NEW RESEARCH

Typical Levels of Eye-Region Fixation in Toddlers With Autism Spectrum Disorder Across Multiple Contexts

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Objective: Unusual eye contact is a common clinical feature in autism spectrum disorder (ASD), yet eye-tracking studies that quantify eye fixation report inconsistent results, possibly because of small samples, varied stimuli, and considerable heterogeneity of eye-region fixation even within typical development. Goals were to examine eye-region fixation levels in a large, very young cohort; the degree to which the presence of speech, hand gestures, and a geometric distractor influence eye-region fixation; and possible developmental changes across time.

Method: In experiment 1, 385 toddlers (143 with ASD, 242 without ASD, 11–47 months old) watched an actress engaging in child-directed speech with hand gestures against a plain background. Ninety-one toddlers participated approximately 8 months later. In experiment 2, another 231 toddlers (74 with ASD, 157 without ASD, 12–47 months old) watched the same video, but with embedded geometric distractors. Total fixation duration on facial and body regions (eg, eyes, hands) and geometric distractor regions (experiment 2 only) while the actress was speaking or silent, with or without gesturing, was examined, as were relations with clinical traits.

Results: Overall, across the 2 experiments and the 2 cross-sectional and longitudinal samples, eye-region fixation duration did not differ between toddlers with and without ASD, although fixation toward the face overall was decreased in toddlers with ASD. This decrease became more apparent with the presence of geometric distractors (experiment 2) as indexed by a geometric preference score, and this score was associated with autism severity.

Conclusion: Within the context of viewing child-friendly vignettes, decreased eye-region fixation does not reliably characterize toddlers with ASD. An index of competition between faces and external distractors might be a more robust measure.

Key words: autism spectrum disorder, eye fixation, eye tracking, visual attention

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Babies are drawn to the human face immediately after birth and even show a preference for fixating on a face with a direct eye gaze over one with an averted gaze.[1] This fascination with the human face, in particular the eye region, is clinically less apparent in individuals with autism spectrum disorder (ASD).[2,3] This deficit is believed to play a major role in the long-term derailment of social development in affected individuals.[4] The ability to accurately characterize and index eye-region fixation in ASD could serve a range of important functions, including the development of diagnostic or prognostic marker tests, tracking changes in social attention in response to treatment, or as a social outcome measure for clinical trials. Understanding eye-region fixation is particularly critical during the toddler years, when experience-dependent mechanisms play an important role in shaping early brain development[5] and treatment participation is just beginning.

Surprisingly, eye-tracking studies of fixation toward the eye region in toddlers and young children with ASD report inconsistent findings; some studies report decreases, whereas others report typical levels (for reviews, see [6-10]). For example, a cross-sectional study of 15 toddlers with ASD reported decreased eye-region fixation while watching a series of 10 child-friendly vignettes compared with typically developing (TD) and developmentally delayed (DD) toddlers.[11] Leveraging the unique strength of the baby-sibling design that allows for the prospective study of ASD from birth, a longitudinal study by this same research group tracked eye movement of 11 ASD and 25 TD infants from 2 to 24 months of age.[12] Results indicated a greater decrease in looking at the eyes across that period in ASD compared with TD children, but noted decreases did not begin to appear until after 2 months of age. However, several studies have failed to replicate the finding of decreased eye-region fixation in toddlers and young children with ASD.[13-17] Interestingly, some of these non-replicating studies also found that children with ASD look less at the mouth region[14,15] or the entire face region[16] or *more* at faces compared with control groups.[17] These widely different findings led two reviews to come to completely opposite conclusions.[7,8]

Context, such as whether the face is still or dynamic, can affect orientation to the face in general and eye-region fixation levels. For instance, a recent study found that among 3 types of face stimuli—a still face, a dynamic face without speech sounds, and a speaking face (ie, dynamic face with speech sounds)—only speaking faces induced children with ASD to look less toward the face than TD children at 6 months.[18] The presence of speech differentially affects heart rate in children with ASD,[19] and abnormal orienting to social linguistic stimuli, such as responding to one's name, is a common feature of the disorder.[20] Therefore, in the present study, we examined fixation levels within vignettes stratified by the presence or absence of speech.

Not only do individuals with ASD display less interest in social stimuli,[21,22] they also are more influenced by nonsocial/low-level stimulus features, such as location, color, intensity, and orientation of objects/scenes compared with TD children, and this frequently results in attending to parts of stimuli that TD children do not.[23,24] However, it is difficult to conclude whether socially relevant content alone or content (social and nonsocial) and low-level visual factors best explains observed differences between looking patterns of those with and without ASD because subjects and stimuli used across prior studies have differed in many ways. Thus, responses to stimuli observed in prior studies could confound contributions of social or communicative content and contributions of other aspects of the stimuli.

To minimize such confounds, we used a single social stimulus (eg, the same actress, actions, facial expressions, voice, and speech) and manipulated it by inserting a single neighboring item in a second experiment. In experiment 1 we used a video composed of 8 different short vignettes in which the same actress produced child-directed speech and gestures (eg, peek-a-boo) and included brief moments when the actress was still. In experiment 2, we added geometric shapes known to draw the attention of children with ASD[25,26] to the experiment 1 video.

There are other possible explanations for the discrepant findings in previous studies, including the wide variation in the characteristics of subjects examined (eg, general population versus baby-sibling cohorts) and ages of subjects (eg, 6 months versus preschool years). Small samples also can contribute to inconsistent findings. For example, as illustrated in a recent review,[7] 63% of studies with infants and young children included fewer than 20 children with ASD and 50% had as few as 9 to 15. Such small samples might not generate reliable results, might provide limited opportunities to detect meaningful subtypes of ASD,[27] and are more susceptible to the influence of heterogeneity owing to the greater impact on sampling variability. Moreover, many studies examine ASD only in comparison with TD subjects, lessening the possibility of understanding fundamental biological processes that can cut across diagnostic boundaries.

