Neural Correlates of Eye Gaze Processing in the Infant Broader Autism Phenotype


Background: Studies of infant siblings of children diagnosed with autism have allowed for a prospective approach to study the emergence of autism in infancy and revealed early behavioral characteristics of the broader autism phenotype. In view of previous findings of atypical eye gaze processing in children and adults with autism, the aim of this study was to examine the early autism phenotype in infant siblings of children diagnosed with autism spectrum disorder (sib-ASD), focusing on the neural correlates of direct compared with averted gaze.

Methods: A group of 19 sib-ASD was compared with 17 control infants with no family history of ASD (mean age = 10 months) on their response to direct versus averted gaze in static stimuli.

Results: Relative to the control group, the sib-ASD group showed prolonged latency of the occipital P400 event-related potentials component in response to direct gaze, but they did not differ in earlier components. Similarly, time-frequency analysis of high-frequency oscillatory activity in the gamma band showed group differences in response to direct gaze, where induced gamma activity was late and less persistent over the right temporal region in the sib-ASD group.

Conclusion: This study suggests that a broader autism phenotype, which includes an atypical response to direct gaze, is manifest early in infancy.

Key Words: Autism, broader autism phenotype, event-related potential, eye gaze processing, infancy

A prominent aspect of the autism phenotype is a qualitatively unusual pattern of eye contact, which may reflect the more widespread deficits in communication and social interaction observed in this population. Behavioral studies with children and adults with autism have demonstrated that the use of gaze cues in social contexts such as joint attention (1–5) or in inferring mental states (4) is an area of difficulty. This does not, however, imply a complete lack of sensitivity to gaze in this clinical group. Individuals with autism are able to estimate gaze direction (5), and they show reflexive orienting toward objects cued by another’s gaze (6,7); however, they are atypical in the context of mutual gaze; that is, when the perceiver makes eye contact (8).

Electrophysiological and neuroimaging studies have documented atypical neural correlates of gaze processing in autism. Using event-related potential (ERP) recording, passive viewing of faces with direct gaze elicited larger occipitoparietal negativity than averted gaze in 4 to 7 year olds with autism, a pattern not seen in typically developing children of the same age (9). In contrast to this study using passive viewing, actively detecting direct versus averted gaze targets elicited an occipitotemporal negativity in children with and without autism. However, the response was stronger and predominantly right lateralized for typically developing children, whereas children with autism showed a bilaterally distributed response (8). Converging evidence for atypical gaze processing comes from functional magnetic resonance imaging (fMRI) studies. In typical individuals, eye gaze is processed in specialized regions including the superior temporal sulcus and the amygdala (10). Furthermore, brain activation patterns are modulated by the referential nature of eye gaze, that is, when gaze shifts are either congruent or incongruent with the appearance of peripheral visual targets. Individuals with autism show brain activity in similar regions, but the modulation of the response as a function of the referential context of eye gaze is reduced (11).

Processing of eye gaze in autism has also been tied to face processing. Although there is some consensus that the neural correlates of face processing in autism are atypical (12), recent evidence suggests that brain activation patterns in response to faces correlate strongly with atypical behavioral scanning patterns in autism. More specifically, a reduction in the typical fusiform and amygdala response to faces seen in autism correlates with reduced fixation to the eye region (13–15).

For genetic reasons (16–18), some of the atypical responses to socially relevant stimuli including gaze found in individuals with autism are also seen in their first-degree relatives who do not have a diagnosis. This broader autism phenotype (BAP) includes several cognitive and neural characteristics (13,19,20). For instance, parents of children on the autistic spectrum show atypical brain activity in response to eyes (21). Also, adult siblings of individuals with autism, who do not themselves have a diagnosis, show diminished fusiform activation correlated with reduced gaze fixation, similar to that seen in their affected siblings (14). Even at the neuroanatomic level, amygdala volume in siblings has been found to be significantly reduced (14).

