Published in final edited form as:
J Autism Dev Disord. 2012 December ; 42(12): 2611–2621. doi:10.1007/s10803-012-1518-8.

Atypical Social Referencing in Infant Siblings of Children with Autism Spectrum Disorders

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Abstract

Social referencing was investigated in 18-month-old siblings of children with autism spectrum disorders (ASD; "high-risk infants"). Infants were exposed to novel toys, which were emotionally tagged via adults' facial and vocal signals. Infants' information seeking (initiation of joint attention with an adult) and their approach/withdrawal behavior toward the toys before versus after the adults' emotional signals was measured. Compared to both typically developing infants and high-risk infants without ASD, infants later diagnosed with ASD engaged in slower information seeking, suggesting that this aspect of referencing may be an early indicator of ASD. High-risk infants, both those who *were* and those who *were not* later diagnosed with ASD, exhibited impairments in regulating their behavior based on the adults' emotional signals, suggesting that this aspect of social referencing may reflect an endophenotype for ASD.

Keywords

Autism; Social referencing; Joint attention; Behavior regulation

Introduction

Autism Spectrum Disorders (ASD) are characterized by impairments in numerous areas of social cognition, communication, and behavior. One important aspect of social communication relevant for the study of ASD is social referencing, a means by which infants obtain social information about objects and events in their environment. Social referencing is comprised of a number of skills, including (a) recognizing that an object or event is ambiguous, (b) looking to another person, presumably in order to seek social and emotional information about the ambiguous object or event, (c) recognizing emotion

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conveyed by the other person, (d) associating the emotional information with the ambiguous object or event, and (e) using the emotional information to appropriately regulate one's behavior toward that object or event. In typical development, social referencing emerges during the end of the first year of life. For instance, previous work has demonstrated that when presented with a novel toy, 10-month-old infants spend more time looking to an experimenter when the experimenter is looking toward rather than away from them, whereas 7-month-olds fail to show this selectivity in their looking behavior (Striano and Rochat 2000). In addition, 12-month-old infants are more likely to crawl across a visual cliff when their mother emits a positive emotional signal and less likely to crawl across when their mother emits a fearful signal (Sorce et al. 1985). Furthermore, 12-month-old infants are more likely to play with a novel toy following a positive signal from their mother and less likely to play with a novel toy following a negative signal from their mother (Stenberg and Hagekull 1997).

Although typically developing infants engage in social referencing by about 12 months of age (Sorce et al. 1985; Stenberg and Hagekull 1997), impaired information seeking and joint attention have been documented in much older children with ASD, suggesting atypical development of social referencing. However, little is known about social referencing in very young children with ASD, or how or whether it might be atypical in unaffected siblings, reflecting a potential ASD endophenotype, defined as a genetically mediated trait appearing in both individuals with ASD and their first-degree relatives. The current study aimed to clarify these questions by investigating social referencing in infant siblings of children with ASD. Infants were tested at 18 months of age, and were assessed for ASD at 36 months of age. Analyses comparing social referencing in infants who were versus were not later diagnosed with ASD were conducted to provide insight into early signs of ASD, and analyses comparing unaffected infants with versus without a family history of ASD were conducted to provide insight into a potential social referencing endo-phenotype. To probe the possibility of dissociable effects on skills that comprise social referencing, we assessed two behaviorally observable aspects of social referencing, specifically (a) social information seeking (looking to an adult when confronted by an ambiguous stimulus); and (b) behavior regulation (altering behavior toward the ambiguous stimulus in accordance with an emotional signal given by the adult).[1]

The social information seeking aspect of social referencing is closely related to joint attention, the act of sharing a common focus with another person. Distinct behaviors comprise joint attention, including following another person's gaze to an object or event, and purposefully directing another person's attention to an object or event (Dawson et al. 2004). The distinction between gaze following and directing another's attention has been conceptualized as responding to joint attention (RJA) versus initiating joint attention (IJA; Seibert et al. 1982). RJA is considered more rudimentary and involves simply following another person's gaze or point, whereas IJA is thought to be more developmentally sophisticated and involves gazing, pointing, and/or other behaviors aimed at engaging another person in shared attention. In most cases, the presumed goal of IJA is to share an interest or experience with another person. In the context of social referencing, however, IJA represents a purposeful action performed with the goal of seeking information about a novel or ambiguous stimulus. Information seeking in social referencing is thus not precisely the same as IJA but, rather, constitutes a special form of IJA. A critical distinction is that, in social referencing, there is intent to seek and obtain information from another individual, which is then typically used by the infant to alter their behavior in accordance with the information provided by that individual.

[1]Note that the use of the term 'behavior regulation' in the context of social referencing is somewhat different from another common use of the term in the ASD literature, where it may refer to behaviors on the part of the child in order to fulfill a goal.

Given the link between social referencing and joint attention, previous research indicating impairments in both RJA and IJA in children with ASD may provide insight into social referencing in ASD. In one study, Bacon and colleagues (Bacon et al. 1998) found that compared to controls, 4- and 5-year-old children with ASD were less likely to initiate joint attention with the experimenter upon hearing an unfamiliar sound. In another study (Adamson et al. 2009), the time that children spent engaged in various types of play-based interactions with a caregiver was measured, and results similarly indicated reduced IJA in 30-month-old children with autism. Additional studies that have employed a standardized assessment, the Early Social and Communication Scales (ESCS), have revealed deficits in both RJA and IJA in young children with ASD (3- to 4-year-olds, Dawson et al. 2004; 3- to 6-year-olds, Mundy et al. 1986). More recently, Landa and colleagues (2007) found that children who met diagnostic criteria for ASD at 30 or 36 months of age initiated joint attention less at 24 months, suggesting that IJA impairments are evident in children with ASD even before a stable diagnosis is currently possible. Although the aforementioned studies have established that children with ASD exhibit impairments in IJA as early as 24 months of age, it is unclear whether these impairments are evident at even younger ages. In addition, as mentioned previously, IJA in contexts in which the infant's goal is presumably to share an experience with an adult is distinct from social referencing contexts, where the infant is presumed to initiate joint attention in order to gain information about how to react to an ambiguous stimulus. Therefore, it is unknown whether infants later diagnosed with ASD exhibit atypical IJA in social referencing contexts.

