Mechanisms of diminished attention to eyes in autism

Jennifer M. Moriuchi, Emory University
Ami Klin, Emory University
Warren R Jones, Emory University

Journal Title: American Journal of Psychiatry
Volume: Volume 174, Number 1
Publisher: American Psychiatric Publishing | 2017-01-01, Pages 26-35
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1176/appi.ajp.2016.15091222
Permanent URL: https://pid.emory.edu/ark:/25593/s8mpz

Final published version: http://dx.doi.org/10.1176/appi.ajp.2016.15091222

Copyright information:
@ 2018 American Psychiatric Association

Accessed June 11, 2018 8:03 PM EDT
Mechanisms of diminished attention to eyes in autism

Jennifer M. Moriuchi, MA1,2, Ami Klin, PhD1,2,3,4, and Warren Jones, PhD2,3,4

1Psychology Department, Emory University, Atlanta, Georgia 30022, USA
2Marcus Autism Center, Children’s Healthcare of Atlanta, Atlanta, Georgia 30329, USA
3Division of Autism & Related Disabilities, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30022, USA
4Center for Translational Social Neuroscience, Emory University, Atlanta, Georgia 30022, USA

Abstract

Objective—Two hypotheses, gaze aversion and gaze indifference, are commonly cited to explain a diagnostic hallmark of autism: reduced attention to others’ eyes. The two posit different areas of atypical brain function, different pathogenic models of disability, and different possible treatments. Existing evidence for and against each hypothesis is mixed but has heretofore focused on older children and adults. We evaluated both mechanistic hypotheses in two sets of experiments at time of initial diagnosis.

Method—Eye-tracking data were collected in 86 two-year-olds: 26 with autism, tested at initial diagnosis; 38 matched typically-developing peers; and 22 matched developmentally-delayed peers. We measured response to direct (Experiment 1) and implicit (Experiment 2) cueing to look at the eyes.

Results—When directly cued to look at the eyes, two-year-olds with autism did not look away faster than their typical peers; their latency varied neither categorically, nor dimensionally, by degree of eyes cueing. Moreover, direct cueing had a stronger sustained effect on their amount of eye-looking than typical peers’. In addition, when presented with implicit social cues for eye-looking, two-year-olds with autism neither shifted their gaze away nor more subtly averted gaze to peripheral locations.

Conclusions—Results falsify the gaze aversion hypothesis; instead, at time of initial diagnosis, diminished eye-looking in autism is consistent with passive insensitivity to the social signals in others’ eyes.

Diminished attention to others’ eyes is a symptom of Autism Spectrum Disorder (ASD) featured in each of the condition’s primary screening (1) and diagnostic (2) instruments. The behavior is among the earliest emerging features of the condition (3), is well-documented in individuals with ASD across the lifespan (4), and contributes to deficits in initiating and
modulating reciprocal social interaction (5). Despite this clinical prominence, the underlying cause of atypical eye contact in ASD remains controversial.

Two explanations for reduced eye-looking in ASD are often advanced. One is that children with ASD purposefully look away from the eyes due to gaze aversion, actively avoiding others’ eyes because the eyes are perceived as having negative affective valence (6,7). The other hypothesis is that children with ASD look less at others’ eyes due to gaze indifference, looking less at others’ eyes because the eyes are not perceived as engaging or adaptively informative (8,9). Whereas gaze aversion indicates specific avoidance with implicit understanding of the social significance of eye contact (10,11), the gaze indifference hypothesis indicates insensitivity to the underlying social signal of others’ eyes, part of a broader insensitivity to social cues in general (12). At a mechanistic level, these hypotheses are mutually exclusive: one posits responses based on distinct and differential awareness of the eyes, the other posits responses based on the relative absence of that awareness.

Existing evidence for and against each hypothesis is mixed. In support of gaze aversion, studies of affective response to gaze have reported increased autonomic arousal (13,14), amygdala activation (15,16), and self-report of threat and anxiety associated with eye-looking in older children and adults with ASD (11,17). Within this model, avoiding others’ eyes represents a motivated response to withdraw in order to prevent negatively-valenced, over-stimulating hyperarousal (13,18). Hyperactivation of the amygdala and of the sympathetic system is thus considered the cause (rather than consequence) of atypical eye-looking in ASD.