Considering the range of factors that could have influenced previous inconsistent findings in eye-region fixation levels, the present study leveraged a large sample containing 616 toddlers that included multiple non-ASD contrast groups (TD, DD, and ASD-features). The ASD-features group was composed of toddlers who showed signs of ASD but not sufficiently to reach diagnostic criteria. Although recruited from the general population and not a high-risk family, toddlers in this category can be similar to children who exhibit the broader autism phenotype found in baby-sibling design studies.[28]

Based on results from prior studies, we tested 4 primary hypotheses. First, although prior studies were inconsistent, we hypothesized that our large sample would allow for the detection of decreased eye-region fixation in toddlers with ASD versus other diagnostic groups. In line with a dimensional, rather than categorical, perspective, we expected that toddlers with mild ASD features (ASD-features group) would exhibit eye-region fixation levels that were better than those of toddlers with ASD but not at typical levels. We further predicted that overall decreases in eye-region fixation would drive an overall abnormal ratio between face and non-face elements (eg, hands and body) in ASD versus non-ASD toddlers. Second, given dysfunctional visual disengagement in individuals with ASD,[29] we expected that eye-region fixation deficits would be more pronounced within the presence of geometric distractors that might differentially draw visual attention and decrease eye-region fixation more in toddlers with ASD versus other toddlers. Third, given previous reports of a relation between symptom severity and eye-fixation abnormalities,[25,26,30] we hypothesized that ASD toddlers with the most severe symptoms as indexed by the Autism Diagnostic Observation Schedule (ADOS) would exhibit the greatest decreases in eye-region fixation and the most abnormal ratio scores with and without the presence of geometric distractors. Fourth, given previous reports[12] on the different developmental trajectories in eye-region fixation level between ASD and TD infants, we examined whether toddlers with ASD show an abnormal decrease in eye-region fixation across time.

METHOD

Experiment 1: Actress Engaging in Hand Gestures Without Distractors

Participants: Cross-Sectional Sample. Three hundred eight-five toddlers whose primary language was English participated (mean age 26.2 months, standard deviation 9.1, range 11–47). An additional 66 toddlers were tested but excluded from the final analyses for a range of issues (eg, failure to attend to the video; Figure S1, available online). All toddlers received psychometric testing by experienced PhD-level psychologists including the ADOS (Module T, 1, or 2),[31] the Mullen Scales of Early Learning (MSEL),[32] and the Vineland Adaptive Behavior Scales[33] at their eye-tracking visits (Table S1, available online). Toddlers younger than 36 months at the time of eye tracking were followed longitudinally until a diagnosis was confirmed at 3 years of age. For analysis purposes, toddlers were categorized into 1 of 4 groups based on their final diagnosis (ASD, n = 143; ASD-features, n = 27; DD, n = 97; TD, n = 118). See Supplement 1 (available online) for more information regarding diagnostic criteria.

Participants: Longitudinal Sample. Ninety-one of the 385 toddlers (46 in ASD group, 45 in TD group; mean age 30 months, standard deviation 7.4, range 18–46) participated in the same eye-tracking test an average of 8 months (4–15 months) later, and changes in fixation levels within areas of interest (AOIs) across time were examined.

Apparatus, Stimuli, and Eye-Tracking Procedure. Eye tracking was conducted in a quiet room at the Autism Center of Excellence at the University of California–San Diego. Eye movements were recorded using a TOBII T120 eye tracker (www.tobii.com; screen size 17 inches, thin-film transistor display, spatial accuracy 0.5°, spatial resolution 0.2°–0.3°, sampling rate 60 Hz).

Each toddler sat on the parent's lap or alone, approximately 60 cm from the Tobii monitor. Each session began with a standard 5-point calibration procedure appropriate for infants and toddlers and repeated as required. For more details on eye-tracking procedures, see Supplement 2 (available online). After the calibration phase, toddlers watched a 43-second video (31° by 22°) showing a close-up image of a woman speaking short common phrases coupled with familiar hand gestures (ie, speech + gesture phase, total 16 seconds). Each of the 8 different vignettes contained 1 to 2 seconds of silence, when the actress was not speaking or gesturing (ie, no speech + no gesture phase, total 22 seconds). Each vignette was separated by a 0.7-second black screen (total 5 seconds; not included in analyses; Figure 1).

Data Processing and Areas of Interest. Fixation was determined by a 35-pixel radius filter (0.88°)[34] using the default fixation algorithm in Tobii Studio 3.2.2 (www.tobii.com). Tobii Studio and custom MATLAB (MathWorks, Natick, MA) scripts were used to create AOIs and to analyze gaze patterns per AOI. Six AOIs covering all locations within the movie, including eyes (upper face), mouth (lower face), nose (center of the face, not overlapping the upper and lower face), hands, and body, and an empty background were created (Figure 1). Although the nose AOI was selected to be consistent with prior studies,[11,12] its size was not significantly larger than the size of stimuli used for our calibration (Supplement 3, available online). Thus, results for the nose AOI are considered only within the context of the entire face but are not reported separately (Tables S2–S8, available online).