Given that, in the context of social referencing, IJA takes the form of purposefully seeking information in order to obtain information regarding how to react in uncertain situations, impaired social referencing in ASD could stem from an underlying impairment in IJA, or, could reflect an impairment in appropriately regulating one's behavior according to the other person's emotional cues (or any of the intermediate skills). Preschool-aged children with ASD have been found to exhibit less concern in response to an adult's distress than controls (Dawson et al. 2004; Sigman et al. 1992); however, their ability to regulate their behavior in the context of a triadic interaction (involving the infant, another person, and an object) is less clear. Sigman et al. (1992) reported that although children with ASD were less likely to respond to adults' toy-directed facial and vocal displays of fear than children with mental retardation or typically developing controls, those children with ASD who *did* respond to these displays were then less likely to play with the toy. In another social referencing study, Warreyn and colleagues reported that preschoolers with ASD were actually *more* likely to show behavior regulation than controls, by avoiding a novel toy toward which their caregiver had reacted fearfully (Warreyn et al. 2005). However, the latter study's results are difficult to interpret because, as the authors acknowledge, the control children may have failed to show behavior regulation because they were too old for the novel toy paradigm and did not modify their behavior toward the toy simply because they did not believe the toy was dangerous. Moreover, in both Sigman's and Warreyn's studies, the emotional signals were provided to the children regardless of whether the children actively sought emotional information. As such, it is not entirely clear whether and how children with ASD regulate their behavior when they must seek emotional information in order to obtain it.

Social referencing is a cornerstone of early social development, because it represents a primary means for navigating a complex world in the absence of sophisticated language ability. Without such a means for learning about the social world early in life, it is easy to imagine far-reaching and cascading detrimental effects on more complex social skills. Therefore, investigating social referencing in ASD, especially early in development, may shed light on potential origins for social deficits evident in individuals with ASD in later childhood. In addition, impaired social referencing during the first 2 years of life may be a predictive marker for subsequent diagnosis of an ASD.

The current study was designed to investigate the social information seeking and behavior regulation aspects of social referencing as they relate to ASD during infancy. To this end, we examined infants who have an older sibling diagnosed with an ASD. These "high-risk" (HR) infants have approximately a 19 % likelihood of developing ASD (Elsabbagh and Johnson 2010; Landa and Garrett-Mayer 2006; Ozonoff et al. 2010, 2011), which is roughly fivefold to tenfold greater than that seen in the general population (Baron-Cohen et al. 2009; Ozonoff et al. 2011). Furthermore, research suggests that, as a group, even those HR infants who do not go on to develop an ASD are likely to exhibit subclinical differences from control infants (without a family history of autism) on social and communicative measures (Cassel et al. 2007; Merin et al. 2007; Presmanes et al. 2007; Yirmiya et al. 2006). Therefore, in the current research, we had two primary goals: (a) to identify the early emergence of social referencing impairments in ASD; and (b) to establish whether subclinical social referencing impairments are apparent in unaffected infant siblings of children with ASD, consistent with an endophenotype. To accomplish this, we utilized a paradigm previously mapped out in typically developing infants (Carver and Vaccaro 2007; Walden and Kim 2005; Walden and Ogan 1988), in which infants are introduced to novel toys in a semi-naturalistic setting in order to elicit referential looking. The caregiver and the experimenter are both present during the experimental session, affording the infant two target adults to whom they could look. The caregiver and experimenter respond to infants' looks by providing an emotional signal directed toward the toy, and infants' looking behavior and their reactions to the toys before versus after the emotional signals are assessed. In the current study, we expected that typically developing low-risk (LR) 18-month-olds would look referentially to adults when novel toys were introduced. However, we predicted that HR infants subsequently diagnosed with ASD would take longer and/or make fewer attempts to seek social information. We also predicted that HR infants who developed ASD would be less apt to use the adults' vocalizations and facial expressions to regulate their behavior. Finally, we hypothesized that, as a group, those HR infants who *did not* subsequently receive an ASD diagnosis would behave in an intermediate manner; specifically, that they would demonstrate more social information seeking and behavior regulation than the HR infants later diagnosed ASD, but less than the LR infants.