In contrast, in support of gaze indifference, many areas of the ‘social brain’ system in individuals with ASD, including the amygdala (19), are hypoactive to social stimuli in general (20) and hyporesponsive to gaze cues in particular (21). Gaze indifference hypothesizes that children with ASD, unlike typically-developing peers (22), are insensitive to underlying social signals from another person and, therefore, do not perceive others’ eyes as adaptively informative or socially salient, leading to passive omission of eye-looking (23). Insensitivity to the social salience of the eyes implicates atypical developmental specialization of the ‘social brain’ network, including right posterior superior temporal sulcus (STS), typically attuned to the inferred communicative intentionality of eye gaze (24), and also of the basolateral nuclei of the amygdala, which typically contribute to attribution of reward value and to processing the relative salience of stimuli (25,26).

Distinguishing between these two hypotheses has critical implications for treatment and for our mechanistic understanding of the condition. If gaze aversion underlies diminished eye contact in ASD, anxiolytics (e.g., 26) or behavioral interventions increasing exposure to others’ eyes (e.g., 27) may be indicated to address social impairments. Alternately, if gaze indifference underlies diminished eye contact in ASD, interventions aimed at enhancing social engagement and reinforcer value/motivation (e.g., 28) may be indicated.

To directly test these two hypotheses, we collected eye-tracking data at time of initial diagnosis in both two-year-olds with ASD and matched peers. In Experiment 1, children were directly cued to look either more or less at the eyes by presenting a target stimulus
directing gaze to specific locations (Figure 1A-B). In Experiment 2, rather than directly cueing a child for eye-looking, we measured levels of eye-looking during periods of time-varying implicit cueing for eye-looking (Figure 1C-D).

**Methods**

**Participants**

Eighty-six children (26 ASD, 38 typically-developing, 22 non-ASD developmentally-delayed) with a mean age of 24.9 months (SD=7.5) participated. Children with ASD were consecutive community referrals to a diagnostic clinic, with experimental procedures collected at time of initial diagnosis. Children with ASD met all of the following diagnostic criteria: (1) met criteria for Autistic Disorder or ASD on the *Autism Diagnostic Observation Schedule* (2), Module 1; (2) met criteria for Autistic Disorder or ASD on the *Autism Diagnostic Interview – Revised* (30); and (3) received a diagnosis of Autistic Disorder (19 of 26 children) or Pervasive Developmental Disorder-Not Otherwise Specified (7 of 26 children) by two experienced clinicians. At time of collection, diagnostic guidelines followed DSM-IV-TR criteria (31); all children also met criteria for ASD per DSM-5 criteria (32). Of the 26 toddlers with ASD, 20 were seen again after the age of 3 years and all still met diagnostic criteria for ASD. Typically-developing children met the following criteria: they (1) exhibited no developmental delays (measured as any single delay of >2SD or as two delays each >1.5SD in the Visual Reception, Receptive, or Expressive Language subscales of the *Mullen Scales of Early Learning* (33)); (2) had no known genetic syndrome; and (3) had no family history of ASD in first- or second-degree relatives. Non-ASD developmentally-delayed children met the following criteria: they (1) did not meet diagnostic criteria for ASD; and (2) exhibited single delays of >2SD or two delays of >1.5SD in the Visual Reception, Receptive, or Expressive Language subscales of the *Mullen*. No participants were receiving intervention services at testing, which was the time of initial diagnosis for all participants with ASD and non-ASD developmental disabilities.

Toddlers with ASD were matched on sex, chronological age, and nonverbal cognitive ability to typically-developing toddlers (Table 1) and were matched on sex, chronological age, and verbal cognitive ability to the developmentally-delayed group (Table S1).

Parents or legal guardians provided written informed consent for participation. The Institutional Review Board at Yale University approved the research protocol. All aspects of the experimental protocol were performed by personnel blinded to diagnostic status of the children. Diagnostic measures were administered by trained clinicians blinded to the results of experimental procedures.

In both experiments, eye-tracking data were collected by a dark pupil/corneal reflection technique (ISCAN, Inc.) while children viewed video scenes of an actress looking directly into the camera, portraying the role of a caregiver and speaking to the viewer in toddler-directed speech. Actresses were filmed in front a background that approximated a child's room, including colorful pictures and shelves of toys. For detailed description of data collection, processing, and stimuli, please see Supplementary Materials.
Experiment 1: Response to Direct Cueing for Eye-Looking

In the first experiment, children were directly cued to look at the eyes, mouth, or non-face regions by a prestimulus cueing target (Figure 1A). The cueing target was standardized at the center of the stimuli presentation monitor, and the location of face stimuli varied with respect to the cueing target location across trials. Trials in which an individual did not look at the cueing target location were excluded from analysis. As dependent variables, we measured each child’s (a) latency to look away and (b) sustained levels of continued eye-looking after cueing and after initial shifts of gaze.

