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New approaches to quantify social development in rhesus macaques (*Macaca mulatta*): Integrating eye tracking with traditional assessments of social behavior

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Abstract

The nonhuman primate provides a sophisticated animal model system both to explore neurobiological mechanisms underlying complex behaviors and to facilitate preclinical research for neurodevelopmental and neuropsychiatric disease. A better understanding of evolutionarily conserved behaviors and brain processes between humans and nonhuman primates will be needed to successfully apply recently released NIMH guidelines (NOT-MH-19–053) for conducting rigorous nonhuman neurobehavioral research. Here, we explore the relationship between two measures of social behavior that can be used in both humans and nonhuman primates – traditional observations of social interactions with conspecifics and eye gaze detection in response to social stimuli. Infant male rhesus macaques (*Macaca mulatta*) serving as controls (N=14) for an ongoing study were observed in their social rearing groups and participated in a noninvasive, longitudinal eye-tracking study. We found significant positive relationships between time spent viewing eyes of faces in an eye-tracker and number of initiations made for social interactions with peers that is consistent with similar observations in human populations. Although future studies are needed to determine if this relationship represents species-typical social developmental processes, these preliminary results provide a novel framework to explore the relationship between social interactions and social attention in nonhuman primate models for neurobehavioral development.

Keywords

Rhesus macaque; eye tracking; animal models; autism spectrum disorder; social development

Introduction

One of the prevailing characteristics of most primates is that they navigate the challenges of survival and reproduction through the creation and maintenance of social relationships. These relationships are critical for early survival of altricial infants and continue to impact juvenile development both through direct maternal effects and through connections in larger social groups that facilitate access to resources (Alberts, 2019; Altmann & Alberts, 2005). As young primates develop, they must learn how to interact with others through shifting contexts and acquire information from others either about conspecifics or about the physical environment. Research using animal models such as nonhuman primates has established the importance of the early environment and social interactions on infant social cognitive development as well as how these early life experiences shape later neuronal connections and capabilities (Fox, Levitt, & Nelson, 2010; Harlow, 1958).

The theoretical endeavor of understanding early behavioral signs of typical social development contributes to our understanding of the brain, but also serves a more pressing need with respect to understanding what is potentially altered in people with neurodevelopmental disorders. For example, autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder that is characterized by impairments in social behavior and increased repetitive and restricted behaviors ("American Psychiatric Association: Diagnostic and statistical manual of mental disorders (DSM-5®)," 2013). Eye-tracking studies that quantify visual attention have provided insight into how children with ASD navigate their social world. For example, when children with ASD are presented with human face stimuli in an eye-tracking paradigm, they show atypical gaze patterns such as reduced gaze directed at the eye region of faces and increased attention to nonsocial components of stimuli (reviewed by Black et al., 2017; Frazier et al., 2017; Papagiannopoulou, Chitty, Hermens, Hickie, & Lagopoulos, 2014). These patterns may be present early on, as six-month-old infants considered at-risk for developing ASD (because an older sibling had an ASD diagnosis) had atypical fixations on faces (Merin, Young, Ozonoff, & Rogers, 2007), although eye-looking behavior may also be typical in the first few months of life and then decline over time in children eventually diagnosed with ASD (Jones & Klin, 2013). These results suggest that there may be differences in social cognitive or attentional mechanisms in people with ASD that can be measured in eye tracking and may compound over development into more pronounced or disabling social behavioral deficits that eventually become observable in social behavior assessments.

Eye-tracking studies may contribute to our understanding of social development, although an important question to address is how eye tracking relates to more ecologically relevant measures of social behavior such as how a person interacts with other people. In typically developing three-to-seven-year-old children, positive correlations were found between looking time for social stimuli in eye-tracking with a structured observation of an interaction with an examiner as well as scores on scales that evaluate social behavior with respect to parents answering how well their child can communicate with them (van Rijn, Urbanus, & Swaab, 2018). Additionally, there was a positive relationship between visual attention to faces measured at 6–12 months of age via eye-tracking with scores

on the Communication and Symbolic Behavior Scales Developmental Profile (CSBS-DP) measured at 18 months (Wagner, Luyster, Yim, Tager-Flusberg, & Nelson, 2013). However, no significant relationship between these measures was detected in children with increased risk for an eventual diagnosis of ASD (Wagner, Luyster, Moustapha, Tager-Flusberg, & Nelson, 2018).

Nonhuman primates provide a unique opportunity to explore the neural underpinnings of social development and provide a model system for neurodevelopmental disorder research. Like humans, rhesus macaques have long gestations with an extended period of maternal care (Phillips et al., 2014) which allows for opportunities to investigate timing of factors in a model more akin to humans than species with shorter gestation and juvenile periods. Macaques also use vision as their primary sensory modality (Ross, 2000) and are highly social. They navigate their social world by rapidly interpreting social information from a variety of signals such as facial expressions, gestures, and vocalizations (Chang et al., 2013). With respect to the neurobiology, many neural circuits implicated in social behavior appear to be similar between human and macaque species (Watson & Platt, 2012).