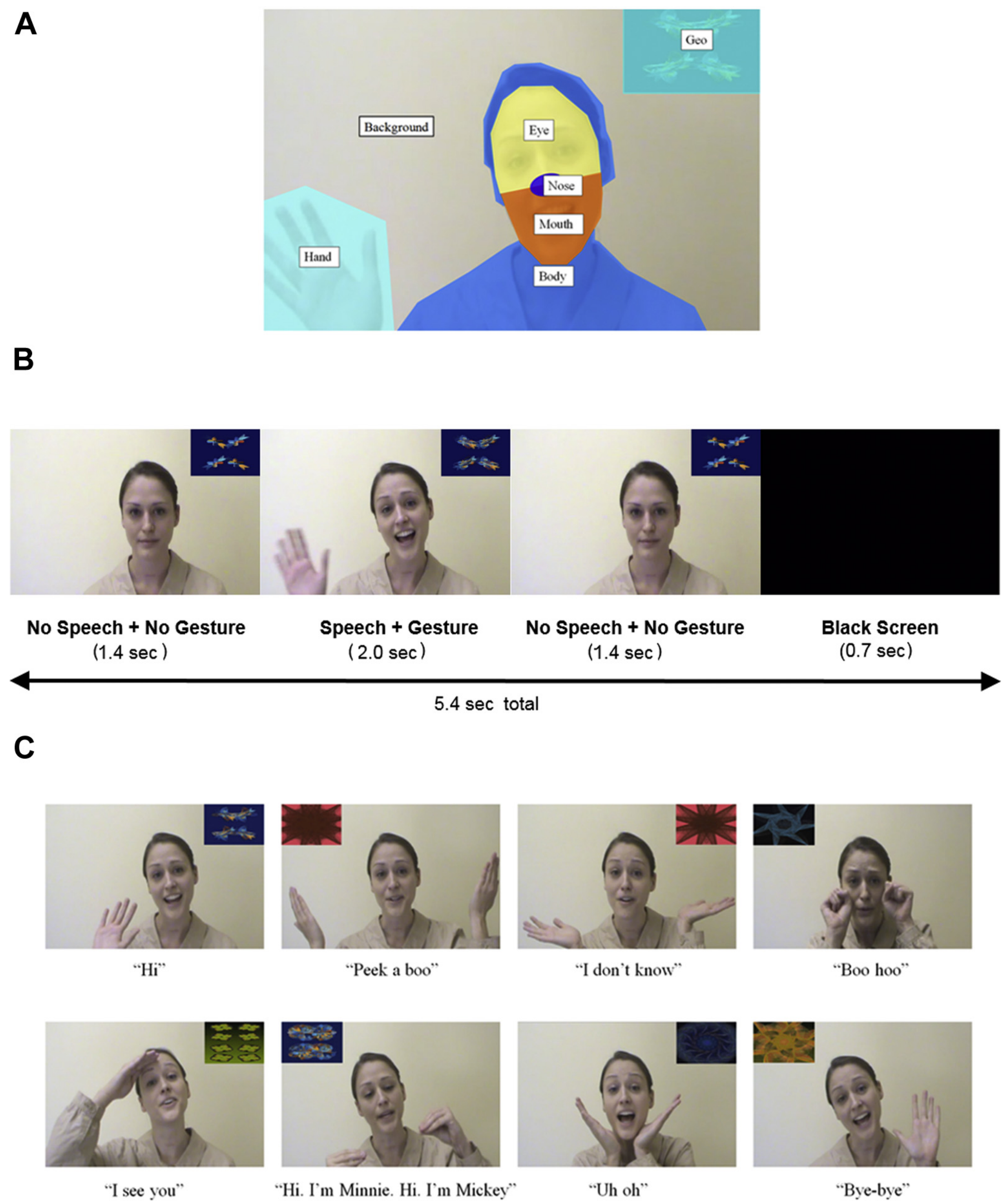
Fixation Within AOIs. Fixation durations were aggregated across vignettes and computed as 3 dependent measures: percentage of fixation, defined as the fixation duration within each AOI divided by total looking time × 100[11,12]; Eye-Mouth Index (EMI), defined as (total fixation time to eyes × 100)/(total fixation time to eyes + total fixation time to mouth); and Non-Face Preference Score (NFPS), defined as (total fixation time to non-face [hands and body] × 100)/(total fixation time to non-face + total fixation time to face [eyes, nose, mouth]). Descriptive statistics are presented in Table S2 and Supplement 4 (available online).

Statistical Analyses

Preliminary Analysis on Sex Differences in ASD. Given prior reports on sex differences in visual fixation patterns in children with ASD, we examined sex differences in our cross-sectional and longitudinal ASD samples. Mixed-effects models with each subject's age at visits 1 and 2 and sex showed the lack of significant sex differences in ASD from all of our longitudinal measures regarding percentages of fixation time on the eyes and the other AOIs and composite scores ($p > .357$ for all comparisons without multiple comparison corrections). Using our cross-sectional ASD sample, analysis of covariance, with sex as a between-subject factor and age as a covariate, also confirmed the lack of significant sex differences in all our dependent measures on percentages of fixation time and composite scores (unadjusted $p > .252$ for all comparisons). Thus, sex was not included in our main analyses, described below.

Developmental Trajectories in Longitudinal Sample. Developmental trajectories were examined across groups by building interaction models using the lme function contained within the R nlme library (http://cran.rproject.org/web/packages). To examine whether changes in each of our dependent measures (eg, eye-region fixation) across time differed between toddlers with ASD and TD toddlers, we ran mixed-effect analyses with our longitudinal sample to model within-individual trajectories and group-level

FIGURE 1 Movie Stimuli



Note: *(A) Areas of interest used for experiments 1 and 2. (B) Structure and length of a single illustrative vignette (ie, the "Hi vignette"). Denoted times represent the average time for each phase across all 8 vignettes. (C) Sample images illustrating the gestures and accompanying speech used in each vignette in experiments 1 and 2. The movies used in the 2 experiments were identical except that dynamic geometric images were presented only during experiment 2.*

trajectories with each subject's age at visits 1 and 2, group (ASD versus TD), and age by group and as fixed variables and subject ID as a random variable. The Benjamini-Hochberg (BH) procedure using a false discovery rate threshold of $q < 0.05$ was used to minimize the potential for type I errors for non-hypothesized contrasts (eg, examining differences in mouth fixation between groups). Test-retest reliability was calculated by correlating percentages of fixation level for each AOI between times 1 and 2.

Developmental Changes and Group Differences in Cross-Sectional Sample. Potential age effects or changes that might occur across time also were examined using our cross-sectional sample by conducting regression models on each dependent measure with group (ASD, ASD-features, DD, TD), age, and age by group as factors. Because most interaction effects were not significant (all regression coefficients and p values are listed in Table S3, available online), we built additional additive regression models with group (ASD, ASD-features, DD, TD) and age as factors to examine differences across groups on each dependent variable. Akaike information criterion and Bayesian information criterion scores also were lower for the additive models than the interaction models, suggesting that the additive models are more efficient for explaining our data (Table S7 and Supplement 5, available online). We also examined differences between toddlers with ASD and each of the 3 contrast groups by conducting planned contrasts directly related to our main hypotheses (eg, examining differences in eye-region fixation between groups) with unadjusted p values and examining effect sizes. The p values corrected by the BH procedure were reported for non-hypothesized contrasts. The results on cross-sectional samples described below were from contrasts from additive models (age + group), unless specifically noted.