Method

Participants

Data from 44 LR (21 female, 29 male) and 38 HR (18 female, 23 male) infants contributed to this study. LR infants were recruited via letters sent to parents, who were screened to ensure no family history of autism. In order to be included in the study, all LR infants were required to have an older sibling living in the same home. HR infants were recruited via referrals from other autism research laboratories at UCSD, the San Diego Regional Center, and the Center for Autism and Related Disorders; advertisements distributed in the community; and a study website. HR infants' older siblings were each diagnosed with an ASD (*Autistic Disorder*; *Asperger's Syndrome*; or *Pervasive Developmental Disorder-Not Otherwise Specified* [*PDD-NOS*]) by a licensed clinical psychologist or medical doctor not associated with this research, based on DSM-IV criteria. The outside ASD diagnoses of the older siblings were verified by a member of our team, a clinical psychologist with expertise in autism (N.A.). This research diagnosis was based on the Autism Diagnostic Observation Schedule (ADOS; Lord et al. 2000) and the Autism Diagnostic Interview-Revised (ADI-R; Lord et al. 1994). Based on these clinical measures, 19 of the older siblings met diagnostic criteria for *Autistic Disorder*, 1 for *Asperger's Disorder*, and 18 for *PDD-NOS*. Older siblings had no known specific neurological or genetic condition (e.g., Fragile X) that could account for their ASD diagnosis. The current study was part of an ongoing longitudinal study in which all children (HR and LR) were assessed for ASD using the ADOS at 36 months of age. If a child's ADOS score was above the ASD cutoff, the ADI-R was administered. A clinical best estimate diagnosis was made by our clinical psychologist (N.A.) based on information from the ADOS and ADI-R and clinical judgment using DSM-IVTR criteria. Clinical best estimate diagnoses from 1 LR infant and 8 HR infants were consistent with an ASD diagnosis (6 *Autistic Disorder*, 2 *PDD-NOS*). Data from these 9 infants (8 male, 1 female) were considered as a separate group ("ASD") during data analysis, in order to distinguish behavioral characteristics that were associated with a subsequent ASD diagnosis from those that were indicative of subclinical features of ASD often observed in first-degree relatives of individuals with ASD, consistent with a potential endophenotype.[2] The remaining HR infants ($n = 30$), whose 36-month screening was not consistent with an ASD diagnosis, are henceforth referred to as the "high-risk non-spectrum" (HR-NS) group.

Infants ranged in age from 17.7 to 20.6 months (LR mean = 18.2; SD = 0.44 HR-NS mean = 18.3, SD = 0.46; ASD mean = 18.3, SD = 0.80). An ANOVA confirmed that the groups did not differ in age, $F(2,78) = 0.38$, $p = 0.688$. Information regarding infants' maturity at birth, (the difference between their birth date and due date) was obtained via parent report. This information was not available for one HR-NS infant, but, for the remaining participants, maturity ranged from −36 to 8 days (LR mean = −4, SD = 7; HR-NS mean = −5, SD = 9; ASD mean = −8, SD = 12). An ANOVA indicated that the groups did not differ in maturity, $F(2,77) = 0.89$, $p = 0.414$.

Stimuli

Stimuli were novel toys chosen to elicit social referencing (Carver and Vaccaro 2007; Fig. 1). Each infant was presented with three toys from a set of six battery-operated moving toys. The toys each child encountered, the order in which they were presented, the emotional valence assigned to each, and the order of the emotional valences were counterbalanced. In addition, it was confirmed with caregivers in advance that the infant had not previously encountered any of the toys assigned to their session; in cases where the infant had previous experience with a given toy, that toy was randomly replaced with another, truly novel toy.

Behavioral Procedure

After providing written informed consent in accordance with UCSD's Institutional Review Board, caregivers were trained using photographs of facial expressions from a standardized set (Tottenham et al. 2009) to produce positive (happy), negative (disgusted), and neutral (calm) expressions. For photographs depicting each of the three emotions, the experimenter demonstrated a corresponding vocalization in the appropriate tone of voice. For the positive condition, this vocalization was, "What a pretty toy!" spoken in a happy and excited tone. For the negative condition, it was, "What a nasty toy," produced in a disgusted tone, and for the neutral condition, it was, "What an ordinary toy," spoken in a calm tone.

The experimental session for each infant took place in the same 10′ by 12′ room. The room contained two adult-sized chairs in which the infant's primary caregiver and an experimenter sat. Infants played on the floor in front of the adult chairs with a small number of common baby toys (e.g., a ball, a toy car, a toy phone) and were permitted to move freely throughout the room. Testing began with a 1-min baseline period, during which the experimenter and caregiver interacted only minimally with the infant. Next, a hidden second experimenter surreptitiously sent one of the novel toys out from behind a curtain, which blocked off a corner of the room, and activated the toy so that it began moving. All infants

[2]Data from the LR infant who was subsequently diagnosed with ASD were included in the ASD group in keeping with the study's goal of investigating the early emergence of social referencing impairments in ASD.

noticed the novel toys, usually immediately after the toy began moving. Each toy was present for 1 min, during which the caregiver and/or experimenter said the appropriate target sentence while producing an emotionally congruent facial expression if and only if the infant looked to them after having noticed the toy. The emotional signal was produced by the caregiver and/or experimenter as many times as the infant looked referentially during that 1-min interval. If the infant referenced either adult at least once during that time, then at the end of the 1-min interval, the experimenter turned off the toy and placed it out of sight. If, however, the infant did not look to either the caregiver or the experimenter during the 1-min interval, the caregiver was cued over a headset by another experimenter and instructed to attract the infant's attention and then provide the emotional signal. When an infant needed a cued prompt to look at an adult, the caregiver attracted the infant's attention by saying the infant's name and/or tapping the infant on the shoulder. The infant's attention was then diverted from the toy to the caregiver's face, and then the emotional signal was given by the caregiver. In cases where an infant needed a cued prompt to look to an adult, the toy was removed 15 s after the emotional signal, allowing the child ample time to react to the toy following the signal. This procedure was repeated for all three toys.