Macaques have been used in eye-tracking studies for decades (Judge, Richmond, & Chu, 1980) and the neurobiology behind visual scanpaths in monkeys is well studied (see Millan & Bales, 2013 for review). Like humans, macaques focus more on the eye region than other parts of the face (Dahl, Wallraven, Bulthoff, & Logothetis, 2009; Gothard, Erickson, & Amaral, 2004). Macaques can use information gained through gaze detection and gaze follow, which along with joint attention is one of the initial mechanisms that ultimately contribute to social cognition and behavior (Leonard, Blumenthal, Gothard, & Hoffman, 2012; Putnam, Roman, Zimmerman, & Gothard, 2016). More recently, eye tracking studies in young macaques have been used to explore the development of species-typical social development. Nursery-reared infant rhesus macaques who are hand-held by an experimenter look longer at face stimuli than nonsocial stimuli (Paukner, Bower, Simpson, & Suomi, 2013; Simpson et al., 2017), preferentially view the eye region of faces (Paukner, Simpson, Ferrari, Mrozek, & Suomi, 2014; Paukner, Slonecker, Murphy, Wooddell, & Dettmer, 2018), and demonstrate a preference for viewing facial stimuli with a direct gaze over those with an averted gaze (Simpson, Paukner, Pedersen, Ferrari, & Parr, 2019). Similar results have been obtained in mother-reared macaques who were evaluated in an eye tracking paradigm while remaining in contact with their sedated mother (Muschinski et al., 2016). In our laboratory, we have recently developed a novel approach for eye tracking infant and juvenile monkeys in which the animals are unconstrained in a modified transport box (Ryan et al., 2019). This noninvasive approach provides an opportunity to integrate longitudinal eye tracking outcome measures into social development assessments in nonhuman primate models for neurodevelopmental disorders and expand on intriguing eye tracking results observed in environmental (Machado, Whitaker, Smith, Patterson, & Bauman, 2015) and genetic (Chen et al., 2017) models.

To effectively apply eye-tracking technology to animal models for neurodevelopmental disorders, it is first essential to understand the typical development of visual attention and social cognition in rhesus macaques. While eye-tracking methods have been used in rhesus macaques, researchers have not addressed the developmental trajectory for viewing

social stimuli in young monkeys as well as how looking tendencies relate to other social behavior measures. In our current study, we collected longitudinal social development data in mother-reared rhesus macaques in which we quantified social behavior and cognition across different measures, including eye-tracking paradigms and social behavior in a group setting. Here, we assessed the developmental trajectory of looking behavior in an eye-tracking paradigm for male macaques from one to six months old. During this same time, we also observed animals in small rearing groups consisting of other mother/infant pairs and an adult male. We quantified their social behavior in the group to confirm that their behavior approximated species-typical social development. Finally, we tested the hypothesis that there is a relationship between our eye-tracking measures and other social behavior measures for rhesus macaque infants between one and six months old in an ongoing study. To reduce the number of comparisons, we focused our analyses on a composite measure of peer social interaction as a global index of sociability. Because a positive relationship was found in the few studies that tested this relationship in humans (van Rijn et al., 2018; Wagner et al., 2013), we predicted that there would be a positive relationship between measures of looking at social stimuli in an eye-tracking paradigm and propensity to be social in a more ecologically relevant group context.

Methods

Subjects and Housing

The 14 male infant rhesus macaques (*Macaca mulatta*) were born to 14 mothers (5–12 years old) and the infants were control subjects for a larger study on the effects of maternal immune activation on offspring brain and behavior development (Bauman, et al., in prep). Pregnancies were coordinated in a time-mated breeding colony at the California National Primate Research Center (CNPRC). As mothers of infants in the control group, ten of the dams received three injections of sterile saline at gestational day 43, 44, and 46 and four of them did not receive any injections. Cytokine analyses of blood samples collected from the saline-injected control dams (N=10) confirmed the absence of an inflammatory response following the saline injection (Bauman et al., in prep). To promote the psychological well-being of all of the animals involved in our study, we developed our experimental procedures in collaboration with veterinary, animal husbandry, and behavioral health staff at the CNPRC in Davis, California, USA. Our protocols were approved by the University of California, Davis Institutional Animal Care and Use Committee.

Once offspring were born, infants were mother-reared and then weaned at six months old when they were transferred to pair-housing with a peer from the control group. In the first six months, the mother-infant pairs were housed in individual cages indoors with visual access to other mother-infant pairs. Starting around one month of age, to facilitate species-typical social development, the infants had additional social enrichment for three hours per day, five days per week, in a large pen-style cage indoors (3m x 1.8m x 2m) in a social group. The social group consisted of one familiar adult male and four familiar mother-infant pairs in which two pairs were from the experimental group and two from the control group. Groups were monitored for stability by trained staff. We recognize that rearing the control animals with experimental animals may influence their social development,

though all animals demonstrated a species-typical repertoire of social behavior and no overt behavioral differences were noted in the experimental animals at these early ages (Bauman, unpublished observations). Throughout the study, infants and juveniles were maintained on a 12-hour light/dark cycle with lights on at 06:00 and lights off at 18:00 and conditions were continually monitored for temperature and humidity. Macaques were fed monkey chow (Lab Diet #5047, St. Louis, MO), daily oat forage enrichment, fresh produce twice a week, and water was available *ad libitum*.

Social Group Behavioral Observations

Group formations were stabilized for all groups by the time the oldest infant in the group was three months old. Starting when infants were three months old, we conducted live behavioral observations while the monkeys were in their social groups (see timeline in Figure 1). We used a focal sampling method (Altmann, 1974) on each infant for a 5-minute continuous observation that was conducted by a trained observer who was familiar to the monkeys. Data were collected on a Dell Inspiron 15 laptop computer using the Noldus Observer program version XT 12. Observations were conducted between 9am and 3pm. We collected ten observations per infant while they were with their mother in the social group from three to six months of age and each observation was conducted at least one week apart. Social groups and data collection continued after weaning, but only data from three to six months while the infants were with their mother are presented here.

