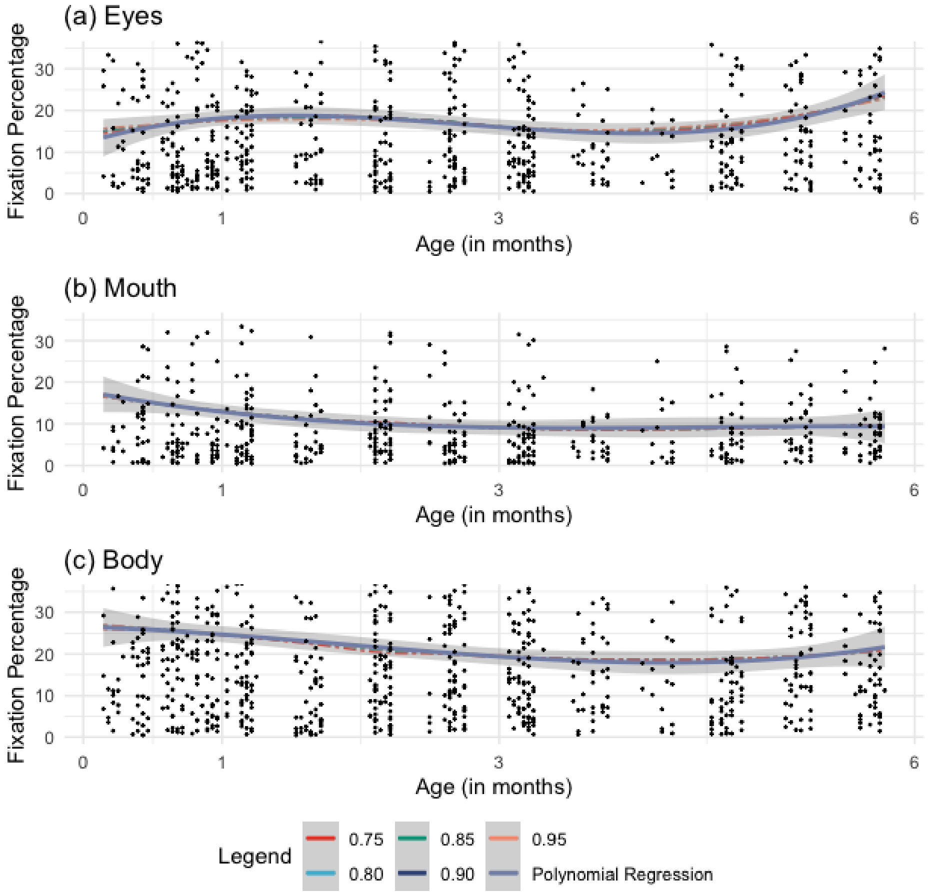
Caption for Figure 1: (a) A sample still from a movie showing the dynamic facial expressions of a single adult macaque. **(b)** The same still with the manually coded ROI imposed showing the definitions of eye (red), mouth (green), head (yellow), and body (blue) regions. **(c)** The example scanpath of one monkey’s viewing of the video at 7 weeks – fixations are represented in red points and saccades are shown as white lines. The scan path represents the viewing of the scan of approximately 1 second before and after the example still.

Figure 2: Moving-block bootstrapping results



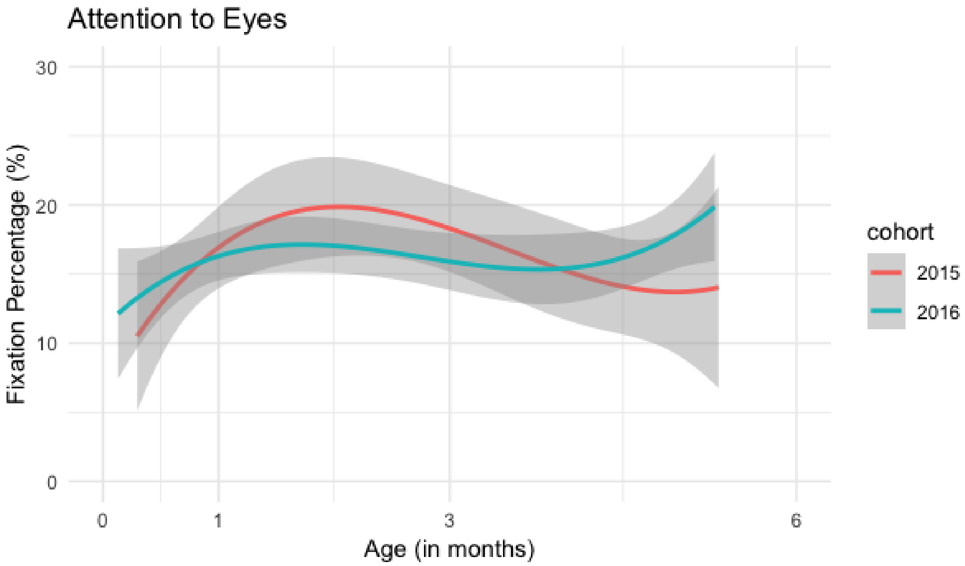
Caption for Figure 2: An example histogram depicting the resulting p-values generated from a moving block bootstrapping of the data. In this example, the fixation percentage to the eye region was bootstrapped, and in each run a polynomial of degree 3 was fit on top of the data and p-value was generated. This was done 5000 times and resulting counts are shown on the y-axis.

Figure 3: Changes in social-visual engagement in infant rhesus macaques



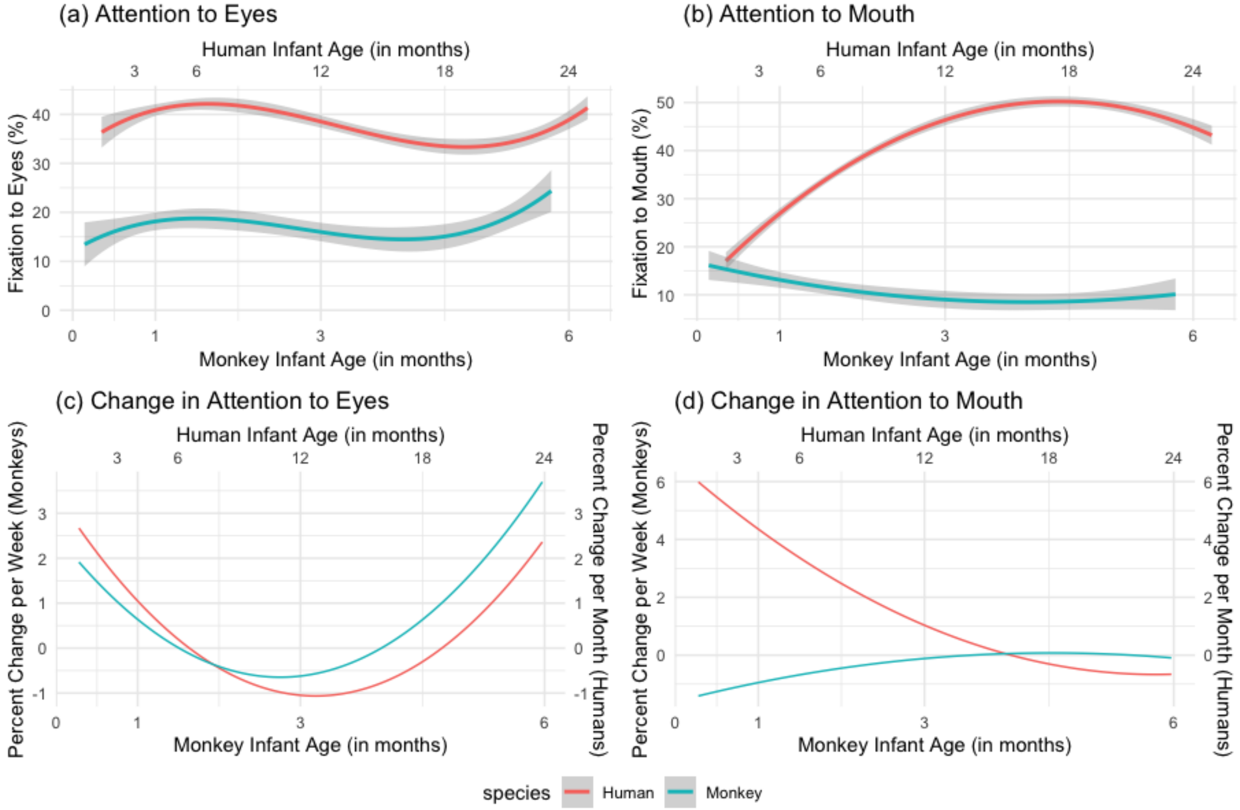
Caption for Figure 3: The fixation percentage to our three regions of interest: **(a)** eyes, **(b)** mouth, and **(c)** body. The dashed lines on each graph represents the LOESS models of varying degrees of α (as indicated in legend) from 0.75 to 0.95. Imposed on top of the LOESS models is a polynomial regression, the shaded gray region surrounding the regression represents the 95% confidence interval for the model.

Figure 4: Comparison between two cohorts (2015, 2016)



Caption for Figure 4: The fixation percentage to the eye region separated between the two cohorts of infants tested over two years, shaded gray regions surrounding the regressions represents the 95% confidence interval for the model each year. The dark gray areas represent the overlap between the two cohorts.

Figure 5: Developmental comparisons between humans and monkeys



Caption for Figure 5: Comparisons between NHP and human infant trajectory of attention to the eye and mouth regions. **(a)** Both human and NHP infants show parallel trajectories for attention to the eye region, though NHPs have lower fixation percentage overall. **(b)** While human and NHP infants have similar levels of attention to the mouth region at birth, human infants begin to pay more attention to the mouth throughout development, whereas monkey attention remains relatively stable. **(c)** Taking the central differential of both human and NHP infant trajectories and adjusting for the difference in developmental timescale, both human and NHP infants have similar rates of change in attention across development, whereas **(d)** attention to the mouth have vastly different rates of change.

References:

Akiyama, T., Kato, M., Muramatsu, T., Umeda, S., Saito, F., and Kashima, H. (2007). Unilateral amygdala lesions hamper attentional orienting triggered by gaze direction. *Cerebral Cortex*, 17, 2593–600.

Arcaro MJ, and Livingstone MS. (2017). A hierarchical, retinotopic proto-organization of the primate visual system at birth. *Elife*. 6:

Arcaro MJ, Schade PF, Vincent JL, Ponce CR, and Livingstone MS. (2017). Seeing faces is necessary for face-domain formation. *Nature Neuroscience.*. 20: 1404-1412.

Baron-Cohen, S, (1992) The theory of mind hypothesis of autism: history and prospects of the idea. *The Psychologist*, 5, 9-12.

Baron-Cohen, S., Wheelwright, S. and Jolliffe, T. (1997). Is there a“ language of the eyes”? Evidence from normal adults, and adults with autism or Asperger syndrome. *Visual Cognition*. doi:10.1080/713756761

Berard, J.D. (1989). Life histories of male Cayo Santiago macaques. *Puerto Rico Health Sciences Journal*, 8, 61-64.

Boothe, R. G., Williams, R. A., Kiorpes, L., & Teller, D. Y. (1980). Development of contrast sensitivity in infant Macaca nemestrina monkeys. *Science*. 208(4449), 1290–2.

Brooks, R. and Meltzoff, A.N. (2008). Infant gaze following and pointing predict accelerated vocabulary growth through two years of age: a longitudinal, growth curve modeling study. *Journal of Child Language*, 35, 207–20.

Chance, M.R.A. and Jolly, C.J. (1970). Social groups of monkeys, apes, and men. London, U.K.: Cape.

Chareyron, L. J., Lavenex, P. B., Amaral, D. G., & Lavenex, P. (2012). Postnatal development of the amygdala: A stereological study in macaque monkeys. *The Journal of Comparative Neurology*, *520*(9), 1965–1984. https://doi.org/10.1002/cne.23023

Cleveland, W. S. (1979). Robust Locally Weighted Regression and Smoothing Scatterplots. *Journal of the American Statistical Association*, *74*(368), 829–836. https://doi.org/10.1080/01621459.1979.10481038

Cleveland, W. S., & Devlin, S. J. (1988). Locally Weighted Regression: An Approach to Regression Analysis by Local Fitting. *Journal of the American Statistical Association*, *83*(403), 596–610. https://doi.org/10.1080/01621459.1988.10478639

Dahl, C. D., Logothetis, N. K., Bülthoff, H. H., & Wallraven, C. (2010). The Thatcher illusion in humans and monkeys. *Proceedings. Biological Sciences*, *277*(1696), 2973–2981. https://doi.org/10.1098/rspb.2010.0438

Deaner, R.O., Khera, A.V., and Platt, M.L. (2005). Monkeys pay per view: adaptive valuation of social images by rhesus macaques. *Current Biology*, 15, 543-548.

Deen, B. et al. (2017) Organization of high-level visual cortex in human infants. Nat. Commun. 8, 13995.

de Waal, F. B. M., & Luttrell, L. M. (1985). The formal hierarchy of rhesus macaques: An investigation of the bared-teeth display. *American Journal of Primatology*, *9*(2), 73–85. https://doi.org/10.1002/ajp.1350090202

Distler C, Bachevalier J, Kennedy C, Mishkin M, Ungerleider LG. (1996). Functional development of the corticocortical pathway for motion analysis in the macaque monkey: a 14C-2-deoxyglucose study. *Cerebral Cortex*, 6, 184-195.

Dunbar, R.I., and Shultz, S. (2007). Evolution of the social brain. *Science*, 317, 1344-1347.

Emery, N.J. (2000). The eyes have it: the neuroethology, function and evolution of social gaze. Neuroscience and biobehavioral reviews, 24(6), 581–604.

Ferrari, P. F., Paukner, A., Ionica, C., and Suomi, S. J. (2009). Reciprocal face-to-face communication between rhesus macaque mothers and their newborn infants. *Current Biology*, 19, 1768–1772.

Goren, C., Sarty, M., and Wu, P. (1975). Visual following and pattern discrimination of face-like stimuli by newborn infants. *Pediatrics*, 56:544-549.

Gottlieb, MD., Pasik, P., & Pasik, T. (1985). Early postnatal development of the monkey visual system. I. Growth of the lateral geniculate nucleus and striate cortex. *Developmental Brain Research* (17). 53-62

Herman, R.A., Jones, B., Mann, D.R., and Wallen, K. (2000). Timing of prenatal androgen exposure: anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Hormones and Behavior*, 38(1), 52.

Hinde & Spencer-Booth (1967). The behavior of socially living rhesus monkeys in their first two and a half years. *Animal Behaviour*, 14(1), 169-196.

Hu, Y.-Z., Jiang, H.-H., Liu, C.-R., Wang, J.-H., Yu, C.-Y., Carlson, S., … Hu, X.-T. (2013). What interests them in the pictures?–differences in eye-tracking between rhesus monkeys and humans. *Neuroscience Bulletin*, *29*(5), 553–564. https://doi.org/10.1007/s12264-013-1367-2

Johnson, M.H. (2005). Subcortical face processing. *Nature Reviews Neuroscience*, 6, 766-774.

Jones, W., and Klin, A. (2013). Attention to eyes is present but in decline in 2-6-month-old infants later diagnosed with autism. *Nature*, 504(7480), 427–31.

Kalin, N. H., Shelton, S. E., & Takahashi, L. K. (1991). Defensive behaviors in infant rhesus monkeys: ontogeny and context-dependent selective expression. *Child Development*, *62*(5), 1175–1183.

Kampe, K. K. W., Frith, C. D., Dolan, R. J., & Frith, U. (2001). Reward value of attractiveness and gaze. *Nature*, *413*(6856), 589. https://doi.org/10.1038/35098149

Kamphuis, S., Dicke, P.W., and Thier, P. (2009). Neuronal substrates of gaze following in monkeys. *The European journal of neuroscience*, 29(8), 1732–8.

Kaburu, S. S., Paukner, A., Simpson, E. A., Suomi, S. J., & Ferrari, P. F. (2016). Neonatal imitation predicts infant rhesus macaque (Macaca mulatta) social and anxiety-related behaviours at one year. *Scientific reports*, 6, 34997. doi:10.1038/srep34997

Klin, A., Lin, D. J., Gorrindo, P., Ramsay, G., & Jones, W. (2009). Two-year-olds with autism orient to non-social contingencies rather than biological motion. *Nature*, *459*(7244), 257–261. https://doi.org/10.1038/nature07868

Kovacs-Balint Z, Feczko E, Pincus M, Howell B, Earl E, Li L, Steele J, Styner M, Bachevalier J, Fair D, Sanchez M (2018). Early developmental trajectories of functional connectivity along the visual pathways in rhesus monkeys. Cerebral Cortex, PMID: 30272135 DOI: 10.1093/cercor/bhy222.

Kuwahata, H., Adachi, I., Fujita, K., Tomonaga, M., and Matsuzuwa, T. (2004) Development of schematic face preferences in macaque monkeys. *Behavioural Processes*, 66:17-21.

LeDoux, J. E. (1996). *The Emotional Brain*. New York, NY: Simon and Schuster.

Livingstone, M. S., Vincent, J. L., Arcaro, M. J., Srihasam, K., Schade, P. F., & Savage, T. (2017). Development of the macaque face-patch system. *Nature Communications*, *8*, 14897. https://doi.org/10.1038/ncomms14897

Lovejoy, J., and Wallen, K. (1988). Sexually dimorphic behavior in group-housed rhesus-monkeys (*Macaca Mulatta*) at 1 year of age. *Psychobiology*, 16, 348

Lutz, C., Lockard, J., Gunderson, V., and Grant, K. (1998). Infant monkeys' visual responses to drawings of normal and distorted faces. *American Journal of Primatology*, 44(2), 169–174.

Machado, C., and Bachevalier, J. (2003). Non-human primate models of childhood psychopathology: the promise and the limitations. *Journal of Child Psychology and Psychiatry*, 44(1), 64–87.

Maestripieri, D., Dee Higley, J., Lindell, S.G., Newman, T.K., McCormack, K.M., and Sanchez, M.M. (2006). Early maternal rejection affects the development of monoaminergic systems and adult abusive parenting in rhesus macaques (*Macaca mulatta*). *Behavioral Neuroscience*, 120(5), 1017.

Maestripieri, D., and Wallen, K. (1997). Affiliative and submissive communication in rhesus macaques. *Primates*, 38, 127–138.

McCormack, K., Newman, T.K., Highley, J.D., Maestripieri, D., and Sanchez, M.M. (2009). Serotonin transporter gene variation, infant abuse, and responsiveness to stress in rhesus macaque mothers and infants. *Hormones and Behavior*, 55(4), 538.

Mendelson, M. J., Haith, M. M., and Goldman-Rakic, P. S. (1982). Face scanning and responsiveness to social cues in infant rhesus monkeys. *Developmental Psychology*, 18, 222–228.

Morrill, RJ, Paukner, A, Ferrari, PF, and Ghazanfar, AA. (2012). Monkey lipsmacking develops like the human speech rhythm. *Developmental Science* 15:4, 557–568.

Mosher, C. P., Zimmerman, P. E., and Gothard, K. M. (2011). Videos of conspecifics elicit interactive looking patterns and facial expressions in monkeys. *Behavioral neuroscience*, 125(4), 639–52.

Muschinski, J., Feczko, E., Brooks, J.M., Collantes, M., and Heitz, T.R. (2016) The development of visual preferences for direct versus averted gaze faces in infant macaques (*Macaca mulatta*). *Developmental Psychology*, 58(8), 926-936.

Nakato, E., Kanazawa, S., & Yamaguchi, M. K. (2018). Holistic processing in mother's face perception for infants. *Infant Behavior and Development*, *50*, 257–263. https://doi.org/10.1016/j.infbeh.2018.01.007

Okada, T., Sato, W., Kubota, Y., Usui, K., Inoue, Y., Murai, T., … Toichi, M. (2008). Involvement of medial temporal structures in reflexive attentional shift by gaze. *Social cognitive and affective neuroscience*, 3(1), 80–8.

Parr, L.A., Murphy, L., Feczko, E., Brooks, J., Collantes, M., and Heitz, T.R. (2016) Experience-dependent changes in the development of face preferences in infant rhesus monkeys. *Developmental Psychobiology*, 58, 1002-1018.

Parr, L.A., Winslow, J.T., and Hopkins, W.D. (1999). Is the inversion effect in rhesus monkeys face-specific? *Animal Cognition*, 2, 123–129.

Paukner, A., Bower, S., Simpson, E. A., & Suomi, S. J. (2013). Sensitivity to first-order relations of facial elements in infant rhesus macaques. *Infant and Child Development*, *22*(3), 320–330. https://doi.org/10.1002/icd.1793

Paul, R., Fuerst, Y., Ramsay, G., Chawarska, K. & Klin, A. (2011). Out of the mouths of babes: Vocal production in infant siblings of children with ASD. *Journal of Child Psychology and Psychiatry*. 52(5): 588-598.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study. *Hippocampus*, *20*(8), 922–935. https://doi.org/10.1002/hipo.20688

Politis, D. N., & Romano, J. P. (1994). The Stationary Bootstrap. *Journal of the American Statistical Association*, *89*(428), 1303–1313. https://doi.org/10.1080/01621459.1994.10476870

Powell, L. J., Kosakowski, H. L., & Saxe, R. (2018). Social Origins of Cortical Face Areas. *Trends in Cognitive Sciences*, *22*(9), 752–763. https://doi.org/10.1016/j.tics.2018.06.009

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raper, J., Stephens, S., Sanchez, M., Bachevalier, J., and Wallen, K. (2014) Neonatal amygdala lesions alter mother-infant interactions in rhesus monkeys living in a species-typical social environment. *Developmental Psychobiology (Special Issue)*. 56(8):1711-22

Reid, V., Dunn, K., Young, R.J., Amu, J., Donovan, T., and Reissland, N. (2017). The Human Fetus Preferentially Engages with Face-like Visual Stimuli. *Current Biology,* 27, 1825–1828.

Riby, D. M., Hancock, P. J., Jones, N., and Hanley, M. (2013). Spontaneous and cued gaze-following in autism and Williams syndrome. *Journal of neurodevelopmental disorders*, 5(1), 13.

Rodman, H. R., & Consuelos, M. J. (1994). Cortical projections to anterior inferior temporal cortex in infant macaque monkeys. *Visual Neuroscience*, *11*(1), 119–133.

Rodman, H. R., Scalaidhe, S. P., & Gross, C. G. (1993). Response properties of neurons in temporal cortical visual areas of infant monkeys. *Journal of Neurophysiology*, *70*(3), 1115–1136. https://doi.org/10.1152/jn.1993.70.3.1115

Rodman, H. R., Skelly, J. P., & Gross, C. G. (1991). Stimulus selectivity and state dependence of activity in inferior temporal cortex of infant monkeys. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(17), 7572–7575. https://doi.org/10.1073/pnas.88.17.7572

Rowell, TE., Hinde, RA., & Spencer-Booth (1964). “Aunt”-infant interactions in captive rhesus macaques. *Animal Behaviour.* 219-226.

Roy, A., Shepherd, S.V., & Platt, M.L. (2014). Reversible inactivation of pSTS suppresses social gaze following in the macaque (*Macaca mulatta*). *Social cognitive and affective neuroscience*, 9(2), 209–17.

Sato, W., Kochiyama, T., Uono, S., Matsuda, K., Usui, K., Inoue, Y., & Toichi, M. (2011). Rapid amygdala gamma oscillations in response to fearful facial expressions. *Neuropsychologia*, *49*(4), 612–617. https://doi.org/10.1016/j.neuropsychologia.2010.12.025

Shepherd, S.V., and Platt, M.L. (2008). Spontaneous social orienting and gaze following in ringtailed lemurs (*Lemur catta*). *Animal cognition*, 11(1), 13–20.

Simpson, EA, Paukner, A, Sclafani, V, Suomi SJ, and Ferrari PF (2013) Lipsmacking Imitation Skill in Newborn Macaques Is Predictive of Social Partner Discrimination. *PLoS ONE.* 8(12): e82921. doi:10.1371/journal.pone.0082921

Sugita, Y. (2008). Face perception in monkeys reared with no exposure to faces. *Proceedings of the National Academy of Sciences*, *105*(1), 394–398. https://doi.org/10.1073/pnas.0706079105

Suomi, S. (1984). Affective development: A psychobiological perspective. In N. Fox & R. Davidson, N. Fox & R. Davidson (Eds.) (p. 119,159). Hillsdale, NJ: Lawrence Erlbaum.

Teller, D.Y., & Boothe R. (1979). Development of vision in infant primates. *Trans optha. Soc*. UK. (333-337) 99

Valenza, E et al (1996). Face preference at birth. *Journal of Experimental Psychology: Human perception and performance,* 22:892-903.

Wallen, K., (2005). Hormonal influences on sexually differentiated behavior in nonhuman primates. *Frontiers in Neuroendocrinology*, 26, 7-26.

Wang, Y., Ramsey, R., & de C. Hamilton, A. F. (2011). The Control of Mimicry by Eye Contact Is Mediated by Medial Prefrontal Cortex. *Journal of Neuroscience*, *31*(33), 12001–12010. https://doi.org/10.1523/jneurosci.0845-11.2011

Webster, M. A., & Mollon, J. D. (1991). Changes in colour appearance following post-receptoral adaptation. *Nature*, *349*(6306), 235–238. https://doi.org/10.1038/349235a0Webster, M. A., & Mollon, J. D. (1994). The influence of contrast adaptation on color appearance. *Vision Research*, *34*(15), 1993–2020. https://doi.org/10.1016/0042-6989(94)90028-0

Wyk, B. C. V., Ramsay, G. J., Hudac, C. M., Jones, W., Lin, D., Klin, A., Lee, S. M. & Pelphrey, K. (2010). Cortical integration of audio-visual speech and non-speech stimuli. *Brain and Cognition.* 74(2): 97-106.

Yin, R. (1969). Looking at upside-down faces. *Journal of Experimental Psychology*, 81(1), 141.

Yovel, G., and Kanwisher, N. (2005). The Neural Basis of the Behavioral Face-Inversion Effect. *Current Biology*, 15(24).

THE DEVELOPMENT OF GAZE FOLLOWING SKILLS IN INFANT RHESUS MACAQUES

***ARICK WANG**[1,2], CHRISTA PAYNE[1], WARREN JONES[3, 4], JOCELYNE BACHEVALIER[1,2]

[1]Yerkes National. Primate Research Ctr., Emory Univ., Atlanta, GA, 30329;

[2]Dept. of Psychology, Emory Univ., Atlanta, GA, 30322;

[3]Dept. of Pediatrics, Emory Univ., Atlanta, GA, 30322;

[4]Marcus Autism Center, Children's Healthcare of Atlanta, Atlanta, GA, 30329;

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Abstract

Gaze following represents a foundational behavior for establishing joint attention and higher-level social cognition (Emery, 2000). Atypical gaze following has been reported in children with Autism Spectrum Disorder and Williams Syndrome (Riby et al., 2013; Lord et al., 2012; Mundy, 2017). To advance our understanding of mechanisms underlying the development of gaze following, rhesus macaques offer an ideal model system: they develop rich and complex social behaviors from infancy onward, similar to humans, and their behavior may help elucidate sensitive periods in the development of gaze following and its neural substrates. Using eye-tracking methodology, this study examines the longitudinal development of gaze following from 1 week to 6 months in mother-reared infant rhesus macaques raised in semi-naturalistic groups that allow for typical emergence of social behavior. The results showed that although gaze following skills emerge around 6 months, more subtle changes in behaviors supporting gaze following occurred earlier. Specifically, we found an increase in mutual eye engagement at 3 months of age followed shortly thereafter by increases in maximum saccade velocities at 4 months. These results corroborate previous findings in the literature (Tomasello et al., 2001; Teufel et al., 2010; Rosati et al., 2016), adding the detection of more subtle developmental visual changes made possible via the use of eye-tracking methodologies.

Gaze following, directing one’s own attention using the gaze of another conspecific, represents a foundational behavior for establishing joint attention and higher-level social cognition in humans (Emery, 2000; Flom, et al., 2007). Baron-Cohen and colleagues (1992) have theorized that gaze following is an essential building block for communicational skills, serving as a basic form of nonverbal communication. The process of gaze following can be operationalized by first making mutual eye contact with another individual, and then following the gaze of the other individual when their attention shifts. Thus, gaze following is a socially specific behavior that is contingent on a number of ongoing perceptual and cognitive processes: (1) detection of another individual (Rutter, 1984), (2) assessing the individual’s attention towards the self (via mutual eye engagement), and (3) locating the individual’s focus of attention when it shifts away from the self.