Relation to Clinical Phenotype. Using the larger cross-sectional dataset from the ASD group, we examined whether any percentage of fixation toward the eyes and NFPS were associated with severity of autism symptoms indexed by ADOS total scores, language ability as indexed by the expressive and receptive components of the MSEL, or cognitive ability as indexed by the Early Learning Composite (ELC) score of the MSEL, in toddlers with ASD. Because age was not significantly correlated with these clinical scores ($p > .090$ for all comparisons), Spearman correlations between each clinical score and each of our measures were performed, collapsing across ages.

Experiment 2: Actress Engaging in Hand Gestures With Geometric Distractor

The somewhat surprising lack of significant differences in eye-region fixation levels in toddlers with ASD compared with toddlers from multiple contrast groups in experiment 1 led to the creation of experiment 2, in which dynamic geometric shapes, which have been shown to attract the attention of a subset of toddlers with ASD,[25,26] were embedded in the corner of the video used in experiment 1. If children with autism show abnormally increased attention to nonsocial stimuli and abnormal visual attention in general,[25,26,35,36] then we hypothesized that including a geometric distractor in this experiment, while holding all other variables constant, would result in a decrease in eye-region fixation in toddlers with ASD compared with other contrast groups and with results from experiment 1.

Participants, Apparatus, Stimuli, Procedure, and Statistical Analysis. Two hundred thirty-one toddlers who were nonoverlapping with toddlers from experiment 1 participated (ASD, n = 74; ASD-features, n = 22; DD, n = 92; TD, n = 43). Table S1 (available online) lists subject characteristics. An additional 79 toddlers were tested but excluded from the final analyses for a range of issues (eg, failure to attend to the video; Figure S1, available online). Apparatus, procedure, stimuli, and statistical analysis were identical to those used in experiment 1 except that small dynamic geometric shapes[25] were displayed continuously at the left or right top corner of the video, with the side alternated between subsequent vignettes.

RESULTS

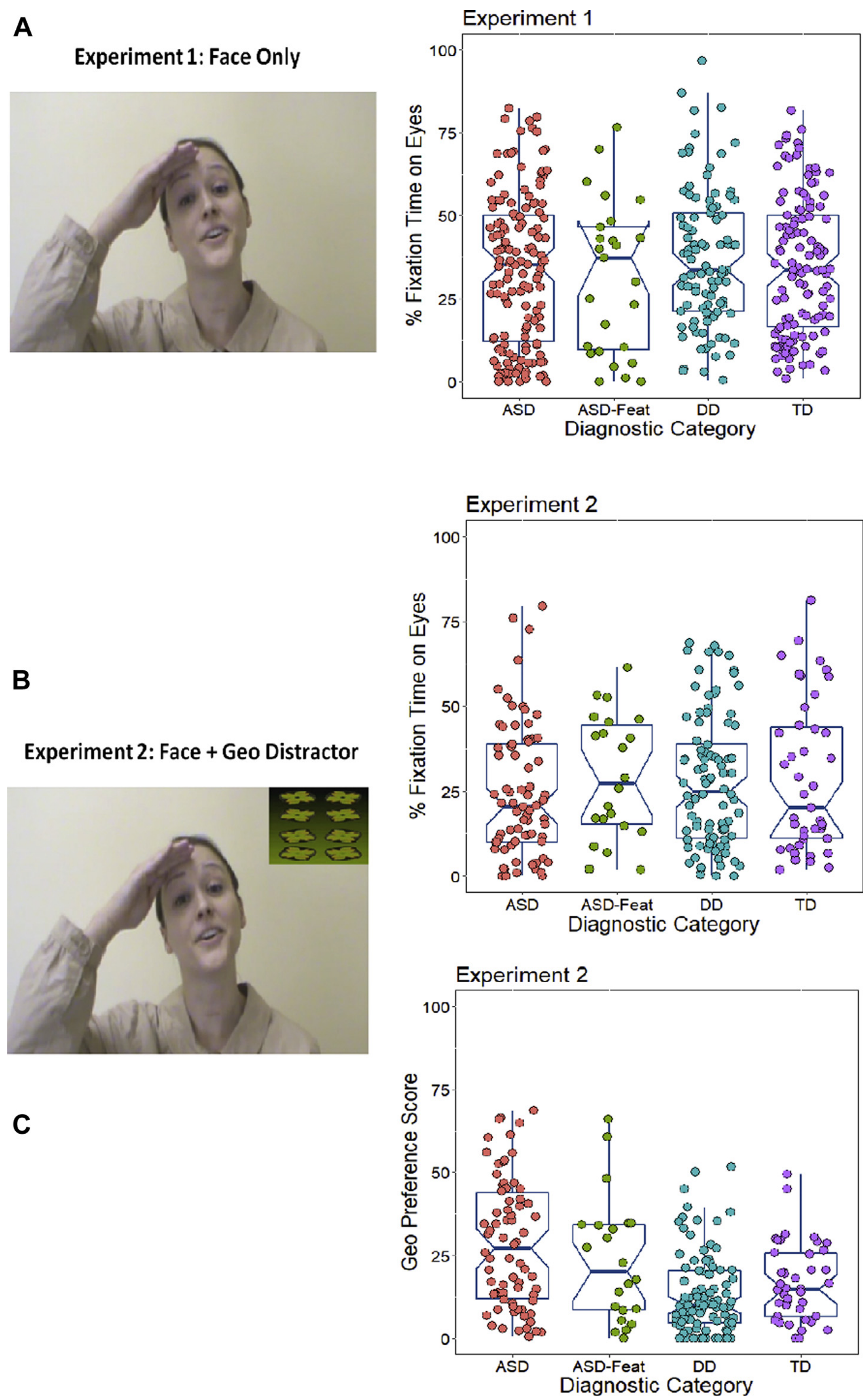
Experiment 1

Results from the speech + gesture and no-speech + no-gesture phases were similar, and for simplicity we focus results on the speech + gestures condition. For more details related to the no-gestures condition, see Tables S2 to S8 (available online).