Our ethogram (see supplemental materials) was designed to capture infant developmental social behavior (Bauman et al., 2013; Bauman, Lavenex, Mason, Capitanio, & Amaral, 2004; Hansen, 1966; Hinde & Spencer-Booth, 1967). Both long-term behavioral states (lasting 1 second or longer) and events (such as facial expressions) were included in the ethogram. Behavioral states were measured by duration, or how long the infant was engaged in the behavior, and events were measured as frequencies, or the number of instances in which the behavior occurred. We recorded the initiator and recipient of social behavior for an interaction that involved the focal infant whether the partner was its mother, another adult, or one of the other infants. The adult males and females were distinguishable from each other and the infants were distinguished from each other with the aid of body dye marks.

Eye Tracking

Materials—We used a Tobii Pro TX300 eye tracker with a sampling rate of 300 Hz and Tobii Studio optical tracking software to record and process eye tracking data (Tobii Technology, Stockholm, Sweden). The hardware and software, including fixation filters, remained in their default settings (I-VT filter with 100ms maximum gap length, 20ms velocity calculator window length, classifier velocity threshold 60 degrees/sec, minimum fixation duration 50ms, maximum time between fixations 75ms, and maximum angle of fixations 0.5 degrees). For eye tracking data collection, we used our non-invasive modified transport box technique (see Ryan, et al., 2019 for further details and specifications). To reduce environmental distractions, black curtains were arranged around the testing space and the room was darkened with the exception of a single lamp that provided 200 lux of light. The modified transport box was placed on a table 55–60 cm in front of the eye

tracking monitor display (1920 × 1080 pixels), which was the distance recommended by the manufacturer.

In each data collection session, we presented the monkeys with four stimulus sets that all contained images of unfamiliar conspecifics, see Table 1 for more details about stimuli. To increase the ability to compare our results to ones conducted in other monkeys, three out of four of our stimulus sets were ones used in previous studies and research groups. Stimulus set 1 (see Table 1) consisted of a video of unfamiliar conspecifics in a social group in a natural setting presented side-by-side with an abstract circle (Dettmer et al., 2016). This stimulus set also has a video of unfamiliar humans presented side-by-side with an abstract circle, however, because all of our other stimulus sets entailed of videos and photos of conspecific monkeys, we excluded the human presentation from our analyses of social stimuli. Stimulus set 2 presented videos of unfamiliar conspecifics alternated with nature scenes of landscapes, land mammals, marine mammals, birds, or insects or other invertebrates (Machado, Bliss-Moreau, Platt, & Amaral, 2011). Stimulus set 3 was a continuously playing video of a mother and infant macaque in a naturalistic social group setting (Ryan et al., 2019). Stimulus set 4 consisted of photos of adult macaque faces engaged in neutral, lipsmacking, fear grimace, or threat facial expressions (Machado et al., 2015).

Procedure—We opportunistically collected longitudinal eye-tracking data on the infants at seven time points in the first six months of age: Day 30, 35, 45, 55, 65, 3-months, and 6-months old (prior to weaning) (Figure 1). Given the young age of the monkeys, infants only participated in these sessions with veterinary staff approval, which resulted in one infant unable to participate in days 30 and 35 eye-tracking sessions. Mothers were lightly sedated so that the infant could be safely removed and then the infant was transferred via a familiar transport box to the eye-tracking room. We conducted one eye-tracking session per monkey and time point.

Before each eye-tracking session, we calibrated the monkey's eyes to the eye tracker using a 5-point calibration procedure in Tobii Studio. Calibration was repeated as needed to improve the error before moving on to data collection. There were five instances in which younger monkeys (Day 65 or less) failed to calibrate (less than 2/5 calibration points could not be detected from at least one eye), and in these cases, we used the monkey's previous calibration from days prior. In the case of a monkey failing to calibrate in its first session, we used its fellow control animal from the social group's calibration. However, no monkey consistently failed to calibrate across multiple time points. Each stimulus set was presented at each time point, with the exception that Stimulus Set 3 was not presented at the 6-month time point due to technical problems. Eye-tracking data was collected opportunistically (when animals chose to view the screen), thus the order of actual viewing often differed from the individual's predetermined presentation order. Our goal was to distribute the order of unique categories or subcategories of stimulus sets across individuals, while allowing the investigator the flexibility to adjust the order as needed to collect as much eye-tracking data as possible within the allotted time. The presentation totaled 914 seconds or 15.23 minutes with all 4 stimulus sets and was 844 seconds at the 6-month time point. The entire session, from calibration through stimuli presentation, lasted no more than one hour. For stimulus

sets 1 and 3, the same stimuli were presented at each time point. For stimulus sets 2 and 4, we presented different versions of the stimuli at each time point with respect to different faces used for facial expressions and monkeys for group interactions, and all infants saw the same version at a specific time point.

Data Analysis

Social Group Data—With respect to understanding social development, we collected data for infant social interactions with their mothers and the three other infants in their social groups. We made no *a priori* assumptions about the frequency, duration, or quality of social interactions with the other infants as recipients based on experimental group membership and thus combined data for all other infants in the social group. There was a low frequency of interactions with the adult male as well as the mothers of other infants in the group, so these interactions were excluded from the analysis to focus on development with respect to mother and peer interactions.