As previously discussed, gaze following is contingent upon the perception of another individual and engaging in mutual eye contact (Rutter, 1984). This early detection of direct gaze is robust at birth as even newborn infants show preferential attention to faces with open eyes and to direct eye contact (Batki et al., 2000; Farroni et al., 2002). These preferences are not present for inverted faces, suggesting an effect specific to face processing and not to low-level perceptual features (Farroni, Menon, & Johnson, 2006). It is believed that this very early detection of direct eye gaze, and preference for making mutual eye contact, acts to accelerate the processing of social information, and provides an innate mechanism for which gaze following and elaborated forms of social cognition are built (Baron-Cohen, 1994). Emphasizing the importance of gaze following in the development of communication, this behavior has been split into two observable categories; (a) contextually gaze following driven by external stimuli presented in a specific series of events (e.g. an attention-grabbing event followed by a head turn from another individual) and (b) gaze following that contains communicative intent, typically operationalized through a triadic interaction and establishing joint attention. In humans, contextually driven gaze following emerges at around six months and appears to be predictive of the development of communicative intent and communication skills in later life (Gredeback et al., 2018; D'Entremont et al., 1997). Shortly thereafter, communicative intent in gaze following (i.e., forming joint attention) begins to develop at around one year of age, with the emergence of self-driven bids for establishing joint attention closely tied to the earlier emergency of contextually driven gaze following (Csibra et al., 2010). Finally, the extent of both contextually driven and communicative intent gaze following in infancy is predictive of the subsequent development of language and communication skills in toddlerhood (Brooks & Melzoff, 2005; Brooks & Melzoff, 2008).

Importantly, disruptions in the development of gaze following is a common symptom seen in pervasive neurodevelopmental disorders, such as Autism Spectrum Disorder (ASD) and Williams Syndrome (Riby et al., 2013; Lord et al., 2012; Mundy, 2017), and often used as an early diagnostic marker of the social disorders in children at risks (APA, 2013). Interestingly, it has been demonstrated that gaze following in children with ASD can be taught in specific laboratory settings, though there may be still a lack of communicative intent in learned gaze following (Baron-Cohen et al., 1995). This suggests that, although children with ASD are capable of perceiving gaze direction and flexibly use it as a directional cue, there may be disruption in the innate, social valence of gaze that is responsible for this disruption in gaze following (Leekam et al., 2000; Volkmar & Mayes, 1990).

Despite our increased knowledge on the developmental trajectory of gaze following in human infants and its derailment in ASD, much less is known on the neural structures supporting these early developing skills and their maturational trajectory patterns in the first few years of life. Thus, the use of an animal model becomes necessary and critical to provide densely sampled longitudinal behavioral and neuroimaging data througheearly infancy. Nonhuman primates (NHPs) have become an invaluable animal model of early developing social skills because they need to learn navigate rich and complex social groups, and, similarly to humans, their social skills develop progressively throughout infancy (Machado and Bachevalier, 2003). Furthermore, the similarity in brain organization and development in rhesus macaques and humans, may provide insights into the developmental critical periods of specific neural networks associated with overt behavioral changes in social-visual attention in humans. Although higher level social cognitive abilities, such as joint attention, are still disputed in monkeys (e.g. Emery, 2000; Ferrari et al., 2000; Itakura, 2004), NHPs act upon ever-changing contingencies and rely on visual information to alter their behavioral responses dependent upon the social environment. For instance, monkeys living in natural habitats use gaze alone as a social signal to help recruit allies and intentional gaze following has been observed in primates in the field in situations such as threat assessment (Maestripieri & Wallen, 1997; Redican, 1975; Shepherd & Platt, 2007). In laboratory settings, gaze following has been observed using eye tracking methodologies in adults viewing dynamic social scenes on a computer monitor (Mosher, Zimmerman, & Gothard, 2011). Additionally, the presence of gaze serves to facilitate responses on tasks probing spatial attention (Emery et al., 1997; Simpson et al., 2016). There has even been evidence of more elaborate social observational learning in rhesus macaques. Specifically, Mineka et al. (1984) conducted a series of experiments demonstrating that fear of snakes is induced through observational learning in rhesus macaques. Young laboratory-reared rhesus macaques previously not afraid of snakes became fearful when observing wild-reared parents showing a fear response towards snakes and snake-like stimuli. In order for the young monkeys to become fearful towards the snakes, they must use the attentional direction of the wild-reared parents to determine the attentional focus of the parents, then notice the fearful response from the wild-reared parents to infer that the fear response is due to the focal object. This form of observational learning requires information about another's direction of attention and understanding the intention of a fear response, and consists of the previously discussed perceptual and cognitive processes required for gaze following in humans: (1) detection of another individual, (2) assessing the individual's attention, and (3) locating the individual's focus of attention. Moreover, this study demonstrates that young monkeys are capable of using gaze for referencing a third object of attention in triadic social referencing. Still, our knowledge of the developmental trajectory of gaze following and associated neural structures throughout infancy in rhesus macaques is limited and not well understood.

Although gaze following has already been studied in a number of nonhuman primate species, most of the studies have focused on juveniles and adult animals and not on infants (see for review Rosati et al., 2009). An earlier study has shown that rhesus monkeys acquire gaze following skills in early infancy, around 5.5 months (Tomasello et al., 2001). Yet, the emergence of these skills is more protracted than in human infants, suggesting that nonhuman primates may require more experience with relevant social interactions than humans (Ferrari et al, 2000, 2009; Teufel et al., 2010). However, several limitations may have affected the results of these studies, such as the rearing conditions and social environment in which the monkeys were raised and the procedures used to promote gaze following. Thus, studies have relyed on either: (a) laboratory eye-tracking methodology rather than live field studies, (b) human experimental actor rather than conspecific actor, and (c) primarily used still images rather than videoclips, that neither may be ecologically relevant for engaging infant monkeys in social-visual behaviors (Rosati et al., 2016; Simpson et al., 2010). Finally, the data collection was done cross-sectionally in most of the studies and with only few developmental time points collected. More recent studies have attempted to remedy to some of these limitations. For example, Teufel and colleagues (2010) collected data cross-sectionally and longitudinally on Barbary macaques living in social groups living in an outdoor enclosure. Data were collected live using conspecific actors within the groups and recorded on a handheld computer in infants of 1-2 months, 3-4 months and 5-6 months, juveniles and adults. Gaze following was present only in the oldest infants of 5-6 months and reached adult levels in the juveniles. In all cases, gaze following was enhanced when the conspecific actors showed some facial expressions. Similarly, Rosati and colleagues (2016) studied gaze following in a group of semi-free-ranging rhesus macaques using a human actor. Gaze following behaviors were video-recorded on a large number of animals grouped into different age cohorts from infants (1 month to 1 year), juveniles (1 to 5 years), to adults (5-15 years) and later coded. Gaze following emerged at around 6 months of age and peaked in juvenile animals (Farroni et al., 2004).

The present study was designed to further gain much needed information on the developmental time course over which gaze following skills emerge in nonhuman primates during early infancy and to remedy some of the main limitations of the previous studies. First, we used semi-free ranging mother-reared rhesus infants to provide subjects with extensive experience in a complex social environment and promote the development of typical social behaviors. Second, the stimuli used to engage gaze follow were short videoclips of conspecifics providing relevant species-dependent social cues. Third, to obtain granular analyses of the behaviors, the subjects were eye-tracked while viewing the videoclips using procedures previously reported in both human infant (Jones & Klin, 2013) and infant rhesus macaque studies (Parr et al., 2016; Muschinski et al., 2016; Wang et al., in prep). Fourth, data were collected longitudinally on infant monkeys every 2 weeks from Week 1 to Week 24. These procedures allowed infants to experience normative social interactions that may be crucial to the development of gaze following while allowing the collection of dense, longitudinal and detailed, behavioral data. In addition, because the same infant monkeys received neuroimaging scans during the same developmental period, this will provide a unique opportunity to examine whether the maturation of certain social visual networks could support the development of gaze following skills (Kovacs-Balint et al., 2018; Wang et al., 2019, in preparation, see chapter 3). Finally, because the procedures to measure gaze following skills in monkeys were similar to those used with human infants, species comparisons could be directly performed.

Methods

Subjects

Thirty-three (33) newborn male monkeys (*Macaca mulatta*) were studied from birth to 6 months of age. All infants were mother-reared and lived in large, socially complex groups at Yerkes National Primate Research Center (YNPRC) Field Station. All infants were estimated full-term (>450g) offspring, and mothers were all mid-ranking and multiparous. Mother-infant pairs lived in large social groups containing over 100 individuals to preserve critical social environment of rhesus macaque colonies. Social groups were housed in outdoor compounds with access to climate-controlled indoor housing, which allows for experimental control typical of less complex social conditions (Berard, 1989; Lovejoy & Wallen, 1988; Wallen, 2005). Each mother-infant pair had at least 3 undisturbed days after birth to establish strong affiliative bonds prior to the first testing session. Infants were tested with their mothers and the pair was returned immediately after testing to their original social groups for the duration of the study. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University and followed the American Psychological Association standards for the ethical treatment of animals. For a summary of testing age distribution and averaged number of potential gaze following instances (see Stimuli below) viewed at each age, see Table 1.

Eye-Tracking Procedures

There were 14 total testing sessions at ages 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 weeks. Animals were tested with previously established procedures allowing direct access from their social groups for short periods of time (Herman et al., 2000; Maestripieri et al., 2006; McCormack et al, 2009). Mother-infant pairs were transferred to an onsite testing facility where the mother was anesthetized (3-5 mg/kg^{-1} telazol, i.m.), then the pair was then carried to a dedicated testing chamber containing a reclining seat onto which the mother could rest comfortably on her back. The infant was placed on her front, ventrum-ventrum, to view experimental stimuli presented on a 19” (62.6 cm, 45.27° diagonal) computer monitor (1024 x 768 pixels). An infrared eye-tracking camera (http://www.iscan.com/ 60 Hz) was mounted underneath the computer monitor on a motorized gimbal used to track the location of the infant’s eye. Following 5-point calibration, infants continued to watch social stimuli (see below) while freely nursing on the ventrum of their mother (*see details in* Parr et al., 2016; Muschinski et al., 2016). The mother’s breathing was monitored throughout the testing session. While the motorized gimbal allowed for minor shifts in infant position, if any large shifts occurred the 5 calibration points were repeated to ensure that reliable and accurate calibration was maintained.

Testing sessions were restricted to 30 min to limit the mother's time under anesthesia. After testing, the mother-infant pair was placed in an adjacent room to allow for recovery of the mother from anesthesia. When the mother was fully alert, the pair was returned to their social group.

Stimuli

Unfamiliar conspecific videos were videotaped on the rhesus monkey breeding colony maintained by the Caribbean Primate Research Center (CPRC) in Cayo Santiago, Puerto Rico to create the high-quality digital videos used as experimental stimuli. Videos were cropped to 10 seconds in length and played with accompanying sound. Videos showed close-up images of a single monkey, with approximately equal representation of female and male, and juvenile and adult monkeys. Videos of juvenile monkeys showed the animal's full body, whereas are those depicting adult monkeys showed the animal's torso, arms, and head only. The subject within the videos covered approximately 40-50% of the screen video surface. Only stimuli with neutral emotional facial expressions were used to avoid emotional reactions from the infant. Each video contained at least one potential instance of gaze following (i.e., the video monkey looks directly into the camera to engage mutual eye contact then looks away). A total of 33 unique videos were created for use in the testing sessions, with an average of 1.91 ± 0.87 potential gaze following instance per clip. In between each video presentation, the 2-second inter-trial interval displayed a centering stimulus (circular, chiming target on an otherwise blank screen) presented to maintain infant attention. For each testing session, a pseudorandom combination of repeated and novel videos was presented with an approximate 4:1 ratio.

Data Analysis

Eye movements and fixation data were analyzed and coded with custom-written software run in MATLAB (MathWorks). Data analysis consisted of (1) an automated identification of non-fixation data, comprising blinks, saccades and fixations directed away from the presented screen; (2) saccades identification by eye velocity using threshold of 30°/s; and finally (3) identification of fixation locations within regions of interest that were defined within each frame of all video stimuli, specifically the eye-region for the detection of mutual eye engagement. The regions of interest were hand traced for all frames of the video and were stored as binary bitmaps (through software written in MATLAB). Automated coding of fixation time to the region of interest consisted of a numerical comparison of each infant's coordinate fixation data with the bitmapped regions of interest (Jones & Klin, 2013).

Frame-by-frame coding of the video monkey's direction of attention was hand-coded as either direct (looking into the camera) or averted. A line was hand drawn during period of averted eye direction, then direction of attention was calculated as radial angle of the line (see Figure 1B). Gaze following was then operationalized as comprising of a fixation to the eyes of the onscreen monkey—a moment of "mutual eye contact" when the onscreen monkey was looking directly at the viewer (Figure A)—followed by a gaze shift in the direction in which the onscreen animal subsequently averted its gaze (Figure 1B). Mathematically, this was defined as saccades that fall within ±30° of the line drawn representing the video monkey's direction of attention (see Figure 2 for an example of distribution of subjects' saccades following the averted gaze of the monkey actor). An analysis of variance (ANOVA) was conducted using linear mixed-effect models for each observed variable (percent of mutual gaze engagement and percent gaze following) using age as a fixed factor and monkey identity as a random factor. Analyses were conducted using R statistical package, version 3.5.0 (Free Software Foundation, Inc., Boston, MA, U.S.A.), followed by Tukey HSD pairwise t-tests for the post-hoc analyses of specific ages and effect sizes determined using Cohen's d.

Results

Mutual eye contact engagement was calculated as the percentage of times mutual eye contact was established between the onscreen monkey and subject monkey compared to the total number of events in which mutual eye contact is possible for each clip (i.e., the number of times the onscreen monkey stared directly at the camera). The results showed a significant increase in mutual gaze from 1 to 6 months as revealed by a significant effect of age [$F(5,113) = 4.12$, $p < 0.01$; see Figure 3a]. Post-hoc t-tests were conducted, controlled for multiple comparisons (see Table 2). There was an increase mutual eye contact engagement at Month 3 ($M = 36.40\%$, $SD = 22.46$) that did not reach significance (Month 1 – 3: $p = 0.09$; Month 2 – 3: $p = 0.09$) but had a large effect size (Month 1 – 3: $d = 0.58$; Month 2 – 3: $d = 0.59$). Mutual eye engagement remained relatively stable with another significant increase at Month 6 ($M = 46.26\%$, $SD = 16.05$), during which mutual eye engagement occurs at level higher than earlier time points (Months 5 vs 6: $p = 0.03$; Months 1 vs 6: $p = 0.002$; Months 2 vs 6: $p = 0.002$; see Table 2).

Gaze following engagement was then calculated as the percentage of time the subject monkey followed the gaze of the onscreen monkey after mutual eye contact was established. The results indicated that there was a significant effect of age on gaze following instances [$F(5,99) =$

3.07, $p < 0.05$; see Figure 3b]. Post-hoc t-test comparisons between ages indicated a slight but nonsignificant decrease in gaze following after Month 1 (M = 18.30%, SD = 6.37), though the effect sizes were fairly high (Month 1 – 2: p = 0.077, d = 0.57; Month 1 – 3: p = 0.066, d = 0.65; see Table 3). Gaze following remained at relatively low rates from months 2 – 5, before increasing again at Month 6 (M = 19.52%, SD = 2.84), with a high effect size in this last increase (Month 5 – 6: d = 1.10). A complete summary of the post-hoc analyses can be found in Table 3.

Finally, the maximum saccade velocities for the 0.5 seconds preceding or following mutual eye contact with the onscreen monkey were also analyzed. A two-way within-subject ANOVA was conducted to determine the effect of infant age on saccade velocities, and whether gaze following occurred after the mutual eye contact. The resulted showed a significant increase in saccade velocities with age both prior to engaging in mutual eye contact [$F(5,1754) = 4.90$, $p < 0.001$; see Figure 4a] and following mutual eye contact [$F(5,1698) = 5.47$, $p < 0.001$; see Figure 4b]. However, there were no effects of the occurrence of gaze following on saccade velocities [Before Fixation: $F(1, 1922) = 2.15$, $p > 0.1$; After Fixation: $F(1, 1914) = 0.61$, $p > 0.1$] and no interactions between gaze following occurrence and age (see Table 4). Additional post-hoc pairwise comparisons between months revealed a significant increase in saccade velocity occurring at Month 4, with significantly higher than maximum saccade velocities to prior time points with moderate effect sizes, for both saccades emitted prior to mutual eye contact (M = 138.8, SD = 48.41; Month 1 – 4: p = 0.003, d = 0.29; Month 2 – 4: p = 0.001, d = 0.29; Month 3 – 4: 0.007, d = 0.29) as well as saccades emitted 0.5 seconds after mutual eye contact (M = 145.9, SD = 52.55; see Table 4). In the maximum saccade velocities leading to the fixation forming mutual eye contact, velocities remain relatively high through Month 5 and Month 6 (M = 134.57, SD = 52.58 and M = 131.72, SD = 52.03, respectively). However, the saccade velocities following fixation drops off significantly with moderate effect size at Month 5 (M = 125.34, SD = 66.04, Month 4 – 5: p = 0.045, d = 0.26), but increase again between Months 5 and 6 though this increase did not reach significance. Individual t-tests at each time point revealed no significant differences between the saccade velocities by event type (p > 0.1 in all comparisons).

Discussion

Establishing mutual eye contact:

Although instances of gaze following remained relatively infrequent in the first 6 months with few noticeable changes, there were more reliable developmental shifts in mutual gaze behaviors upon which gaze following is contingent. In this study, mutual eye contact, which is a requisite for the engagement in gaze following (Baron-Cohen 1994; Rutter 1984), was operationalized as the infant monkey fixating in the eye-region of the video while the video monkey was looking directly at the camera. The data showed a progressive increase in the infants' engagements in mutual eye contact across the first six months of age, indicating that the social significance of establishing eye contact increases gradually throughout this period of infancy. This increase in mutual eye contacts began around 2 to 3 months and strengthened between 5 to 6 months. The early slight increase in mutual eye contact engagement also closely follows a peak in mutual mother-infant face-face interactions observed in field studies at 2 months (Ferrari et al., 2009). Because mother-infant face-face interactions are generally mother-initiated, the increase at 3 months may indicate an increase in voluntary engagement of the infants to make in eye contacts and self-initiated social behaviors. Given that gaze following is contingent upon mutual eye contact, it is unsurprising to see that the sharp increase in mutual gaze by 6 months of age coincides with the slight rise in the number of gaze following instances at this same time point. The early emergence of mutual gaze is supported by other nonhuman primate and human studies reporting that in the few weeks of life infants are attracted to the eye region of faces (Mendelson et al., 1982; Gliga et al., 2007; Farroni et al., 2002) and that increased visual attention to eyes is associated with other social skills such as neonatal imitation (Paukner at al., 2013). Neonatal imitation, like gaze following, is a social skill known to require the interest and ability to track another individual's behavior (Carpenter et al., 1998) and has been shown to be a strong predictor of gaze following abilities (Simpson et al., 2016).

Interestingly, the shifts in the trajectory of mutual gaze appear to parallel the shifts in the same infants reported when assessing their preference to look at the eyes of a face (Wang et al., 2019, in prep; see chapter 1). In that later study, we also showed an increase in looking towards the eye region of faces by 2 to 3 months of age followed by a decline and then a second rise between 5 and 6 months. These shifts in the developmental trajectories of early social experience may be associated with the maturation of specific neural networks (see below).

Gaze following:

Overall, the data indicated few instances of gaze following (~10-20%) during this early period of development with a slight rise beginning between Month 5 and Month 6 (see Figure 3b). The low levels of gaze following could be due to the social constraints of the stimuli used, as the video monkeys were passive and not intentionally directing their attention to aspecific object or conspecific in the environment. Moreover, stimuli consisted of neutral facial expression with no monkey vocalizations; the presence of a socially salient facial expression (i.e., lipsmack or fear grimace) or of monkey vocalizations could be necessary to initiate contextually driven gaze following at this early age (before 6 months). The observed onset of gaze following abilities around 5-6 months parallels earlier findings showing that rhesus macaques begin to follow the gaze of a human experimenter at a similar age (Tomasello et al., 2001). Furthermore, two recent reports indicated similar developmental trajectory of gaze following abilities in infant monkeys. First, Teufel and colleagues (2010), combining longitudinal and cross-sectional observational field data on Barbary macaques with live conspecifics emitting directional cues, showed relatively low gaze following prior to five months with a similarly rapid increase in the number of gaze following instances between 5 and 6 months to progressively reach adult-like levels by 1 year of age (Teufel et al., 2010). Second, Rosati and colleagues (2016) found that gaze following in infant monkeys leaving in semi-free-ranging social groups does not emerge until 6 months using the eye gaze from a masked human experimenter as the directional cue. The current study is the first to show a similar developmental trajectory in gaze following instances using controlled laboratory eye-tracking procedures that provide more precise automated identification of gaze follows while maintaining species-relevant dynamic social stimuli. It should be noted that this study used only faces presenting with neutral facial expressions and it is possible that the use of faces with facial expressions could have enhanced the ability to follow gaze in the infants. Such facilitation of gaze following by emotional expressions had already been reported in human adults (Adams & Kleck, 2003; Mathews et al., 2003; Tipples, 2006; Hietanen and Leppänen, 2003) as well as in long-tailed adult macaques (Goosens et al., 2008), and Barbary infant macaques (Teufel et al., 2010). Thus, early gaze following prior to 6 months may be contingent upon emotional saliency and the use salient facial expressions cues (e.g. lipsmacking) could hasten these developmental changes.

Saccade velocities:

Following the increase in voluntary mutual eye contact at 3 months was a significant increase in maximum saccade velocities from Months 3 to 4 that had never been measured and reported in earlier studies. This increase in saccade velocities occurred both prior to and after mutual eye contact engagement. These changes in the saccade velocities indicate subtle behavioral changes that could depend on the social information gained from directed mutual gaze attention. The faster saccade velocities towards engaging in mutual eye contact points to faster detection of direct eye contact as a socially salient event to attend to, whereas faster maximum saccade velocities following mutual eye contact points to faster visual search following an important social eye-to-eye interaction. The current findings also provide some indirect evidence that by 4 months of age, infant rhesus macaques may be able to begin developing some unsophisticated understanding of attention or perception in others and not simply co-orient with conspecifics in a more egocentric and reflexive manner. Indeed, these developmental changes by 4 months coincide with the onset of weaning. Beginning around 4 months of age, mothers begin to wean their infants by resisting attempts of feeding (Fooden, 2000), and infants begin to explore their surroundings off their mothers and seek out attention from aunts and other familial members (Hinde & Spencer-Booth, 1967; Rowell et al., 1964). Additionally, 4 months marks affective changes and the emergence of fear grimace into the infants' behavioral repertoire (Suomi, 1984). Given that direct eye contact in rhesus macaques increases gestural display and socially appeasing behaviors (Linnankoski et al., 1993; Emery et al., 1997), the combined changes in affective fear grimace displays and increased exploration of social environments suggest that the 4 month time point in rhesus macaques represents a critical developmental period for becoming self-aware of the social contingencies surrounding the infants, and a need for directing attention in a socially relevant manner rather than responding more reflexively.

Neural basis of socially-directed attention:

The neural networks supporting the neonatal development of mutual eye gaze and gaze following are currently poorly understood. Neuroanatomical studies and more recent neuroimaging studies in monkeys have shown that within the first 6 months of infancy for rhesus macaques, there is substantial development and maturation of cortical visual systems and affective neural networks that help support perception of social cues and social attention and gaze monitoring (Kovacs-Balint et al., 2018, see Chapter 3). Growing evidence tends to support the view that the early emergence of infant primates to prefer looking at the eyes and to initiate mutual eye gaze involves the early maturation of a subcortical network, including the amygdala. Specifically, the subcortical network may provide infants with a reflexive behavioral mechanism to direct attention to social cues that is separate from an understanding of mutual gaze as a communicative signal (see for review, Johnson 2005). Indeed, developmental neurobehavioral studies in infant rhesus macaques have shown that neonatal insults to the amygdala before 2 weeks age yield decreased mother-infant mutual gaze and an inability to establish a preference for their mothers over unfamiliar females (Bauman et al., 2004), and reduced contacts towards the mothers (Goursaud et al., 2014). Similarly, Raper and colleagues (2014) showed subtle alterations in infant-mother interactions following neonatal amygdala lesions, further highlighting the importance of the amygdala in the development of early social skills, including social-visual engagement. Developmentally, there are specific regional volumetric increases within the lateral, basal, and accessory basal nuclei of the amygdala that are due to increased interconnectivity with neocortical areas occuring between 3-4 months of age (Chareyron et al., 2012). Recent studies have also shown that the functional connectivity between cortical area TE and the amygdala increases steadily during the first 3 months of age (Kovacs-Balint et al., 2018). In this study, we do see significant increases in both mutual eye contact engagement and increases in visual detection of mutual eye contact (i.e., increase maximum saccade velocities) occuring at 4 months, coinciding with these regional volumetric changes and changes in functional connectivity in the amygdala.

In adults, cortical areas in the anterior portion of the ventral visual stream (TEO, TE) have been shown to be critically important for evaluating face identity and facial expressions in rhesus macaques (Gothard et al., 2007; Hoffman et al., 2007; Mosher et al., 2010). Additionally, attention to eye gaze direction and gaze following in humans is known to activate cortical areas within the ventral motion pathway along superior temporal sulcus (STS), and the STS is crucially important for evaluating social context of gaze shift (Puce et al., 2003; Puce and Perrett, 2003; Pelphrey et al., 2003, 2004). A recent functional neuroimaging has highlighted the importance of the STS in actively follow the eye-gaze of a conspecific (Kamphuis et al., 2009). However, these cortical areas in the ventral visual pathway and ventral motion pathway are not fully developed at birth. It is believed that as these cortical visual systems develop, infant social attention moves away from a reflexive model as volitional attention towards socially relevant information develops (see for review, Johnson 2005).

There are progressive developments between area TE and STS as well as between extrastriate cortex and the parietal zones responsible for the emergence of voluntarily directing visual attention until around 6 months of age, coinciding with the latter rise in the number of gaze following instances suggesting a role in volitional attentional networks in gaze following

(Rodman & Consuelos, 1994). Additional support for this proposal comes from our DTI study on the infant monkeys of the present study indicating an increase in fractional anisotropy (FA, i.e. increase myelination) around 4 months of age between parietal areas and the frontal eye field region of the prefrontal cortex responsible for monitoring saccadic eye movements and around 5-6 months between areas within the superior temporal sulcus responsible for the detection of gaze motion (Wang et al., 2019, in preparation, see chapter 3).

Although the present results indicate an interesting parallel between developmental trajectories of mutual gaze and gaze following abilities during the first six months of age in monkeys and the progressive maturation of cortical visual and attentional networks, they also point to some limitations. In particular, stimuli that would elicit infant reactivity and/or body movements were avoided because our methodological approach allowed for the infant to freely move around the testing apparatus. Thus, only stimuli of neutral facial expression were used. Additionally, these stimuli were filmed at a close-up camera angle which removed contextual information about the surroundings of the video monkey that may be necessary for the social processing of meaningful person-object-person triadic interactions, facilitating gaze following. In order to overcome these study limitations, further studies should be conducted using both stimuli featuring varying facial expressions and those that contain more of the surrounding environment to establish contextual information.

Summary:

Ultimately, our main findings are consistent with previous studies of gaze following in NHPs with the use of eye tracking methodologies in semi-free ranging rhesus macaques, showing an early emergence of mutual gaze followed several months later by increase in gaze following instances around 6 months of age. Through leveraging the benefits of laboratory eye-tracking methodologies while keeping the infants in a rich and complex social environment, we also found increases in maximum saccade velocities, suggesting the continued development of socially guided behaviors at 4 months of age.

When comparing these developmental shifts in gaze following skills between human and NHP, there does appear to be some degrees of similarity in developmental milestones that suggest some phylogenetic conservation of these basic social cognitive abilities. Although human newborns are sensitive to eye contact, they do not appear to have any social expectations behind mother-infant face-face interaction (Rochat & Striano, 1999; Lavelli & Fogel, 2005). Contextually driven gaze following emerges relatively early in development at around 6 months in human infants (Gredeback et al., 2018; D'Entremont et al., 1997), which should coincide with a similar emergence of a contextually driven gaze following between 1 and 2 months in NHPs. Intriguingly, the NHP data actually showed a decrease in gaze following instances at 2 months. These developmental shifts in gaze following in the monkeys could suggest more contextually driven, reflexive, gaze following present at 1 month that is then diminished as more elaborate volitional social cognitive abilities develop and functionally come online later. Finally, socially meaningful gaze following in triadic person-object-person interactions has a more prolonged development emerging at about 12 months in human infants (Carpenter et al., 1998; Tomasello et al., 2005; Csibra et al., 2010). We were unable to measure these instances as our stimuli did not include referencing objects, but we do see significant changes in gaze monitoring occurring at 3 months in this study and increases in maximum saccade velocities at 4 months that suggest a similar increase in the social saliency of mutual gaze that increases aspects of visual search behavior (i.e., saccade velocities). The present findings together with previous developmental

NHP data, showed that gaze following in NHPs emerges later in development compared to humans (6 months in NHPs being approximately similar to 24 months in humans). This species differences may be due to the added importance of gaze following in language development for humans (Brooks & Meltzoff, 2005). Still, even with such differences in developmental timing, NHP studies are still invaluable to further our understanding of the neural basis underlying the development of gaze following skills.