Behavioral Coding and Measures of Interest

Testing sessions were videotaped using two cameras to ensure that the infants and adults were both visible, and coders knowledgeable about social referencing later rated the infants' behavior. Coders were led to believe that the aim of the study was to investigate social referencing in 18-month-old infants and were not told that some of the infants had an older sibling with ASD and were at increased risk for ASD. Measures of interest were chosen to index infants' social information seeking and behavior regulation in response to social information. Information seeking was assessed via two measures: (1) whether or not the infant spontaneously looked at either their caregiver or the experimenter after first looking at the stimulus toy; and (2) the infant's latency to reference (the number of seconds that elapsed between when the infant first noticed the stimulus toy and his/her first look to the caregiver or the experimenter). Behavior regulation was assessed by comparing the infant's initial approach/withdrawal behavior with respect to each novel toy (i.e., before either adult provided emotional signals about the toy) to the infant's approach/withdrawal behavior following the signals. Coders rated infants' approach/withdrawal behavior on the following 5-point scale: (1) withdraws/pushes away; (2) ignores; (3) looks at/points; (4) approaches/reaches; and (5) touches/explores. Coders made a separate rating for each 10-s increment in the before-signal interval and the after-signal interval, and ratings were averaged over the 10-s increments within each interval. Finally, in order to ensure that they adequately expressed the target emotions, caregivers' signals were rated on a five-point scale that ranged from very non-prototypical (1) to very prototypical (5) emotion.

The aforementioned measures of interest were the same as in Carver and Vaccaro's (2007) social referencing study involving 12-month-olds; however, new guidelines were established based on pilot data from typically developing 18-month-olds in order to ensure that the coding system was suited to this age group. Two original coders helped to establish these guidelines; and, following extensive training, subsequent coders were required to attain a minimum of 85 % agreement with both original coders on three consecutive sessions before contributing ratings to be included in data analyses. Because the current study was part of a larger longitudinal study and data collection occurred over several years, ratings were contributed by a total of 10 coders. However, preliminary analyses did not indicate any systematic differences between ratings made by individual coders. Coders were randomly assigned behavioral sessions and overlapped on 12 % of sessions to provide an index of agreement. Intraclass correlations indicated a high degree of overlap between coders' ratings on all variables of interest. The mean intraclass correlation for the information seeking

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variables was 0.97 (SD = 0.04), and the mean intraclass correlation for the behavior regulation variables was 0.91 (SD = 0.10). A *t* test confirmed that intraclass correlations did not significantly differ between the information seeking and behavior regulation variables, $t(6) = 0.67$, $p = 0.530$.

Results

Emotion Order

Infants were randomly assigned to one of six possible orders of emotion condition (whether the positive, negative, and neutral emotional signals were provided in reference to the first, second, or third stimulus toy presented). Initial analyses were conducted to verify that emotion order did not impact variables of interest. First, a Chi Square test indicated that the proportion of infants assigned to each emotion order did not differ between the LR and HR-NS groups, $\chi^2(5, N = 73) = 7.32$, $p = 0.198$.[3] Next, a Chi Square test confirmed no significant effect of emotion order on whether infants spontaneously looked to their caregiver or the experimenter after noticing the stimulus toys, $\chi^2(5, N = 82) = 4.92$, $p = 0.426$. Finally, ANOVAs with group and emotion order as between-subjects factors indicated neither a significant main effect of emotion order, nor a significant group by emotion order interaction for infants' latency to reference or behavior regulation for any emotion condition (all $F < 1.88$, $p > 0.081$). Therefore, subsequent analyses were conducted without emotion order as a factor.

Caregiver Emotional Signals

Ratings indicated that caregivers generally portrayed each emotion accurately: For the LR, HR-NS and ASD groups, respectively, median ratings for each signal were: 5, 4, and 5 (positive), 4, 4, and 5 (negative), and 5, 4, and 5 (neutral). Kruskall-Wallis tests demonstrated that the accuracy of caregivers' emotional signals did not significantly differ between groups (all $\chi^2 < 5.01$, $p > 0.082$).

Referencing Behavior

Information Seeking

Spontaneous Referencing: For each emotion condition, whether or not infants spontaneously referenced either adult within the first 60 s of noticing the novel stimulus toy was coded dichotomously. Because the infants' first referential looks preceded the emotional signals, the data were combined across conditions to indicate whether infants failed to spontaneously reference in any of the three conditions. In the LR group, 9 of the 43 infants (21 %) failed to spontaneously reference in at least one emotion condition. In the HR-NS group, 12 of the 30 infants (40 %) failed to spontaneously reference in at least one condition. Finally, in the ASD group, 6 of the 9 infants (67 %) failed to spontaneously reference in at least one condition. A Chi Square test yielded a significant difference across groups in spontaneous referencing, $\chi^2(2, N = 82) = 8.12$, p(2-tailed) = 0.017. Additional Chi Square tests were conducted to elucidate the nature of the group difference, and revealed that spontaneous referencing was significantly more frequent in the LR than in the ASD group, $\chi^2(1, N = 52) = 7.59$, p(2-tailed) = 0.006 and marginally more frequent in the LR than in the HR-NS group, $\chi^2(1, N = 73) = 3.14$, p(2-tailed) = 0.077. The HR-NS and ASD groups did not significantly differ from one another, $\chi^2(1, N = 39) = 1.98$, p(2-tailed) = 0.159.

[3]The ASD group was not included in this analysis because there were too few values in each cell to meet the assumptions of a Chi Square test. In addition, at the time of testing, it was unknown which HR infants would later meet ASD diagnostic criteria. Nevertheless, the emotion orders of the ASD infants were distributed, with two infants assigned to order 1, zero to order 2, two to order 3, three to order 4, and one each to orders 5 and 6.