We combined duration data from the behavioral states (proximity, contact, grooming, and extended play) both initiated by the infant or initially started by its mother or peers to generate a global measure of the total time, in seconds, that each infant spent interacting with its mother or other infants. To describe how time spent with its mother and other infants changed from three to six months and whether this mirrored species-typical social development, we used our combined duration data for social interactions within each sampling session and calculated the percentage of time that the infant spent with its mother or other infants within a 5-minute focal sample. We also used our combined duration data to examine the relationship between infant sociality and propensity to view social stimuli in an eye-tracking paradigm. For this question, we combined our duration data for each infant across the 10 focal samples to generate a total duration of social behavior measure for interactions with its mother and another measure for interactions with its peers.

We also sought to generate a measure that could gauge infant propensity to interact with others. In addition to a measure of duration for behavioral states, we also quantified frequency of events initiated by the focal infant towards its mother and peers, including facial expressions, vocalizations, play behaviors, social interest behaviors, sexual behavior events, and agonistic interaction. We generated a mean number of initiations for each behavior across sessions for each infant and then summed the means across different behaviors. We did this for all social events. In order to generate a measure of more specifically prosocial tendencies, we also generated a separate measure for play behaviors with peers. We used the global measure of initiations with its mother and peers and examined the relationship between these measures and looking times in our eye-tracking paradigm.

Eye-tracking Data—In Tobii Studio, areas of interest (AOI) within a presented stimulus can be isolated by drawing a shape around an object or individual of interest in the scene. These AOIs can be adjusted in a video so that a monkey moving in a video can be tracked across the screen. Within our four stimulus sets, we thus created AOIs (see Table 1 for examples) so that we could more specifically measure whether our subject was looking at

the social component of our stimuli, such as a monkey, rather than a nonsocial part of the video, such as trees or objects.

Our calibration data, which provides error vectors for the eye-tracker's estimation for where on the screen the monkey is looking, suggested that the combined current method of unrestrained subjects and the eye-tracking technology are possibly not sensitive enough to reliably measure whether our infants are looking at the eyes of a monkey in a video, for this area on the computer screen is too small and would discount monkeys that are social but did not calibrate as well to the eye-tracker. In stimulus sets 1 and 2, we thus generated AOIs for whole bodies of monkeys. For stimulus set 3, the monkeys in the video were large enough on the computer screen so that we could generate AOIs for the more specific region of the face. Finally, for Stimulus Set 4, the faces were sufficiently large enough so that we could reliably generate AOIs for the face, eyes, and mouth regions.

Statistical Analysis

We used the total fixation duration measure generated from Tobii Studio as our measure of looking time. Total fixation duration is the sum of the duration of all fixations during a stimulus presentation in seconds. We generated this measure for each AOI in our stimulus sets. To describe how overall looking time towards social stimuli changed over the course of the first six months of life, we summed total fixation durations for AOIs for each stimulus set that monkeys viewed in a testing session from Day 30 through the six-month eye tracking session. We thus generated a total looking time across the four stimulus sets (for the six-month time point, it was three stimulus sets). Because Stimulus Set 3 was not presented at the six-month time point, we determined the overall percentage of time that the monkeys viewed available stimuli rather than comparing total fixation seconds across time points. We generated these percentages by dividing the total monkey looking times by the number of possible seconds they could have viewed social stimuli.

In addition, we sought to test how looking times towards social stimuli in an eye tracking paradigm relate to social behavior measured around a similar period of time. In order to limit the number of comparisons, we utilized a composite measure peer social interaction (duration and frequency) as our primary outcome measure. For comparison, we also generated a composite measure of maternal social interaction (duration and frequency). We used the looking time data from the three- and six-month eye tracking sessions to coincide with the social group data collected between three and six months of age. We used two different metrics to determine the propensity for infants to view social stimuli in the eye-tracker. The first metric was the total looking time across all of the social AOIs in our four stimulus sets. We generated a mean looking time score across the three- and six-month time points. Secondly, we used the AOIs generated from Stimulus set 4 facial stimuli to more closely assess the relationship between group sociality and how the infants attend to eyes, mouth, and faces in an eye-tracking paradigm. We generated mean looking time scores across the three- and six-month time points for looking time at faces, eyes, and mouths in Stimulus Set 4.

To assess the relationship between the tendency to view social stimuli in an eye-tracking paradigm and social group behavior, we used Spearman Rank correlations with an alpha

of 0.05 as our criterion for statistical significance. To control the false positive rate when conducting multiple correlations, we used the Benjamini-Hochberg false discovery rate control method and set our false discovery rate at 10% (Benjamini & Hochberg, 1995). Our Benjamini-Hochberg adjusted p-values (q) are included in Table 2.

Data Availability Statement—The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Descriptive Social Development Data

Social Group Behavior—Our infants social behavior in the group setting proceeded in a species-typical fashion, for as the infants matured, they spent less time with their mother and more time with other infants during focal sample observation sessions (Figure 2). In the first session, the infants spent the majority of their 5-minute focal observation session with their mothers (78.91% ±6.39) rather than their peers (6.28% ±2.47). By the tenth observation session which took place around 6 months of age, the infants spent less time with their mother (39.34% ±6.36) and more time with their peers (16.13% ±3.52).