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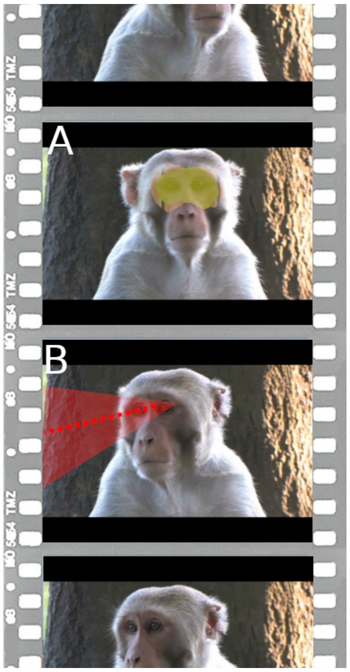
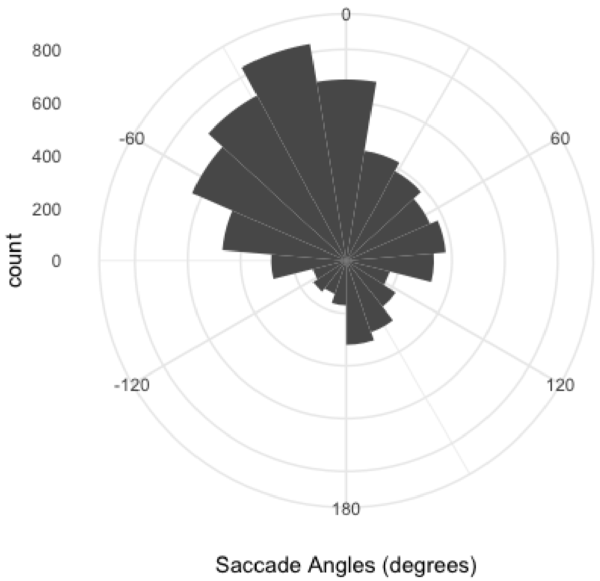
Figures and Tables

Figure 1: Operationalization of gaze following



Caption for Figure 1: Example of consecutive video frames from one of the stimuli. In all stimuli, a single close-up monkey was shown of various ages and of both sexes. Gaze following occurred when **(A)** the video monkey first made direct eye contact to the camera. A region of interest encompassing the eyes were used to detect if the subject infant made a fixation establishing mutual eye contact. Following, **(B)** the direction of the video monkey's attention was coded, and saccades were considered gaze following if the saccade path followed was within the video monkey's attention.

Figure 2: Example of saccade directions following mutual eye contact



Caption for Figure 2: An example plot showing directions for all saccades following mutual eye contact engagement at the first month across all monkeys. All angles are adjusted so that 0° indicates the direction of the movie monkey's attention. For each clip, a percentage was calculated of all saccades falling within ±30° with respect to all available saccades following mutual eye contact.

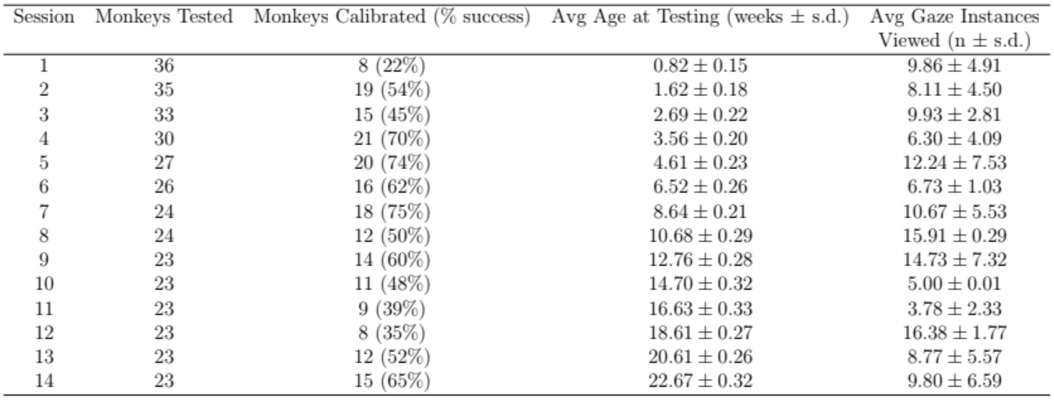
Figure 3: Development of mutual eye contact and gaze following

Caption for Figure 3: (A) The percentage of mutual gaze cues during which infants engaged in mutual eye contact (mean ± sem). **(B)** From the instances in which mutual eye contact is established between the movie monkey and infant monkey, the percentage of gaze following events during which the infant would shift attention in the same direction as the movie monkey (mean ± sem). A summary statistical pairwise comparisons can be seen in Table 2 for mutual eye contact and Table 3 for gaze following.

Figure 4: Changes in saccade velocities

Figure 4: The maximum saccade velocities (in ° visual angle/second, mean ± sem) both (a) before and (b) after engaging in mutual eye contact, separated by whether the mutual eye contact resulted in gaze following (teal) or not (orange). Significant indicators indicate $p < 0.05$ from t-tests of adjacent months in development. There is a significant increase in maximum saccade velocities between months 3 and 4 both before and after engaging in mutual eye contact. This increase is only apparent when the mutual eye contact resulted in gaze following (saccade velocities after eye fixation).

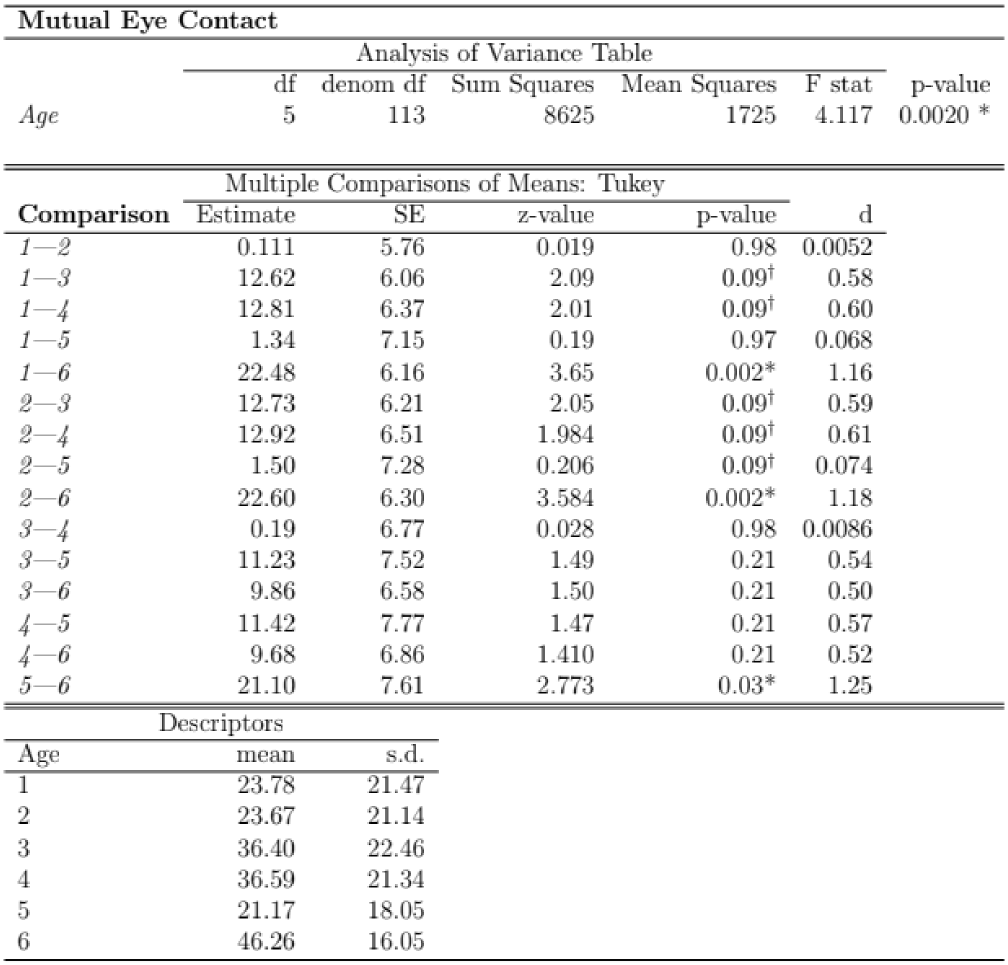
Table 1: Summary of data collection



Session	Monkeys Tested	Monkeys Calibrated (% success)	Avg Age at Testing (weeks $\pm$ s.d.)	Avg Gaze Instances Viewed (n $\pm$ s.d.)
1	36	8 (22%)	0.82 ± 0.15	9.86 ± 4.91
2	35	19 (54%)	1.62 ± 0.18	8.11 ± 4.50
3	33	15 (45%)	2.69 ± 0.22	9.93 ± 2.81
4	30	21 (70%)	3.56 ± 0.20	6.30 ± 4.09
5	27	20 (74%)	4.61 ± 0.23	12.24 ± 7.53
6	26	16 (62%)	6.52 ± 0.26	6.73 ± 1.03
7	24	18 (75%)	8.64 ± 0.21	10.67 ± 5.53
8	24	12 (50%)	10.68 ± 0.29	15.91 ± 0.29
9	23	14 (60%)	12.76 ± 0.28	14.73 ± 7.32
10	23	11 (48%)	14.70 ± 0.32	5.00 ± 0.01
11	23	9 (39%)	16.63 ± 0.33	3.78 ± 2.33
12	23	8 (35%)	18.61 ± 0.27	16.38 ± 1.77
13	23	12 (52%)	20.61 ± 0.26	8.77 ± 5.57
14	23	15 (65%)	22.67 ± 0.32	9.80 ± 6.59

Caption for Table 1: A summary of the amount of data collected from infants at each testing session. There is a large degree of variation in the number of clips infants payed attention to at each session, as seen in the large standard deviation in the number of gaze instances viewed.

Table 2: Statistical summary for mutual eye contact



Mutual Eye Contact

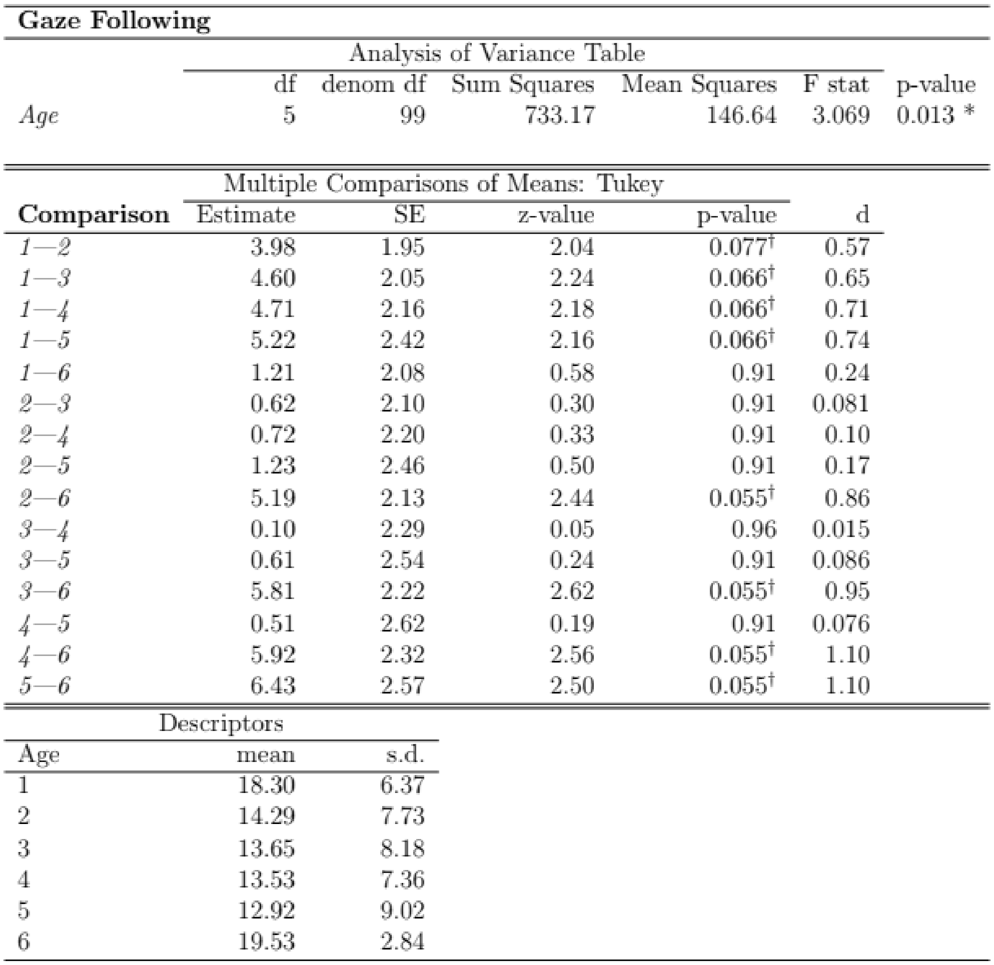
	Analysis of Variance Table					
	df	denom df	Sum Squares	Mean Squares	F stat	p-value
Age	5	113	8625	1725	4.117	0.0020 *

	Multiple Comparisons of Means: Tukey				
Comparison	Estimate	SE	z-value	p-value	d
1—2	0.111	5.76	0.019	0.98	0.0052
1—3	12.62	6.06	2.09	$0.09^{\dagger}$	0.58
1—4	12.81	6.37	2.01	$0.09^{\dagger}$	0.60
1—5	1.34	7.15	0.19	0.97	0.068
1—6	22.48	6.16	3.65	0.002*	1.16
2—3	12.73	6.21	2.05	$0.09^{\dagger}$	0.59
2—4	12.92	6.51	1.984	$0.09^{\dagger}$	0.61
2—5	1.50	7.28	0.206	$0.09^{\dagger}$	0.074
2—6	22.60	6.30	3.584	0.002*	1.18
3—4	0.19	6.77	0.028	0.98	0.0086
3—5	11.23	7.52	1.49	0.21	0.54
3—6	9.86	6.58	1.50	0.21	0.50
4—5	11.42	7.77	1.47	0.21	0.57
4—6	9.68	6.86	1.410	0.21	0.52
5—6	21.10	7.61	2.773	0.03*	1.25

	Descriptors	
Age	mean	s.d.
1	23.78	21.47
2	23.67	21.14
3	36.40	22.46
4	36.59	21.34
5	21.17	18.05
6	46.26	16.05

Caption for Table 2: A summary of statistical analysis for mutual eye contact (ANOVA, Tukey HSD, and descriptive statistics). An ANOVA with using linear mixed-effect models yielded a significant effect of Age on mutual eye contact. Post-hoc Tukey HSD test showed a significant increase from Month 5 to Month 6, at which it is also significantly higher than Month 1 and 2. There was trending increase at Months 3, with fairly high effect size.

Table 3: Statistical summary for gaze following



Gaze Following

	Analysis of Variance Table					
	df	denom df	Sum Squares	Mean Squares	F stat	p-value
Age	5	99	733.17	146.64	3.069	0.013 *

	Multiple Comparisons of Means: Tukey				
Comparison	Estimate	SE	z-value	p-value	d
1—2	3.98	1.95	2.04	0.077†	0.57
1—3	4.60	2.05	2.24	0.066†	0.65
1—4	4.71	2.16	2.18	0.066†	0.71
1—5	5.22	2.42	2.16	0.066†	0.74
1—6	1.21	2.08	0.58	0.91	0.24
2—3	0.62	2.10	0.30	0.91	0.081
2—4	0.72	2.20	0.33	0.91	0.10
2—5	1.23	2.46	0.50	0.91	0.17
2—6	5.19	2.13	2.44	0.055†	0.86
3—4	0.10	2.29	0.05	0.96	0.015
3—5	0.61	2.54	0.24	0.91	0.086
3—6	5.81	2.22	2.62	0.055†	0.95
4—5	0.51	2.62	0.19	0.91	0.076
4—6	5.92	2.32	2.56	0.055†	1.10
5—6	6.43	2.57	2.50	0.055†	1.10

	Descriptors	
Age	mean	s.d.
1	18.30	6.37
2	14.29	7.73
3	13.65	8.18
4	13.53	7.36
5	12.92	9.02
6	19.53	2.84

Caption for Table 3: A summary of statistical analysis for gaze following (ANOVA, Tukey HSD, and descriptive statistics). An ANOVA with using linear mixed-effect models yielded a significant effect of Age on gaze following. Post-hoc Tukey HSD test showed several changes trending towards significance with very high effect sizes. There was an initially high degree of gaze following at Month 1 that decreases at Month 2. The gaze following at Month 6 was substantially higher than Months 2-5.

Table 4: Statistical summary for saccade velocities

Maximum Saccade Velocities

Analysis of Variance Table

Before	df	denom df	Sum Squares	Mean Squares	F stat	p-value
Age	5	1755	74125	14825	4.90	0.00019 *
Gaze Followed	1	1922	6491	6491	2.15	0.14
Age x Followed	5	1926	6196	1239	0.41	0.84
After	df	denom df	Sum Squares	Mean Squares	F stat	p-value
Age	5	1698	107014	21402	5.46	5.38 e-5 *
Gaze Followed	1	1914	2379	2379	0.61	0.44
Age x Followed	5	1925	8772	1754	0.45	0.81

Multiple Comparisons of Means: Tukey

Before Fixation

Comparison	Estimate	SE	z-value	p-value	d
1—2	0.76	3.84	0.20	0.84	0.012
1—3	1.77	4.06	0.44	0.76	0.030
1—4	15.70	4.44	3.53	0.0031*	0.29
1—5	13.68	5.20	2.63	0.026*	0.22
1—6	9.30	4.52	2.06	0.074†	0.17
2—3	2.53	3.78	0.67	0.63	0.042
2—4	16.46	4.18	3.94	0.0012*	0.31
2—5	14.44	5.02	2.88	0.015*	0.24
2—6	10.07	4.28	2.35	0.046*	0.19
3—4	13.93	4.37	3.19	0.0071*	0.26
3—5	11.91	5.18	2.30	0.046*	0.19
3—6	7.53	4.48	1.68	0.15	0.15
4—5	2.02	5.48	0.37	0.76	0.070
4—6	6.40	4.81	1.33	0.27	0.12
5—6	4.38	5.53	0.79	0.58	0.05

After Fixation

Comparison	Estimate	SE	z-value	p-value	d
1—2	6.56	4.35	1.51	0.20	0.11
1—3	8.87	4.60	1.93	0.10	0.14
1—4	22.97	5.03	4.57	7.49e-05*	0.37
1—5	8.29	5.90	1.41	0.22	0.12
1—6	19.77	5.12	3.86	0.00084*	0.32
2—3	2.32	4.29	0.54	0.68	0.041
2—4	16.42	4.75	3.46	0.0027*	0.29
2—5	1.74	5.70	0.31	0.81	0.020
2—6	13.21	4.86	2.72	0.020*	0.23
3—4	14.10	4.96	2.84	0.017*	0.23
3—5	0.58	5.88	0.10	0.92	0.020
3—6	10.89	5.07	2.15	0.068†	0.18
4—5	14.68	6.22	2.36	0.045*	0.26
4—6	3.21	5.45	0.59	0.68	0.057
5—6	11.47	6.27	1.83	0.11	0.20

Descriptors

Before Fixation			After Fixation		
Age	mean	s.d.	Age	mean	s.d.
1	121.95	58.59	1	117.01	68.97
2	121.29	55.28	2	124.10	59.84
3	123.67	57.58	3	126.66	64.22
4	138.16	51.08	4	141.04	56.89
5	134.57	52.58	5	125.34	66.04
6	131.72	52.03	6	137.75	59.61

Caption for Table 4: A summary of statistical analysis for maximum saccade velocities (ANOVA, Tukey HSD, and descriptive statistics). Both BEFORE and AFTER establishing mutual eye contact, Age showed had a significant effect on saccade velocities, however there was no effect of gaze following or interaction of the two variables. Posthoc Tukey HSD showed a significant increase in maximum velocities from Month 3 to Month 4 both before and after mutual eye gaze. For saccades following mutual eye contact, saccade velocities continued to increase from Month 4 to 5.

References

Adams, R. B., & Kleck, R. E. (2003). Perceived gaze direction and the processing of facial displays of emotion. *Psychological Science*, *14*(6), 644–647. https://doi.org/10.1046/j.0956-7976.2003.psci_1479.x

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders(5th ed.). Arlington, VA: Author. Baron-Cohen, S. (1992). The theory of mind hypothesis of autism: history and prospects of the idea. *The Psychologist*, *5*, 9–12.

Baron-Cohen, S. (1992). The theory of mind hypothesis of autism: history and prospects of the idea. *The Psychologist*, *5*, 9–12.

Baron-Cohen, S. (1994). How to build a baby that can read minds: cognitive mechanisms in mindreading. *Cahiers de Psychologie Cognitive/Current Psychology of Cognition*, *13*(5), 513–552.

Baron-Cohen, S., Campbell, R., Karmiloff-Smith, A., Grant, J., & Walker, J. (1995). Are children with autism blind to the mentalistic significance of the eyes? *British Journal of Developmental Psychology*, *13*(4), 379–398. https://doi.org/10.1111/j.2044-835x.1995.tb00687.x

Bauman, M. D., Lavenex, P., Mason, W. A., Capitanio, J. P., & Amaral, D. G. (2004). The development of social behavior following neonatal amygdala lesions in rhesus monkeys. *Journal of Cognitive Neuroscience*, *16*(8), 1388–1411. https://doi.org/10.1162/0898929042304741

Batki, A., Baron-Cohen, S., Wheelwright, S., Connellan, J., & Ahluwalia, J. (2000). Is there an innate gaze module? Evidence from human neonates. *Infant Behavior and Development*, *23*(2), 223–229. https://doi.org/10.1016/s0163-6383(01)00037-6

Berard, J. D. (1989). Life histories of male Cayo Santiago macaques. *Puerto Rico Health Sciences Journal*, *8*(1), 61–64.

Brooks, R., & Meltzoff, A. N. (2005). The development of gaze following and its relation to language. *Developmental Science*, *8*(6), 535–543. https://doi.org/10.1111/j.1467-7687.2005.00445.x

Brooks, R., & Meltzoff, A. N. (2008). Infant gaze following and pointing predict accelerated vocabulary growth through two years of age: a longitudinal, growth curve modeling study. *Journal of Child Language*, *35*(1), 207–220.

Carpenter, M., Nagell, K., & Tomasello, M. (1998). Social cognition, joint attention, and communicative competence from 9 to 15 months of age. *Monographs of the Society for Research in Child Development*, *63*(4), i–vi,1-143.

Csibra, G., Johnson, M. H., & Tucker, L. A. (1997). Attention and oculomotor control: a high-density ERP study of the gap effect. *Neuropsychologia*, *35*(6), 855–865.

D’Entremont, B., Hains, S. M. J., & Muir, D. W. (1997). A demonstration of gaze following in 3- to 6-month-olds. *Infant Behavior and Development*, *20*(4), 569–572. https://doi.org/10.1016/s0163-6383(97)90048-5

Emery, N. J. (2000). The eyes have it: the neuroethology, function and evolution of social gaze. *Neuroscience and Biobehavioral Reviews*, *24*(6), 581–604.

Emery, N. J., Lorincz, E. N., Perrett, D. I., Oram, M. W., & Baker, C. I. (1997). Gaze following and joint attention in rhesus monkeys (Macaca mulatta). *Journal of Comparative Psychology*, *111*(3), 286–293. https://doi.org/10.1037/0735-7036.111.3.286

Farroni, T., Csibra, G., Simion, F., & Johnson, M. H. (2002). Eye contact detection in humans from birth. *Proceedings of the National Academy of Sciences*, *99*(14), 9602–9605. https://doi.org/10.1073/pnas.152159999

Farroni, T., Massaccesi, S., Pividori, D., & Johnson, M. H. (2004). Gaze Following in Newborns. *Infancy*, *5*(1), 39–60. https://doi.org/10.1207/s15327078in0501_2

Farroni, T., Menon, E., & Johnson, M. H. (2006). Factors influencing newborns’ preference for faces with eye contact. *Journal of Experimental Child Psychology*, *95*(4), 298–308. https://doi.org/10.1016/j.jecp.2006.08.001

Ferrari, P. F., Kohler, E., Fogassi, L., & Gallese, V. (2000). The ability to follow eye gaze and its emergence during development in macaque monkeys. *Proceedings of the National Academy of Sciences*, *97*(25), 13997–14002. https://doi.org/10.1073/pnas.250241197

Ferrari, P. F., Paukner, A., Ionica, C., & Suomi, S. J. (2009). Reciprocal Face-to-Face Communication between Rhesus Macaque Mothers and Their Newborn Infants. *Current Biology*, *19*(20), 1768–1772. https://doi.org/10.1016/j.cub.2009.08.055

Flom, R., Lee, K., & Muir, D. (2007). *Gaze Following: Its Development and Significance*. Erlbaum Associates.

Fooden, J. (2000). Systematic review of the rhesus macaque, Macaca mulatta (Zimmermann, 1780). *Fieldiana Zoology*, *96*, 1–180.

Gliga, T., & Csibra, G. (2007). Seeing the face through the eyes: a developmental perspective on face expertise. In *Progress in Brain Research* (pp. 323–339). Elsevier. https://doi.org/10.1016/s0079-6123(07)64018-7

Goossens, B. M. A., Dekleva, M., Reader, S. M., Sterck, E. H. M., & Bolhuis, J. J. (2008). Gaze following in monkeys is modulated by observed facial expressions. *Animal Behaviour*, *75*(5), 1673–1681. https://doi.org/10.1016/j.anbehav.2007.10.020

Gothard, K. M., Battaglia, F. P., Erickson, C. A., Spitler, K. M., & Amaral, D. G. (2007). Neural responses to facial expression and face identity in the monkey amygdala. *Journal of Neurophysiology*, *97*(2), 1671–1683. https://doi.org/10.1152/jn.00714.2006

Goursaud, A.-P. S., Wallen, K., & Bachevalier, J. (2014). Mother recognition and preference after neonatal amygdala lesions in rhesus macaques (Macaca mulatta) raised in a semi-naturalistic environment. *Developmental Psychobiology*, *56*(8), 1723–1734. https://doi.org/10.1002/dev.21233

Gredebäck, G., Astor, K., & Fawcett, C. (2018). Gaze Following Is Not Dependent on Ostensive Cues: A Critical Test of Natural Pedagogy. *Child Development*, *89*(6), 2091–2098. https://doi.org/10.1111/cdev.13026

Herman, R. A., Jones, B., Mann, D. R., & Wallen, K. (2000). Timing of prenatal androgen exposure: anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Hormones and Behavior*, *38*(1), 52–66. https://doi.org/10.1006/hbeh.2000.1608

Hietanen, J. K., & Leppänen, J. M. (2003). Does facial expression affect attention orienting by gaze direction cues? *Journal of Experimental Psychology. Human Perception and Performance*, *29*(6), 1228–1243. https://doi.org/10.1037/0096-1523.29.6.1228

Hinde, R. A., & Spencer-Booth, Y. (1967). The behaviour of socially living rhesus monkeys in their first two and a half years. *Animal Behaviour*, *15*(1), 169–196. https://doi.org/10.1016/s0003-3472(67)80029-0

Hoffman, K. L., Gothard, K. M., Schmid, M. C., & Logothetis, N. K. (2007). Facial-expression and gaze-selective responses in the monkey amygdala. *Current Biology : CB*, *17*(9), 766–772. https://doi.org/10.1016/j.cub.2007.03.040

Itakura, S. (2004). Gaze-following and joint visual attention in nonhuman animals. *Japanese Psychological Research*, *46*(3), 216–226. https://doi.org/10.1111/j.1468-5584.2004.00253.x

Johnson, M. H. (2005). Subcortical face processing. *Nature Reviews. Neuroscience*, *6*(10), 766–774. https://doi.org/10.1038/nrn1766

Jones, W., & Klin, A. (2013). Attention to eyes is present but in decline in 2-6-month-old infants later diagnosed with autism. *Nature*, *504*(7480), 427–431. https://doi.org/10.1038/nature12715

Kamphuis, S., Dicke, P. W., & Thier, P. (2009). Neuronal substrates of gaze following in monkeys. *European Journal of Neuroscience*, *29*(8), 1732–1738. https://doi.org/10.1111/j.1460-9568.2009.06730.x

Kovacs-Balint, Z., Feczko, E., Pincus, M., Earl, E., Miranda-Dominguez, O., Howell, B., … Sanchez, M. (2018). Early Developmental Trajectories of Functional Connectivity Along the Visual Pathways in Rhesus Monkeys. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhy222

Lavelli, M., & Fogel, A. (2005). Developmental Changes in the Relationship Between the Infant's Attention and Emotion During Early Face-to-Face Communication: The 2-Month Transition. *Developmental Psychology*, *41*(1), 265–280. https://doi.org/10.1037/0012-1649.41.1.265

Leekam, S. R., López, B., & Moore, C. (2000). Attention and joint attention in preschool children with autism. *Developmental Psychology*, *36*(2), 261–273.