The significance of these findings in formulating a developmental model of autism has been recognized. The reduced sensitivity to gaze in the social context seems to suggest that the early developmental course in autism contrasts with that in typical development. Infants display very early sensitivity to gaze (22,23), which develops rapidly in the first few years in the form of social referencing, joint attention, and communication.
autism, it has been suggested that a form of neurodevelopmental deficit in brain networks subserving social cognition leads to decreased attention to, or interest in, the social world (12,19,24). This early lack of attention to or interest in social stimuli, including gaze, may interfere with the emergence of critical developmental milestones relevant for social cognition such as shared attention. These cascading influences may eventually preclude the typical development of sociocommunicative skills.

Compelling as they are, such developmental accounts face serious empirical challenges. Because a confirmed and reliable diagnosis of autism is usually only made from approximately 3 years of age (25), our knowledge of the early neural, behavioral, and cognitive profile in autism is limited. Retrospective studies indicate that subtle deficits are present before the typical age of diagnosis in autism. This evidence relates to early differentiation between social and nonsocial stimuli, which continues into adulthood. Home videos of the first 2 years of life of infants later diagnosed with autism show less orienting toward social stimuli from as early as 9 months (26–29) or even younger (30) compared with infants later diagnosed with developmental delay. However, these findings remain limited as far as controlled experimental assessment. Furthermore, it is unclear whether these early differences in social orienting in general, or in eye gaze processing in particular, found in infants later diagnosed with autism extend to the BAP.

An emerging area of research focusing on infants at genetic high risk for autism has begun to address the emergent nature of autism symptoms more directly. Research on infant siblings of children diagnosed with autism spectrum disorders (ASD; hereafter, “infant siblings”) offers this opportunity because the recurrence rate of ASD is significantly elevated above the general population (31) and has been even higher in recent studies (32,33). Research on infant siblings may clarify why autism emerges in some cases and not in others, as well as help to explain variations associated with the autism phenotype.

Some studies have followed up infant siblings from 4 or 6 months of age to the stage when some received a formal diagnosis. These studies have confirmed that the expression of autism in the first year is often subtle, at least as far as overt symptoms or delays in the expected developmental milestones (32–35). Furthermore, several studies have documented differences in groups of siblings of children with autism, relative to matched groups of infants who do not have affected siblings. One study examined gaze fixation in infant siblings as young as 4–6 months and reported that they looked more to the mother’s mouth relative to her eyes (36). Other studies did not find differences in visual fixation but reported subtle differences in affect (37,38). Slightly older infant siblings (> 14 months) show somewhat clearer differences in a number of cognitive and motor measures as well as sociocommunicative measures (39–41). For instance, in a study examining infant’s response to different combinations of joint attention cues (e.g., eye gaze or eye gaze and head movement), siblings showed less responding to joint attention cues relative to a low-risk control group (41). Although follow-up data are essential in understanding the functional impact of such early behavioral patterns on later development (e.g., 42), these findings nonetheless suggest that early atypical gaze behavior may form part of the BAP (38).

Despite the behavioral results just described, as yet nothing is known about the early neural basis of gaze processing in the BAP, including in infant siblings at risk for autism. Thus, the aim of this study was to explore differences in response to gaze in infant siblings as a group using electrophysiological measures. We examined the neurophysiological correlates of gaze processing in infant siblings while they viewed photographs of females displaying direct or averted gaze. The stimuli and paradigm we employed have previously been used with typically developing infants (43,44) as well as young children diagnosed with autism (9). We used two established techniques for analysis of brain activity during this task. First, we measured ERPs that are phase-locked to stimulus onset. ERP relies on contrasting average neurophysiological activity in response to direct gaze relative to averted gaze in infant siblings relative to the control group. Second, we analyzed high-frequency oscillatory activity in the gamma band (20–60 Hz), which is thought to reflect synchronization of brain activity in response to the task (44–46). Activity in the gamma band can be evoked, that is, time locked to the eliciting stimulus, or induced, that is, it jitters in its latency from one trial to the next and cannot be detected after the averaging process used in ERP analysis. Detecting the latter form of brain activity relies on observed variation in spectral power over time (time-frequency analysis; TFA). Hence, the two techniques, ERP and TFA, provide converging sources of evidence, each measuring a different form of neural activity associated with the eliciting stimuli.