Eye Region, Mouth Region, and EMI. Toddlers with ASD did not show any decrease in their eye-region fixation compared with any other group ($p > .122$ for all comparisons; see Table S4 for regression coefficients and Table S2 for means, available online). Toddlers with ASD fixated on the eye region an average of 34% of the time compared with TD, DD, and ASD-features toddlers who fixated an average of 35%, 37% and 32%, respectively. The raw data plotted in Figure 2 illustrate the wide range of eye-region fixation levels across toddlers in all groups and lack of group differences, which is echoed in the effect size table (Figure 3). Toddlers with ASD also did not show differences in levels of mouth-region fixation (p with BH correction for 6 contrasts > .208 for all comparisons) or in the ratio between eye and mouth fixation as determined by the EMI (p with BH correction for 6 contrasts > .208 for all comparisons) compared with the other groups. See Table S4 (available online) for all regression coefficient and p values.

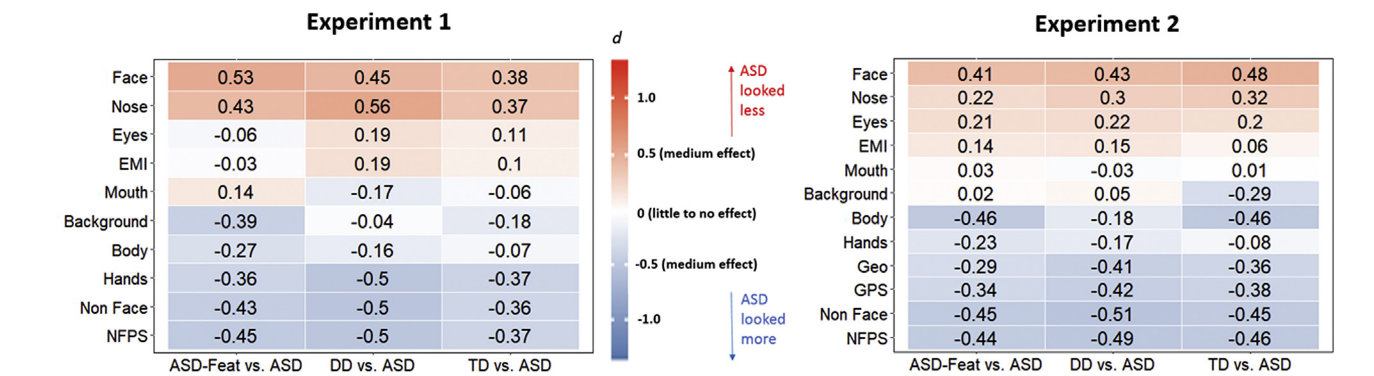
Face, NFPS, and Other Scene Elements. Although there were no differences in eye or mouth fixation among groups while the actress was talking and gesturing, toddlers with ASD exhibited decreased face fixation overall, defined as fixation toward the eyes plus mouth plus nose (versus ASD-features group, $\beta = 5.345$, $p = .009$; versus DD group, $\beta = 4.676$, $p < .001$; versus TD group, $\beta = 4.067$, $p = .001$). Cohen d effect sizes (Figure 3) showed this effect as small to moderate. Toddlers with ASD also showed an overall significantly increased NFPS, which was defined as percentage of fixation time toward the non-face regions (hands and body) × 100 divided by percentage of fixation time

FIGURE 2 Percentage of Fixation Time on Eyes



Note: Scatterplots illustrating the distribution of percentage of fixation on the eyes in experiments 1 (A) and 2 (B) and geo preference score in experiment 2 (C). Although the medians are extremely similar, note the heterogeneity in fixation levels evident across all groups. For example, fixation levels on the eyes ranged from 0% to approximately 80% for the autism spectrum disorder (ASD) and typically developing (TD) groups. The middle line of each box indicates a group median, and the 2 ends of each line represent (Q3 + [1.5 × IQR]) and (Q1 – [1.5 × IQR]), respectively, where Q1 represents the 25th percentile, Q3 represents the 75th percentile, and IQR represents Q3 to Q1. Dots represent individual scores. ASD-Feat = autism spectrum disorder features; DD = developmentally delayed; IQR = interquartile range.

FIGURE 3 Cohen's d Effect Sizes



Note: *Tables illustrate the magnitude of difference in percentage of fixation between toddlers with autism spectrum disorder (ASD) and those with ASD features (ASD-Feat), delayed development (DD), or typical development (TD) within each area of interest or area-of-interest ratio. The largest effect sizes were seen within the overall face area of interest, but not within the eyes or mouth per se, which had small effect sizes. EMI = Eye-Mouth Index; Geo = geometric distractor; GPS = Geo Preference Score; NFPS = Non-Face Preference Score.*

toward the non-face regions and face (eyes + mouth + nose) regions compared with the other groups (versus ASD-features group, $\beta = -4.206$, $p = .024$; versus DD group, $\beta = -4.839$, $p < .001$; versus TD group, $\beta = -3.589$, $p = .001$), also with small to moderate effect sizes. Additional analyses showed that toddlers with ASD looked significantly more at the hands than did toddlers in the DD and TD groups (versus DD group, $\beta = -3.918$, p with BH correction for 6 contrasts $< .001$; versus TD group, $\beta = -2.985$, p with BH correction for 6 contrasts $= .009$).