Linnankoski, I., Gronroos, M., & Pertovaara, A. (1993). Eye Contact as a Trigger of Male Sexual Arousal in Stump-Tailed Macaques (Macaca arctoides). *Folia Primatologica*, *60*(3), 181–184. https://doi.org/10.1159/000156689

Lord, C., Luyster, R., Guthrie, W., & Pickles, A. (2012). Patterns of developmental trajectories in toddlers with autism spectrum disorder. *Journal of Consulting and Clinical Psychology*, *80*(3), 477–489. https://doi.org/10.1037/a0027214

Lovejoy, J., & Wallen, K. (1988). Sexually dimorphic behavior in group-housed rhesus monkeys (Macaca mulatta) at 1 year of age. *Psychobiology*, *16*(4), 348–356.

Machado, C. J., & Bachevalier, J. (2003). Non-human primate models of childhood psychopathology: the promise and the limitations. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *44*(1), 64–87.

Mathews, A., Fox, E., Yiend, J., & Calder, A. (2003). The face of fear: Effects of eye gaze and emotion on visual attention. *Visual Cognition*, *10*(7), 823–835. https://doi.org/10.1080/13506280344000095

Maestripieri, D., Higley, J. D., Lindell, S. G., Newman, T. K., McCormack, K. M., & Sanchez, M. M. (2006). Early maternal rejection affects the development of monoaminergic systems and adult abusive parenting in rhesus macaques (Macaca mulatta). *Behavioral Neuroscience*, *120*(5), 1017–1024. https://doi.org/10.1037/0735-7044.120.5.1017

Maestripieri, D., & Wallen, K. (1997). Affiliative and submissive communication in rhesus macaques. *Primates*, *38*(2), 127–138. https://doi.org/10.1007/bf02382003

McCormack, K., Newman, T. K., Higley, J. D., Maestripieri, D., & Sanchez, M. M. (2009). Serotonin transporter gene variation, infant abuse, and responsiveness to stress in rhesus macaque mothers and infants. *Hormones and Behavior*, *55*(4), 538–547. https://doi.org/10.1016/j.yhbeh.2009.01.009

Mendelson, M. J., Haith, M. M., & Goldman-Rakic, P. S. (1982). Face scanning and responsiveness to social cues in infant rhesus monkeys. *Developmental Psychology*, *18*(2), 222–228. https://doi.org/10.1037/0012-1649.18.2.222

Mineka, S., Davidson, M., Cook, M., & Keir, R. (1984). Observational conditioning of snake fear in rhesus monkeys. *Journal of Abnormal Psychology*, *93*(4), 355–372.

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2010). Response characteristics of basolateral and centromedial neurons in the primate amygdala. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(48), 16197–16207. https://doi.org/10.1523/JNEUROSCI.3225-10.2010

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2011). Videos of conspecifics elicit interactive looking patterns and facial expressions in monkeys. *Behavioral Neuroscience*, *125*(4), 639–652. https://doi.org/10.1037/a0024264

Mundy, P. (2017). A review of joint attention and social-cognitive brain systems in typical development and autism spectrum disorder. *European Journal of Neuroscience*, *47*(6), 497–514. https://doi.org/10.1111/ejn.13720

Muschinski, J., Feczko, E., Brooks, J. M., Collantes, M., Heitz, T. R., & Parr, L. A. (2016). The development of visual preferences for direct versus averted gaze faces in infant macaques (Macaca mulatta). *Developmental Psychobiology*, *58*(8), 926–936. https://doi.org/10.1002/dev.21421

Parr, L. A., Murphy, L., Feczko, E., Brooks, J., Collantes, M., & Heitz, T. R. (2016). Experience-dependent changes in the development of face preferences in infant rhesus monkeys. *Developmental Psychobiology*, *58*(8), 1002–1018. https://doi.org/10.1002/dev.21434

Paukner, A., Bower, S., Simpson, E. A., & Suomi, S. J. (2013). Sensitivity to first-order relations of facial elements in infant rhesus macaques. *Infant and Child Development*, *22*(3), 320–330. https://doi.org/10.1002/icd.1793

Pelphrey, K. A., Morris, J. P., & McCarthy, G. (2004). Grasping the intentions of others: the perceived intentionality of an action influences activity in the superior temporal sulcus during social perception. *Journal of Cognitive Neuroscience*, *16*(10), 1706–1716. https://doi.org/10.1162/0898929042947900

Pelphrey, K. A., Singerman, J. D., Allison, T., & McCarthy, G. (2003). Brain activation evoked by perception of gaze shifts: the influence of context. *Neuropsychologia*, *41*(2), 156–170.

Puce, A., & Perrett, D. (2003). Electrophysiology and brain imaging of biological motion. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *358*(1431), 435–445. https://doi.org/10.1098/rstb.2002.1221

Puce, A., Syngeniotis, A., Thompson, J. C., Abbott, D. F., Wheaton, K. J., & Castiello, U. (2003). The human temporal lobe integrates facial form and motion: evidence from fMRI and ERP studies. *NeuroImage*, *19*(3), 861–869. https://doi.org/10.1016/s1053-8119(03)00189-7

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raper, J., Wallen, K., Sanchez, M. M., Stephens, S. B. Z., Henry, A., Villareal, T., & Bachevalier, J. (2013). Sex-dependent role of the amygdala in the development of emotional and neuroendocrine reactivity to threatening stimuli in infant and juvenile rhesus monkeys. *Hormones and Behavior*, *63*(4), 646–658. https://doi.org/10.1016/j.yhbeh.2013.01.010

Redican, W. K. (1975). Facial expressions in nonhuman primates. *Primate Behavior*, 103–194.

Riby, D. M., Hancock, P. J., Jones, N., & Hanley, M. (2013). Spontaneous and cued gaze-following in autism and Williams syndrome. *Journal of Neurodevelopmental Disorders*, *5*(1), 13. https://doi.org/10.1186/1866-1955-5-13

Rochat, P., & Striano, T. (1999). Early social cognition: Understanding others in the first months of life. In P. Rochat, P. Rochat (Ed.) (pp. 3–34). Mahwah, NJ, US: Lawrence Erlbaum Associates Publishers.

Rodman, H. R., & Consuelos, M. J. (1994). Cortical projections to anterior inferior temporal cortex in infant macaque monkeys. *Visual Neuroscience*, *11*(1), 119–133.

Rosati, A. G., Arre, A. M., Platt, M. L., & Santos, L. R. (2016). Rhesus monkeys show human-like changes in gaze following across the lifespan. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1830), 20160376. https://doi.org/10.1098/rspb.2016.0376

Rosati, A. G., & Hare, B. (2009). Looking past the model species: diversity in gaze-following skills across primates. *Current Opinion in Neurobiology*, *19*(1), 45–51. https://doi.org/10.1016/j.conb.2009.03.002

Rowell, T., Hinde, R., & Spencer-Booth. (1964). “Aunt”-infant interactions in captive rhesus macaques. *Animal Behaviour*, 219–226.

Rutter, D. R. (1984). *Looking and seeing: The role of visual communication in social interaction*. Chichester, UK: Wiley.

Shepherd, S. V., & Platt, M. L. (2007). Spontaneous social orienting and gaze following in ringtailed lemurs (Lemur catta). *Animal Cognition*, *11*(1), 13–20. https://doi.org/10.1007/s10071-007-0083-6

Simpson, E. A., Paukner, A., Sclafani, V., Kaburu, S. S. K., Suomi, S. J., & Ferrari, P. F. (2016). Acute oxytocin improves memory and gaze following in male but not female nursery-reared infant macaques. *Psychopharmacology*, *234*(3), 497–506. https://doi.org/10.1007/s00213-016-4480-x

Simpson, E. A., Varga, K., Frick, J. E., & Fragaszy, D. (2010). Infants Experience Perceptual Narrowing for Nonprimate Faces. *Infancy*, *16*(3), 318–328. https://doi.org/10.1111/j.1532-7078.2010.00052.x

Suomi, S. (1984). Affective development: A psychobiological perspective. In N. Fox & R. Davidson, N. Fox & R. Davidson (Eds.) (p. 119,159). Hillsdale, NJ: Lawrence Erlbaum.

Teufel, C., Gutmann, A., Pirow, R., & Fischer, J. (2010). Facial expressions modulate the ontogenetic trajectory of gaze-following among monkeys. *Developmental Science*, *13*(6), 913–922. https://doi.org/10.1111/j.1467-7687.2010.00956.x

Tipples, J. (2006). Fear and fearfulness potentiate automatic orienting to eye gaze. *Cognition & Emotion*, *20*(2), 309–320. https://doi.org/10.1080/02699930500405550

Tomasello, M., Carpenter, M., Call, J., Behne, T., & Moll, H. (2005). Understanding and sharing intentions: the origins of cultural cognition. *The Behavioral and Brain Sciences*, *28*(5), 675-91-735. https://doi.org/10.1017/S0140525X05000129

Tomasello, M., Hare, B., & Fogleman, T. (2001). The ontogeny of gaze following in chimpanzees, Pan troglodytes, and rhesus macaques, Macaca mulatta. *Animal Behaviour*, *61*(2), 335–343. https://doi.org/10.1006/anbe.2000.1598

Volkmar, F. R., & Mayes, L. C. (1990). Gaze behavior in autism. *Development and Psychopathology*, *2*(1), 61–69. https://doi.org/10.1017/s0954579400000596

Wallen, K. (2005). Hormonal influences on sexually differentiated behavior in nonhuman primates. *Frontiers in Neuroendocrinology*, *26*(1), 7–26. https://doi.org/10.1016/j.yfrne.2005.02.001

DEVELOPMENTAL TRAJECTORIES OF WHITE MATTER MICROSTRUCTURE ALONG THE VISUAL PATHWAYS OF THE INFANT RHESUS MACAQUES

***ARICK WANG**[1,2], ZSOFIA KOVACS-BALINT[1], MAR SANCHEZ[1,3], JOCELYNE BACHEVALIER[1,2], LONGCHUAN LI[4,5]

[1]Yerkes National. Primate Research Ctr., Emory Univ., Atlanta, GA, 30329;

[2]Dept. of Psychology, Emory Univ., Atlanta, GA, 30322;

[3]Dept. of Psychiatry and Behavioral Sciences, Emory Univ., Atlanta, GA, 30322;

[4]Dept. of Pediatrics, Emory Univ., Atlanta, GA, 30322;

[5]Marcus Autism Center, Children's Healthcare of Atlanta, Atlanta, GA 30322

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Abstract

The underlying neurodevelopmental processes that support the development of social behaviors in infancy are not well understood. Here, we studied developmental changes in the neural pathways underlying visual social engagement in the translational rhesus macaque model and correlated these changes with developmental trajectories of social-visual behavior (i.e., changes in attention to the faces of others, mutual eye contact, and gaze following). Changes in the structural connectivity along the ventral object and motion pathways and the dorsal attention/visuo-spatial pathway were studied longitudinally using DTI in infant rhesus macaques, from birth until 6 months. The same infants were used in studies of the development of social-visual engagement longitudinally using eye-tracking methodologies during the same time period. Our results revealed that (1) maturation along the cortical visual pathways extended throughout the first 6 months with continual changes in FA and MD; (2) robust changes in streamline count plateaued between 8 and 12 weeks in each cortical pathways; and (3) moderate to strong correlations between DTI measures and behavioral measures. Our findings suggest that visual pathways in infant macaques undergo extensive changes in WM properties in the first 6 months, and that the measured changes along these cortical visual pathways support the development of social-visual engagement in infants.

Keywords

Macaque, Infant Development, Social Visual Engagement, Gaze Following

Research examining the precursors of human social abilities during early infancy has significantly increased in the last few years, yet our understanding of the early neural changes that underlie these behavioral changes remains limited. Work in this area is important not only for testing theories of typical social development, but also for identifying new ways to study the neural mechanisms underlying the emergence of social and communication difficulties in infants who develop social maladaptation, such as infants with Autism Spectrum Disorders (ASD, Klin et al., 2015). The main limitations in this field of research relates to the difficulty of gaining neuroimaging of the brain of human babies and of acquiring densely-sampled longitudinal brain-behavior relationship across development. Thus, knowledge in this domain must emerge from translational research examining both human populations and animal models. Rodent models offer suitable and robust models for many human neurodevelopmental disorders since they display a large variety of social behaviors (Homberg et al. 2016) and their genes can be manipulated to examine the genetic bases of social behavior. However, rodent models also have inherent limitations in that their social repertoire, especially in early infancy, are limited compared to those of primates. Non-human primates (NHP), in contrast, have great degree of similarity to humans (1) in genetic composition and physiology, (2) in the rich and complex social structure in which they develop and navigate, and (3) in brain development (Dettmer et al., 2014). Recent developmental studies in rhesus monkeys have shown that early developing social skills are phylogenetically well conserved. Thus, as human infants, infant rhesus monkeys display preferences to faces in the first week of life (Parr et al., 2016) and engage in complex face-to-face interactions including bouts of mutual gaze (Ferrari et al., 2009; Wang et al., 2019a, in preparation, see chapter 1) and gaze following skills (Tomasello et al., 2001; Ferrari et al., 2000; Teufel et al., 2010; Rosati et a., 2016; Wang et al., 2019b, in preparation, see chapter 2).

These few studies have critically furthered our understanding of the developmental trajectories of early social skills although they provided very little information on the neural networks supporting these cognitive functions.

It has been proposed that early attraction to faces at birth may be driven by a subcortical system, including the superior colliculus, pulvinar, and amygdala that is activated by the visual/sensory properties present in faces. In the following months after birth, this system appears to give way to a more cortically mediated system (Johnson, 2005; Morton & Johnson, 1991) that becomes strengthened with experience due to both regressive (synaptic pruning) and progressive (synaptogenesis/ myelination) events that increase the efficiency with which relevant and frequently encountered information is processed (de Haan, Humphreys, & Johnson, 2002; Lourenco & Casey, 2013; Morton & Johnson, 1991; Scott, Pascalis, & Nelson, 2007). This cortical system directs attention to faces and includes three parallels visual stream processing pathways: the ventral temporal pathway mediating object perception, such as face and identity perception and facial expression, discrimination and recognition (Ungerleider and Bell, 2011), the motion pathway along the superior temporal sulcus (STS) mediating body, facial and eye motion perception (Baylis et al., 1987; Boussaoud et al., 1990; Furl et al., 2012), and the dorsal cortical pathway in the dorsal parietal areas mediating visuo-spatial processing and spatial attention (Ungerleider and Mishkin, 1982; Dickinson et al., 2003; Liu et al., 2010). Functional neuroimaging studies in infant rhesus macaques viewing faces have shown a robust early activation of the lateral geniculate nucleus as early as 1 week of age, suggesting an early reliance on subcortical visual processing (Livingstone et al., 2017; Arcaro & Livingstone, 2017, Arcaro et al., 2017). In addition, sharp increase in amygdala volume, most likely resulting from increased interconnectivity of the amygdala with neocortical areas, occurs within the first six weeks of age in monkeys (Payne et al., 2010; Chareyon et al., 2012) and may help tag perceptual face processing with emotional content of a face. The visual cortical pathways by contrast seem to have a more protracted maturation. Earlier histological and electrophysiological studies in infant monkeys have shown that cortical areas along these three visual streams mature progressively during the first 4 months, from the most posterior towards the most anterior cortical areas (Rodman et al., 1991; Webster et al., 1991; Distler et al., 1996). Recent blood-volume fMRI studies in infant monkeys have revealed the presence of a retinotopic proto-organization of the visual system as early as 1 month of age that preceded the face-patch system as early as 1 month of age and that provides the scaffolding for the subsequent development of visual face processing cortical areas through experiences with faces (Livingston et al, 2017; Arcaro et al., 2017, Arcaro & Livingstone, 2017). Yet, because behavioral measures of face processing abilities were limited to face preferences versus objects and neuroimaging data included only cortical areas within the visual temporal pathways, the data can tell us very little on the maturation of the motion and attention pathways involved in the development of mutual eye gaze and gaze following.

The goal of the present study was to obtain addition information on the early maturational trajectories of the three visual cortical processing streams in infant rhesus monkeys using neuroimaging tools. The study was performed on a group of infants that were reared with their mothers in large social groups. These infants were longitudinally tested from 1 week to 6 months and showed developmental behavioral shifts on their preferences from looking at conspecific faces versus heterospecific faces (Parr et al., 2016), looking at averted versus direct faces (Muschinski et al., 2016), on the attention to the eye-regions of conspecifics (Wang et al., 2019, in preparation see chapter 1), and on the emergence of gaze following skills (Wang et al., 2019, see chapter 2). To achieve our goal of understanding the early infant development of the three visual cortical streams, infant macaques were scanned longitudinally from 2 weeks to 6 months to obtain structural magnetic resonance imaging scans (sMRI) for volumetric analyses, resting state functional magnetic imaging (rsFMRI) scans for functional connectivity (FC) analyses, and diffusion tensor imaging (DTI) scans for anatomical connectivity analyses. We have recently reported preliminary findings on FC between cortical areas within the three visual cortical pathways indicating stronger FC between the most posterior cortical areas of the three visual cortical streams than in the more anterior cortical areas with age, and FC between the anterior portion of ventral visual stream and the amygdala increased progressively in the first 12 weeks of age (Kovatz-Balint et al., 2018).

In the current study, DTI was used to examine developmental changes in infant rhesus macaque monkeys by measuring changes in white matter microstructure between cortical regions along each of the three pathways, based on DTI measures of fractional anisotropy (FA), mean diffusivity (MD) and streamline count. Since behavioral and neural data were obtained on the same animals and at similar time points, this allowed us to make direct correlations between the behavioral and neural findings. To our knowledge, this is the first systematic DTI study of visual cortical pathway in the nonhuman primate from birth to six months.

Methods

Fifteen mother-reared, socially housed male monkeys (*Macaca mulatta*) were studied. Both mother and infant lived in social compounds with over 100 individuals, preserving critical aspects of the social environment of native rhesus monkey groups (Berard, 1989; Lovejoy & Wallen, 1988; Wallen, 2005). All infants were estimated full-term (>450g), and mothers were all mid-ranking and multiparous. The infants were studied as part of a larger project studying the development of social-visual attention using eye-tracking methodologies. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University and followed the American Psychological Association standards for the ethical treatment of animals.

Structural MRI and DTI Image Acquisition

Neuroimaging data were collected from infants longitudinally at weeks 2, 4, 8, 12, 16, 20, and 24; see Table 1 for a detailed breakdown of detailed distribution of age at each time point. Infants with their mother were removed from their social groups and transported from Yerkes Field Station (Lawrenceville, GA) to theYerkes National Primate Research Imaging Center (Emory University, Atlanta, GA) on the day of the scan. Scans were acquired in a single session, including T1- and T2-weighted structural scans and DTI scans. Animals were scanned supine in the same orientation through placement and immobilization of the head in a custom-made head holder via ear bars and mouthpiece. A vitamin E capsule was placed on the right temple to mark the right side of the brain. Following initial telazol induction (2.89 ± 0.60 mg/kg BW) and intubation to minimize motion artifacts, scans were collected under anesthesia kept at the lowest possible level (isoflurane 0.8-1%). Physiological parameters were monitored using an oximeter, ECG, rectal thermistor, and blood pressure monitor. An i.v. catheter was used to administer dextrose/NaCl (0.45%) to maintain normal hydration, and MRI-compatible heating pad helped maintain the animals’ body temperature. Upon completion of the scans and full recovery from anesthesia, infants were returned to their mother and the pairs returned to their social group the following day.

Scans were acquired using a 3T Siemens Magnetom Trio Tim system scanner (Siemens Med. Sol., Malvern, PA, USA), and an 8-channel phase array coil. T1-weighted structural scans were acquired for registration of DTI scans using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) sequence (voxel size = 0.5x0.5x0.5 mm^3, TI/TR/TE = 950/3000/3.48 ms, 8 averages, GRAPPA, R=2). T2-weighted scans were collected in the same direction as the T1 to aid with the delineation of anatomical borders of regions of interest by improving GM/WM/CSF borders and segmentations (voxel size = 0.5x0.5x0.5 mm^3, TR/TE = 3200/373 ms, 3 averages, GRAPPA, R=2). DTI scans were obtained using a single-shot double spin-echo diffusion-weighted EPI sequence (voxel = 1.3x1.3x1.3 mm^3, TR/TE 5000/86 ms, b:0, 1000 s/mm^2, 128 directions, 10 averages, GRAPPA, R=3).

DTI Data Processing

Image pre-processing (e.g. artifact correction), tensor reconstruction and streamline reconstruction were performed using FSL (FMRIB, Oxford, UK). After images were quality controlled using DTI Prep (Oguz et al., 2014), diffusion tensors models were fitted using ‘dtifit’ (FSL) with remaining volumes to generate FA and MD values for each infant.

T1-weighted rhesus infant brain structural MRI atlases developed in-house at YNPRC (Shi et al., 2017) were used for regions of interests (ROIs) creation and propagation, these infant atlases were previously registered to the 112RM_SL atlas in F99 space (McLaren et al., 2009; 2010) and to templates of scans acquired longitudinally at 2, 12, and 24 weeks of age on 40 infant rhesus macaque monkeys from the YNPRC social colony, balanced by sex and social rank. First, individual T1-weighted structural images were transformed to conform to these age-specific infant atlases using nonlinear registration methods (FNIRT). Based on best match of neuroanatomical characteristics, the earliest scans (2, 4 weeks) were registered to the 2 weeks atlas, later scans (8, 12, 16 weeks) were registered to the 12 weeks atlas, and the latest scans (20, 24 weeks) were registered to the 24-week atlas. Regions of interests (ROIs) were drawn within atlas space and inverse-transformed back into each individual T1-weighted structural space where it was manually inspected and finally transformed using affine matrixes between individual T1-weighted structural images to images from the diffusion weighted imaging (DWI).

Pathway specific ROI-ROI streamlines were then reconstructed. First local diffusion orientation distribution functions (dODFs) of principal, secondary and tertiary fibers were estimated using the ‘bedpostx’ function in FSL. Next, ROI-ROI streamlines were generated from the dODFs using ‘probtrackx’ for each pathway of interest. These streamlines were then normalized based upon waytotal values and seed ROI size (in pixels), and individually thresholded between 2 and 6 x 10^{-6} to generate masks of ROI-ROI pathways. These masks were then used to calculate mean FA and MD with the tensor models previously generated by ‘dtifit’ along each ROI-ROI pathway.

Fractional anisotropy (FA) is a local metric derived from water molecules confined within an image and is modulated by intra-voxel orientation dispersion, myelination, and packing density. DTI measures is currently the only method of mapping changes in white matter (WM) architecture *in vivo*. Specifically, DTI data are modeled by a diffusion tensor and used to reconstruct the trajectories of WM tracts using local, discrete estimates of fiber orientation (Mori & van Zijl, 2002; Mori et al., 1999; Tournier et al., 2011), and measures can reflect changes in fiber diameter, fiber density, and myelination (Beaulieu, 2002). Generally, FA changes are believed to reflect changes in aspects important to connectivity, though it is best supported with other scalar metrics derived from DTI (Cohen & Assaf, 2002). Mean diffusivity (MD) is derived from the three eigenvalues of reconstructed tensors and decreases can be due to a number of changes in WM microstructure, including higher density, stronger myelination, or loss of certain axonal fibers resulting in more coherent organization (synaptic pruning). Finally, streamline count is a derived through probabilistic tractography and provides an estimation of connectivity strength (i.e., number of axons).

Data Analysis

Data between adjacent cortical areas within each of the visual pathways were used to analyze ROI-ROI anatomical connectivity (See Figure 1). Developmental changes in ROI-ROI connectivity were statistically analyzed using ANOVA with a linear mixed-effect model; AGE and HEMISPHERE were used as factors and the monkey identity as grouping factors in R Studio (Rstudio Inc, Boston, MA). When a main effect or interaction effect was detected, post-hoc analyses were conducted using Tukey HSD tests in R Studio. Significance level was set at $p < 0.05$ and data are reported as means, across subjects ± SEM. In order to prevent failed convergence in the models, streamlines and mean MD were log-transformed.

To assess the correlations between the developmental changes in white matter microstructure with developmental shifts in behaviors (Attention to Eyes/Mouth, Mutual Eye Gaze, and Gaze Following), linear mixed-effect models were generated for each ROI-ROI pathway of interest. Mean FA, inverse MD, log-transformed streamline counts, and AGE were used as factors; the monkey identity was set as the grouping factor; and the behavioral measure (as reported in chapters 1 and 2, percentages) as the dependent variables. Because the behavioral measures were taken at a higher temporal resolution than neuroimaging scans, the middle-mean interpolation of the behavior was taken to correspond with the neuroimaging timeline (e.g. Week

2 scan was a mean of weeks 1, 2, and 3 of behavioral measures; Week 8 scan was a mean of Weeks 7 and 9 behavioral measures). Correlation coefficients were calculated from these models using a pseudo-R^2 method developed for the analysis of linear mixed-effect models, reported as marginal R^2 values (Nakagawa, Johnson, & Schielzeth, 2017). All analyses were conducted in R Studio.

Results

Ventral Object Pathway: Overall, maturational changes between cortical areas along the ventral object pathway can be seen for mean FA (Figure 2A), MD (Figure 2B) and streamline counts (Figure 2C).

Connections between V4 and TEO (Figure 2, left column): As seen in Figure 1A, FA values increased progressively throughout development from Weeks 2 through 24 ($F(6, 172) = 13.24$, $p < 0.001$), and were generally higher in the left hemisphere when compared to the right ($F(1,172) = 52.25$, $p < 0.001$). The interaction between AGE and HEMISPHERE was not significant ($F(6, 172) = 0.59$, $p > 0.1$). Post-hoc Tukey HSD tests showed that FA values at the last 2 time points were significantly higher than those at the first 2 time points (Week 2 vs 20: $p < 0.001$; Week 2 vs 24: $p < 0.001$; Week 4 vs 20: $p < 0.005$; Week 4 vs 24, $p < 0.001$) and there was a significant FA increase between time points from Week 4 to Week 8 ($p = 0.049$). Separated by HEMISPHERE, there was a significant difference between Weeks 2 and 24 in the Left hemisphere ($p = 0.0445$), but not the Right ($p = 0.139$). Weeks 4 and 24 in the Right hemisphere was trending towards significance ($p = 0.0784$). Finally, although the mean FA values were generally lower in the Right hemisphere across development, post-hoc analyses did not show any significant difference between Left and Right at a single time point.

Correspondingly, this increase in FA values was associated with a progressive decrease in MD values as reflected by a significant effect of AGE ($F(6,172) = 526.12$, $p < 0.001$, see Figure 1B) with slightly higher values on the right hemisphere than on the left hemisphere ($F(1,172) = 4.96$, $p < 0.05$). Post-hoc Tukey HSD tests showed that MD values were significantly different from each other between all timepoints ($p < 0.005$ in all comparisons). However, when separated by HEMISPHERE, the pattern of development showed slight changes. Both Left and Right hemispheres showed significant changes up until 12 weeks ($p < 0.01$ in all comparisons of adjacent time points in both Left and Right). However, after 12 weeks, the change was not significant to 16 weeks in either hemisphere (Left 12v16: $p = 0.238$; Right: $p = 0.280$). Week 24 was still significantly lower than week 16 (Left: $p = 0.00118$; Right: $p < 0.0001$) in both hemispheres, though the Right hemisphere was the only one that showed a significant difference between Week 20 and Week 16 (Left: $p = 0.497$; Right: $p = 0.0114$). Finally, although the Left hemisphere had lower MD values overall, there were no significant pairwise difference at any single time point.

Streamline counts also increased from 2 weeks to 24 weeks (AGE: $F(6,172) = 188.65$, $p < 0.001$). Post-hoc Tukey HSD tests showed that the most significant increase in streamline counts was prior to 8 weeks; specifically, Weeks 2 and 4 had significantly fewer streamline counts than all other time points ($p < 0.001$ in all comparisons) and no significant differences between time points from 8 weeks onward. These comparisons remained similar when separated between Left and Right hemispheres. There was also a significant difference of streamline counts between HEMISPHERE ($F(1,172) = 17.28$, $p < 0.001$) with the Left hemisphere having slightly less streamlines at each time point, though the post-hoc pairwise comparisons showed no significant differences at any single time point.