Eye-tracking—We were able to successfully collect eye-tracking data from infants using our unrestrained and noninvasive eye-tracking method from Day 30 through 6 months of age. The average percent of time that the infants fixated on presented social stimuli was consistent between Day 30 (6.19% ±1.57; 42.94 ± 10.88 seconds) and Day 65 (6.12% ±1.21; 42.39 ±8.37 seconds) (Figure 3) and while some data were collected, the infants watched little of the presented stimuli. At 3 months, infants increased their looking at social stimuli (11.01% ±2.21; 76.43 ±15.30 seconds), and this further increased across the group at 6 months of age (18.51% ±2.03; 115.50 ±12.69 seconds) (Figure 3).

Relationship between eye tracking at 3 and 6 months peer social interaction
—A significant positive relationship was detected for the amount of time spent viewing eyes using our mean measure for the three and six month time points in the facial stimulus set 4 and the amount of time spent interacting with peers with behavioral states such as proximity, contact, grooming, or extended play ($r(12)$=.604, p=0.022, q=0.088) (Figure 4a, Table 2). There were no other positive relationships detected between looking time towards social stimuli and duration of peer interactions (Table 2). There was a significant positive relationship between the time spent viewing eyes during the three and six month time points in the facial stimulus set 4 and the number of peer social interactions initiated ($r(12)$=.625, p=0.017, q=0.088) (Figure 4b, Table 2). Yet, there was no significant relationship between the number of initiations and the tendency to look at the mouth area in facial stimuli ($r(12)$=.378, p=0.182, q=0.261) or faces overall ($r(12)$=.475, p=0.086, q=0.206) (Table 2). We further examined the relationship between the number of initiations for play behavior by the infants, which may be considered a prosocial behavior, and looking time for social stimuli. There was a significant positive relationship between number of play initiations with time spent looking at the eye region in facial stimuli ($r(12)$=.614, p=0.020, q=0.088) (Figure 4c, Table 2), although not with the time spent viewing the face ($r(12)$=.484,

p=0.079, q=0.206) or mouth region (t(12)=.367, p=0.196, q=0.261) (Table 2). There was no significant relationship between the number of initiations for play and the tendency to view all of the monkey photo and video AOIs across stimulus sets (t(12)=.295, p=0.306, q=0.306).

Relationship between eye-tracking and maternal social interaction—As for the relationship between social behavior with the infant's mom and the looking time towards social stimuli in an eye-tracking paradigm, there was no significant relationship between an infant's time spent with its mother and how long it viewed social stimuli (Table 2). Furthermore, there was no significant relationships between the number of social interactions initiated by the infant towards its mother and the time it spent looking at social stimuli, or more specifically, facial stimuli (Table 2).

Discussion

Here, we collected longitudinal data on male rhesus macaque infants using traditional social group focal observations paired with eye-tracking technology to assess social development. Our results demonstrated that social attention, as indexed via an eye-tracking paradigm, was positively correlated with how infants interacted with their peers as measured across similar time points. This relationship was significant between infant propensities to look at the eyes of faces in an eye-tracking paradigm and time spent with peers and initiations of social interactions with their peers (Table 2). Notably, the relationship between social attention and behavior existed outside of the mother-infant dyad, in which there was no significant statistical relationship between the infants' interactions with their mothers and measures of social attention.

The positive relationship found between interest in the eye region and social interactions with peers aligns with studies performed in human children, as both studies demonstrated that an increase in looking time towards eyes in an eye-tracking paradigm had a positive relationship with social behavior metrics (van Rijn et al., 2018; Wagner et al., 2013). While most of the social propensities were measured via parent ratings on scales, van Rijn et al. included social behavior from a structured interaction between the participant (3–7 years old) and an experimenter (van Rijn et al., 2018). Similar to our results, van Rijn et al. (2018) found a significant association between the percent of time children spent attending to eyes in an eye-tracking paradigm and their tendency to initiate social interactions with an experimenter. These cross-species comparisons highlight the use of eye-tracking approaches as a means to improve translation between human and nonhuman primate studies as findings in nonhuman primates can support biological explanations for phenomena observed in humans.

In both human and nonhuman primates, faces are salient social stimuli (macaques: Gothard et al., 2004; Keating & Keating, 1982; Mosher, Zimmerman, & Gothard, 2011; Nahm, Perret, Amaral, & Albright, 1997; chimpanzees: Hirata, Fuwa, Sugama, Kusunoki, & Fugita, 2010; Kano & Tomonaga, 2009; humans: Farah, Wilson, Drain, & Tanaka, 1998; Goren, Sarty, & Wu, 1975). The eye region is of particular importance to primates as demonstrated in eye-tracking studies in humans (Birmingham & Kingstone, 2009), chimpanzees (Hirata et

al., 2010), rhesus macaques (Dahl et al., 2009; Gothard et al., 2004), and recently found in a more distantly related monkey, the common marmoset (Kotani et al., 2017).

Eye contact with a recipient animal presents a meaningful social cue about where the recipient animal's attention is located and thus the potential for shared communication. In species with the ability to engage in gaze following and joint attention, sensitivity to the eye region and eye contact is an important step to develop the social cognitive skill of sharing attention with another. People diagnosed with ASD demonstrate reduced visual attention to the eye region of faces in eye tracking paradigms (reviewed by Black et al., 2017; Frazier et al., 2017; Papagiannopoulou, et al., 2014) as well as impairments in joint attention (Dawson et al., 2004). One proposed mechanism for the progression of ASD is that infants with ASD may initially have impairments in social attention that deprive them of social information and this deprivation compounds into an altered social development trajectory from typically developing children (Mundy & Neal, 2001). Given that our population of infant rhesus macaques displayed variation in their tendencies to look at the eye region of faces in an eye-tracking paradigm, it is possible that we can explore how variation in social attention mechanisms relate to the development of social cognition. Notably, our more broad social AOIs had a positive but not statistically significant relationship with social interactions. More research will determine whether these social AOIs can be effective proxies for the eye region when monkeys are viewing videos of monkeys as opposed to a static image of an enlarged face.