Cueing effects on latency to first saccade—For latency to look away, the gaze aversion hypothesis predicted a significant negative association: if children with ASD were averse to eye-looking, latency to first saccade would decrease (looking away more quickly) as direct cueing for eye-looking increased (Figure 2A). In contrast, gaze indifference predicted no relationship: if indifferent to eye-looking, latency to look away would remain unchanged as direct cueing for eye-looking increased. (Figure 2B).

Comparisons were made both categorically (cued to eyes, mouth, or non-face regions) and continuously (closer to or farther from the eyes, measured in degrees of visual angle; see Figure 1b). Categorical hypotheses were tested by within-group one-way ANOVAs, evaluating latency to look away as a function of cueing target region (eyes, mouth, and non-face). Dimensional hypotheses were tested by Pearson correlation, measuring the association between latency and degree of cueing.

Post-cueing effects on sustained levels of eye-looking—Participants’ initial fixation location was determined by cueing target location, but subsequent fixation locations and sustained levels of eye-looking were determined by the participants themselves, freely shifting their gaze. The gaze aversion hypothesis predicted a weaker association in the ASD group between initial degree of direct cueing and subsequent levels of eye-looking: after being cued to look at the eyes, children with ASD, even if not initially faster to look away, would still avoid the eyes over time, using subsequent saccades to look away (Figure 3A). In contrast, gaze indifference predicted a stronger association in the ASD group between initial degree of direct cueing and subsequent levels of eye-looking: after being cued for eye-looking, children with ASD would continue to look more at the eyes (Figure 3B).

We tested these hypotheses by measuring the association, at each moment in time, between degree of initial direct cueing for eye-looking and subsequent percentage of actual eye-looking. Associations were measured by Pearson correlation coefficient, with between-group analyses conducted by comparison of bootstrapped means and 95% confidence intervals calculated across 5000 resamplings.

Experiment 2: Response to Implicit Cueing for Eye-Looking

In the second experiment, we measured levels of eye-looking during periods of time-varying implicit cueing for eye-looking.
Implicit cueing for eye-looking—Given previous research in typically-developing children demonstrating intrinsic engagement and responsiveness to the underlying social cueing of the eyes (34,35), we quantified the strength of implicit cueing for eye-looking in our video stimuli by measuring typically-developing children's data. Specifically, after the period in which direct cueing was no longer associated with fixation location (τ >1,500ms), we measured the time-varying probability that typically-developing toddlers would look at the eyes (Figure 1C). This moment-by-moment variation in probability of eye-looking reflects, for typically-developing toddlers, their normative response to cues within the video (i.e., naturally-occurring social cues proffered by each actress while speaking to the viewer in toddler-directed speech). Probability of eye-looking varied between 0 and 1, measured in 33.3 ms intervals. This probability, ranked by quartiles, served as our index of implicit cueing (Figure 1D). To test the validity of these cues, we used leave-one-out cross validation (LOOCV; 36). A within-group repeated measures ANOVA across all LOOCV comparisons showed high predictive validity for increased eye-looking (F_{3,111} = 34.30, p < 0.001).

As dependent variables, in relation to strength of implicit cueing, we then measured each child's (a) probability of eye-looking and (b) density of fixation locations.

Implicit cueing effects on probability of eye-looking—The gaze aversion hypothesis predicted a decline in probability of eye-looking as the strength of implicit cueing increased: children with ASD would avoid the eyes specifically at moments with strongest implicit cueing for increased eye-looking (Figure 4A). In contrast, gaze indifference predicted no change in the probability of eye-looking as a function of implicit cueing; children with ASD would be insensitive to implicit cues (Figure 4B). Effects of implicit cueing on probability of eye-looking were measured by within-group repeated measures ANOVA.

Implicit cueing effects on fixation density—Rather than fully shifting gaze away from the eyes, it was also possible that children with ASD might more subtly re-orient gaze towards peripheral locations. The gaze aversion hypothesis predicted a decline in viewers' fixation density relative to level of implicit cueing: at times of high implicit cueing, children with ASD would shift gaze to the periphery (37), resulting in more widely distributed fixations (Figure 4E). Alternaately, the gaze indifference hypothesis predicted no relationship: children with ASD would not reorient their gaze specifically in response to implicit cues, resulting in unchanged fixation density (Figure 4F). Fixation density was measured by kernel density analysis (38) in 33.3 ms intervals. The effect of implicit cueing for increased eye-looking on fixation density was assessed using within-group repeated measures ANOVAs.