Previous studies employing the same paradigm have established differentiation of response to direct versus averted gaze in typically developing infants using both methods (43,44). On the basis of previous work suggesting that older children and adults with autism as well as their adult siblings exhibit atypical neural correlates of eye gaze, we hypothesized that a similar pattern would be observed in a group of infant siblings. More specifically, we expected atypical differentiation of direct as opposed to averted gaze to be reflected in ERP and electroencephalogram (EEG) components sensitive to face and gaze processing in infancy. Based on previous findings from autism, we anticipated that early visual processing of eye gaze in the occipital region would not differentiate high-risk from low-risk infants but that differences might be observed in later components in that region, particularly those sensitive to attentional modulation and top-down visual processing (47) or components that are sensitive to the detection of mutual eye gaze and sensitive to its referential context found over central and right anterior regions (44). Because infant waveforms may differ substantially from those found in children and adults, no specific predictions were made in relation to whether any differences would be reflected in terms of amplitude or latency between the groups.

Methods and Materials

Participants

Sixty-two infants were recruited for this study, including 31 infant siblings of children with ASD (sib-ASD; 17 male, mean age = 10.1 months, SD = 1.5) and 31 infants who have no family history with ASD (control; 18 male, mean age = 10.1 months, SD = 1.6). All infants except one from the sib-ASD group were born full term. Informed consent was obtained from the parents. All infants in the sib-ASD group had an older brother or sister who had received a clinical diagnosis of an autism spectrum disorder from a U.K. clinician based on ICD-10 criteria (48). All but six infants from the control group had at least one or more siblings. The infants in the sib-ASD group fell within the average range of functioning as verified by the Mullen Scales for Early Development (mean = 104, SD = 9.6). Mullen scores were not available for the control group.

Apparatus and Stimuli

The infants sat on their parents’ laps at 60-cm distance from a 40 × 29 cm computer screen. The infants’ gaze during stimulus...
presentation was recorded using a video camera. Each trial began with a static colorful fixation stimulus followed by a color image of a female face. In these images, gaze was either direct or averted. Four faces were presented in a pseudorandom order. The faces were aligned with the center of the screen so that the eyes appeared at a location where the fixation stimuli had been presented. The faces subtended 21.3 × 13.9 degrees of visual angle, and one eye subtended to about 1.6 × 2.7 degrees. Fixation stimuli subtended approximately 1.6 × 1.6 degrees and were presented for a variable duration of 800–1200 msec. The face stimuli were presented for 1000 msec followed by a 500-msec interval without a visual stimulus.

**Procedure**

A 63-channel Geodesic Sensor Net was mounted on the infants’ heads while they sat on their parents’ laps in front of the stimulus screen. When the baby was attending toward the screen, trials were presented continuously for as long as the infant attended. The brain electrical activity was measured simultaneously. The reference electrode was the vertex (Cz in the conventional 10/20 system). The electrical potential was amplified with .1–100 Hz bandpass, digitized at 250-Hz sampling rate.

**Behavioral and Electrophysiological Data Analysis.** First, participants’ overall behavior was initially coded from the videotape, including any gaze shifts during trial presentation (see Supplement 1 for further details regarding gaze shifting during the task). For electrophysiological data analysis, trials were included only if the infants were fixating the center of the screen at target onset, without any gaze shifts, blinking, or head movements at any time during the 800-msec segment following the onset of the face. Furthermore, data from individual sensors were excluded if they contained artifacts created by movement or poor contact. The entire trial was excluded if data from more than 12 sensors were removed or if the trial contained blinks or other artifacts, and missing data for trials with 12 or fewer bad channels, irrespective of their location, were interpolated. Infants with less than 10 artifact-free trials in any condition were excluded. Data were then referenced to the average.

The same EEG data were further analyzed using two techniques, ERP and TFA. For the ERP analysis, the 1000-msec segments were baseline corrected, with the baseline a 200-msec segment before stimulus onset. Individual participant averages were computed for each trial type. The same ERP analysis was conducted over the filtered raw data (using a 30-Hz low-pass filter) to check whether the same results held after filtering.