The integration of eye tracking with traditional behavioral observations provides new insight into the development of social behavior and opens new translational research opportunities. Although reciprocal social interactions remain the gold standard for evaluating species-typical social interactions (Bauman, Crawley, & Berman, 2019; Silverman, Yang, Lord, & Crawley, 2010), there are challenges in quantifying social interactions. First of all, it is difficult to accurately quantify social behavior, especially for primates, where social interactions entail subtle social cues that may be missed by observers. Moreover, the nature of a social interaction is dependent on the social paradigm and/or choice of recipient, both with respect to humans interacting with a novel experimenter or monkey interacting with an experimenter or other monkey. Eye-tracking studies compliment the information gleaned from observations of social interactions and provide an opportunity to evaluate social development in a more objective paradigm that allows for more experimental control. Stimulus presentation in an eye-tracking paradigm furthermore provides time-sensitive gaze information as well as what components of stimuli are meaningful to the viewer. These methods can also be carried out in nonhuman animals with no previous habituation or training to the experimental paradigm (Ryan et al., 2019).

The positive correlation between eye tracking and social behavior is also a promising step towards understanding the development of individual differences in social behavior. Much like humans, rhesus macaques demonstrate individual differences in sociability with some animals readily engaging in social interactions while others spend more time alone (Capitanio, 1999, 2002). Although the present study does not address the issue of whether social experiences drive social attention or if heightened social attention leads to more interactions with peers, nonhuman primates provide an opportunity to explore these

questions. Indeed, results from a recent study with human twins suggest that preferential attention, timing, and direction of individual eye movements in an eye-tracking paradigm are highly influenced by genetics (Constantino et al., 2017). However, it may be difficult to examine these mechanisms in human studies alone as human infant development is subject to many social and cultural factors. There is an opportunity to study these mechanisms in nonhuman primates because we can control for or manipulate more factors that influence infant social development.

In the present study, we utilized control animals from an ongoing experiment to explore social development in male rhesus macaques. Limitations include a relatively modest sample size and the use of only male subjects. Previous studies in 2–3 week old rhesus macaque infants raise the possibility for sex differences in eye-tracking behavior, since female infants looked significantly longer than male infants at conspecific faces in an eye-tracking paradigm (Simpson et al., 2016). However, these infants were indoor-housed and nursery-reared, whereas sex differences in eye-tracking behavior were not detected in infants of a similar age that were mother-reared, lived in social groups, and had outdoor access (Paukner et al., 2018). Our rhesus infants were indoor-housed, mother-reared, and had intermittent access to social groups, so it is possible that female infants would behave differently than males in an eye-tracking paradigm. Social behavior and development in group settings can also differ between male and female infants as females will ultimately remain in their natal group and males will emigrate into a new group when they are older (Amici, Kulik, Langos, & Widdig, 2019; Lonsdorf, 2017). It appears that early on in life there are no sex differences in the infant macaque proximity, grooming, and nursing to mothers, but males decrease time spent with their mothers earlier than female infants (Kulik, Langos, & Widdig, 2016) and begin to play earlier and more often than female infants (Kulik, Amici, Langos, & Widdig, 2015). It is possible that the relationships we observed in male infants between eye-tracking and social behavior would differ in female infants either with respect to the nature of the relationship itself or the timing during development.

Future studies are also needed to explore the relationship between social attention and social interactions to determine if similar relationships exist for animals reared in a more naturalistic environment than the laboratory setting. Social groups take on characteristics of their own with such factors as how interactive individuals in a particular group are compared with those in other groups. Larger sample sizes can help researchers address how the nature of the social group itself impacts the relationship between eye tracking and social behavior. Studies conducted at our facility support the notion that adult animals have varying propensities of sociability in group settings and respond differently to stimulus animals in experimental paradigms (Capitanio, 1999, 2002). Furthermore, social information processing as represented by facial recognition memory and gaze aversion to aggressive faces during infancy predicted high and low sociability, respectively, in juvenile monkeys (Sclafani et al., 2016). The current study provides a framework to explore the relationship between social interactions and social attention in our nonhuman primate models of neurobehavioral development, including ongoing studies evaluating the effects of maternal immune activation on offspring development. Decreased visual attention to social stimuli in eye-tracking paradigms for people with neurodevelopmental disorders (Black et al., 2017; Frazier et al., 2017; Papagiannopoulou et al., 2014) demonstrate an opportunity for eye