Results

Throughout free-viewing of the video scenes, we replicated a reduction in eye-looking in the ASD group compared to typically-developing and developmentally-delayed peers (39) (Table 1, Figure 1E), indicating diminished visual attention to others' eyes, the basic behavioral criterion for either gaze aversion or indifference. Across all analyses, results in the developmentally-delayed group were comparable to the typically-developing group, providing evidence that differences observed in the ASD group were due to diagnostic status.
rather than to verbal functioning. For detailed description of results in the developmentally-delayed group, please refer to Supplemental Materials.

Response to direct cueing for eye-looking

As controls, we tested whether typically-developing and ASD groups differed in any of the following respects: in successful direct cueing (Figure 1F; typically-developing, 92%; ASD, 92%: \(\chi^2 = 0, p > 0.999\)), in time spent freely watching the stimuli (Figure 1G; typically-developing, 65.8% (13.9); ASD, 65.6% (13.1), data presented as mean (standard deviation); \(t_{1,62} = 0.05, p = 0.96, d = 0.01\)), and in integrity of extraocular muscle movements (for control of saccade amplitude and velocity; Figure 1H-I). In all cases, there were no significant between-group differences.

Given prior studies of reaction time to disengage in children with ASD (40), reaction time following direct cueing was measured across all cueing trials. Latency to first saccade was not significantly different between groups (Figure 1J; typically-developing, 0.86 s (0.52); ASD, 0.76 s (0.48); \(t_{1,361} = 1.42, p = 0.16, d = 0.15\)), indicating that typically-developing and ASD groups were equally capable of rapidly shifting gaze.

We then measured latency to first saccade as a function of cueing target location. There was no significant relationship between latency to first saccade and degree of eyes cueing in the typically-developing (Figure 2C, 2E) or ASD group (Figure 2D, 2F), measured either categorically (Figure 2C-D; typically-developing, \(F_{2,197} = 1.13, p = 0.33, \eta^2_p = 0.01\); ASD, \(F_{2,160} = 0.65, p = 0.52, \eta^2_p = 0.01\)) or continuously (Figure 2E-F; typically-developing, \(r = -0.40, p = 0.28\); ASD, \(r = -0.39, p = 0.30\)).

Next, we examined the effects of direct cueing for eye-looking on sustained (post-cueing) levels of eye-looking. At trial onset, level of eye-looking was significantly associated with degree of eyes cueing in both the typically-developing (Figure 3C; \(r = 0.88, p = 0.002\) and ASD groups (\(r = 0.85, p = 0.004\)), indicating that participants in both groups had been successfully cued by the prestimulus target. For between-group analyses, we compared bootstrapped 95% confidence intervals of the \(r\) value of the correlation over time. At trial onset, as expected, bootstrapped confidence intervals for each group were overlapping, but beginning at 470 ms after cueing, the association between degree of initial cueing and level of eye-looking was significantly greater in the ASD than in the typically-developing group (Figure 3D). The significant association between eyes cueing and level of eye-looking persisted in the ASD group until 1,233 ms, a more than 2-fold increase in duration of the effect of direct cueing in the ASD as compared to the typically-developing group (for whom the effect lasted 500 ms).

The observation of stronger association between eye-looking and initial direct cueing in the ASD group (Figure 3D) occurred despite equivalent latencies to first saccade; stated differently, the persistence of this association required both additional fixations on the eyes when cued for eye-looking as well as additional fixations on the mouth when cued for mouth-looking, an indication of relative insensitivity to the content of either cued target location.
Response to implicit cuing for eye-looking

We measured probability of eye-looking as a function of implicit cueing for eye-looking. In the typically-developing group, by leave-one-out cross validation, we observed a significant main effect of strength of implicit cueing (Figure 4C; $F_{3,111} = 34.30, p < 0.001, \eta^2_p = 0.48$). There was also a significant main effect in the ASD group (Figure 4D; $F_{3,75} = 12.78, p < 0.001, \eta^2_p = 0.34$). In contrast to our predictions based on the gaze aversion and indifference accounts, children with ASD were more likely to look at the eyes at times with stronger implicit cueing. Critically, however, children with ASD were not less likely to look at the eyes in response to the strongest cueing for eye-looking, evidence that is inconsistent with the gaze aversion hypothesis.