For the TFA, a Morlet wavelet transformation (45,46) was applied using 1-Hz intervals for oscillatory activity in the gamma range. To avoid distortion caused by this transformation, 100-msec segments at the beginning and end of each trial were removed so that the resulting segment length was 800-msec long. For analysis focusing on condition differences between the groups, the Morlet transformation was applied with baseline correction either to single EEG trials to compute induced oscillatory gamma activity or the infants’ average ERPs to compute evoked activity. Another analysis was conducted to assess any overall group differences during a baseline period, which was 100 msec before stimulus onset. This was done by applying the Morlet transformation to single uncorrected baseline EEG segments in each trial.

**Regions of Interest and Statistical Analysis.** Four channel groups (Figure 1) were selected on the basis of visual inspection of grand averages for both groups. Furthermore, the selection of these regions was based on previous research (reviewed in 24) showing that face- and gaze-sensitive ERP and EEG components are found over occipital, central, and right temporal channel groups. These were a posterior group, a right temporal group, a left temporal group, and an anterior central group. For components of interest, statistical models focused on the interaction of Group × Condition separately for each channel group. Only when this was significant were effects explored further.

**Results**

Twelve infants from the sib-ASD group and 14 from the control group were excluded because of excessive artifacts or for completing too few trials because of fussiness or fatigue. The final sample consisted of 19 sib-ASD and 17 control infants. The groups did not differ on a number of baseline measures, including the total number of trials or the number of valid trials retained for analysis (Table 1).

| Table 1. Characteristics of Participants Included in the Analysis and Their Behavior During the Task |
|-------------------------------------------------|--------|--------|
| **Group** | **Control** | **Sib-ASD** |
| N | 17 | 19 |
| Male:Female | 10:7 | 10:9 |
| Age (SD) | 297.64 (55.05) | 291.94 (39.07) |
| Total | 119.42 (49.49) | 131.68 (38.76) |
| Valid trials (SD) | 44.36 (25.96) | 43.21 (22.42) |
| Direct Gaze | | |
| Number of trials (SD) | 66.01 (28.81) | 65.79 (19.51) |
| Valid trials (SD) | 22.11 (12.94) | 21.42 (11.25) |
| Averted Gaze | | |
| Number of trials (SD) | 53.42 (22.92) | 65.89 (19.26) |
| Valid trials (SD) | 22.26 (13.27) | 21.79 (11.43) |

sib-ASD, siblings of children diagnosed with autism spectrum disorder.
ERP Analysis of Gaze Effects

The ERP effects were observed in the posterior channel group but not in any of the other channel groups. The corresponding waveform is displayed in Figure 2A. Mean amplitude and latency of three components sensitive to face and gaze processing in infancy (49–52) were averaged for each of the channel groups. The components analyzed were the P1 around 100–199 msec, N290 around 200–319 msec, and P400 around 320–539 ms. The ERP effects were assessed using analysis of variance (ANOVA) with Condition (Direct, Averted) as a within-subject factor and Group (sib-ASD, control) as a between-subject factor for the amplitude and latency of each ERP component.

There were no significant main effects or interactions for the amplitude of any of the components. On the other hand, there was a main effect of Condition on the latency of the P1 \( F(1,34) = 6.1, p = .01 \) and N290 \( F(1,34) = 12.0, p = .001 \) in the posterior channel group; in both groups, the response to Direct gaze was faster than to Averted gaze. Furthermore, the interaction of Condition and Group was significant for the latency of the P400 \( F(1,34) = 6.4, p = .01 \) over the posterior region. Figure 2B shows the difference in latency between the two conditions for each group, and Figure 2C is a scatter plot of individual data points (see Supplement 1 for examples of waveforms for individual infants). Further analysis revealed that the sib-ASD group showed a slower P400 response to Direct gaze \( F(1,34) = 10.1, p = .003 \), but the P400 latency in the two groups did not differ for Averted gaze \( F(1,34) = 1.8, p = .81 \). Within-group comparisons indicated that whereas the control group showed no latency difference between Direct and Averted gaze \( p = .1 \), the difference between the two conditions in the sib-ASD group approached significance \( p = .05 \). The sib-ASD group tended to respond faster to the Averted relative to the Direct gaze condition. Analysis of peak amplitude for the three components revealed no significant interactions between the two groups. Reanalysis of the raw data after applying a 30-Hz low-pass filter had negligible effects on the level of significance of any of the results reported here, indicating that potential noise from high frequencies had little impact on the findings.
TFA Analysis of Gaze Effects