Latency to Reference: The number of seconds between when an infant first noticed a novel toy and her first referential look to either the caregiver or experimenter (defined as looking first to the toy and then shifting gaze to either adult) was used to index infants' readiness to seek social information about the toy. In cases where the infant did not spontaneously reference either adult, the latency to reference was recorded as the number of seconds elapsed between noticing the toy and when the infant looked to the caregiver in response to the caregiver's cued prompt. Several infants (particularly in the HR-NS and ASD groups) did not respond to their caregiver's prompt within 15 s, leading to latencies longer than 75 s. However, in an effort to reduce the likelihood of Type I error, a conservative approach was adopted, such that these infants' latencies were recorded as a predetermined maximum of 75 s. As would be expected, since the infants' first referential look in each condition preceded the emotional signal, an ANOVA revealed no effect of emotion condition on latency to reference, $F(2,74) = 0.03$, $p = 0.973$. Therefore, each infant's latencies were averaged across positive, negative, and neutral conditions to provide a more stable measure of information seeking. Average latencies for each group were as follows: LR, 24 s (SD = 16); HR-NS, 28 s (SD = 16); and ASD, 45 s (SD = 18). An ANOVA revealed a significant difference across the three groups, $F(2,79) = 6.35$, $p = 0.003$, $\eta_p^2=0.14$ (Fig. 2). Bonferroni-corrected post hoc tests indicated that infants in the ASD group had longer latencies to reference than LR and HR-NS infants (*p*s = 0.002 and 0.029, respectively), who did not differ from each other (p = 0.662).

Behavior Regulation: Infants' reactions to the novel toys before and after the first emotional signal in each condition were rated on a scale from 1 (withdrawing from/pushing away the toy) to 5 (touching/exploring the toy). Because infants' behavior before an emotional signal served as a baseline response to a given toy, we computed difference scores to account for pre-signal behavior, including that which might be due to an infant's inherent preference for a given toy. Log scores were used so that 0 would represent no change in behavior before versus after an emotional signal, and positive and negative scores would represent equally strong (although opposite) changes in behavior as a result of the emotional signal. Scores were calculated as the log ratio of the reaction to the toy rating for two emotion conditions following the emotional signal minus the log ratio of the ratings for the same two emotion conditions before the emotional signal. For instance, for the comparison between "positive" and "neutral," the difference score is: log(positive toy: reaction after signal/neutral toy: reaction after signal) — log(positive toy: reaction before signal/neutral toy: reaction before signal), which indexes whether the infant's relative reaction to the "positive" and "neutral" toys changed as a result of the emotional signals. A difference score greater than zero indicates that, as a result of the adults' emotional cues, the infant's desire to approach/explore the positive toy increased relative to their desire to approach/explore the neutral toy, suggesting appropriate behavior regulation. Likewise, for the comparison between "positive" and "negative," the difference score is: log(positive toy: reaction after signal/negative toy: reaction after signal) — log(positive toy: reaction before signal/negative toy: reaction before signal), where a score greater than zero indicates that as a result of the adults' emotional cues, the infant's desire to approach/explore the positive toy increased relative to their desire to approach/explore the negative toy, suggesting appropriate behavior regulation. Finally, for the comparison between "negative" and "neutral," the difference score is: log(negative toy: reaction after signal/neutral toy: reaction after signal) — log(negative toy: reaction before signal/ neutral toy: reaction before signal), where a score *less* than zero indicates that as a result of the adults' emotional cues, the infant's desire to approach/explore the negative toy decreased relative to their desire to approach/explore the neutral toy, suggesting appropriate behavior regulation.

Behavior regulation difference scores for each group in each emotion comparison are presented in Table 1. To test for group differences as well as effects of emotion comparison in behavior regulation, the "negative versus neutral" difference scores were first multiplied by −1 (so that in all three conditions, a score greater than 0 would indicate appropriate behavior regulation). A repeated measures ANOVA was conducted, with emotion comparison (positive vs. negative, positive vs. neutral, negative vs. neutral) as a within-subjects factor and group as a between-subjects factor. This ANOVA revealed a significant main effect of group, $F(2, 75) = 3.17$, $p = 0.048$, $\eta_p^2=0.08$, but no main effect or interaction involving emotion comparison was observed (*p*s > 0.43). Post hoc Tamhane tests (for unequal variances) revealed marginally significantly greater behavior regulation in the LR group compared to both the HR-NS and the ASD groups (*p*s = 0.097 and 0.085, respectively). The HR-NS and ASD groups did not differ from one another ($p = 0.998$).

Because there was no significant effect of emotion on behavior regulation, and in order to provide a cleaner metric of infants' behavior regulation capabilities, a composite behavior regulation score was computed for each infant by averaging over the three comparisons (positive vs. negative, positive vs. neutral and negative vs. neutral). Because the behavior regulation metric is meaningful in relation to zero, each group's behavior regulation capabilities were directly assessed by comparing composite scores to zero using one-sample t-tests. One-tailed t-tests were conducted, as only composite difference scores significantly greater than zero would indicate appropriate behavior regulation. For LR infants, the mean composite difference score was 0.043 (SD = 0.127), which was significantly greater than 0, $t(42) = 2.21$, $p(1\text{-tailed}) = 0.016$, $d = 0.33$. For HR-NS infants, the mean composite difference score was −0.03 (SD = 0.15), which was not significantly greater than 0, $t(30) = -1.19$, $p(1\text{-tailed}) = 0.758$. Similarly, for ASD infants, the mean composite difference score was −0.04 (SD = 0.08), which was also not significantly greater than 0, $t(8) = -1.46$, $p(1\text{-tailed}) = 0.811$ (Fig. 3). Results from this family of analyses indicated that LR infants appropriately regulated their behavior toward the novel toys in response to the emotional signals, whereas HR-NS and ASD infants failed to do so. Independent samples t-tests were conducted to assess whether infants' behavior regulation differed depending on whether or not they spontaneously referenced an adult within the first 60 s of noticing a novel stimulus toy (collapsed across groups). Results revealed no effects of spontaneous referencing on behavior regulation (*p*s > 0.265).