Next, we examined fixation density as a function of implicit cueing. In the typically-developing group, there was a significant main effect of implicit cueing (Figure 4G; $F_{3,111} = 23.69, p < 0.001, \eta^2_p = 0.39$). In contrast, in the ASD group, there was no change in fixation density based on strength of implicit cueing (Figure 4H; $F_{3,75} = 1.60, p = 0.20, \eta^2_p = 0.06$).

Discussion

We studied two mechanistic hypotheses for diminished eye-looking in ASD: gaze aversion and gaze indifference. The distinction between these hypotheses is critical because the two posit different causal mechanisms and different target areas of atypical brain function. Ultimately, the two hypotheses present different conceptions of ASD and indicate different approaches to behavioral and pharmacological treatment.

In the current study, across all analyses, results were inconsistent with the gaze aversion hypothesis. At time of initial diagnosis, two-year-olds with ASD did generally look less at the eyes, but they looked less at the eyes in a fashion that could be overridden by direct cueing for eye-looking and in a fashion that was not systematically or consistently driven by implicit social cueing. When directly cued to look at the eyes (Experiment 1), two-year-olds with ASD did not look away faster than typically-developing peers. The effects of direct cueing were actually stronger in two-year-olds with ASD than in typically-developing peers (when cued to look at the eyes, they looked longer at the eyes; when cued to look at the mouth, they looked longer at the mouth). Moreover, when presented with implicit social cues for eye-looking (Experiment 2), there was no evidence of two-year-olds with ASD shifting their gaze away from the eyes at specific moments of high implicit eyes salience.

These results contradict the hypothesis that children with ASD actively avoid looking at the eyes in early life. Instead, the results are consistent with gaze indifference and indicate passive insensitivity to social signals in others’ eyes coupled with intact sensitivity to non-social, physical cues (41). Our results are also consistent with existing evidence on response to directional eye gaze cues in toddlers with ASD. Similar to typically-developing toddlers, toddlers with ASD orient their attention based on eye gaze cues in structured experimental situations (i.e., Posner-like cueing tasks) (42). However, unlike typically-developing toddlers, toddlers with ASD do not show a differential response or distinct sensitivity to eye gaze cues versus non-social cues (e.g., arrows) (35). Even when toddlers with ASD exhibit superficially-typical responses to gaze cues in experimental settings, they do not
demonstrate spontaneous response to gaze cues (i.e., response to joint attention) in less structured interactions (35). Similarly, even at older ages when children with ASD more consistently demonstrate response to joint attention, which is associated with developmental level (42), they show consistent impairments in initiating joint attention with others (43) and in inferring others' mental states from gaze cues (44), providing additional evidence of gaze indifference.

In finding that the results of the current experiments are inconsistent with and cannot support the gaze aversion hypothesis, we do not suggest that all other evidence supportive of gaze aversion is necessarily invalid. Previous findings of atypical autonomic response (13,14), atypical amygdala activation (15,16), and increased anxiety (11,17) in response to gaze have focused on older children and adults with ASD. Similarly, clinical observations (6) and first-person reports (45) of anxiety or aversion in response to others' eyes are generally from later childhood or adulthood. The age contrast between the current study and later-life accounts is an important source of information. In older children and adults, atypical affective response to eye gaze may be a developmental consequence rather than cause of atypical eye-looking. Consistent with this, the prevalence of anxiety symptoms in ASD is positively associated with age (46) and moderated by cognitive functioning (47), suggesting that anxiety symptoms are late-emerging and do not underlie core social deficits in ASD. Additionally, EEG frontal asymmetry, which provides evidence of approach- withdrawal responses (48), suggests that hyperactivation and hyperarousal to the eyes in older individuals with ASD may not be negatively valenced (23,49), arguing against the interpretation of atypical autonomic and amygdala activity as indicating aversion.

If at the time of initial diagnosis, toddlers with ASD passively omit eye contact and do not show clear signs of actively avoiding the eyes of others (as in the current experiments), and if, at a later time point, some individuals with ASD either exhibit or self-report anxiety-related reactions to eye-looking (which result in their actively avoiding the eyes of others), what changes transpired between the early and later time points? The answer to that question may help shed light on learning processes underlying the natural history of secondary symptomatology in ASD, that is, symptoms that emerge as a result of compensatory processes rather than mechanisms that play a causative role in pathogenesis (please see Supplementary Materials for an extended discussion).