Evoked gamma activity in the lower-frequency band (20–30 Hz) and induced activity in the higher-frequency band (30–50 Hz) were assessed by calculating mean amplitude of spectral power over 100-msec segments over the selected channel groups. ANOVA was used to assess the relationship between evoked and induced gamma and Condition (two levels) × Time (seven levels) × Group (two levels). Evoked gamma activity was observed in the posterior region, but none of the main effects or interactions were significant. In contrast, clear condition differences were observed for induced gamma activity (30–50 Hz) in the right temporal region, where there was a significant main effect of Condition \( F(1,34) = 5.8, p = 0.02 \) and a two-way interaction between Time and Group \( F(6,29) = 3.1, p = 0.01 \). Furthermore, the three-way interaction between Condition, Time, and Group approached significance \( F(6,29) = 2.3, p = 0.06 \). The time-frequency plot for this region is shown in Figure 3. The effects observed did not extend above 50 Hz and could thus not have been due to muscular activity. Subsequent analyses were conducted separately for each time segment, and pairwise \( t \) tests were used to assess differences between the two conditions within each group. As Figure 3 illustrates, the control group showed clear differentiation of the Direct relative to the Averted gaze condition around 200 msec, and induced gamma effect persists during later time periods. Pairwise comparisons for the control group showed that the response to Direct gaze was significantly different from that to Averted gaze within the following time bins: 100–200, 200–300, 300–400, and 600–700 msec (all \( p < 0.05 \)). However, the differentiation of the two conditions in the sib-ASD group began approximately 200 msec later than the control group and was less persistent over time (\( p < 0.05 \) only in the 300–400-msec time bin, \( p = 0.06 \) in the 500–600-msec time bin). Consistent with the ERP results, time-frequency analysis also indicated that the two groups differed mainly in their processing of the direct gaze condition (Figure 3).

Analysis of Baseline Gamma Activity

In view of these results, we also explored potential group differences during the baseline interval of 100 msec before stimulus onset while the infants were viewing different, randomly presented, color cartoons. This analysis focused on absolute differences in gamma activity between the two groups, irrespective of condition, and was conducted without baseline correction of the data. Oscillatory activity in the lower (20–30 Hz) and higher (30–50 Hz) frequency band during the baseline period was assessed over single trials and averaged over all scalp electrodes except peripheral ones (channels 10, 23, 26, 35, 51, 59). There was a near significant effect of Group in both frequency ranges (20–30 Hz, \( p = 0.06 \); 30–50 Hz, \( p = 0.05 \)). Separate ANOVAs for each of the four regions showed a significant main effect of Group for the central anterior region (20–40 Hz, \( p = 0.04 \); 30–50 Hz, \( p = 0.03 \)) and right temporal region (20–40 Hz, \( p = 0.02 \); 30–50 Hz, \( p = 0.01 \)) but not for the two other regions. Hence, irrespective of the condition, the sib-ASD group showed increased gamma oscillatory activity relative to the control group in central and right temporal regions. This is despite the fact that the central anterior region showed no group differences in relation to the eliciting gaze stimuli.

Discussion

Developmental models of autism have focused on understanding the precursors of the observed social difficulties includ-
ing orienting to social stimuli and events, joint attention, imitation, and social interactions. Although various models propose alternative explanations of the origins of these difficulties (12,53,54), all agree that differences in attention to, or preference for, socially relevant information has an important contribution. Our findings are consistent with results indicating atypical gaze processing in children and adults with autism (8,9,11) as well as in unaffected siblings of individuals with autism (14) and offer some insights into how the developmental process is altered in infant siblings of children with autism relative to infants who have no family history of autism.

In this study, siblings of children diagnosed with autism were indistinguishable from the control group in their response to direct relative to averted gaze in early posterior ERP components. In contrast, the later P400 component differentiated the two groups, with the sib-ASD group showing prolonged latency in responding to direct gaze. This P400 ERP component is sensitive to face processing in infants and, together with the N200, is thought to be a precursor to the face-sensitive adult N170 (51). This component is also sensitive to attentional modulation, or top-down visual processing in infants (47). Consistent results were found using TFA of high-frequency oscillatory activity in the gamma band. The sib-ASD and control groups did not differ in early phase-locked evoked gamma activity, but they differed in induced gamma activity. Whereas the control group showed a clearly differentiated and temporally persistent induced response to direct relative to averted gaze, the response in the sib-ASD was delayed and less persistent.