Changes in Eye-Region Fixation Across Time. To examine whether the ASD group show a greater decrease compared with the TD group in eye-region fixation as a function of age and group, we used our longitudinal samples. We did not find any evidence of different developmental trajectories in percentage of looking toward the eyes ($p = .640$) across 2 longitudinal visits. In fact, developmental changes in eye-region fixation levels were heterogeneous in the ASD and TD groups (Figure 4); approximately half the toddlers with ASD showed increased looking time toward the eyes on a per-unit basis across time, whereas the other half showed decreased fixation (Figure S2, available online). A similar tendency was observed with TD toddlers. Additional analyses excluded alternative explanations that the ASD group showed different developmental trajectories in mouth-region fixation (p with BH correction for 9 contrasts $= .762$) or in relative fixation of the eye region to the mouth region defined as EMI (p with BH correction for 9 contrasts $= .762$). Coefficients and p values from mixed models are presented in Table S5 (available online).

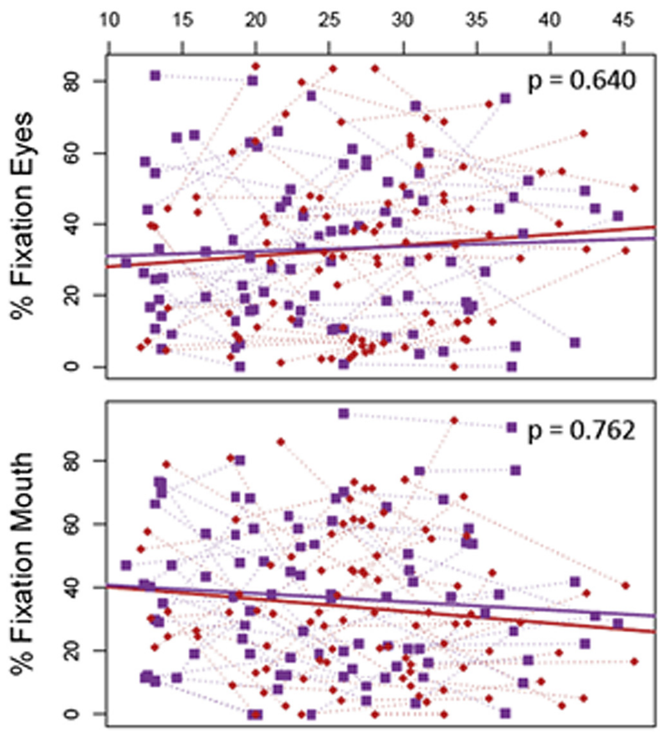
Test-Retest Reliability. Although the second eye-tracking test visit occurred an average of 8 months after the first, intraclass correlations showed significant correlations in the percentage of looking toward the eye region between 2 visits in the ASD ($r = 0.746$, $p < .001$) and TD ($r = 0.652$, $p < .001$) groups. Similarly, significant correlations were found in the percentage of looking toward the mouth and EMI between the 2 visits in each group ($p < .001$ for all comparisons).

Relationships Between Fixation and Clinical Phenotype in ASD. Spearman correlations showed that none of eye-region fixation levels and NFPSs were associated with clinical symptoms as indexed by ADOS scores ($p > .544$ for all comparisons), receptive and expressive language ability, and cognitive ability score as indexed by the ELC score of the MSEL ($p > .341$ for receptive language; $p > .162$ for expressive language; $p > .512$ for ELC) in the ASD group alone.

Experiment 2

Consistent with experiment 1, we started by examining interaction effects of age and group for each measure and did not find any significant effects overall. There also were no age interactions for our main measures of interest, eyes and mouth, for any between-group planned contrasts. Therefore, an age-by-group interaction term was not included in further analyses (Table S3, available online). For simplicity, we focus results on the speech + gestures condition. See Tables S2 to S8 for more details related to the no-gestures condition. As in experiment 1, we reported unadjusted p values for planned contrasts and adjusted p values using the BH procedure for post hoc contrasts on 7 other AOIs/scores.

FIGURE 4 Percentage of Fixation on Eyes and Mouth Across 2 Time Points



Note: *Longitudinal data illustrating percentages of fixation time in toddlers with autism spectrum disorder and typically developing toddlers in experiment 1. The solid red and blue lines in each graph represent model fits for toddlers with autism spectrum disorder and typical development, respectively. Red diamonds and purple squares represent percentages of fixation time of toddlers with autism spectrum disorder and typical development at their first and second visits, respectively, and each child's percentages of fixation time are connected by a dotted line. Age, in months, is plotted on the x-axis. As illustrated, percentage of fixation on the eyes or mouth did not change significantly across time.*

Eye Region, Mouth Region, and EMI When a Distractor Is Present. Despite the inclusion of a distractor image, consistent with experiment 1, there were no significant differences between toddlers with ASD and those in the contrast groups in eye-region fixation ($p > .204$ for all comparisons; Figure 2 and Table S4, available online). Toddlers with ASD did not look at the mouth region more than other groups (p with BH correction for 7 contrasts $>$ .828 for all comparisons) or show different EMIs from other groups (p with BH correction for 7 contrasts $> .512$ for all comparisons).

Face, Non-Face, and Geo Preference Scores When a Distractor Is Present. Also similar to experiment 1, ASD toddlers showed a decrease in face-fixation levels overall (mean 67% in ASD group versus 72% in ASD-features group, $p = .044$; 76% in DD group, $p = .003$; 73% in TD group, $p = .006$). Next, we examined attention to nonsocial stimuli as indexed not only by the NFPS but also by the geo preference score (GPS). As with the NFPS, the GPS was calculated by dividing the time spent looking at the geometric shapes $\times$ 100 by the time spent looking at the inner features of the face (eyes, mouth, and nose) and geometric shapes. Regression analyses showed that the NFPS in toddlers with ASD was significantly higher than in all 3 control groups (versus ASD-features group, $\beta = -6.841$, $p = .024$; versus DD group, $\beta = -6.722$, $p < .001$; versus TD group, $\beta = -6.568$, $p = .006$). Differences in the GPS between the ASD group and the non-ASD groups were significant compared with the DD group ($\beta = -6.272$, $p = .003$) and the TD group ($\beta = -5.787$, $p = .024$) and marginally significant compared with the ASD-features group ($\beta = -5.572$, $p =$ .084). To examine what could have contributed to the high NFPS and GPS scores in the ASD and control groups, separate analyses on percentage of fixation time for each non-face AOI were conducted. Results showed that toddlers with ASD spent a large amount of time fixating on the geometric shapes compared with the DD group ($\beta = -5.355$, p with BH correction for 7 contrasts = .07), but not compared with the TD group (p with BH correction for 7 contrasts = .147) and ASD-features group (p with BH correction for 7 contrasts = .651).