tracking to be additionally integrated into studies in which nonhuman primates serve as animal models for neurodevelopmental disorders such as ASD and schizophrenia (Millan & Bales, 2013; Qin, Wu, Chen, & Hu, 2019; Ryan, Berman, & Bauman, 2018).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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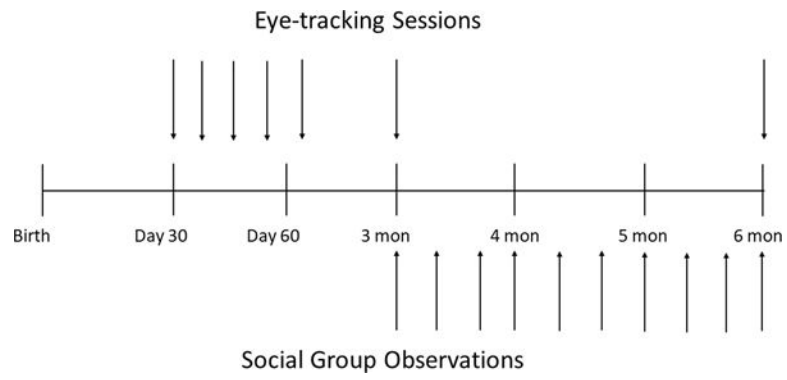
Figure 1.
Timeline of rhesus macaque infants' first six months of life and when we collected eye-tracking data and social group observations.
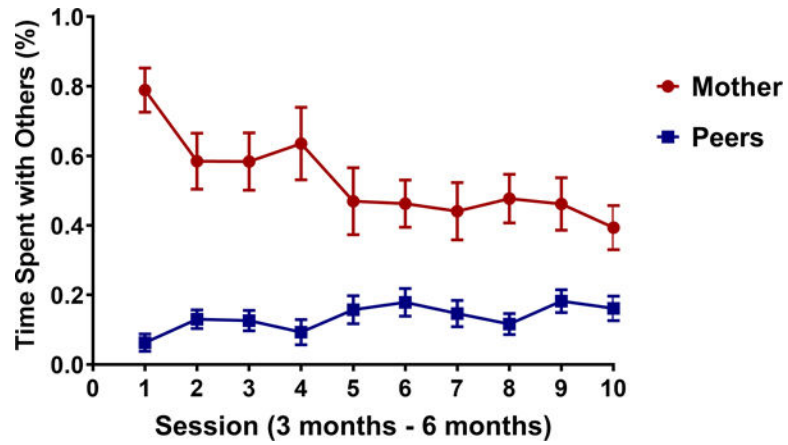
Figure 2.
The mean percent of time (± standard error of individual variation in the group) each infant spent with either its mother (red circle) or peers (blue square) in a 5-minute focal sampling session. Ten focal sample sessions were conducted on each infant starting at 3 months of age through 6 months old.
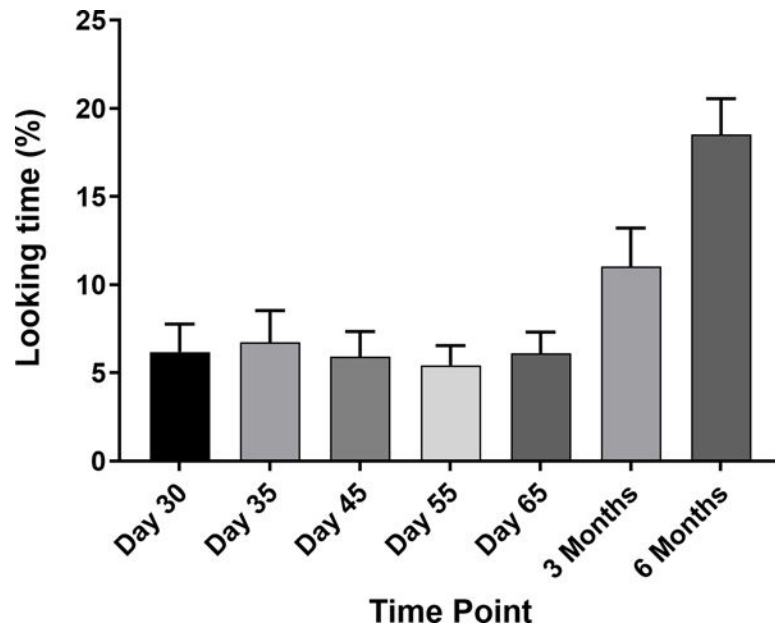
Figure 3.
The mean percent of time (± standard error of individual variation in the group) that monkeys viewed all social stimuli (monkey photos and videos) out of the total time that social stimuli were displayed on the eye-tracking screen. For each monkey, there was one eye tracking session at each time point.
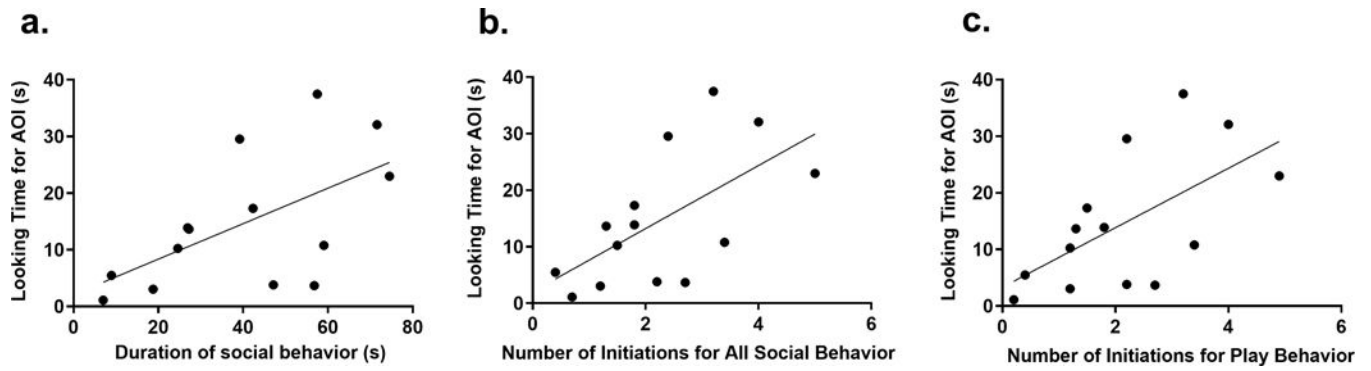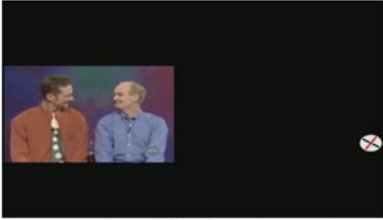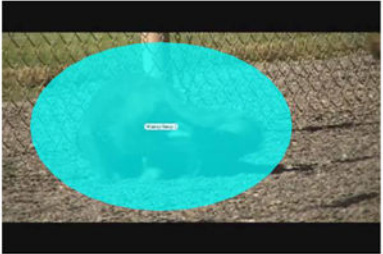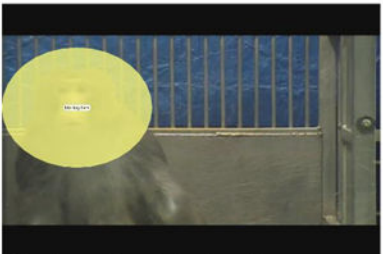
Figure 4.
Scatterplots with lines of best fit for the average fixation duration in seconds for the three and six month time points on the Eye AOIs in the facial stimulus set. Fixation duration was compared with the total duration of social behavior with peers (a), the number of initiations made towards peers for all social behaviors (b), and more specifically for the number of initiations for play behaviors made towards peers in their social groups (c).