These converging results suggest that early visual processing of eye gaze is not atypical in infant siblings at-risk for autism, because both ERP analysis and TFA showed that early ERP components and phase-locked gamma activity were similar in the two groups. However, the two groups differed mainly in their response to the direct gaze condition in later occipital ERP components and in right temporal induced gamma activity. The latter components are thought to reflect neural processes specific to the detection of mutual gaze and sensitive to its referential context (44). These findings complement recent behavioral evidence indicating that infant siblings are less able to integrate social cues, including gaze, relevant in the context of joint attention (41).

In addition to these differences in treatment of direct relative to averted gaze, the sib-ASD group showed increased baseline oscillatory activity in the gamma band relative to the control group in central and right temporal regions, but not in other brain regions. A recent study that reported increased gamma band oscillatory activity in children diagnosed with autism (55) has suggested this may reflect atypical patterns of cortical connectivity (55,56). Excess gamma oscillations have also been tied to several psychiatric disorders, and there are a variety of interpretations of the source and functional relevance of this pattern (for a review, see 57). The extent to which these baseline differences relate to eye gaze processing is less clear. In our study, the increase in high-frequency oscillatory activity was observed in the right temporal region where the two groups showed differences in their treatment of direct relative to averted gaze. Yet a similar increase was also seen in the anterior central region, where the two groups did not differ in their response to the two conditions. It is still possible that such baseline differences may indirectly affect processing of eye gaze. For instance, anterior central components were found to be sensitive to the referential nature of eye gaze in typical infants in a task in which the infant uses eye gaze cues to predict the appearance of peripheral targets (8), as well as in the context of joint attention (58).

It is generally acknowledged that electrophysiological methods in infancy are challenging and confer some limitations including relatively small samples, reduction in the number of valid trials, and individual variability in responses. To what extent do these limitations relate to the key results and interpretation in our study? Given that this is one of the first reports using neurophysiological methods in at-risk infants, we opted for conservative procedures based on previous studies using the same task and analysis methods. Despite their general limitations, these procedures allow for comparison between our results and previous findings in low-risk, typically developing infants. Confidence in our results comes from our findings indicating that significant differences between the groups were specific to certain neural components and conditions but not to others. The use of two relatively independent analysis techniques also yielded converging results.

A more general challenge to this area of research also relates to limitations in the interpretation of EEG and ERP components—not only in infants but also in adults. For instance, it has recently been suggested that some induced gamma activity effects reported in adults may be due to small involuntary saccades time-locked to the onset of a stimulus presentation (59). These small involuntary eye movements are associated with “spike potentials” that may contaminate high-frequency EEG (such as within the gamma range) in certain paradigms. We do not believe that our induced gamma effects can be explained in this way because infants under 12 months are known not to have recordable spike potentials (60). Further, our gamma effects are not broadband (characteristic of the saccade-related spike potential) but are restricted in frequency range. Finally, removing the infants who showed measurable saccades during stimulus presentation did not significantly change our main results (see Supplement 1), and it seems unlikely that viewing a face with direct gaze would elicit less foveation (more involuntary saccades) than a face with averted gaze (which may induce gaze following). Hence, studies with larger samples and with methods specifically targeting individual correlations with behavioral patterns including saccadic movements during the task (see also Supplement 1) will be important future directions for this line of work.

Taken together, our results suggest that the broader autism phenotype, including an atypical response to eye gaze, is manifest early in infancy. Although we found no evidence of differences in the infant BAP in terms of early visual processing of eye gaze, the neural patterns associated with later attentional modulation and those sensitive to referential aspects of eye gaze are comparable to those reported in children and adults diagnosed with autism. This early atypical response to eye gaze is likely to combine with other risk factors, resulting in a later diagnosis for some individuals (61). Differences also encompass an increase in baseline oscillatory activity, likely to reflect atypical neural connectivity, as well as atypical mechanisms for processing of eye gaze within the first year of life.

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Supplementary material cited in this article is available online.