Discussion

Here we report an investigation of social referencing in 18-month-old infants at high risk for autism compared with typically developing controls. Our goals were twofold: to identify early manifestations of ASD, and to investigate the potential presence of subclinical atypicalities in HR infants who are not later diagnosed with ASD. Infants were introduced to three novel toys in a semi-naturalistic play setting, and their social information seeking behavior, as well as their behavior regulation following adults' emotional signals about the toys, were assessed.[4] Results demonstrated that HR infants who met diagnostic criteria for ASD at 36 months of age (ASD infants) sought social information more slowly at 18 months compared to both LR infants and HR infants who *did not* later meet ASD diagnostic criteria (HR-NS infants). This finding is consistent with studies in which older children already

[4]Infants' looking behavior is referred to as information seeking throughout this paper because our paradigm was designed to study social referencing, of which information seeking is a crucial part. Nevertheless, the motivation driving infants' looking behavior when confronted by an ambiguous stimulus is uncertain. When infants' referential looking is followed by appropriate behavior regulation toward the stimulus in accordance with the emotional signals, it is reasonable to infer that their looking was motivated by information seeking. However, in cases where infants looked referentially to adults but did not appropriately regulate their behavior, it is possible that they were not in fact seeking information.

diagnosed with ASD were found to be less likely than typically developing children to initiate bids for joint attention (three- and four-year-olds; Dawson et al. 2004) or to seek information from an adult when confronted with a novel stimulus (4- and 5-year olds; Bacon et al. 1998). Of particular note is that, in the present study, atypical information seeking behavior was evident at 18 months of age, suggesting that this aspect of social referencing has predictive value for later ASD diagnosis. This result corroborates a previous report that at 24 months of age, HR children given an outcome diagnosis of ASD at 30 or 36 months of age initiated joint attention less than control children and HR children who were not later diagnosed with ASD (Landa et al. 2007), and extends this finding to younger infants and to the domain of social referencing, in which joint attention is initiated specifically in order to seek information about a novel or ambiguous stimulus. Therefore, the social information seeking aspect of social referencing should be a key behavior of interest for clinicians attempting to identify the early manifestations of ASD.

Interestingly, the LR and HR-NS infants in the current study did not significantly differ on measures of information seeking (although HR-NS infants exhibited marginally lower rates of spontaneous looking than LR infants), suggesting that impaired information seeking in the context of social referencing is not a candidate endophenotype, or genetically mediated characteristic seen in unaffected family members, at 18 months of age. This observed lack of a difference in IJA between LR and HR-NS infants is consistent with the results from another study in which the ESCS was administered to 14-month-old HR-NS infants (Yirmiya et al. 2006). Two additional studies reported less initiation of joint attention in HR infants compared to LR controls during the second year of life (Cassel et al. 2007; Goldberg et al. 2005); however, in both of these studies the effect may have been driven by HR infants who later met ASD diagnostic criteria, since the HR infants were not subdivided based on subsequent ASD diagnostic outcomes and the HR sample sizes were small (*N*s = 5 and 8, respectively).

The aforementioned results raise the question of the mechanism underlying reduced social information seeking in infants later diagnosed with ASD. One potential mechanism is impaired disengagement of visual attention early in life. Zwaigenbaum and colleagues (2005) reported that HR infants' latency to disengage attention from one visual stimulus to a competing visual stimulus at 12 months of age was correlated with ADOS scores at 24 months. In addition, HR infants who met diagnostic criteria for ASD at 24 months of age exhibited longer latencies to disengage at 12 months than at 6 months, whereas typically developing infants demonstrated the opposite pattern. Given the triadic nature of initiating joint attention in the context of social referencing, difficulty in disengaging visual attention from a stimulus of interest (in the current study, a novel toy) in order to attend to another person could ostensibly lead to prolonged latencies to reference in infants later diagnosed with ASD, as observed here.

Another potential visual mechanism that might contribute to ASD-related impairments in initiating joint attention is the development of the magnocellular pathway, which subserves visual orienting in the first few months of life. Six-month-old HR infants have been shown to exhibit enhanced magnocellular sensitivity, which may serve to bias their visual attention toward certain types of stimuli, including moving stimuli (McCleery et al. 2007). This type of visual bias early in life has been proposed to underlie later-emerging abnormalities in social processing in ASD, in areas such as joint attention (Mundy et al. 2009) and face processing (McCleery et al. 2007; Mundy et al. 2009). An additional possibility is that a primary social impairment accounts for reduced information seeking in infants later diagnosed with ASD. For instance, if ASD infants do not attend to faces in the same way, or do not glean the same social-emotional information from faces as their typically developing counterparts, then looking to others when confronted by ambiguous stimuli would not serve the same important function early in life.

To our knowledge, the current study is the first to investigate behavior regulation in HR infants in the context of social referencing. LR infants were more likely to approach a toy following positive facial and vocal expressions and withdraw from a toy following negative expressions. In contrast, HR infants, irrespective of whether they subsequently met ASD diagnostic criteria, failed to show evidence of this type of behavior regulation. Therefore, in addition to impaired information seeking, impaired behavior regulation characterizes infants who later meet diagnostic criteria for an ASD. Interestingly, the finding that infants' behavior regulation did not depend on whether or not they spontaneously looked to their caregiver or the experimenter when presented with a novel toy suggests that the observed group differences in behavior regulation cannot be accounted for by the decreased frequency of spontaneous looking in HR infants (particularly the ASD group). In addition, the group differences in behavior regulation were evident even though parents of infants in the ASD group demonstrated the emotions with high accuracy, particularly in the negative condition, where a marginally significant effect suggested that they were more accurate than parents of LR and HR-NS infants. Although this effect only approached statistical significance, perhaps parents of infants in the ASD group tended to exaggerate their emotional expressions. This kind of exaggeration might be necessary in working with their older, affected child to help the child learn to identify others' emotions, and might carry over into experimental settings. Although we did not collect the treatment histories of older siblings of HR infants in the current study, we are now obtaining this treatment information in our ongoing longitudinal study of HR infants. This information, and particularly involvement in parent training, will be useful for investigating the trend toward group differences in parents' emotional signals observed here.