Table 1

Stimulus Sets used in Eye-tracking Data Collection

Example Stimulus with AOI	Presentation Length	Source
Stimulus Set 1		
Human stimuli (left) abstract shape (right) Monkey stimuli (left) abstract shape (right)	4 pairs of nonsocial and social stimuli (2 with social on left, 2 on right) shown for 36 seconds each	Donated by Dr. Paukner Cited in Dettmer et al., 2016
Stimulus Set 2		
Monkey group stimuli Monkey directed stimuli	10 stimuli (5 nature, 3 monkey group, 2 monkey directed) shown for 30 seconds each	Donated by Dr. Machado Cited in Machado et al., 2011
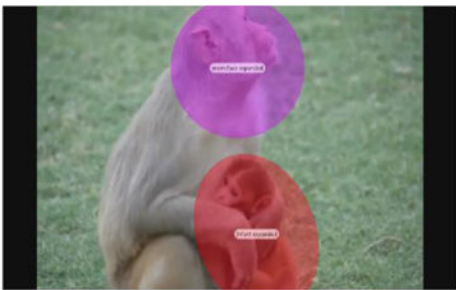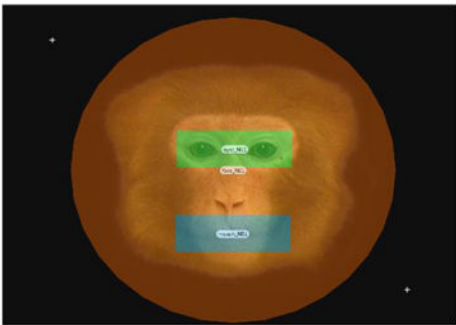
Nature Stimuli		
Stimulus Set 3		
	70 seconds continuously playing video	Cited in Ryan et al., 2019
Stimulus Set 4		
Neutral Expression	12 images (3 Threat; Neutral; Fear Grimace, and Lipsmack) shown for 15 seconds each	Donated by Dr. Machado Cited in Machado et al., 2015 (note: our current methods omitted the fixation requirement and juice reward described in the previous study)

Table 2

Spearman correlation coefficient (r) for AOIs in Eye-tracking and Measures of Social Group Behavior

	Eye-tracking AOIs for Mean of 3 -and 6-Month Time Points			
	All Social AOIs	**Facial Stimuli: Face AOI**	**Facial Stimuli: Eye AOI**	**Facial Stimuli: Mouth AOI**
	Social behavior with peers			
Duration spent in social interactions (s)	r=.327 p=.253 (q=.295)	r=.437 p=.118 (q=.236)	**r=.604** * **p=.022 (q=.088)**	r=.407 p=.149 (q=.255)
Number of initiations all social behavior	r=.317 p=.270 (q=.295)	r=.475 p=.086 (q=.206)	**r=.625** * **p=.017 (q=.088)**	r=.378 p=.182 (q=.261)
Number of initiations play behavior	r=.295 p=.306 (q=.306)	r=.484 p=.079 (q=.206)	**r=.614** * **p=.020 (q=.088)**	r=.367 p=.196 (q=.261)
	Social behavior with mother			
Duration spent in social interactions (s)	r= −.156 p=.594 (q=.950)	r= −.196 p=.503 (q=.950)	r= −.393 p=.164 (q=.864)	r= −.213 p=.464 (q=.950)
Number of initiations	r=.027 p=.927 (q=.976)	r= −.076 p=.796 (q=.976)	r=−.353 p=.216 (q=.864)	r= −.009 p=.976 (q=.976)

Bolded values represent

[*] p< 0.05, adjusted Benjamini-Hochberg p-values (q) in parentheses

N=14, df=12 for all analyses