Relations Between Fixation and Symptom Severity in ASD. None of the clinical traits were correlated with eye-fixation levels in ASD ($p > .567$ for all comparisons). However, unlike experiment 1, which showed no relation between fixation levels and clinical traits in toddlers with ASD, the inclusion of a distractor in this experiment resulted in a range of significant correlations, as we hypothesized. For example, greater symptom severity as indexed by the ADOS was correlated with increased fixation toward geometric images (Spearman $\rho = 0.366$, $p < .001$). A similar pattern was found between the ADOS score and the NFPS (Spearman $\rho = 0.331$, $p =$ .004) and the GPS (Spearman $\rho = 0.371$, $p = .001$).

Examination of Impact of Presence of Geometric Distractor on Eye-Fixation Levels: Experiment 1 Versus 2. We hypothesized that the presence of a geometric distractor would affect eye-fixation levels in toddlers with ASD to a greater degree than the other diagnostic groups. Results indicated that the presence of a single geometric distractor decreased eye-region fixation by approximately 10% for all diagnostic groups; however, this effect was not selectively greater for toddlers with ASD at the group level. Analyses of covariance conducted on percentage of fixation toward the eye regions, with experiment (1 and 2) and group (ASD, TD, DD, and ASD-features) as between-subject factors and age as a covariate, showed that the presence of geometric shapes decreased children's looking time toward the eyes ($F_{1,607} = 14.445$, $p < .001$, eta-squared $= 0.023$). None of the main effects of experiment ($p > .101$ for all comparisons) and interactions of experiment and group were statistically significant ($p > .077$ for all comparisons). Figure 5 and Supplement 6 (available online) present more information.

DISCUSSION

Abnormalities in eye contact and difficulty in following the eye gaze of others are among the most striking clinical features of ASD[37] and key evaluation items in modern ASD diagnostic tools.[31] Establishing the degree to which this clinical feature can be easily captured using eye-tracking technology is essential for scientists and clinicians seeking to discover clinical biomarkers of ASD, developing individually tailored treatments, and engaging in clinical trial research attempting to use eye-gaze fixation as a clinical end point. Surprisingly, across multiple contexts, including when an actress was speaking or silent, gesturing or not, present alone or with geometric distractors, toddlers with ASD did not exhibit a robust decrease in eye-region fixation compared with other contrast groups. Overall, decreases in eye-region fixation levels were not found even at the group level when geometric distractors were embedded, although a small percentage of toddlers with ASD were differentially distracted by the presence of geometric images. Moreover, there were no significant relations between percentage of looking time at eyes or EMI and clinical measures, including severity of autism, expressive and receptive language, and cognitive ability.

Because this study is the largest to date with a sample exceeding 600 toddlers across experiments 1 and 2, insufficient power to detect effects is unlikely to explain the negative result. Consistent with other studies that have failed to find differences in eye-region fixation in toddlers with ASD,[13-15] this study suggests that eye-region fixation decreases in ASD are not robust features of ASD, at least as indexed by eye tracking using stimuli with no background distractors (experiment 1) or a single distractor (experiment 2). In a recent baby-sibling study, Young *et al.*[13] sampled eye-region fixation levels in 6-month-old infants at risk for ASD and found that none of those who showed decreased eye-region fixation levels showed any signs of ASD at 2 years of age.

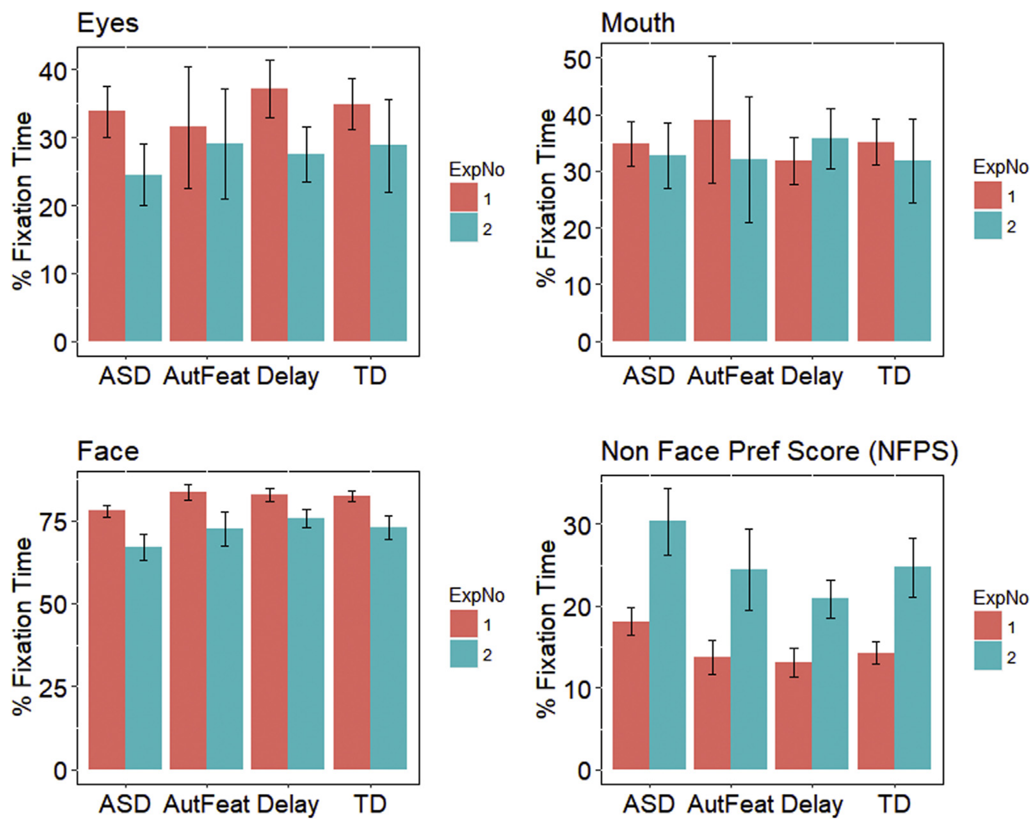
A lack of group differences in eye-region fixation makes sense when considered within a developmental framework and the fact that most toddlers in the study were 12 to 36 months old, precisely when most toddlers speak their first words and craft their first sentences.[38] To become a master of speech, a toddler must focus attention not only on the eyes but also on the mouth, because relevant information is gleaned from these 2 sources. Each child has a particular timetable for making this shift, with considerable variability within even typical toddlers. Another recent eye-tracking study that examined eye and mouth fixation in a cohort of 1-year-old typical toddlers concluded that "despite consistent results within subjects, there was considerable variation between subjects. This raises the question of whether a developmental 'norm' of face scanning in infancy ought to be pursued."[39]