HR infants, whether later diagnosed with ASD or not, showed atypical behavior regulation, indicating that this aspect of social referencing does not seem to reflect ASD per se but rather a more subtle impairment consistent with a possible ASD endophenotype. Abnormal behavior regulation despite partially intact information seeking in HR-NS infants, coupled with the finding that infants' behavior regulation did not differ as a function of spontaneous referencing, suggests that these two aspects of social referencing are at least partially dissociable. This dissociation is consistent with findings from a previous study of typically developing 12-month-olds (Carver and Vaccaro 2007) and also with a proposed developmental progression of social referencing, according to which referential looking paves the way for behavior regulation through repeated experience (Carver and Cornew 2009). Therefore, the current results suggest that this trajectory may be disrupted in infants with familial risk for autism. Although the current findings implicate a breakdown between the social information seeking and behavior regulation aspects of social referencing in HR-NS infants, it is not possible to discern from our results whether the breakdown occurs in behavior regulation per se or in an intermediate aspect of social referencing, such as emotion recognition.

Given that HR infants exhibit several endophenotypes (here, a failure to use adults' emotional signals to regulate their behavior) irrespective of later ASD diagnosis, perhaps those not later diagnosed with ASD possess attributes that protect them from developing ASD despite the presence of the endophenotype (see McCleery et al. 2007 for an expansion of this argument). The results of the present study, together with those of Yirmiya et al. (2006), implicate preserved IJA as one such potential protective factor. Joint attention interventions are successfully implemented with young children with ASD (Ingersoll and Dvorcsak 2010; Kasari et al. 2010; Whalen and Schreibman 2003) and other at risk populations (Olafsen et al. 2006). Extending these kinds of interventions to younger HR infants may help to bolster their development of social referencing. This pursuit is of the utmost importance, especially because there are likely sensitive periods in social-cognitive development, during which the infant is maximally responsive to experiences that promote development in this domain (Fox et al. 2010). If a sensitive period characterizes the development of social referencing, then a failure to develop this set of skills during the first 18 months of life, which the current study suggests is the case in ASD, may have cascading detrimental effects on more sophisticated social capabilities, including intersubjectivity and theory of mind.

In sum, the current findings suggest that social referencing behavior provides insight into the early emergence of autism as well as its subclinical manifestations early in life. In addition, findings demonstrate that social referencing is not a unitary construct and suggest that IJA and behavior regulation skills are differentially impacted by familial ASD risk and ASD diagnosis. Although a limitation of our study is the small sample size of the ASD group, and future studies are needed to replicate and extend the findings reported here, we hope that the findings reported here will contribute to earlier ASD diagnosis and a better understanding of social development in infants with increased ASD risk.

Acknowledgments

This research was supported by NIH (R01 HD052804-01A2), the MIND Institute, and Autism Speaks (KRD and LJC). We thank the families that participated; Annette Cluver, Rebecca Cunningham, Kelly Deegan, Elizabeth Dohrmann, Sara Geal-Touhy, Fiona Ma, Michelle Peltz, Kellie Swayne, Margaret Swingler, Lisa Tully, and Faye Van der Fluit for their contributions to the project; and members of the Developmental Cognitive and Social Neuroscience Lab for assistance with data collection and coding.

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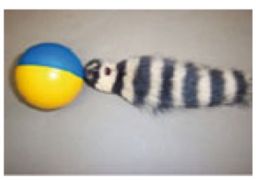
Toy	Dimensions	Description
	30 x 30 x 28 cm	RC dragon; moves forward, backward, and turns
	11.78 cm (diameter)	Battery operated ball; bounces in random patterns
	12 x 18.5 x 27 cm	RC dinosaur; moves, spins and beeps
	11 x 11 x 15.5 cm	RC robot; eyes light up and beeps
	29 x 7 x 6 cm	Battery operated ball with furry stuffed animal attached; moves in random patterns
	13 x 20 x 8.5 cm	RC spider; moves and beeps

Fig. 1.
Stimulus toys. Each infant was presented with three of the six toys (counterbalanced across participants in each group). RC = Remote Controlled