Connections between TEO and TE (Figure 2, center column): FA values between these temporal cortical areas also varied according to AGE ($F(6,373) = 25.90$, $p < 0.001$) and HEMISPHERE ($F(1,372)=114.47$, $p < 0.001$). FA values between TEO-TE connections decreased in the first 12 weeks before increasing again from 16 to 24 weeks. Analyses of data from adjacent time points (Tukey HSD) revealed that the most significant decrease in FA was from Weeks 4 to 8 ($p = 0.00633$). The decrease in FA continues from Weeks 8 to 12, though not at significant levels ($p = 0.0974$). Thereafter, FA values significantly increased from 12 to 16 weeks of age ($p = 0.00336$). Separated by HEMISPHERE, developmental changes remained relatively similar. Although there were no significant differences between adjacent time points within each hemisphere, Week 2 was significantly higher than Weeks 8 through 24 in both hemispheres ($p < 0.001$ in all comparisons in both hemispheres). Overall, the Left hemisphere had higher FA values than the Right across development, with pairwise comparisons reaching significant levels only at Weeks 4 ($p = 0.0050$), 16 ($p = 0.0275$), and 24 ($p = 0.00097$).

In contrast to the FA values, MD values decreased progressively and continuously across age and similarly for both hemispheres (AGE: $F(6,372) = 961.77$, $p < 0.001$, and HEMISPHERE: $F(1,372) = 1.46$, $p > 0.1$). This decrease in MD values was significant between each time point ($p < 0.001$ in all comparisons). Separated by HEMISPHERE, the development slowed slight differences between the Left and Right in the last 12 weeks. Specifically, the Left hemisphere showed no significant change from Weeks 12 to 16 ($p = 0.106$), or Weeks 20 to 24 ($p = 0.417$), but a significant decrease from Weeks 16 to 20 ($p = 0.00592$). In contrast, the Right hemisphere did not show a significant decrease from Weeks 16 to 20 ($p = 0.999$), but instead showed significant decrease from Weeks 12 to 16 ($p < 0.0001$) and Weeks 20 to 24 ($p = 0.0147$).

Streamline counts between TEO and TE increased with age ($F(6,372) = 351.21$, $p < 0.001$) with the right hemisphere having more streamlines than the left ($F(1, 372) = 4050.89$, $p < 0.001$). Additionally, the interaction between AGE and HEMISPHERE was significant ($F(6, 372) = 9.45$, $p < 0.001$). Post-hoc Tukey HSD tests showed a significant increase from Week 2 until Week 12 ($p < 0.001$ in all comparisons), but no significant pairwise differences from Weeks 12 through 24. When separated by HEMISPHERE, the Right hemisphere showed significant changes up until Week 8, whereas the Left hemisphere showed a more prolonged development, increasing up until 12 weeks before leveling off (Weeks 8-12, Left: $p < 0.001$; Right: $p = 0.843$). Pairwise comparisons between the two hemispheres were significant at all time points ($p < 0.001$ in all comparisons).

<u>Connections between TE and Amygdala (Figure 2, right column)</u>: Mean FA values for the TE-amygdala connections showed a significant developmental shift similar in both hemispheres as revealed by a significant effect of AGE ($F(6,372) = 19.91$, $p < 0.001$) and HEMISPHERE ($F(1,372) = 16.15$, $p < 0.001$). The interaction between AGE and HEMISPHERE was not significant, but certainly trending towards significance ($F(6, 372) = 2.12$, $p = 0.0501$). Following an initial decrease in FA values from Weeks 2 to 4 ($p < 0.001$), there were slight, but not significant, increases in FA from Week 8 onward. Thus, post-hoc analyses revealed that Week 2 had the highest FA values than any other time points ($p < 0.05$ in all comparisons). When looking at each HEMISPHERE separately, both showed a significant decrease from Week 2 to Week 4 (Left: $p = 0.00021$; Right: $p = 0.0037$). In addition, the interaction indicates that the left hemisphere showed lower FA values from Weeks 2 to 4, and this pattern reversed (e.g. higher FA values than the right) starting at about 8 weeks. This is reflected by the fact that, although FA values at Week 2 were significantly higher than at all other time points in the Right

hemisphere ($p < 0.0001$ in all comparisons), within the Left hemisphere FA values increased such that the difference between Weeks 2 and 24 was no longer not significant ($p = 0.248$).

MD values between TE and Amygdala decreased progressively across age ($F(6, 373) = 681.06$, $p < 0.001$) with the right hemisphere having slightly, but not significantly, higher values than the left ($F(1, 372) = 3.14$, $p = 0.0771$). There was also an interaction of both variables ($F(6, 372) = 2.72$, $p < .05$). All values were significant from each other in the post-hoc analyses, except for no significant changes from Weeks 12 – 16 ($p = 0.280$) and Weeks 20 – 24 ($p = 0.317$). Within the individual hemispheres, however, both Weeks 12 – 16 and 20 – 24 had significant decreases in the Right hemisphere (12v16: $p = 0.00789$; 20v24: $p = 0.000683$), but not in the Left (12v16: $p = 0.998$; 20v24: $p = 0.999$). In contrast, the Left hemisphere decreased from Weeks 16 – 20, but the Right hemisphere did not (Left: $p = 0.00315$; Right: $p = 0.982$). Generally, the Left hemisphere had lower MD values across development, though pairwise comparisons did not reveal any significant differences at any single time point.

Finally, streamline counts from TE to the amygdala increased significantly across AGE ($F(6, 373) = 143.65$, $p < 0.001$), and were higher in right HEMISPHERE ($F(1, 372) = 380.60$, $p < 0.001$). The interactions between the two factors also reached significance ($F(6, 372) = 4.99$, $p < .001$). Post-hoc analyses showed a significant increase from Week 2 until Week 8 ($p < 0.01$ in all comparisons), and then no significant increases after 8 weeks of age. These changes were reflected in both the Right and Left hemispheres. The Left hemisphere, however, showed continual development with streamline counts increasing throughout development with a significant difference between Weeks 8 and 24 ($p = 0.00370$) that was not present in the Right hemisphere ($p = 0.843$). Finally, the Right hemisphere had significantly more streamlines than the Left at all time points ($p < 0.001$ in all comparisons).

Ventral Motion Pathway: Maturational changes between cortical areas along the ventral motion pathway can be seen for mean FA (Figure 3A), MD (Figure 3B) and streamline counts (Figure 3C).

<u>Connection between V4 and MT (Figure 3, left column):</u> Mean FA values seem to slightly increase in the later ages as reflected by a significant effect of AGE ($F(6, 172) = 6.54$, $p < 0.001$) with higher FA values in the left hemisphere than the right (HEMISPHERE: $F(1, 172) = 104.66$, $p < .001$). Post-hoc analyses showed total pairwise differences between Weeks 4 to 24 ($p = 0.0296$) and between 12 and 24 ($p = 0.0158$). Within each hemisphere, however, there were no significant pairwise difference between age groups. At 8 weeks, the Left hemisphere had significantly higher FA than the Right ($p = 0.00589$), though this difference disappeared at 12 weeks.

There was a progressive and continuous decrease in MD, with significant effects of both AGE ($F(6, 172) = 508.85$, $p < 0.001$) and HEMISPHERE ($F(1, 172) = 7.86$, $p < 0.01$). Post-hoc Tukey HSD tests showed that MD values decrease was significantly different between each time points ($p < 0.001$ in all comparisons). Separated by HEMISPHERE, however, the MD decrease ceases to be significant at 12 weeks in both Left and Right, with the Right hemisphere showing another decrease from 20 week to 24 weeks ($p = 0.0184$) that

was not present in the Left ($p = 0.376$), suggesting that changes in the Right hemisphere is driving the overall MD decreases in this pathway.

Similar to both FA and MD values, streamline counts showed significant effects of AGE ($F(6, 172) = 188.65$, $p < .001$) and HEMISPHERE ($F(1, 172) = 17.28$, $p < .001$), with higher counts in the right hemisphere than the left. Post-hoc Tukey HSD tests showed that the most significant increases in streamline counts occurred in the first 4 weeks, with Weeks 2 and 4 being significantly different from each other and between all other time points ($p < 0.001$ in all comparisons). These changes were the same when analyzing each hemisphere separately. The Left hemisphere had fewer streamlines overall throughout development, though post-hoc pairwise comparisons did not show any significant difference at any given time point.

Connection between MT and MST (Figue 3, center column): Mean FA values showed a significant effect of AGE ($F(6, 117) = 3.04$, $p < .01$) and of HEMISPHERE ($F(1, 116) = 7.12$, $p < 0.01$). FA values remained relatively stable in the first few weeks with a slight dip at 12 weeks followed by a slight but progressive increase between Weeks 12 and 24. Tukey HSD tests showed that Week 12 was the lowest mean FA with significantly lower values than Week 4 ($p = 0.017$) and Week 24 ($p = 0.024$). The Right hemisphere had lower FA values than the left, but pairwise comparisons at each time point did not yield any significant results.

Mean MD values showed a significant effect of AGE ($F(6, 172) = 538.70$, $p < .001$), but no significant effect of HEMISPHERE ($F(1, 172) = 0.42$, $p > 0.1$). Post-hoc analyses revealed a significant and steeper decrease from 2 weeks through 12 weeks, followed by a shallower decrease from 12 weeks to 24 weeks, with each time point being significantly different from each other ($p < 0.001$ in all comparisons). When separated by HEMISPHERE, both Left and Right hemispheres showed significant decreases up until 12 weeks, but the pairwise comparisons between adjacent months were not significant thereafter. However, there was evidence of progressive decreases in MD values, as MD values in Week 24 was significantly lower than in Week 12 (Right: $p < 0.0001$; Left: $p < 0.0001$).

Lastly, the effects of AGE ($F(6, 173) = 87.62$, $p < 0.001$) and HEMISPHERE ($F(1, 172) = 115.80$, $p < 0.001$) were both significant for streamline counts. Post-hoc comparisons showed that there was a significant increase in streamline counts until Week 8 ($p < 0.005$ in all comparisons) with no further significant changes thereafter. These changes were similarly reflected when analyzing each hemisphere separately in the post-hoc comparisons. Although the Right hemisphere had lower streamline counts compared to the Left, post-hoc comparisons did not show any significant hemispheric difference at any time point.

<u>Connection between MST and FST:</u> FA values for connections between MST-FST indicate significant differences with AGE ($F(6, 172) = 44.18$, $p < .001$) and HEMISPHERE ($F(1, 172) = 29.09$, $p < .001$). This increase was steeper between Week 2 and Week 8 ($p < 0.001$ in all comparisons) but continues thereafter, with a significant increase from Week 8 to Week 24 ($p = 0.041$). Post-hoc analyses of each hemisphere separately showed a significant increase for 4 to 8 weeks in the Left ($p = 0.049$), but not in the right ($p = 0.159$). However, both hemispheres showed an increase from Week 2 and Week 8 (Left: $p = 0.00203$; Right: $p = 0.000278$). Although the Right hemisphere had higher FA values than the Left, the post-hoc analyses did not reveal any significant hemispheric difference at each time point.

Mean MD values showed a significant effect of AGE ($F(6, 172) = 854.43$, $p < .001$) and of HEMISPHERE ($F(1, 172) = 9.75$, $p < .01$). Similar to the other two connections along this pathway, post-hoc Tukey HSD tests showed a significant decrease from one time point to the next, with all time points being significantly different from each other ($p < 0.001$ in all comparisons). When analyzing the hemispheres separately post-hoc, there were slight differences in the development of the Right and Left hemispheres. Specifically, the Left hemisphere has significant decreases until 12 weeks, but no other significance differences between adjacent time points, though overall the decrease from 12 to 24 weeks was significant ($p < 0.0001$). In contrast, the Right hemisphere, MD values continued to decrease from Week S12 to 16 ($p = 0.00535$) and from Week 20 to 24 ($p = 0.0355$).

Lastly, streamline counts showed significant effects of AGE ($F(6, 173) = 117.32$, $p < .001$) and HEMISPHERE ($F(1, 172) = 138.12$, $p < .001$). Post-hoc Tukey HSD tests indicated significant increase in streamline counts from Week 2 until Week 8 ($p < 0.001$ in all comparisons) but remained relatively stable with one last significant increase from Weeks 16 to 20 ($p = 0.00941$). When the HEMISPHERES were analyzed separately, there was a similar increase in streamline counts from Week 2s until 8 ($p < 0.001$ in all comparisons in both hemispheres), but no significant increases thereafter. The Left hemisphere had higher streamline counts than the Right overall. Post-hoc pairwise comparisons showed that this difference became significant at 12 weeks and continued until the last time point (Week 12: $p = 0.0128$; Week 16: $p = 0.0268$; Week 20: $p = 0.000220$; Week 24: $p = 0.0127$).

Dorsal Attention/Visujo-Spatial Pathway: Overall, developmental changes between cortical areas along the dorsal attention/visuo-spatial pathway can be seen for mean FA (Figure 4A), MD (Figure 4B) and streamline counts (Figure 4C).

Connection between V4 and LIP (Figure 4, left column): There was a general significant increase in FA throughout development (AGE: $F(6, 372) = 31.23$, $p < .001$), with higher FA values in the left hemisphere than in the right hemisphere (HEMISPHERE:$F(1, 372) = 99.62$, $p < .001$). Post-hoc Tukey HSD tests revealed significant increase in mean FA from Week 4 to Week 8 ($p < 0.001$), followed by a significant decrease from Week 8 to Week 12 ($p=0.0370$) and a second gradual and significant increase from Week 12 to Week 24 (Weeks 12 vs 20: $p < 0.001$; 12 vs 24: $p < 0.001$). Separated out by HEMISPHERE, the Left hemisphere showed significant pairwise differences between Week 2 and Weeks 8, 20, and 24 (2v8: p = 00670; 2v20: 0.00085; 2v24: $p < 0.0001$), between Week 4 and Weeks 8 and 24 (4v8: p = 0.0330; 4v24: 0.00040), and between Weeks 12 and 24 (p = 0.0304). In the Right hemisphere, there were similar differences between Weeks 2 and 4 between Weeks 20 and 24 (2v20: p = 0.0002; 2v24: p = 0.0003; 4v20: p = 0.0002; 4v24: p = 0.0004). Although the Right hemisphere had generally lower FA values than the Left, the only point at which Left was significantly different from Right was at Week 8 (p = 0.00448).

There was a corresponding and continuous decrease in MD values across age ($F(6, 372) = 1188.02$, $p < 0.001$) with higher values in the right hemisphere than in the left ($F(1, 372) = 9.01$, $p < 0.01$). The interaction between AGE and HEMISPHERE also reached significance ($F(6, 372) = 2.94$, $p = < 0.01$). Post-hoc Tukey HSD tests showed that MD values decrease was significantly different between each time points ($p < 0.001$ in all comparisons), and similarly within both Right and Left hemisphere each time point was significantly different from each other ($p < 0.001$), with the exception of Week 16 to Week 20 in the Right hemisphere (p = 0.752). There were no significant differences between Left and Right hemispheres at each time point, though the Left had generally lower MD values, with a trending towards significant change at 20 weeks (p = 0.0692).

Finally, streamline counts at V4-LIP significantly increased with AGE ($F(6, 372) = 407.71$, $p < .001$), with slightly greater increase for the right than the left HEMISPHERE ($F(1, 372) = 37.38$, $p < .001$), especially at Week 8 and Week 18 (AGE and HEMISPHERE: $F(6, 372) = 2.71$, $p < 0.0138$). Post-hoc Tukey HSD tests showed that for both hemispheres, the most significant increases in streamline count occurred in the first 8 weeks ($p < 0.001$ in all comparisons). Following 8 weeks, streamline count remains stable until another decrease from Weeks 12 to 16 ($p = 0.0045$) for the Left hemisphere only. The Left hemisphere had generally lower streamline counts but was not significantly different from the right at any single time point.

Connection between LIP and FEF (Figure 4, right column): Mean FA values at LIP-FEF also increased throughout development (AGE: $F(6, 373) = 90.07$, $p < 0.001$) with higher values in the right than the left hemispheres (HEMISPHERE: $F(1, 372) = 38.97$, $p < 0.001$). Post-hoc analyses revealed that the most steeper increase in FA occurred from Weeks 4 to 8 ($p < 0.001$), with a second increase from Weeks 12 to 16 ($p = 0.0120$) that continued to gradually increase (Weeks 12 vs 20: $p < 0.001$; 12 vs 24: $p < 0.001$), though the increase in adjacent time points between Weeks 12 and Weeks 16 and 20 only trending towards significant (12 vs 16: $p = 0.0692$; 16 vs 20: $p = 0.0593$). Separated by HEMISPHERE, both Left and Right had a significant increase from Weeks 4 to 8 (Left: $p = 0.0003$; Right: $p = 0.0166$). Additionally, Weeks 2 and 4 were significantly different from Weeks 8 through 24 ($p < 0.001$ in all comparisons) in both hemispheres. Although the Left side had generally lower mean FA values, the two hemispheres were not significantly different from each other at any single time point.

Accompanying these changes in FA, mean MD values decreased significantly with age ($F(6, 372) = 2510.77$, $p < .001$) and were slightly lower in the right than the left hemispheres ($F(1, 372) = 66.29$, $p < .001$). All post-hoc tests revealed significant differences for all pair-wise comparisons ($p < 0.001$ in all comparisons), and was similarly reported when separated by HEMISPHERE, with the exception of no significance between Weeks 16 and 20 in the Right hemisphere ($p = 0.223$). Mean MD values were similar between both left and right, with the

Right hemisphere decreasing and becoming lower than the Left. The Right had significantly lower MD at 16 weeks ($p = 0.0165$), but the difference was not significant at any other time points.

Finally, streamline counts had significant effects of AGE ($F(6, 372) = 187.06$, $p < .001$) and HEMISPHERE ($F(1, 372) = 163.21$, $p < .001$), with increases in streamline count through development and higher streamline count in the left hemisphere than in the right. Tukey HSD tests conducted post-hoc showed significant increases in the first 8 weeks of life ($p < 0.001$ in all comparisons). After 8 weeks, streamline counts remained relatively stable with only one significant comparison between Weeks 16 and 24 ($p = 0.0293$) indicated slight increase in streamline counts in the last few time points. Separated out by HEMISPHERE, both Right and Left hemispheres showed a significant increase in streamlines in the first 8 weeks, with Weeks 2 and 4 significantly different from every other time point and with each other ($p < 0.001$ in all comparisons). The Right hemisphere had lower streamline counts. This difference was significant at week 2, 4, and 20 weeks (2 weeks: $p = 0.00317$; 4 weeks: $p = 0.00110$; 20 weeks: $p = 0.0126$).

WM microstructure and behavioral measures: Comparisons between the white matter measures and the behavioral measures were performed with marginal R^2 values using a modified Cohen approach for linear mixed-effect models (Cohen, 1988; Nakagawa, Johnson, & Schielzeth, 2017).

Social-visual attention measures: Comparisons between WM maturation (FA, MD, and streamline count as factors) and Fixation Percentage to the Eyes and Fixation Percentage to

Mouth (see Chapter 1) are depicted on Figure 5. Along the ventral object pathway, there was no correlations between WM measures and fixation percentage to the eyes, with the exception of connections between V4-TEO that showed moderate effects on eye fixation percentage (Left: marginal $R^2 = 0.14$; Right: marginal $R^2 = 0.14$). For the ventral motion pathway, moderate (marginal $R^2 > 0.13$) effects of WM microstructure measures on fixation percentage to the eye region were present throughout the ventral motion pathway, with smaller effects at MT-MST (Left: marginal $R^2 = 0.12$; Right: marginal $R^2 = 0.12$) but larger effects (marginal $R^2 = 0.24$) for the development of WM microstructures in the Right MST-FST connection. Finally, maturation along the dorsal attention network was moderate (marginal $R^2 > 0.13$) at all tracts for both hemispheres.

In contrast, the vast majority of WM tracts had a small effect (marginal $R^2 < 0.12$) on fixation percentage to the mouth region. However, the maturation of MT-MST (Left: marginal $R^2 = 0.17$; Right: marginal $R^2 = 0.17$) and the left MST-FST (marginal $R^2 = 0.15$), but not the right MST-FST (marginal $R^2 = 0.12$), had moderate effects on changes in percentage fixation to the mouth.

Gaze following skills: Overall, effects of WM maturation on Gaze Following and Mutual Eye Engagement (see Chapter 2) are depicted in Figure 6. WM maturation had moderate effects (marginal $R^2 > 0.13$) on the development of gaze following. The largest effects were seen for the Right V4-MT (marginal $R^2 = 0.23$), and MST-FST bilaterally (Left: marginal $R^2 = 0.21$, Right: marginal $R^2 = 0.22$) within the ventral motion pathway; and an especially large effects within the right V4-TEO (marginal $R^2 = 0.29$) in the ventral object pathway.

All connections in the three pathways had large effects on engaging in mutual eye contact (marginal $R^2 > 0.27$). Finally, with the exception of very high effect of V4-TEO WM changes on mutual eye engagement (Left: marginal $R^2 = 0.40$, Right: marginal $R^2 = 0.39$), WM maturation in the ventral object pathway showed the lowest marginal R^2 correlation effect on mutual eye contact.

By contrast, WM maturation in the ventral motion pathway had a fairly high effects on mutual eye gaze, with large marginal R^2 values at V4-MT (Left: marginal $R^2 = 0.38$; Right: marginal $R^2 = 0.38$), MT-MST (Left: marginal $R^2 = 0.39$; Right: marginal $R^2 = 0.39$), and MST-FST (Left: marginal $R^2 = 0.42$, Right: marginal $R^2 = 0.41$). Finally, although the correlations were weaker, there were still large effects of WM maturation with mutual eye gaze along the dorsal attention pathway at V4-LIP (Left: marginal $R^2 = 0.32$; Right: marginal $R^2 = 0.32$) and LIP-FEF (Left: marginal $R^2 = 0.31$; Right: marginal $R^2 = 0.29$).

Discussion

To our knowledge, this is the first DTI-based study to characterize normal development of the visual cortical networks that support social visual engagement in the early months of life in nonhuman primates. It provided indispensable information on the developmental trajectories of brain maturation for rhesus monkeys that is crucial as a baseline in investigating disease models of neuropathology and the effects of perturbations during early rearing and environmental insults. DTI properties (FA, MD, streamline counts) revealed critical information reflective of WM integrity. Overall, all the DTI properties indicated that there were major maturational changes in white matter (WM) microstructures of the three visual processing pathways (i.e., ventral object and motion pathways, dorsal attention/visuo-spatial pathways) and these WM changes appeared to parallel the developmental changes in all behavioral measures of social visual engagement.

WM maturation of the ventral object pathway:

WM microstructure changes along this pathway occur largely in the first 8 weeks of age, before slowing and becoming relatively stable thereafter. Specifically, streamline counts suggest an increase in fiber growth within the first 8 weeks across all connections along the pathway that slowed down and stabilized by 24 weeks. As fiber growth stabilizes by 8 weeks, increases in FA and decreases in MD along V4-TEO throughout the 24 weeks suggest a general increase in microstructural integrity via either increased myelination or axonal density. Both TEO-TE and TE-amygdala show initial decreases in FA before increasing again at either 8 (TE-amygdala) or 12 weeks (TEO-TE). A decrease in FA accompanied with increased streamline counts can be associated either with an overall increase in fiber growth or with lower packing density (Takahashi et al., 2002). The inflection point at which FA begins to increase along TEO-TE and TE-amygdala suggests that the slowed fiber growth is accompanied by synaptic pruning to increase microstructural integrity. Interestingly, these developmental changes in WM integrity parallels the significant pruning of connections between these temporal cortical areas as well as between the temporal cortical areas and the amygdala that had already been demonstrated in histological studies (Webster et al., 1991). Thus, the continual increase in FA, decrease in MD, and relatively stable streamline count following 8 months suggest improved connectivity that leads to an associated increase in functional connectivity between some of the areas within the ventral object pathway found earlier in the same animals (Kovacs-Balint, 2018). Since the cortical areas along the ventral object pathway are known to mediate face perception and facial features detection in adulthood (Tsao et al., 2006; Van Essen et al., 2001; Tootell, Tsao, &

Vanduffel, 2003; Sereno & Huang, 2006), the changes of WM integrity from birth to six months are likely to support the emergence of facial feature detections and preference for looking at the eye region of a face (see further discussion below).

WM maturation in the ventral motion pathway:

Connections between the most posterior part of the pathway (V4-MT) developed very gradually throughout the first 6 months of age. Although the streamline counts showed a sharp increase until 8 weeks, suggesting an increase in fibers up to this age, FA values only slightly increased with significant decreases in mean MD values suggesting that WM between V4-MT continues to develop through infancy and may continue to develop after 24 weeks. Connections in the most anterior portions of the motion pathway, MT-MST and MST-FST, showed a shallower increase in streamline counts from 2 to 8 weeks with overall subtle increases in FA and more pronounced continuous decreases in MD in both pathways. These data suggest that although connectivity strength increased only slightly during this developmental period, there is a continual development of myelination and axonal density during this early period of development. Cortical areas in the motion pathway along the superior temporal sulcus (STS) has been shown to be critically involved in the perception of gaze and face direction in both humans and rhesus macaques (Hoffman & Haxby, 2000; Puce et al., 2000; Kamphuis et al., 2009, Roy et al., 2014). Thus, it is likely that increases in WM integrity during this early period of development may be associated with the emergence of gaze monitoring behaviors (see further discussion below).

WM maturation in the dorsal attentional pathway (V4-LIP, LIP-FEF):

WM development betweenV4-LIP indicated an increase in streamline counts from 2 to 8 weeks that remained relatively stable thereafter. Finally, WM between LIP-FEF showed a continual development throughout the first 24 weeks with a continual increase in FA up until 24 weeks. Given the critical role of the dorsal visual pathway for oculomotor control, spatially directed attention, and volitional attention (Milner & Goodale, 1995), WM development along this pathway may be responsible for the emergence and maintenance of skills such as gaze following that requires direction of attention within the environment to important social cues as well as the use of volitional saccades to follow the gaze of a peer.

Associations between Behavioral changes and WM maturation

One critical component of our large longitudinal study on the development of early social skills in infant macaques was the assessment of both critical basic social skills (see chapter 1, chapter 2) and of maturation of visual cortical networks known to support these skills in the same animals and at approximately the same time points from 2 to 24 weeks. Thus, in the present study, we also reported an analysis on how the maturational changes in WM integrity along cortical areas of the three visual streams were related to the developmental behavioral changes obtained in the same animals.

Marginal R^2 values of a series of linear mixed-effect models showed that the combined developmental changes in FA, MD, and streamline count along these cortical pathways of interest have varying degrees of correlational strength with behaviors, such as preference to the eyes, preference to the mouth (see Figure 4), mutual eye gaze and gaze following (Figure 5).

The maturation of the ventral object pathway showed generally moderate correlational strength with the measured behaviors. This effect was stronger for the WM the maturation of the most posterior cortical areas V4-TEO than the more anterior areas, i.e. between TE and TEO and TE vs Amygdala. Areas within V4-TEO mediate visual perception and contained the face area critical for developing preference for the eyes, mutual eye gaze, and gaze following (Desimone et al. 1984; Perrett et al. 1985; Tsao et al. 2003; Hadj-Bouziane et al. 2008; Pinsk et al. 2009; Ungerleider and Bell 2011). Thus, the larger correlational strength of WM maturation of V4-TEO with fixation to the eyes, mutual eye contact, and in gaze following suggests that, at this early age, the development of the pathway connecting visual cortex to the temporal cortex may be more crucial to the processing of facial information than the development of associated temporal structures themselves. Finally, TE-AMY interactions are also critical for face identity and facial expressions (Gothard et al. 2007; Hoffman et al. 2007; Mosher et al. 2010; Schwiedrzik et al. 2015), which emerge in the first months in monkeys (Lutz et al. 1998; Kuwahata et al. 2004; Sugita 2008; Muschinski et al. 2016; Parr et al. 2016). Thus, all WM measures of TE-amygdala indicated that maturation of this WM tract extends beyond 24 weeks and parallel the strong functional TE-AMY coupling we already found in the same animals in the first 12 weeks of age (Kovacs-Balint et al., 2018). Thus, the anatomical and functional interactions between the temporal lobe areas and the amygdala may enable the development of continuous precision in the evaluation of social stimuli and faces in particular.