Even when we tried to minimize variability in our data associated with age by parsing subjects into narrow 6-month-old bands, considerable variability and a lack of group differences remained (Supplement 7, available online). As another attempt to decrease variability, we examined eye-region fixation using AOIs that were more tightly drawn around the eyes and still found no significant group differences (Figure S3 and Supplement 8, available online). We also examined whether boys with ASD show different looking patterns than girls with ASD and did not find any significant sex differences in eye-region fixation.

The toddlers with ASD in this study, some of whom showed eye-region fixation levels that exceeded 75%—which parallels the highest levels found in typical toddlers—chose to look at the actress' eyes when they could have looked somewhere else on the human body or at the background. Although mutual eye gaze with an actress in a video is somewhat lacking in ecologic validity, this finding does suggest that eye-gaze aversion is not a defining feature of ASD. This conclusion is consistent with a recent study on this topic.[40] However, because "eye contact" in the present study was indexed by 1-way passive viewing of an actress on a video monitor, and not a live person, less complex neural systems might have been engaged and the stress load might have been lessened, thus allowing more typical responding in the toddlers with ASD. Also, because all participants in our study watched the 8 gestures in the same order, we could not completely exclude the possibility of a particular order effect. Further research should consider these issues.

Given prior findings that stress the importance of examining developmental *changes* in children's looking preferences across time[12,41,42] rather than a sole examination of eye-region fixation within a single snapshot in time, we also examined whether changes in percentage of looking time at the eyes across time distinguished toddlers with ASD from TD toddlers. Similar to the lack of eye- and mouth-region fixation differences, results indicated no differences in changes in eye- or mouth-region fixation across

FIGURE 5 Mean Changes in Fixation Due to the Presence of a Distractor: Experiment 1 Versus Experiment 2



Note: Each error bar represents the 95% CI of a mean. ASD = autism spectrum disorder; AutFeat = autism spectrum disorder features; Delay = developmentally delayed; TD = typically developing.

time in toddlers with ASD compared with TD toddlers. In fact, eye- and mouth-fixation levels were relatively stable across time for these 2 groups. However, because our longitudinal sample was relatively small (ASD, n = 46; TD, n = 45), we also examined age-related changes within our much larger cross-sectional cohort that contained many more subjects (N = 385) and 3 non-ASD groups and did not find group differences.

Quantification of fixation levels toward the eye region is only one, and perhaps the most straightforward, method for examining unusual eye gaze in ASD. What makes a person with ASD stand out often is not a decrease in eye contact per se or a lack of willingness to engage in eye contact, but rather more complex abnormalities in the timing, "naturalness," and fluidity of their gaze. Approaches used by other researchers, and used in the present study, that might better capture such nuanced abnormalities include examining the balance in time spent fixating between the eyes and mouth (EMI) and/or examining fixation times on body regions outside the face relative to fixation levels within the face (NFPS). Although the EMI did not show differences between toddlers with ASD and other toddlers, there was a significant difference in the NFPS in children with ASD in experiments 1 and 2. This was likely driven by the fact that when social vignettes were presented in isolation (experiment 1), toddlers with ASD looked less at the face and more at the hands than TD and DD toddlers, but when the dynamic colorful geometric patterns were added to the scene (experiment 2), children with ASD looked more at the geometric shapes. Adding the dynamic nonsocial geometric images doubled the NFPS (Figure 5), the measure reflecting children's preferential looking for non-faces over faces and the strength of association between the NFPS and the severity of autism. The differences in experiments 1 and 2 cannot be explained by the effect of social content alone and are better explained by the competition between social and nonsocial geometric images for attention[24,35,36] (but also see Freeth *et al.*[43] for negative findings on the effect of competition on high-functioning adolescents with ASD). This multi-factor competition view also provides potential explanations about inconsistent prior findings observed by using stimuli that differed in many ways. Such ratio differences found across experiments 1 and 2 also are consistent with the fact that toddlers with ASD looked slightly less at the face overall (∼5% decrease) compared with other toddlers, a finding reported by many other researchers.[16,18]

Our study also raises important considerations about how toddlers with ASD might be perceived and characterized. More than 7 decades have passed since Leo Kanner first described autism, and through this time considerable changes have occurred in how the disorder is conceptualized and diagnosed. Children with ASD from the 1950s through the 1990s were regularly described as having a severe form of psychopathology that included social aloofness and avoidance combined with extremely poor eye contact and comorbid mental retardation.[44,45] Currently, the Centers for Disease Control and Prevention reports that 68% of individuals with ASD have an IQ that falls within the typical or borderline IQ range.[46] Although a subset of individuals with ASD do avoid eye contact, abnormalities in timing and fluidity and contextual appropriateness of eye-gaze alterations might more appropriately characterize eye contact deficits in ASD.

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