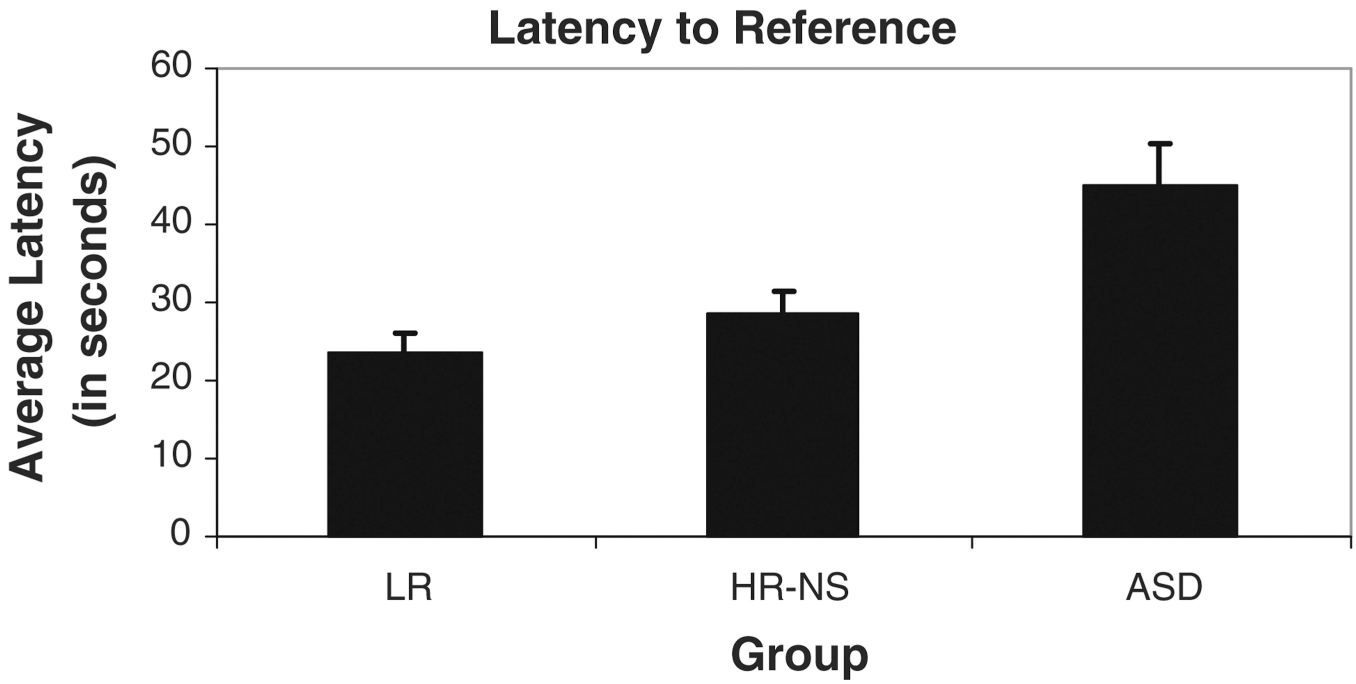


Fig. 2.
Latency to reference (averaged across the positive, negative, and neutral conditions) in low-risk infants (LR), high-risk infants who did not develop ASD (HR-NS), and infants later diagnosed with ASD (ASD)

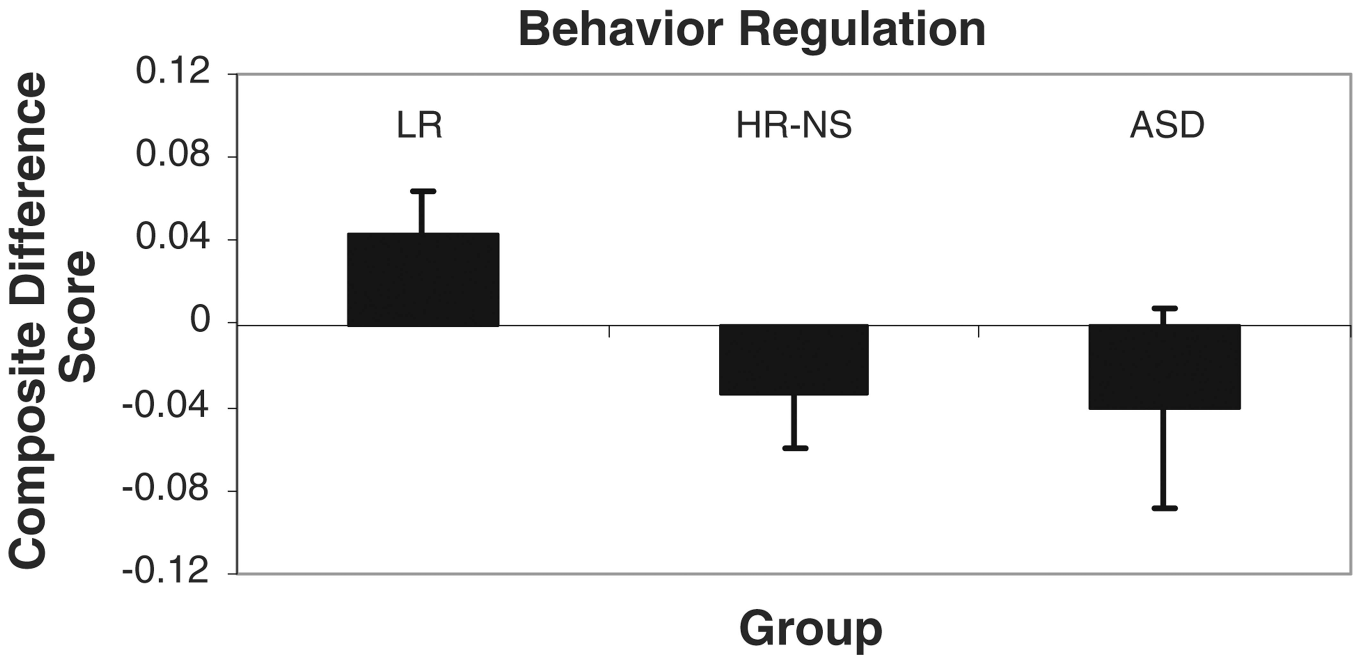


Fig. 3.
Behavior regulation (averaged across the positive, negative, and neutral conditions) in low-risk infants (LR), high-risk infants who did not develop ASD (HR-NS), and infants later diagnosed with ASD (ASD). Scores greater than zero indicate successful behavior regulation

Table 1

Mean (SD in parentheses) log difference behavior regulation scores for each emotion comparison in low-risk infants (LR), high-risk infants who did not develop ASD (HR-NS), and infants later diagnosed with ASD (ASD)

	Positive versus negative	Positive versus neutral	Negative versus neutral
LR	0.06 (0.19)	0.02 (0.20)	0.04 (0.15)
HR-NS	−0.05 (0.23)	−0.05 (0.16)	0.00 (0.18)
ASD	−0.06 (0.12)	−0.01 (0.11)	−0.05 (0.07)

Positive scores indicate appropriate behavior regulation