Changes in these measures of WM microstructure along the ventral motion pathway seemed to have an effect on all measured behaviors, with the maturation of these tracts having particularly high correlational strength in fixation towards the eyes and in mutual eye contact in moments of direct gaze. Both these supports previous literature highlighting the importance of cortical areas within the STS in monitoring the gaze of others. Previous electrophysiological studies in adult rhesus macaques have shown differential firing with respect to gaze in the STS with a subset of STS neurons being particularly important for establishing mutual eye gaze (Perret et al., 1985; Perret & Mistlin, 1990; Perret et al., 1992). Additionally, reversible inactivation of the STS disrupts the use of gaze as a directional cue (Roy et al., 2014). Thus, the present results indicate that the ventral motion pathway is similarly important for the support of gaze monitoring behaviors in infant development. The WM changes between MT-MST changes align with the early emergence of motion detection at 2 weeks of age that becomes more refined throughout infancy and continues to develop past 1 year of life in monkeys (Kiorpes & Movshon, 2004; Kiorpes et al., 2012). More importantly, given that rapid evaluation of direct-gaze faces emerges at around 3 months of age in infant macaques (Mendelsen et al., 1982; Muschinski et al., 2016), the rapid early development of MST-FST may help support this form of early social-visual attention that, with further WM maturation may aid in the strengthening of gaze monitoring behaviors in childhood.

White matter maturation within areas of the dorsal attention pathway had moderate correlational strength with fixation percentage to the eyes, but a larger strength with changes in engaging in mutual eye contact. These cortical areas within the lateral intraparietal sulcus (LIP) are critical for mediating spatial attention and largely responsible for attentional shifts in response to salient stimuli (Ungerleider and Mishkin, 1982; Dickinson et al., 2003; Liu et al., 2010). Additionally, the LIP has shown activation while subjects are monitoring the gaze of other subjects (Okada et al., 2008; Akiyama et al., 2007). Although some aspects of this pathway seem to have continual anatomical development that extends beyond 24 weeks (i.e., FA values along LIP-FEF), the early changes in this attentional pathway seems to play a role in directing attention towards socially salient cues, such as the eyes of a conspecific. These changes in white matter microstructural integrity in this cortical pathway were also associated with similar developmental changes in functional connectivity within the pathway (Kovacs-Balint et al., 2018).

Study Limitations

There are several limitations in interpreting WM microstructural development using DTI methodologies. FA and MD provide measurable changes in WM microstructure commonly associated with myelination, axonal density, and direction coherency of axon paths. However, multiple crossing fibers can influence these measures and their interpretation. Although the use of higher resolution images with multiple directions (i.e., 1.3 mm^3 isotropic, 128 directions) ameliorates these issues, it is still unlikely that voxels contain axons entirely of a single directional orientation. Additionally, streamline counts calculated from probabilistic tractography can be sensitive to gross differences in ROIs and the geometry of specific WM tracts (Kaden et al. 2007). Thus, the analyses of streamline count were only made within a pathway and not between pathways, as comparisons of streamline count between pathways cannot be readily interpreted.

Summary

In sum, this study reports the development of WM tracts along three visual pathways that are believed to be essential to support social-visual engagement in early infancy. Rapid changes were seen in the connections between the extrastriate cortical area V4 and cortical areas in temporal lobe (MT, TEO) and parietal lobe (LIP) in the first few months of life in macaques. ROI-ROI connections along each cortical visual pathway show slower and longer development. In addition, the findings showed robust effects of these white matter developmental changes with the maturation of social visual engagement, i.e. eye preference, mutual eye engagement, and gaze following, thus providing evidence that the visual cortical pathways studied in adult social-visual attention do indeed serve to support basic behaviors of social-visual engagement even in infancy. Specifically, the ventral motion pathway along the STS seems to be critically important in establishing mutual eye contact in infancy, paralleling its importance in monitoring eye gaze in adulthood. Although large effects in the development of the dorsal attention network on mutual eye gaze were observed, it is possible that more robust changes could occur after 24 weeks when the prefrontal areas (FEF) become more crucial for volitional attentional mechanisms. Also, our findings support previous literature showing the importance of TE-amygdala interactions in facial processing, especially when emotional information needs to be decoded from faces. More importantly, although recent blood-volume fMRI studies in infant monkeys have revealed the presence of a retinotopic proto-organization of the visual system as early as 1 month (Livingston et al, 2017; Arcaro et al., 2017, Arcaro & Livingstone, 2017), the present findings demonstrate that significant maturation of WM microstructures, together with the maturation of functional connectivity (Kovacs-Balint et al., 2018) during the first six months of age within the cortical visual pathways is critical for the emergence and strengthening of the early visual face processing abilities early after birth.

Finally, given the difficulty in scanning longitudinally typically developing human infants, the present findings are highly valuable to better understand the normative development of social visual networks in humans. Cortical visual pathways are evolutionarily well-conserved between NHPs and humans. Thus, the emergence of functional connectivity between primary visual cortex and higher-order visual areas during infancy -neonates to 2 years- (Lin et al. 2008) suggest that visual neural networks are just beginning to develop during infancy as they do in monkeys, and, as our study suggests, this ongoing development supports measures of social-visual attention and social behavior through infant development.

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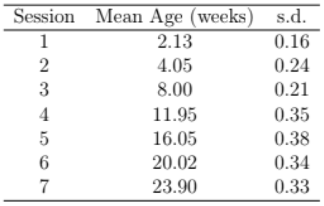
Figures and Tables

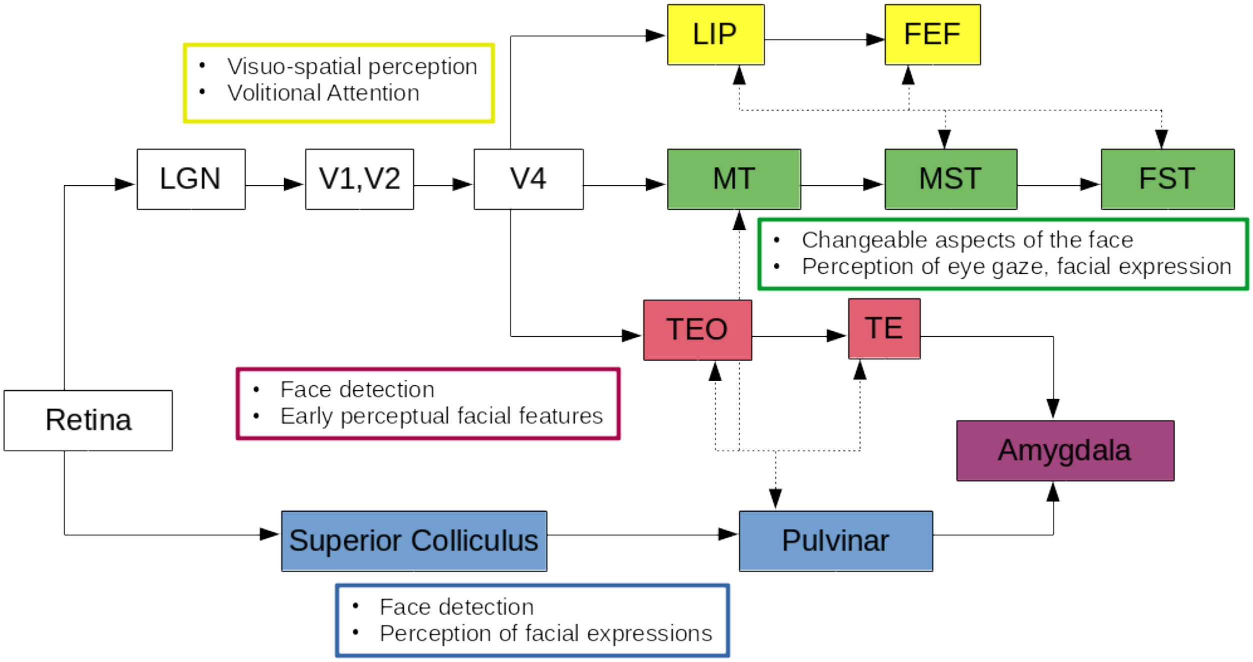
Table 1: Summary of scan ages



Session	Mean Age (weeks)	s.d.
1	2.13	0.16
2	4.05	0.24
3	8.00	0.21
4	11.95	0.35
5	16.05	0.38
6	20.02	0.34
7	23.90	0.33

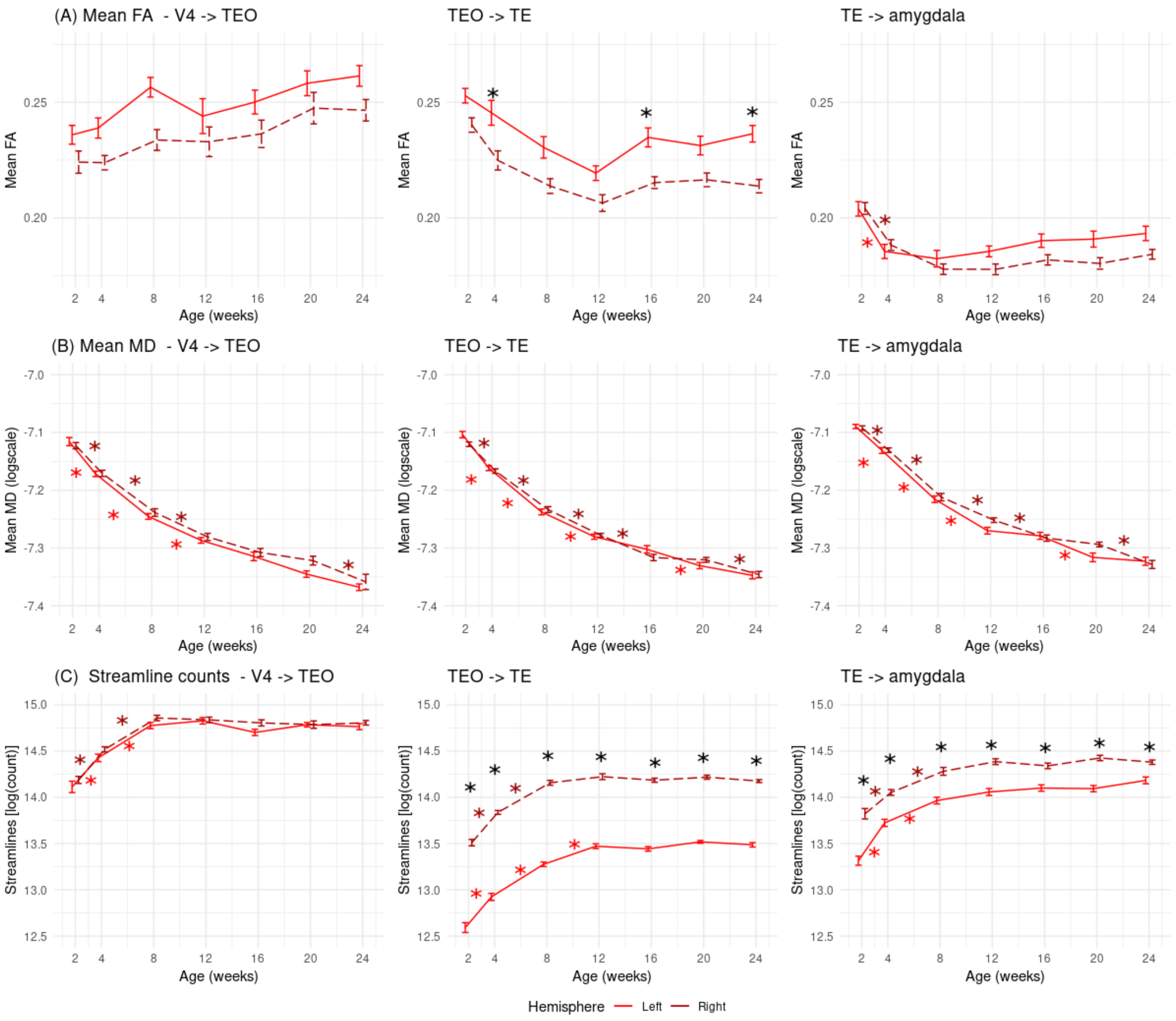
Caption for Table 1: A summary of the ages for each of the 7 longitudinal timepoints for the neuroimaging scans, taking place at approximately 2, 4, 8, 12, 16, 20, and 24 weeks of age.

Figure 1: Overview of cortical visual pathways



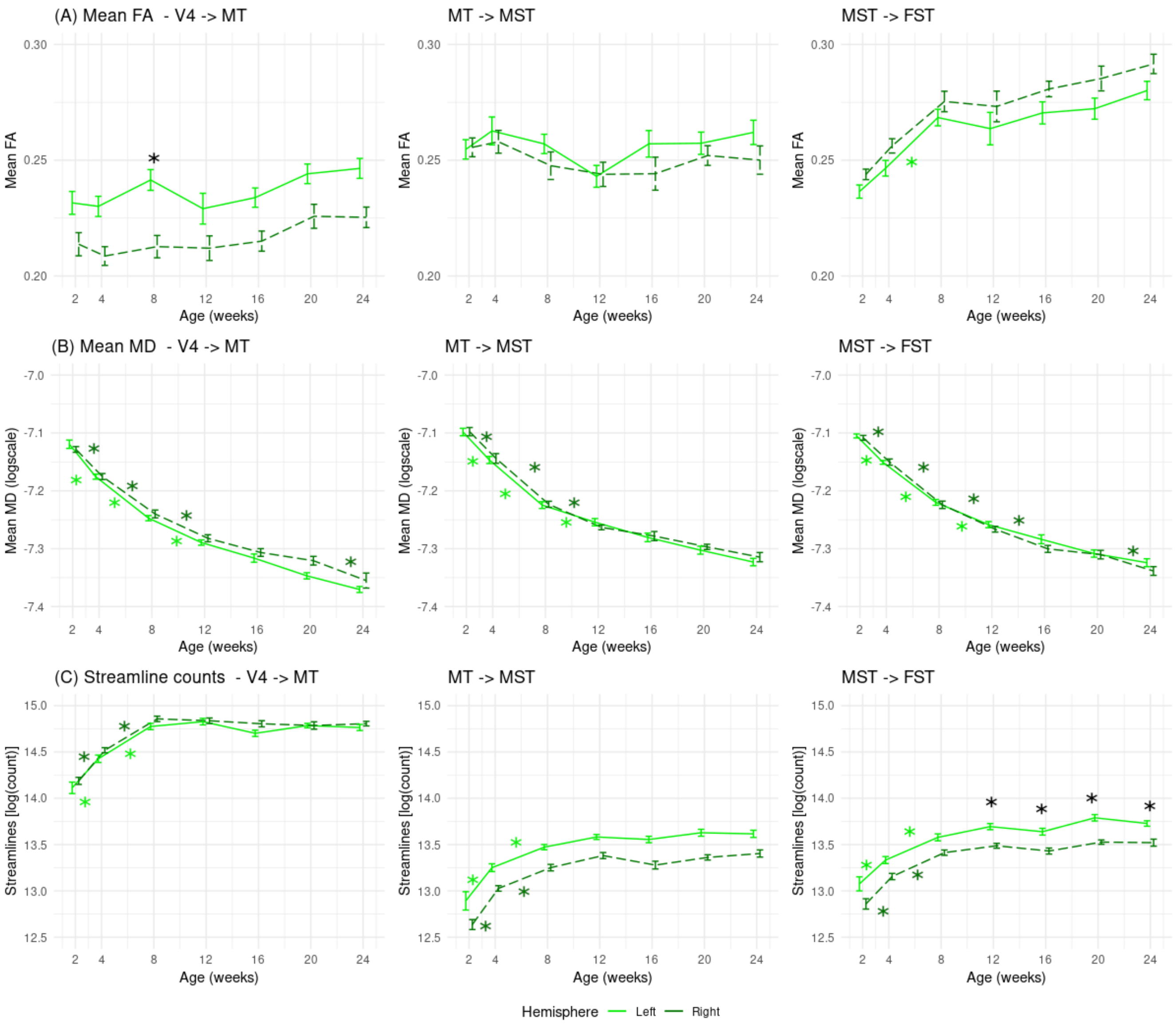
Caption for Figure 1: Summary of the visual pathways implicated in social-visual engagement. Those analyzed in this study include the ventral motion pathway (in green) along the superior temporal sulcus (STS) encompassing areas MT, MST and FST; the ventral object pathway (in red) along the inferior temporal (IT) cortex encompassing areas TEO, TE and the amygdala; and the dorsal attention pathway including areas LIP and FEF. Included in this figure, though not analyzed in this study, the subcortical visual pathway (in blue) that includes the superior colliculus, pulvinar, and the amygdala.

Figure 2: WM maturation along the ventral object pathway



Caption for Figure 2: Developmental changes along the ventral object pathways. The three tracts that were analyzed included the connection from V4 to TEO (left column), TEO to TE (center column), and finally the connection from TE to the amygdala (right column). WM metrics analyzed included **(A)** mean FA along the tract that decreases along TEO-TE and TE-AMY before increasing again; **(B)** mean MD along the tract that shows continual decreases throughout development, and **(C)** streamline counts that show initial increases to around 8 weeks along each pathway before leveling off. Black asterisks indicate significant differences between hemispheres at the indicated timepoint. Colored asterisks indicate significant pairwise difference for adjacent timepoints in the associated hemisphere.

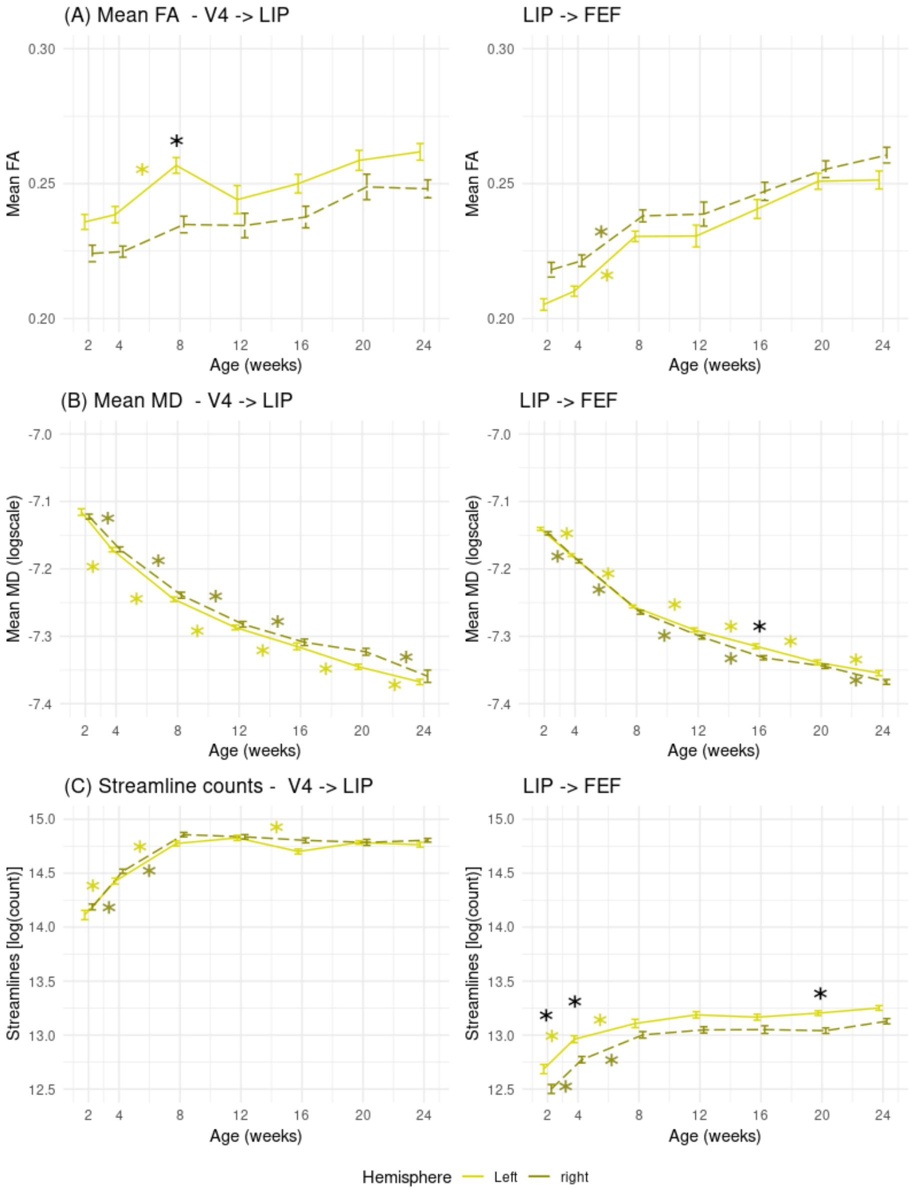
Figure 3: WM maturation along the ventral motion pathway



Caption for Figure 3: Developmental changes along the ventral object pathways. The three tracts that were analyzed included the connection from V4 to MT (left column), MT to MST (center column), and finally the connection from MST to the FST (right column). WM metrics analyzed included **(A)** mean FA along the tract that showa subtle changes in V4-MT and MT-

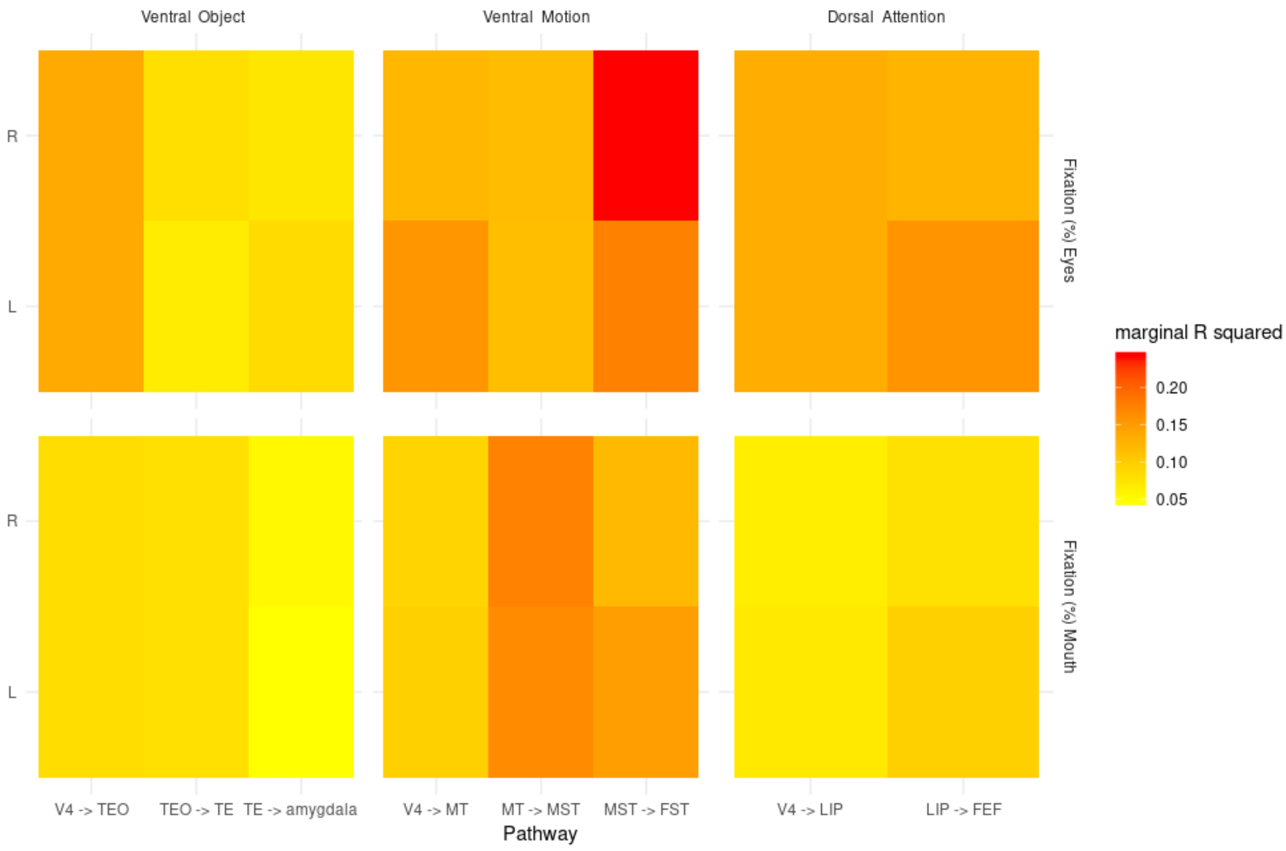
MST, and a gradual increase along MST-FST; **(B)** mean MD along the tract that shows continual decreases throughout development, and **(C)** streamline counts that show initial increases to around 8 weeks along each pathway before leveling off, with hemisphere differences appearing in MST-FST at 12 weeks. Black asterisks indicate significant differences between hemispheres at the indicated timepoint. Colored asterisks indicate significant pairwise difference for adjacent timepoints in the associated hemisphere.

Figure 4: WM maturation along dorsal attention pathway



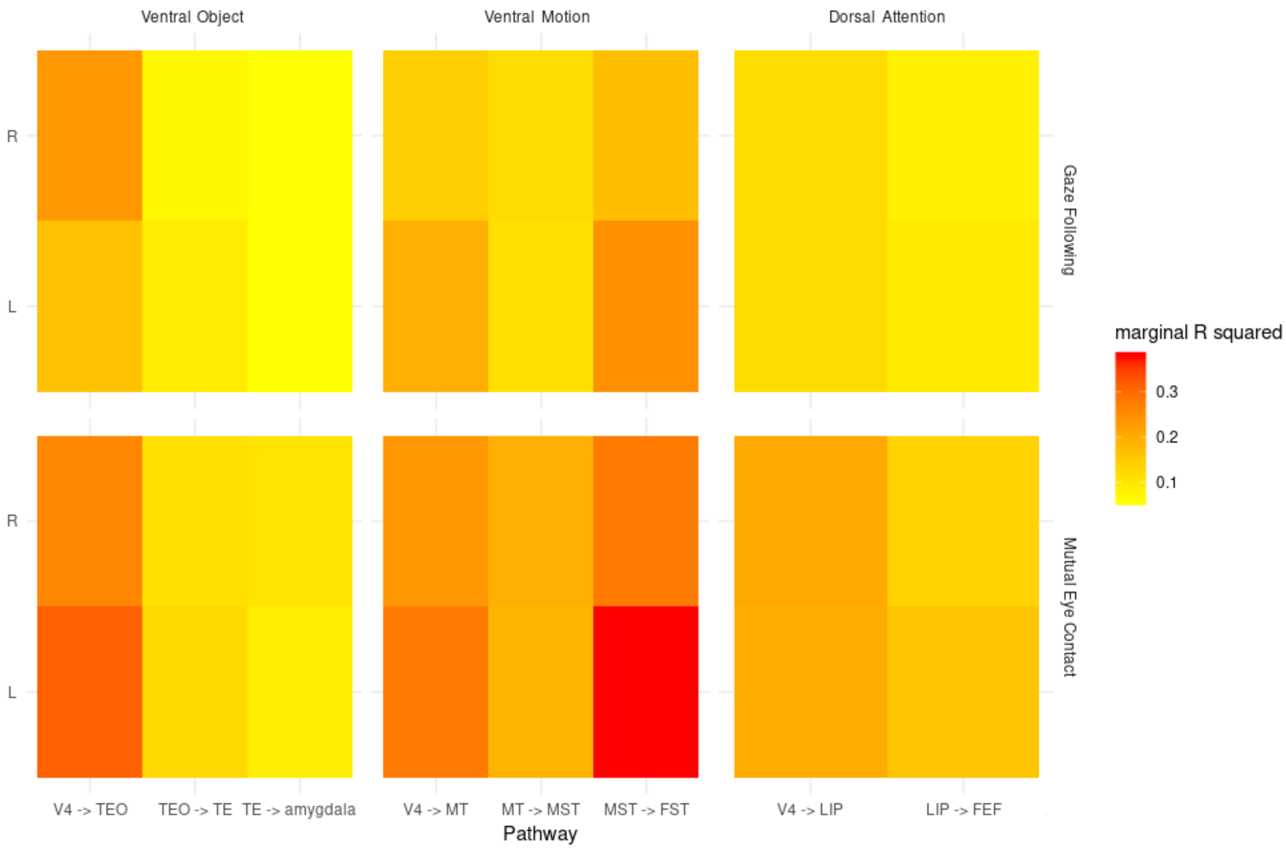
Caption for Figure 4: Developmental changes along the ventral object pathways. The two tracts that were analyzed included the connection from V4 to LIP (left column) and LIP to FEF (right column). WM metrics analyzed included **(A)** mean FA along the tract that increases gradually along LIP-FEF throughout development; **(B)** mean MD along the tract that shows continual decreases throughout development, and **(C)** streamline counts that show initial increases to around 8 weeks along each pathway before leveling off. Black asterisks indicate significant differences between hemispheres at the indicated timepoint. Colored asterisks indicate significant pairwise difference for adjacent timepoints in the associated hemisphere.

Figure 5: Correlation of WM development with social-visual attention



Caption for Figure 5: Correlational strengths between maturational changes in each of the tracts analyzed in this study and developmental changes in social-visual attention (see chapter 1). WM metrics analyzed included FA, MD, and streamline count along the ventral object (left column), ventral motion (center column) and dorsal attention (right column) as fixed factors, correlated to behavior of interest: % fixation to the eyes (top) or mouth (bottom). The most robust correlation was with the development of Right MST-FST and the attention to the eyes. Overall, the developmental changes correlated more strongly with attention to the eyes than to the mouth.

Figure 6: Correlation of WM development with gaze following behaviors



Caption for Figure 6: Correlational strengths between maturational changes of each of the tracts analyzed in this study and developmental changes in gaze following behaviors and mutual gaze (see chapter 2). WM metrics analyzed included FA, MD, and streamline count along the ventral object (left column), ventral motion (center column) and dorsal attention (right column) as fixed factors, correlated to behavior of interest: % gaze following (top) or % engaging in mutual eye contact (bottom). The most robust correlation was with the development of Left MST-FST and engaging in mutual eye contact, though there were high effects throughout. Overall, the developmental WM changes correlated more strongly with mutual eye gaze than with gaze following, with high effects for V4-TEO and all throughout the ventral motion pathway.

References:

Akiyama, T., Kato, M., Muramatsu, T., Umeda, S., Saito, F., & Kashima, H. (2007). Unilateral amygdala lesions hamper attentional orienting triggered by gaze direction. *Cerebral Cortex (New York, N.Y. : 1991)*, *17*(11), 2593–2600. https://doi.org/10.1093/cercor/bhl166

Arcaro, M. J., & Livingstone, M. S. (2017). A hierarchical, retinotopic proto-organization of the primate visual system at birth. *eLife*, *6*. https://doi.org/10.7554/eLife.26196

Arcaro, M. J., Schade, P. F., Vincent, J. L., Ponce, C. R., & Livingstone, M. S. (2017). Seeing faces is necessary for face-domain formation. *Nature Neuroscience*, *20*(10), 1404–1412. https://doi.org/10.1038/nn.4635

Baylis, G. C., Rolls, E. T., & Leonard, C. M. (1987). Functional subdivisions of the temporal lobe neocortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *7*(2), 330–342.

Beaulieu, C. (2002). The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR in Biomedicine*, *15*(7–8), 435–455. https://doi.org/10.1002/nbm.782

Berard, J. D. (1989). Life histories of male Cayo Santiago macaques. *Puerto Rico Health Sciences Journal*, *8*(1), 61–64.

Boussaoud, D., Ungerleider, L. G., & Desimone, R. (1990). Pathways for motion analysis: cortical connections of the medial superior temporal and fundus of the superior temporal visual areas in the macaque. *The Journal of Comparative Neurology*, *296*(3), 462–495. https://doi.org/10.1002/cne.902960311

Chareyron, L. J., Lavenex, P. B., Amaral, D. G., & Lavenex, P. (2012). Postnatal development of the amygdala: A stereological study in macaque monkeys. *The Journal of Comparative Neurology*, *520*(9), 1965–1984. https://doi.org/10.1002/cne.23023

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*. Taylor and Francis Inc.

Cohen, Y., & Assaf, Y. (2002). High b-value q-space analyzed diffusion-weighted MRS and MRI in neuronal tissues - a technical review. *NMR in Biomedicine*, *15*(7–8), 516–542. https://doi.org/10.1002/nbm.778

de Haan, M., Humphreys, K., & Johnson, M. H. (2002). Developing a brain specialized for face perception: a converging methods approach. *Developmental Psychobiology*, *40*(3), 200–212.

Desimone, R., Albright, T. D., Gross, C. G., & Bruce, C. (1984). Stimulus-selective properties of inferior temporal neurons in the macaque. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *4*(8), 2051–2062.

Dettmer, A., Suomi, S., & Hinde, K. (2014). Ancestral Landscapes in Human Evolution: Culture, Childrearing and Social Wellbeing. In Narvaez, Valentino, Fuentes, McKenna, & Gray, Narvaez, Valentino, Fuentes, McKenna, & Gray (Eds.) (pp. 42–58). Oxford University.

Dickinson, A. R., Calton, J. L., & Snyder, L. H. (2003). Nonspatial saccade-specific activation in area LIP of monkey parietal cortex. *Journal of Neurophysiology*, *90*(4), 2460–2464. https://doi.org/10.1152/jn.00788.2002

Distler, C., Bachevalier, J., Kennedy, C., Mishkin, M., & Ungerleider, L. (1996). Functional development of the corticocortical pathway for motion analysis in the macaque monkey: A 14C-2-deoxyglucose study. *Cerebral Cortex*, *6*(2), 184,195.

Ferrari, P. F., Kohler, E., Fogassi, L., & Gallese, V. (2000). The ability to follow eye gaze and its emergence during development in macaque monkeys. *Proceedings of the National Academy of Sciences*, *97*(25), 13997–14002. https://doi.org/10.1073/pnas.250241197

Ferrari, P. F., Paukner, A., Ionica, C., & Suomi, S. J. (2009). Reciprocal Face-to-Face Communication between Rhesus Macaque Mothers and Their Newborn Infants. *Current Biology*, *19*(20), 1768–1772. https://doi.org/10.1016/j.cub.2009.08.055

Furl, N., Hadj-Bouziane, F., Liu, N., Averbeck, B. B., & Ungerleider, L. G. (2012). Dynamic and static facial expressions decoded from motion-sensitive areas in the macaque monkey. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(45), 15952–15962. https://doi.org/10.1523/JNEUROSCI.1992-12.2012

Gothard, K. M., Battaglia, F. P., Erickson, C. A., Spitler, K. M., & Amaral, D. G. (2007). Neural responses to facial expression and face identity in the monkey amygdala. *Journal of Neurophysiology*, *97*(2), 1671–1683. https://doi.org/10.1152/jn.00714.2006

Hadj-Bouziane, F., Bell, A. H., Knusten, T. A., Ungerleider, L. G., & Tootell, R. B. H. (2008). Perception of emotional expressions is independent of face selectivity in monkey inferior temporal cortex. *Proceedings of the National Academy of Sciences*, *105*(14), 5591–5596. https://doi.org/10.1073/pnas.0800489105

Hoffman, K. L., Gothard, K. M., Schmid, M. C., & Logothetis, N. K. (2007). Facial-expression and gaze-selective responses in the monkey amygdala. *Current Biology : CB*, *17*(9), 766–772. https://doi.org/10.1016/j.cub.2007.03.040

Hoffman, E. A., & Haxby, J. V. (2000). Distinct representations of eye gaze and identity in the distributed human neural system for face perception. *Nature Neuroscience*, *3*(1), 80–84. https://doi.org/10.1038/71152

Homberg, J. R., Kyzar, E. J., Scattoni, M. L., Norton, W. H., Pittman, J., Gaikwad, S., … Kalueff, A. V. (2016). Genetic and environmental modulation of neurodevelopmental disorders: Translational insights from labs to beds. *Brain Research Bulletin*, *125*, 79–91. https://doi.org/10.1016/j.brainresbull.2016.04.015

Johnson, M. H. (2005). Subcortical face processing. *Nature Reviews. Neuroscience*, *6*(10), 766–774. https://doi.org/10.1038/nrn1766

Kamphuis, S., Dicke, P. W., & Thier, P. (2009). Neuronal substrates of gaze following in monkeys. *European Journal of Neuroscience*, *29*(8), 1732–1738. https://doi.org/10.1111/j.1460-9568.2009.06730.x

Kaden, E., Knösche, T. R., & Anwander, A. (2007). Parametric spherical deconvolution: inferring anatomical connectivity using diffusion MR imaging. *NeuroImage*, *37*(2), 474–488. https://doi.org/10.1016/j.neuroimage.2007.05.012

Kiorpes, L., & Movshon, J. A. (2004). Development of sensitivity to visual motion in macaque monkeys. *Visual Neuroscience*, *21*(6), 851–859. https://doi.org/10.1017/s0952523804216054

Kiorpes, L., Price, T., Hall-Haro, C., & Movshon, J. A. (2012). Development of sensitivity to global form and motion in macaque monkeys (Macaca nemestrina). *Vision Research*, *63*, 34–42. https://doi.org/10.1016/j.visres.2012.04.018

Klin, A., Shultz, S., & Jones, W. (2015). Social visual engagement in infants and toddlers with autism: Early developmental transitions and a model of pathogenesis. *Neuroscience & Biobehavioral Reviews*, *50*, 189–203. https://doi.org/10.1016/j.neubiorev.2014.10.006

Kovacs-Balint, Z., Feczko, E., Pincus, M., Earl, E., Miranda-Dominguez, O., Howell, B., … Sanchez, M. (2018). Early Developmental Trajectories of Functional Connectivity Along the Visual Pathways in Rhesus Monkeys. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhy222

Kuwahata, H., Adachi, I., Fujita, K., Tomonaga, M., & Matsuzawa, T. (2004). Development of schematic face preference in macaque monkeys. *Behavioural Processes*, *66*(1), 17–21. https://doi.org/10.1016/j.beproc.2003.11.002

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

Liu, Y., Yttri, E. A., & Snyder, L. H. (2010). Intention and attention: different functional roles for LIPd and LIPv. *Nature Neuroscience*, *13*(4), 495–500. https://doi.org/10.1038/nn.2496

Livingstone, M. S., Vincent, J. L., Arcaro, M. J., Srihasam, K., Schade, P. F., & Savage, T. (2017). Development of the macaque face-patch system. *Nature Communications*, *8*, 14897. https://doi.org/10.1038/ncomms14897

Lourenco, F., & Casey, B. J. (2013). Adjusting behavior to changing environmental demands with development. *Neuroscience and Biobehavioral Reviews*, *37*(9 Pt B), 2233–2242. https://doi.org/10.1016/j.neubiorev.2013.03.003

Lovejoy, J., & Wallen, K. (1988). Sexually dimorphic behavior in group-housed rhesus monkeys (Macaca mulatta) at 1 year of age. *Psychobiology*, *16*(4), 348–356.

Lutz, C. K., Lockard, J. S., Gunderson, V. M., & Grant, K. S. (1998). Infant monkeys' visual responses to drawings of normal and distorted faces. *American Journal of Primatology*, *44*(2), 169–174. https://doi.org/10.1002/(sici)1098-2345(1998)44:2<169::aid-ajp7>3.0.co;2-u

Mendelson, M. J., Haith, M. M., & Goldman-Rakic, P. S. (1982). Face scanning and responsiveness to social cues in infant rhesus monkeys. *Developmental Psychology*, *18*(2), 222–228. https://doi.org/10.1037/0012-1649.18.2.222

Milner, A. D., & Goodale, M. A. (1995). *The Visual Brain in Action*. Oxford: Oxford University Press.

Mori, S., Crain, B. J., Chacko, V. P., & van Zijl, P. C. (1999). Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Annals of Neurology*, *45*(2), 265–269.

Mori, S., & van Zijl, P. C. M. (2002). Fiber tracking: principles and strategies - a technical review. *NMR in Biomedicine*, *15*(7–8), 468–480. https://doi.org/10.1002/nbm.781

Mori, S., Crain, B. J., Chacko, V. P., & van Zijl, P. C. (1999). Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Annals of Neurology*, *45*(2), 265–269.

Morton, J., & Johnson, M. H. (1991). CONSPEC and CONLERN: a two-process theory of infant face recognition. *Psychological Review*, *98*(2), 164–181.

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2010). Response characteristics of basolateral and centromedial neurons in the primate amygdala. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(48), 16197–16207. https://doi.org/10.1523/JNEUROSCI.3225-10.2010

Muschinski, J., Feczko, E., Brooks, J. M., Collantes, M., Heitz, T. R., & Parr, L. A. (2016). The development of visual preferences for direct versus averted gaze faces in infant macaques (Macaca mulatta). *Developmental Psychobiology*, *58*(8), 926–936. https://doi.org/10.1002/dev.21421

Nakagawa, S., Johnson, P. C. D., & Schielzeth, H. (2017). The coefficient of determination R 2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of The Royal Society Interface*, *14*(134), 20170213. https://doi.org/10.1098/rsif.2017.0213

Okada, T., Sato, W., Kubota, Y., Usui, K., Inoue, Y., Murai, T., … Toichi, M. (2008). Involvement of medial temporal structures in reflexive attentional shift by gaze. *Social Cognitive and Affective Neuroscience*, *3*(1), 80–88. https://doi.org/10.1093/scan/nsm027

Parr, L. A., Murphy, L., Feczko, E., Brooks, J., Collantes, M., & Heitz, T. R. (2016). Experience-dependent changes in the development of face preferences in infant rhesus monkeys. *Developmental Psychobiology*, *58*(8), 1002–1018. https://doi.org/10.1002/dev.21434

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study. *Hippocampus*, *20*(8), 922–935. https://doi.org/10.1002/hipo.20688

Perrett, D. I., Hietanen, J. K., Oram, M. W., & Benson, P. J. (1992). Organization and functions of cells responsive to faces in the temporal cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *335*(1273), 23–30. https://doi.org/10.1098/rstb.1992.0003

Perrett, D. I., & Mistlin, A. J. (1990). Wiley series in neuroscience, Vol. 2. Comparative perception, Vol. 2. Complex signals. In W. C. Stebbins & M. A. Berkley, W. C. Stebbins & M. A. Berkley (Eds.) (pp. 187–215). Oxford, England: John Wiley and Sons.

Perrett, D. I., Smith, P. A., Potter, D. D., Mistlin, A. J., Head, A. S., Milner, A. D., & Jeeves, M. A. (1985). Visual cells in the temporal cortex sensitive to face view and gaze direction. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *223*(1232), 293–317. https://doi.org/10.1098/rspb.1985.0003

Pinsk, M. A., DeSimone, K., Moore, T., Gross, C. G., & Kastner, S. (2005). Representations of faces and body parts in macaque temporal cortex: a functional MRI study. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(19), 6996–7001. https://doi.org/10.1073/pnas.0502605102

Puce, A., Smith, A., & Allison, T. (2000). ERPS evoked by viewing facial movements. *Cognitive Neuropsychology, 17*(1–3), 221–239. https://doi.org/10.1080/026432900380580

Rodman, H. R., Skelly, J. P., & Gross, C. G. (1991). Stimulus selectivity and state dependence of activity in inferior temporal cortex of infant monkeys. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(17), 7572–7575. https://doi.org/10.1073/pnas.88.17.7572

Rosati, A. G., Arre, A. M., Platt, M. L., & Santos, L. R. (2016). Rhesus monkeys show human-like changes in gaze following across the lifespan. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1830), 20160376. https://doi.org/10.1098/rspb.2016.0376

Roy, A., Shepherd, S. V., & Platt, M. L. (2012). Reversible inactivation of pSTS suppresses social gaze following in the macaque (Macaca mulatta). *Social Cognitive and Affective Neuroscience*, *9*(2), 209–217. https://doi.org/10.1093/scan/nss123

Schwiedrzik, C. M., Zarco, W., Everling, S., & Freiwald, W. A. (2015). Face Patch Resting State Networks Link Face Processing to Social Cognition. *PLOS Biology, 13*(9), e1002245. https://doi.org/10.1371/journal.pbio.1002245

Scott, L. S., Pascalis, O., & Nelson, C. A. (2007). A Domain-General Theory of the Development of Perceptual Discrimination. *Current Directions in Psychological Science, 16*(4), 197–201. https://doi.org/10.1111/j.1467-8721.2007.00503.x

Sereno, M. I., & Huang, R.-S. (2006). A human parietal face area contains aligned head-centered visual and tactile maps. *Nature Neuroscience*, *9*(10), 1337–1343. https://doi.org/10.1038/nn1777

Sugita, Y. (2008). Face perception in monkeys reared with no exposure to faces. *Proceedings of the National Academy of Sciences*, *105*(1), 394–398. https://doi.org/10.1073/pnas.0706079105

Takahashi, M., Hackney, D. B., Zhang, G., Wehrli, S. L., Wright, A. C., O'Brien, W. T., … Selzer, M. E. (2002). Magnetic resonance microimaging of intraaxonal water diffusion in live excised lamprey spinal cord. *Proceedings of the National Academy of Sciences*, *99*(25), 16192–16196. https://doi.org/10.1073/pnas.252249999

Teufel, C., Gutmann, A., Pirow, R., & Fischer, J. (2010). Facial expressions modulate the ontogenetic trajectory of gaze-following among monkeys. *Developmental Science*, *13*(6), 913–922. https://doi.org/10.1111/j.1467-7687.2010.00956.x

Tomasello, M., Hare, B., & Fogleman, T. (2001). The ontogeny of gaze following in chimpanzees, Pan troglodytes, and rhesus macaques, Macaca mulatta. *Animal Behaviour*, *61*(2), 335–343. https://doi.org/10.1006/anbe.2000.1598

Tootell, R. B. H., Tsao, D., & Vanduffel, W. (2003). Neuroimaging Weighs In: Humans Meet Macaques in Primate Visual Cortex. *The Journal of Neuroscience*, *23*(10), 3981–3989. https://doi.org/10.1523/jneurosci.23-10-03981.2003

Tournier, J.-D., Mori, S., & Leemans, A. (2011). Diffusion tensor imaging and beyond. *Magnetic Resonance in Medicine*, *65*(6), 1532–1556. https://doi.org/10.1002/mrm.22924

Tsao, D. Y., Freiwald, W. A., Knutsen, T. A., Mandeville, J. B., & Tootell, R. B. H. (2003). Faces and objects in macaque cerebral cortex. *Nature Neuroscience*, *6*(9), 989–995. https://doi.org/10.1038/nn1111

Tsao, D. Y., Freiwald, W. A., Tootell, R. B. H., & Livingstone, M. S. (2006). A cortical region consisting entirely of face-selective cells. *Science (New York, N.Y.)*, *311*(5761), 670–674. https://doi.org/10.1126/science.1119983

Ungerleider, L. G., & Bell, A. H. (2011). Uncovering the visual "alphabet": advances in our understanding of object perception. *Vision Research*, *51*(7), 782–799. https://doi.org/10.1016/j.visres.2010.10.002

Ungerleider, L. G., & Mishkin, M. (1982). Analysis of Visual Behavior. In D. J. Ingle, M. A. Goodale, & R. J. . Mansfield, D. J. Ingle, M. A. Goodale, & R. J. . Mansfield (Eds.) (pp. 549–586). Cambridge, MA: MIT press.

Van Essen, D. C., Lewis, J. W., Drury, H. A., Hadjikhani, N., Tootell, R. B. ., Bakircioglu, M., & Miller, M. I. (2001). Mapping visual cortex in monkeys and humans using surface-based atlases. *Vision Research*, *41*(10–11), 1359–1378. https://doi.org/10.1016/s0042-6989(01)00045-1

Wallen, K. (2005). Hormonal influences on sexually differentiated behavior in nonhuman primates. *Frontiers in Neuroendocrinology*, *26*(1), 7–26. https://doi.org/10.1016/j.yfrne.2005.02.001

Webster, M. A., & Mollon, J. D. (1991). Changes in colour appearance following post-receptoral adaptation. *Nature*, *349*(6306), 235–238. https://doi.org/10.1038/349235a0

General Discussion

Social-visual engagement and attention encompass an important set of behaviors that allow both humans and nonhuman primates (NHPs) to navigate their respective social environments, including gaze behavior such as attention to the eyes of others, gaze following, and (in humans) establishing joint attention. Deficits in these behaviors are hallmark traits in human neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) and Rett's Syndrome (APA, 2013), including measurable differences in gaze behavior at early infancy via eye-tracking methodologies (Frazier et al., 2017). Indeed, Jones and Klin (2013) found reliable differences in the development of gaze behavior detectable within the first 2 years of life between typically developing infants and those who are later diagnosed with ASD. These early detectable changes in gaze behavior associated with neurodevelopmental disorders affecting social cognitive abilities has led to the further exploration of gaze behavior as a possible biomarker for ASD (Pierce et al., 2016). Although the development of the social-visual engagement behaviors is further studied, our understanding of the underlying neural changes that support these behaviors in early infancy remains sparse. The vast majority of our understanding is inferred from studies of adult social-visual engagement, these serve only to provide an approximation of infant neural substrates. Because of the ethical and technical limitations in obtaining densely-sampled, longitudinal neuroimaging data in human infants, the use of animal models become necessary to better understand the neural contributions that support social-visual engagement in infancy.

Whereas mouse models have proven useful in identifying various genetic and molecular components of social behavior that may be implicated in ASD (Bey & Jiang, 2014), attempts to bridge these genetic models to neural substrates have had limited success (Ellegood & Crawley, 2015). Additionally, significant difference is brain structures and relatively restricted social repertoire compared to humans makes establishing direct comparisons between rodent models and human brain and behaviors difficult (Watson & Platt, 2012). In contrast, NHPs have great degree of similarity to humans (1) in genetic composition and physiology, (2) in the rich and complex social structure in which they develop and navigate, and (3) in brain development (Dettmer et al., 2014). Most importantly, recent studies in rhesus monkeys have shown that early developments in social-visual engagement are phylogenetically well conserved: displaying preferences to faces in the first week of life (Parr et al., 2016), engaging in complex face-to-face interactions including bouts of mutual gaze (Ferrari et al., 2009) and gaze following skills (Tomasello et al., 2001; Ferrari et al., 2000; Teufel et al., 2010; Rosati et a., 2016).

In this dissertation, I examined both the behavioral and neuroanatomical development of social-visual attention and engagement in infant rhesus macaques that were mother-reared in a semi-naturalistic setting. The findings from this dissertation provide evidence of the phylogenetic conservation of the development of social-visual attention between humans and NHPs while highlighting the development of neuroanatomical connections that support these behaviors in infancy. These provide a meaningful advancement in our ability to use NHPs as a meaningful and critical animal model for basic social cognitive abilities, especially in infant development, and provide some advances in our understanding of neural underpinnings behind infant social-visual engagement.

Summary of the findings:

Developmental trajectories of social visual engagement: In Chapter 1, I described the longitudinal development of social-visual attention in rhesus macaques and compared them to human infant development. We report that rhesus macaques showed developmental shifts to the attention to the eyes that were similar to humans. Specifically, our findings indicated an increase in attention to the eyes that reached a peak around 5-6 weeks of age, followed by a decrease that reached a trough around 16 weeks before a continuous rise thereafter until the last age point assessed (i.e. 24 weeks). Although we observed species-differences in overall fixation percentage that matched previous literature assessing eye-tracking methodologies in NHPs (Dahl et al., 2009; Hu et al., 2013), there were striking similarities between the two species' trajectory of attention to the eyes. There was a first rise in fixation percent towards the eyes for the first 6-8 weeks in monkeys and 6-8 months in humans, followed by a slow decline until 16 weeks for monkeys and 18 months in humans, and then a second rise until 24 weeks for monkeys and 24 months in humans. Such remarkable similarities suggest that the eyes provide crucial social information for face processing in both monkeys and humans (Nakato et al., 2018), and that cross-species shifts in attention to the eyes in early infancy is well conserved across monkeys and humans.

In contrast, we reported species differences in the attention to the mouths of others. In rhesus macaques, we found that attention to the mouth started relatively high at birth and declined to reach a trough around 15-16 weeks, then increasing slightly thereafter. This trajectory is nearly inverted from what has been reported in human infants that display a sharp and continuous rise in fixation to the mouth until 14 months. This species difference highlights the different ethological relevance of the mouth region between monkeys and humans. Specifically, the overall increase observed in human infants is likely associated with the beginning of language acquisition (Klin et al., 2009; Paul et al., 2011; Wyk et al., 2010), in which attention to the mouth region provides information on specific mouth movements for the formation of sounds and syllables. Although rhesus macaque lipsmacking shares a homologous developmental mechanism as human speech (Paukner et al., 2013), the developmental timing may be shifted due to differences in the relative demands of the infant's social environment. Specifically, the earlier attention to the mouth region may be driven by an early emergence of reciprocal lipsmacking (Kaburo et al., 2016), which is later overshadowed by increase mother face-face interactions driving on the increased attention to the eyes (Ferrari et al., 2009). Additionally, the mouth region of the rhesus macaque provides important social information regarding the affective attitudes of others (i.e., fear grimace, threat bark, lipsmacking) and is crucially important for establishing and enforcing social hierarchies (de Waal & Luttrell, 1995). Thus, when infants are beginning to leave their mothers at 15-16 weeks (Suomi, 1984), they must begin to assess the social attitudes of others within the social group, driving the later increase in attention to the mouth.

These main findings from Chapter 1 indicate that infant development of social-visual attention is conserved in primates, with important species differences that are likely guided by ethological constraint. For example, although both species have a similar developmental trajectory of interest to the eyes, fixation to the mouth, has different developmental trajectories for monkeys and humans, reflecting the information provided by mouth movements that are related to language acquisition in human infants but not in monkeys. Despite these limitations, rhesus macaques may provide a critically needed NHP model to further examine developmental changes in neural systems engaged in social-visual attention.

Developmental trajectories of gaze following and mutual eye gazes: In Chapter 2, I described the longitudinal development of gaze following and associated social-visual engagement skills (i.e., establishing mutual eye contact) using the same animals while looking at the same videoclips. We reported fairly low levels of gaze following during the first 6 months of infant development with a rise in gaze following instances occurring at month 6. This was consistent with earlier studies reporting rhesus macaques begin to follow the gaze of human experimenters at a similar age in both laboratory-reared infants (Tomasello et al., 2001) and mother-reared semi-free-ranging infants (Rosati et al., 2016). Additionally, observational field data in Barbary macaques likewise showed relatively low levels of gaze following prior to 5 months with a similar increase at 6 months that continues to develop until 1 year (Teufel et al., 2010). However, gaze following has been shown to be facilitated by emotional expressions in human adults (Adams & Kleck, 2003; Mathews et al., 2003; Tipples, 2006; Hietanen and Leppänen, 2003), long-tailed adult macaques (Goosens et al., 2008), and Barbary infant macaques (Teufel et al., 2010). It is possible that our reported measures from Chapter 2 are limited using emotionally neutral stimuli.

Although gaze following remained relatively infrequent in the first 6 months, there were more reliable developmental shifts in mutual gaze behaviors upon which gaze following is contingent. In Chapter 2, we showed a progressive increase in the infants’ engagements in mutual eye contact, suggesting a gradual increase in the social significance of establishing eye contact throughout this period of infancy. We reported that increases in mutual eye contacts began at around 2 to 3 months and strengthened between 5 to 6 months.

The earlier increase in mutual eye contact engagement closely follows a peak in mutual mother-infant face-face interactions that has been reported in field studies at 2 months (Ferrari et al., 2009), suggesting that an increase in mutual eye contact at 3 months in our eye-tracking paradigm is due to an increase in volitional mutual eye engagement. This early emergence of mutual gaze is supported by other studies in both NHPs and human reporting an attraction to the eye region of faces that is present in the very first few weeks of life (Mendelson et al., 1982; Gliga et al., 2007; Farroni et al., 2002). Additionally, the increased visual attention to eyes is associated with other social skills such as neonatal imitation (Paukner at al., 2014), that, like gaze following, is a social skill known to require the interest and ability to track another individual's behavior (Carpenter et al., 1998) and has been shown to be a strong predictor of gaze following abilities (Simpson et al., 2016). Interestingly, the shifts in the trajectory of mutual gaze appear to parallel the shifts in the same infants reported when assessing their preference to look at the eyes of a face from Chapter 1. These shifts in the developmental trajectories of early social experience may be associated with the maturation of specific neural networks.

We also reported subtle developmental changes in the saccade velocities when engaging in mutual eye contact. Specifically, we found a significant increase in maximum saccade velocities both prior to and after engaging in mutual eye contact with another individual occurring between 3 and 4 months. The faster saccade velocities towards engaging in mutual eye contact suggest a faster detection of direct eye contact, perhaps due to increased social saliency. Faster maximum saccade velocities following mutual eye contact points to faster visual search following an important social eye-to-eye interaction. These results indicate that by 4 months, infant rhesus macaques may be developing an understanding of the visual attention of others and not simply co-orienting with conspecifics in a reflexive manner. Coinciding with this change at 4 months, infants are being weaned from their mothers (Fooden, 2000), have increased exploration of their surroundings and seek out attention from familial members (Hinde & Spencer-Booth, 1967; Rowell et al., 1964), and begin to show affective changes and the emergence of fear grimace into the infants' behavioral repertoire (Suomi, 1984). Altogether, these imply a critical period at 4 months, during which rhesus macaques become increasingly self-aware of the social contingencies surrounding themselves and begin to direct their attention in more socially relevant manners.

Compared to humans, however, gaze following emerges later developmentally in rhesus macaques, with 6 months in NHPs being approximately similar to 24 months in human infant neural and behavioral development. This species differences may be due to the added importance of gaze following in language development for humans (Brooks & Meltzoff, 2005), increasing the ethological relevance of social referencing and person-object-person triadic interactions in early infancy. In turn, the earlier need for the development of social referencing may drive an earlier development of gaze following skills in humans compared to NHPs. Still, even with such differences in developmental timing, NHP studies are still invaluable to further our understanding of the neural basis underlying the development of gaze following skills.

Developmental trajectories of white matter in three visual cortical networks: Finally, in Chapter 3, I described developmental changes in the white matter (WM) of pathways important to socially-directed visual behavior and correlated those changes with the behavioral developments seen in Chapter 1 and Chapter 2.

Along the ventral object pathway, general increases in FA and decreases in MD along V4-TEO throughout the first 24 weeks of infant development suggested continual development of WM microstructure during this time period. WM tracts along TEO-TE and TE-amygdala had initial decreases in FA before increasing again at either 8 (TE-amygdala) or 12 weeks (TEO-TE). This decrease in FA accompanied with increased streamline counts can be associated with an overall increase in fiber growth with a lower packing density (Takahashi et al., 2002). The inflection point at which FA begins to increase along TEO-TE and TE-amygdala suggested a period of synaptic pruning. Interestingly, such pruning of anatomical connections between the temporal lobe and the amygdala has already been reported between TEO and the amygdala (Webster et al., 1991). Thus, the continual increase in FA, decrease in MD, and relatively stable streamline count following 8 months suggest improved strengthening of connectivity that leads to an associated increase in functional connectivity between the two regions (Kovacs-Balint, 2018; Distler et al., 1996). Cortical areas in the ventral object pathways have been shown to be sensitive to face perception and the detection of facial features (Tsao et al., 2006; Van Essen et al., 2001; Tootell, Tsao, & Vanduffel, 2003; Sereno & Huang, 2006). Indeed, we reported a large effect of WM maturation along TE-amygdala on mutual eye contact, with a moderate effect on gaze following, supporting the critical role of TE-amygdala in the processing of facial cues (Gothard et al., 2007; Hoffman et al., 2007; Mosher et al., 2010; Sugita et al., 2008). Specifically, the largest effect we reported along the ventral object pathway was at V4-TEO on fixation percentage to the eyes, engaging in mutual eye contact, and in gaze following. At this early stage of development, the maturation of WM tracts connecting visual cortex to the temporal cortex and ventral object pathway may be more crucial to the processing of facial information than the development of associated temporal structures themselves.

Along the ventral pathway, we reported a gradual WM development that may extend beyond 24 weeks. V4-MT had significant increases in streamline counts up until 8 weeks, although the in the increase in mean FA remained nonsignificant. WM tracts along MT-MST had similar increases streamline counts from 8 weeks onward with overall subtle changes in FA and continued decreases in MD. Although these findings suggest a significant amount of fiber growth within the first 8 weeks of age, they also indicate a continual development of myelination and axonal density as reflected in the decrease in mean MD values from 8 weeks onward. Both these

V4-MT and MT-MST changes align with an early emergence of motion detection at 2 weeks of age that continues to become more refined throughout infancy, continuing to develop past 1 year of life (Kiorpes & Movshon, 2004; Kiorpes, 2012). Finally, significant developmental changes within MST-FST occurred in the first 8 weeks with significant increases in FA and streamline count, followed by a slower increase in FA and continued decrease in MD up until 24 weeks. These areas along the ventral motion pathway within the superior temporal sulcus (STS) are reported to be important for the perception of gaze and face direction in both humans and rhesus macaques (Hoffman et al., 2000; Puce et al., 2000; Kamphuis et al., 2009, Roy et al., 2014). Indeed, our reported changes in WM microstructure had particularly high correlational effects on the fixation percentage towards the eyes and in establishing mutual eye contact. Our findings support previous electrophysiological studies in adult rhesus macaques demonstrating the importance of the STS in the active monitoring of the gaze from others, and for establishing mutual eye contact in moments when direct gaze was present (Perret et al., 1985; Perret & Mistlin, 1990; Perret et al., 1992). Importantly, structures along the STS are also evolutionarily conserved between humans and NHPs; human studies additionally emphasize the critical importance of the STS is the processing and monitoring of gaze direction (Pelphrey et al., 2004; Itier et al., 2006) and in the flexible use of gaze direction as directional cue (Mosconi et al., 2005; Pelphrey et al., 2003).

Lastly, we reported in Chapter 3 the development of dorsal attentional networks that are important for oculomotor control, spatially directed attention, and volitional attention (Milner & Goodale, 1995). Specifically, we found a rapid development of WM tracts connecting V4 and LIP in the first 8 weeks which remains relatively stable for the first 24 weeks reflects similar developmental changes in functional connectivity (Kovacs-Balint et al., 2018). We found that

WM tracts from LIP-FEF showed continual development throughout the first 24 weeks, suggesting that the pathway continues to develop 24 weeks, which would support previous findings that report the majority of cortical visual fields (with the exception of MT) develop after birth, developing in a hierarchical manner following the development of primary visual cortex (Conde et al., 1996). This slow, gradual development is consistent with the maturation of dorsal attentional networks seen in humans (Braddick & Atkinson, 2011). Similarly, maturation of the dorsal attention pathway had moderate effects on fixation percentage to the eyes and large effects on engaging in mutual eye contact. These cortical areas are critical to mediate spatial attention and are responsible for attentional shifts in response to salient stimuli (Ungerleider and Mishkin, 1982; Dickinson et al., 2003; Liu et al., 2010). There were generally moderate effects of WM maturation in this network on measures of social-visual engagement; this pathway seems particularly important for the developmental changes in fixation percentage to the eyes, with some effect on mutual eye contact as well. These moderate effects support studies demonstrating the importance of LIP in monitoring the gaze of others (Okada et al., 2008; Akiyama et al., 2007). It is possible that this connection becomes more engaged in active gaze following beyond our final timepoint (i.e., 24 weeks), but our findings provide some preliminary evidence that developments along the dorsal attentional network plays a role in the directed attention in infants, in particular to the eyes of others.

Clinical and behavioral relevance to human social development and future studies

In this dissertation I describe 3 studies assessing the longitudinal development of social-visual engagement in infant rhesus macaques, using both behavioral measures obtained via eye-tracking methodology and neuroanatomical measures obtained via noninvasive neuroimaging procedures.

In Chapter 1, I investigated development of social-visual engagement in rhesus macaques, in particular the amount of time infants spent fixating on the eyes, body, and mouth of another conspecific. We found that the attentional shifts in the fixation on the eye regions closely paralleled developmental shifts observed in human infants, establishing a phylogenetic conservation in attention to the eyes. In addition, we noted important species differences in the attention to the mouth, highlighting the convergence of socially directed attention based upon ethological needs of the species (i.e., language development in humans). Further studies should continue describing shifts in social-visual attention using stimuli with increasing social factors (i.e., social grooming, juvenile play behavior, and emotionally salient facial features) in order to assess how increased sociality of the stimuli may impact the attentional shifts observed.

In Chapter 2, I investigated the development of gaze following skills in infant rhesus macaques. We reported low levels of gaze following until our last reported time point (6 months). However, we found subtle changes in behavior upon which gaze following is contingent. Specifically, we found increases in the percentage of times infants engaged in mutual eye contact, suggesting increasing social saliency of the eyes that reflects changes seen in Chapter 1. We also found significant changes in saccade behavior that may indicate better detection of the eyes of others. Although the developmental pattern of mutual eye gaze is similar to that describe in humans, the developmental pattern of gaze following seems to be more protracted in infant monkeys than in human infants. These species differences may arise from the types of stimuli used in the monkey studies where faces were devoid of expressions. Future studies would benefit from the use of emotionally engaging facial features, as they have been shown to facilitate both mutual eye engagement and gaze following. In addition, stimuli could be created in which the video monkey specifically fixates on an object to better simulate triadic person-object-person interactions that facilitate gaze following.

Finally, in Chapter 3, I investigated the development of WM tracts along three cortical visual pathways and correlated these structural changes to the behavioral developmental trajectories found in Chapters 1 and 2. We found rapid changes within the first 8 weeks of development, followed by slow, gradual changes that likely extend beyond 24 weeks. These WM changes appeared to be linked with developmental shifts in attention to the eyes, engagement in mutual eye contact, and gaze following behaviors. Specifically, white matter changes in the ventral motion pathway, which had previously been implicated in gaze monitoring and had a strong correlational effect with our behavioral measures. Developmental changes in WM within cortical areas along the STS likely are likely to support the developmental trajectories of infant social-visual attention. In addition, the particularly strong correlational effect between the development of the V4-TEO connection and the behavioral measures suggest the significant functional connections between visual cortex and temporal cortical areas for the development of social-visual attention.

However, there are several limitations to these studies that need to be discussed. First, only male infants were used across all three studies. Ultimately, males were chosen over females because of the higher prevalence of ASD in males, and to allow for the use of a large sample size and increase the statistical power of the findings. Macaque mothers differentially engage with their infants in other ways depending on their own experience as well as their infant’s sex (Wallen, 1996). For example, as compared to mothers of daughters, mothers of sons engage in more frequent mutual gaze and increase the rates of grooming responses they direct to their infants over time (Dettmer et al., 2016). Nonhuman primates also show other forms of sex-biased investment (Berkovitch et al., 2002), such as differential social interactions (Murray et al., 2014) and milk production (Hinde, 2007, 2009; Hinde et al., 2015) for sons versus daughters. These early sex differences in early social interactions are likely to impact on the trajectory of social visual engagement development in the first months of life. Second, the stimuli presented could also have impacted the development of social visual engagement. None had true vocalizations that may be important in directing attention; all stimuli were of neutral facial expressions, which may lack the salient information required for directing social attention; and all were of unfamiliar monkeys, whereas familiar monkeys may be more important subjects for an infant to socially attend to.

Despite these limitations, these sets of studies help establish the feasibility of using NHPs as a model of infant development of social-visual behaviors, using methodologies that closely parallel those in the human literature. Although species-specific differences in attention to the mouth and development of gaze following were noted due to ethological constraint, they do not overshadow the overall conserved mechanisms of social-visual attention. The similarities in brain and behavior between NHPs and humans, along with the use of highly similar eye-tracking methodologies, allow for more readily applicable results yielded compared to other animal models. It is my hope that further NHP study may be used to assess how genetic variations and manipulations (molecular and/or experimental) of social neural networks may serve to alter the normative development of social-visual engagement described in this dissertation. As recent discoveries in humans point to the importance of early-emerging and highly-conserved social phenotypes, NHP models could aid to advance our understanding of the brain-behavior pathogenesis of ASD.

References:

Adams, R. B., & Kleck, R. E. (2003). Perceived gaze direction and the processing of facial displays of emotion. *Psychological Science*, *14*(6), 644–647. https://doi.org/10.1046/j.0956-7976.2003.psci_1479.x

Akiyama, T., Kato, M., Muramatsu, T., Umeda, S., Saito, F., & Kashima, H. (2007). Unilateral amygdala lesions hamper attentional orienting triggered by gaze direction. *Cerebral Cortex (New York, N.Y. : 1991)*, *17*(11), 2593–2600. https://doi.org/10.1093/cercor/bhl166

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

Bey, A. L., & Jiang, Y. (2014). Overview of mouse models of autism spectrum disorders. *Current Protocols in Pharmacology*, *66*, 5. https://doi.org/10.1002/0471141755.ph0566s66

Braddick, O., & Atkinson, J. (2011). Development of human visual function. *Vision Research*, *51*(13), 1588–1609. https://doi.org/10.1016/j.visres.2011.02.018

Brooks, R., & Meltzoff, A. N. (2005). The development of gaze following and its relation to language. *Developmental Science*, *8*(6), 535–543. https://doi.org/10.1111/j.1467-7687.2005.00445.x

Carpenter, M., Nagell, K., & Tomasello, M. (1998). Social cognition, joint attention, and communicative competence from 9 to 15 months of age. *Monographs of the Society for Research in Child Development*, *63*(4), i–vi,1-143.

Condé, F., Lund, J. S., & Lewis, D. A. (1996). The hierarchical development of monkey visual cortical regions as revealed by the maturation of parvalbumin-immunoreactive neurons. *Brain Research. Developmental Brain Research*, *96*(1–2), 261–276.

Dahl, C. D., Logothetis, N. K., Bülthoff, H. H., & Wallraven, C. (2010). The Thatcher illusion in humans and monkeys. *Proceedings. Biological Sciences*, *277*(1696), 2973–2981. https://doi.org/10.1098/rspb.2010.0438

Dettmer, A., Suomi, S., & Hinde, K. (2014). Ancestral Landscapes in Human Evolution: Culture, Childrearing and Social Wellbeing. In Narvaez, Valentino, Fuentes, McKenna, & Gray, Narvaez, Valentino, Fuentes, McKenna, & Gray (Eds.) (pp. 42–58). Oxford University.

de Waal, F. B. M., & Luttrell, L. M. (1985). The formal hierarchy of rhesus macaques: An investigation of the bared-teeth display. *American Journal of Primatology*, *9*(2), 73–85. https://doi.org/10.1002/ajp.1350090202

Dickinson, A. R., Calton, J. L., & Snyder, L. H. (2003). Nonspatial saccade-specific activation in area LIP of monkey parietal cortex. *Journal of Neurophysiology*, *90*(4), 2460–2464. https://doi.org/10.1152/jn.00788.2002

Distler, C., Bachevalier, J., Kennedy, C., Mishkin, M., & Ungerleider, L. (1996). Functional development of the corticocortical pathway for motion analysis in the macaque monkey: A 14C-2-deoxyglucose study. *Cerebral Cortex*, *6*(2), 184,195.

Ellegood, J., & Crawley, J. N. (2015). Behavioral and Neuroanatomical Phenotypes in Mouse Models of Autism. *Neurotherapeutics*, *12*(3), 521–533. https://doi.org/10.1007/s13311-015-0360-z

Farroni, T., Csibra, G., Simion, F., & Johnson, M. H. (2002). Eye contact detection in humans from birth. *Proceedings of the National Academy of Sciences*, *99*(14), 9602–9605. https://doi.org/10.1073/pnas.152159999

Ferrari, P. F., Kohler, E., Fogassi, L., & Gallese, V. (2000). The ability to follow eye gaze and its emergence during development in macaque monkeys. *Proceedings of the National Academy of Sciences*, *97*(25), 13997–14002. https://doi.org/10.1073/pnas.250241197

Ferrari, P. F., Paukner, A., Ionica, C., & Suomi, S. J. (2009). Reciprocal Face-to-Face Communication between Rhesus Macaque Mothers and Their Newborn Infants. *Current Biology*, *19*(20), 1768–1772. https://doi.org/10.1016/j.cub.2009.08.055

Fooden, J. (2000). Systematic review of the rhesus macaque, Macaca mulatta (Zimmermann, 1780). *Fieldiana Zoology*, *96*, 1–180.

Frazier, T. W., Strauss, M., Klingemier, E. W., Zetzer, E. E., Hardan, A. Y., Eng, C., & Youngstrom, E. A. (2017). A Meta-Analysis of Gaze Differences to Social and Nonsocial Information Between Individuals With and Without Autism. *Journal of the American Academy of Child & Adolescent Psychiatry*, *56*(7), 546–555. https://doi.org/10.1016/j.jaac.2017.05.005

Gliga, T., & Csibra, G. (2007). Seeing the face through the eyes: a developmental perspective on face expertise. In *Progress in Brain Research* (pp. 323–339). Elsevier. https://doi.org/10.1016/s0079-6123(07)64018-7

Goossens, B. M. A., Dekleva, M., Reader, S. M., Sterck, E. H. M., & Bolhuis, J. J. (2008). Gaze following in monkeys is modulated by observed facial expressions. *Animal Behaviour*, *75*(5), 1673–1681. https://doi.org/10.1016/j.anbehav.2007.10.020

Gothard, K. M., Battaglia, F. P., Erickson, C. A., Spitler, K. M., & Amaral, D. G. (2007). Neural responses to facial expression and face identity in the monkey amygdala. *Journal of Neurophysiology*, *97*(2), 1671–1683. https://doi.org/10.1152/jn.00714.2006

Hietanen, J. K., & Leppänen, J. M. (2003). Does facial expression affect attention orienting by gaze direction cues? *Journal of Experimental Psychology. Human Perception and Performance*, *29*(6), 1228–1243. https://doi.org/10.1037/0096-1523.29.6.1228

Hinde, R. A., & Spencer-Booth, Y. (1967). The behaviour of socially living rhesus monkeys in their first two and a half years. *Animal Behaviour*, *15*(1), 169–196. https://doi.org/10.1016/s0003-3472(67)80029-0

Hoffman, K. L., Gothard, K. M., Schmid, M. C., & Logothetis, N. K. (2007). Facial-expression and gaze-selective responses in the monkey amygdala. *Current Biology : CB*, *17*(9), 766–772. https://doi.org/10.1016/j.cub.2007.03.040

Hoffman, E. A., & Haxby, J. V. (2000). Distinct representations of eye gaze and identity in the distributed human neural system for face perception. *Nature Neuroscience*, *3*(1), 80–84. https://doi.org/10.1038/71152

Hu, Y.-Z., Jiang, H.-H., Liu, C.-R., Wang, J.-H., Yu, C.-Y., Carlson, S., … Hu, X.-T. (2013). What interests them in the pictures?–differences in eye-tracking between rhesus monkeys and humans. *Neuroscience Bulletin*, *29*(5), 553–564. https://doi.org/10.1007/s12264-013-1367-2

Itier, R. J., & Batty, M. (2009). Neural bases of eye and gaze processing: the core of social cognition. *Neuroscience and Biobehavioral Reviews*, *33*(6), 843–863. https://doi.org/10.1016/j.neubiorev.2009.02.004

Jones, W., & Klin, A. (2013). Attention to eyes is present but in decline in 2-6-month-old infants later diagnosed with autism. *Nature*, *504*(7480), 427–431. https://doi.org/10.1038/nature12715

Kaburu, S. S. K., Paukner, A., Simpson, E. A., Suomi, S. J., & Ferrari, P. F. (2016). Neonatal imitation predicts infant rhesus macaque (Macaca mulatta) social and anxiety-related behaviours at one year. *Scientific Reports*, *6*, 34997. https://doi.org/10.1038/srep34997

Kamphuis, S., Dicke, P. W., & Thier, P. (2009). Neuronal substrates of gaze following in monkeys. *European Journal of Neuroscience*, *29*(8), 1732–1738. https://doi.org/10.1111/j.1460-9568.2009.06730.x

Kiorpes, L., & Movshon, J. A. (2004). Development of sensitivity to visual motion in macaque monkeys. *Visual Neuroscience*, *21*(6), 851–859. https://doi.org/10.1017/s0952523804216054

Kiorpes, L., Price, T., Hall-Haro, C., & Movshon, J. A. (2012). Development of sensitivity to global form and motion in macaque monkeys (Macaca nemestrina). *Vision Research*, *63*, 34–42. https://doi.org/10.1016/j.visres.2012.04.018

Klin, A., Lin, D. J., Gorrindo, P., Ramsay, G., & Jones, W. (2009). Two-year-olds with autism orient to non-social contingencies rather than biological motion. *Nature*, *459*(7244), 257–261. https://doi.org/10.1038/nature07868

Kovacs-Balint, Z., Feczko, E., Pincus, M., Earl, E., Miranda-Dominguez, O., Howell, B., … Sanchez, M. (2018). Early Developmental Trajectories of Functional Connectivity Along the Visual Pathways in Rhesus Monkeys. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhy222

Liu, Y., Yttri, E. A., & Snyder, L. H. (2010). Intention and attention: different functional roles for LIPd and LIPv. *Nature Neuroscience*, *13*(4), 495–500. https://doi.org/10.1038/nn.2496

Mathews, A., Fox, E., Yiend, J., & Calder, A. (2003). The face of fear: Effects of eye gaze and emotion on visual attention. *Visual Cognition*, *10*(7), 823–835. https://doi.org/10.1080/13506280344000095

Mendelson, M. J., Haith, M. M., & Goldman-Rakic, P. S. (1982). Face scanning and responsiveness to social cues in infant rhesus monkeys. *Developmental Psychology*, *18*(2), 222–228. https://doi.org/10.1037/0012-1649.18.2.222

Milner, A. D., & Goodale, M. A. (1995). *The Visual Brain in Action*. Oxford: Oxford University Press.

Mosconi, M. W., Mack, P. B., McCarthy, G., & Pelphrey, K. A. (2005). Taking an “intentional stance” on eye-gaze shifts: a functional neuroimaging study of social perception in children. *NeuroImage*, *27*(1), 247–252. https://doi.org/10.1016/j.neuroimage.2005.03.027

Mosher, C. P., Zimmerman, P. E., & Gothard, K. M. (2010). Response characteristics of basolateral and centromedial neurons in the primate amygdala. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(48), 16197–16207. https://doi.org/10.1523/JNEUROSCI.3225-10.2010

Nakato, E., Kanazawa, S., & Yamaguchi, M. K. (2018). Holistic processing in mother’s face perception for infants. *Infant Behavior and Development*, *50*, 257–263. https://doi.org/10.1016/j.infbeh.2018.01.007

Okada, T., Sato, W., Kubota, Y., Usui, K., Inoue, Y., Murai, T., … Toichi, M. (2008). Involvement of medial temporal structures in reflexive attentional shift by gaze. *Social Cognitive and Affective Neuroscience*, *3*(1), 80–88. https://doi.org/10.1093/scan/nsm027

Parr, L. A., Murphy, L., Feczko, E., Brooks, J., Collantes, M., & Heitz, T. R. (2016). Experience-dependent changes in the development of face preferences in infant rhesus monkeys. *Developmental Psychobiology*, *58*(8), 1002–1018. https://doi.org/10.1002/dev.21434

Paukner, A., Bower, S., Simpson, E. A., & Suomi, S. J. (2013). Sensitivity to first-order relations of facial elements in infant rhesus macaques. *Infant and Child Development*, *22*(3), 320–330. https://doi.org/10.1002/icd.1793

Paul, R., Fuerst, Y., Ramsay, G., Chawarska, K., & Klin, A. (2011). Out of the mouths of babes: vocal production in infant siblings of children with ASD. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *52*(5), 588–598. https://doi.org/10.1111/j.1469-7610.2010.02332.x

Pelphrey, K. A., Singerman, J. D., Allison, T., & McCarthy, G. (2003). Brain activation evoked by perception of gaze shifts: the influence of context. *Neuropsychologia*, *41*(2), 156–170.

Pelphrey, K. A., Morris, J. P., & McCarthy, G. (2004). Grasping the intentions of others: the perceived intentionality of an action influences activity in the superior temporal sulcus during social perception. *Journal of Cognitive Neuroscience*, *16*(10), 1706–1716. https://doi.org/10.1162/0898929042947900

Perrett, D. I., Hietanen, J. K., Oram, M. W., & Benson, P. J. (1992). Organization and functions of cells responsive to faces in the temporal cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *335*(1273), 23–30. https://doi.org/10.1098/rstb.1992.0003

Perrett, D. I., & Mistlin, A. J. (1990). Wiley series in neuroscience, Vol. 2. Comparative perception, Vol. 2. Complex signals. In W. C. Stebbins & M. A. Berkley, W. C. Stebbins & M. A. Berkley (Eds.) (pp. 187–215). Oxford, England: John Wiley and Sons.

Perrett, D. I., Smith, P. A., Potter, D. D., Mistlin, A. J., Head, A. S., Milner, A. D., & Jeeves, M. A. (1985). Visual cells in the temporal cortex sensitive to face view and gaze direction. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *223*(1232), 293–317. https://doi.org/10.1098/rspb.1985.0003

Pierce, K., Marinero, S., Hazin, R., McKenna, B., Barnes, C. C., & Malige, A. (2016). Eye Tracking Reveals Abnormal Visual Preference for Geometric Images as an Early Biomarker of an Autism Spectrum Disorder Subtype Associated With Increased Symptom Severity. *Biological Psychiatry*, *79*(8), 657–666. https://doi.org/10.1016/j.biopsych.2015.03.032

Puce, A., Smith, A., & Allison, T. (2000). ERPS evoked by viewing facial movements. *Cognitive Neuropsychology*, *17*(1–3), 221–239. https://doi.org/10.1080/026432900380580

Rosati, A. G., Arre, A. M., Platt, M. L., & Santos, L. R. (2016). Rhesus monkeys show human-like changes in gaze following across the lifespan. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1830), 20160376. https://doi.org/10.1098/rspb.2016.0376

Rowell, T., Hinde, R., & Spencer-Booth. (1964). “Aunt”-infant interactions in captive rhesus macaques. *Animal Behaviour*, 219–226.

Roy, A., Shepherd, S. V., & Platt, M. L. (2012). Reversible inactivation of pSTS suppresses social gaze following in the macaque (Macaca mulatta). *Social Cognitive and Affective Neuroscience*, *9*(2), 209–217. https://doi.org/10.1093/scan/nss123

Sereno, M. I., & Huang, R.-S. (2006). A human parietal face area contains aligned head-centered visual and tactile maps. *Nature Neuroscience*, *9*(10), 1337–1343. https://doi.org/10.1038/nn1777

Simpson, E. A., Miller, G. M., Ferrari, P. F., Suomi, S. J., & Paukner, A. (2016). Neonatal imitation and early social experience predict gaze following abilities in infant monkeys. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep20233

Sugita, Y. (2008). Face perception in monkeys reared with no exposure to faces. *Proceedings of the National Academy of Sciences*, *105*(1), 394–398. https://doi.org/10.1073/pnas.0706079105

Suomi, S. (1984). Affective development: A psychobiological perspective. In N. Fox & R. Davidson, N. Fox & R. Davidson (Eds.) (p. 119,159). Hillsdale, NJ: Lawrence Erlbaum.

Takahashi, M., Hackney, D. B., Zhang, G., Wehrli, S. L., Wright, A. C., O’Brien, W. T., … Selzer, M. E. (2002). Magnetic resonance microimaging of intraaxonal water diffusion in live excised lamprey spinal cord. *Proceedings of the National Academy of Sciences*, *99*(25), 16192–16196. https://doi.org/10.1073/pnas.252249999

Teufel, C., Gutmann, A., Pirow, R., & Fischer, J. (2010). Facial expressions modulate the ontogenetic trajectory of gaze-following among monkeys. *Developmental Science*, *13*(6), 913–922. https://doi.org/10.1111/j.1467-7687.2010.00956.x

Tipples, J. (2006). Fear and fearfulness potentiate automatic orienting to eye gaze. *Cognition & Emotion*, *20*(2), 309–320. https://doi.org/10.1080/02699930500405550

Tomasello, M., Hare, B., & Fogleman, T. (2001). The ontogeny of gaze following in chimpanzees, Pan troglodytes, and rhesus macaques, Macaca mulatta. *Animal Behaviour*, *61*(2), 335–343. https://doi.org/10.1006/anbe.2000.1598

Tootell, R. B. H., Tsao, D., & Vanduffel, W. (2003). Neuroimaging Weighs In: Humans Meet Macaques in Primate Visual Cortex. *The Journal of Neuroscience*, *23*(10), 3981–3989. https://doi.org/10.1523/jneurosci.23-10-03981.2003

Tsao, D. Y., Freiwald, W. A., Knutsen, T. A., Mandeville, J. B., & Tootell, R. B. H. (2003). Faces and objects in macaque cerebral cortex. *Nature Neuroscience*, *6*(9), 989–995. https://doi.org/10.1038/nn1111

Tsao, D. Y., Freiwald, W. A., Tootell, R. B. H., & Livingstone, M. S. (2006). A cortical region consisting entirely of face-selective cells. *Science (New York, N.Y.)*, *311*(5761), 670–674. https://doi.org/10.1126/science.1119983

Ungerleider, L. G., & Mishkin, M. (1982). Analysis of Visual Behavior. In D. J. Ingle, M. A. Goodale, & R. J. . Mansfield, D. J. Ingle, M. A. Goodale, & R. J. . Mansfield (Eds.) (pp. 549–586). Cambridge, MA: MIT press.

Van-Essen, D. C., Lewis, J. W., Drury, H. A., Hadjikhani, N., Tootell, R. B. ., Bakircioglu, M., & Miller, M. I. (2001). Mapping visual cortex in monkeys and humans using surface-based atlases. *Vision Research*, *41*(10–11), 1359–1378. https://doi.org/10.1016/s0042-6989(01)00045-1

Watson, K. K., & Platt, M. L. (2012). Social Signals in Primate Orbitofrontal Cortex. *Current Biology*, *22*(23), 2268–2273. https://doi.org/10.1016/j.cub.2012.10.016

Webster, M. A., & Mollon, J. D. (1991). Changes in colour appearance following post-receptoral adaptation. *Nature*, *349*(6306), 235–238. https://doi.org/10.1038/349235a0

Wyk, B. C., Ramsay, G. J., Hudac, C. M., Jones, W., Lin, D., Klin, A., … Pelphrey, K. A. (2010). Cortical integration of audio-visual speech and non-speech stimuli. *Brain and Cognition*, *74*(2), 97–106. https://doi.org/10.1016/j.bandc.2010.07.002