These findings provide important points for future study of the neuropathology of ASD. The current results indicate that reduced eye-looking in ASD is not, at time of initial diagnosis, an anxiety-related response, and that reduced eye-looking is unlikely to be caused by hyperarousal or amygdala hyperactivation. Instead, because reduced attention to the eyes appears to be due to passive insensitivity to the social signals of a conspecific, observed amygdala dysfunction in ASD is more likely due to atypical development of neural networks involving the basolateral amygdala, including circuits associated with social gaze perception and with frontal cortex-associated attribution of reward value to social interaction (25,26,50). Thus, rather than focusing on emotion regulation and threat processing pathways, future studies may focus more productively on developmental specialization of social brain networks subserving engagement with and valuation of socially-relevant stimuli, in hopes of better understanding and ultimately treating the neural mechanisms of gaze
indifference as well as associated, and more general, impairments in social adaptive action in ASD.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

The authors gratefully acknowledge all of the children and families for their participation and thank Laura Edwards, Sarah Shultz, Katherine Rice, Jessie Northrup, and Gordon Ramsay for discussions of data analysis as well as Casey Zampella and Tawny Tsang for assistance with measures of extraocular muscle movements.

This work was supported by grants to A.K. and W.J. from the Simons Foundation and the National Institute of Mental Health (P50 MH081756-01, U54 MH66494). Additional support was provided by the Marcus Foundation, the Whitehead Foundation, and the Georgia Research Alliance.

**References**

3. Jones, W., Klin, A. Nature Publishing Group; 2013. Attention to eyes is present but in decline in 2-6-month-old infants later diagnosed with autism.


Figure 1. Experimental design for direct (Experiment 1) and implicit (Experiment 2) cueing for increased eye-looking: reduction in eye-looking in toddlers with ASD at time of initial diagnosis; and completion-of-protocol controls

(A) In Experiment 1, toddlers with and without ASD, matched on age and cognitive function, were directly cued to look either more or less at the eyes by means of a prestimulus cueing target presented on an otherwise blank screen. The cueing target was presented and then replaced with video of a face looking directly at the child or with video presenting non-face information. (B) Location of the cueing target varied with respect to the eyes and mouth of each face. Degree of direct cueing for eye-looking was measured relative to the center of the nose. (C) In Experiment 2, the same children were implicitly cued to look either more or less at the eyes. (D) The timeline of implicit cueing for eye-looking was defined by normative data. We measured the probability of eye-looking at each moment in time in typically-developing toddlers, and then tested the validity of these implicit cues by measuring how successfully they predicted the eye-looking of independent typically-developing toddlers. (E) Toddlers with ASD displayed a reduction in attention to others’ eyes, consistent with either hypothesis being tested. (F) As a control for completion of protocol, we measured the percentage of trials successfully cued in Experiment 1 within each group. (G) As a control for completion of protocol in Experiment 2, we measured the percentages of time spent fixating, saccading, blinking, and looking offscreen while video
trials were presented. (H, I) As a control for the capacity to execute saccadic eye movements, we measured the main sequence relationship between saccade velocity and amplitude in (H) typically-developing toddlers and (I) in toddlers with ASD. (J) As a control for differences in reaction time, we measured latency to first saccade across all trials. ASD = autism spectrum disorder; TD = typically developing.
Figure 2. Following direct cueing to look at the eyes, two-year-olds with ASD do not look away more rapidly than their typically-developing peers

(A, B) Expected results for (A) the gaze aversion hypothesis and (B) gaze indifference hypothesis. (C) In typically-developing toddlers, latency to first saccade did not vary categorically as a function of the content of cued target region: eyes, mouth, or non-face. (D) In toddlers with ASD, latency to first saccade did not vary categorically as a function of the content of cued target region: eyes, mouth, or non-face. (E) In typically-developing toddlers, latency to first saccade did not vary dimensionally by degree of physical cueing for eye-looking: closer to or farther from the eyes. (F) In toddlers with ASD, latency to first saccade did not vary dimensionally by degree of physical cueing for eye-looking: closer to or farther from the eyes. ASD = autism spectrum disorder; TD = typically developing.

\[ r = -0.40, p = 0.28 \]
\[ r = -0.39, p = 0.30 \]
Figure 3. After direct cueing, despite equivalent latencies to first saccade, two-year-olds with ASD show higher levels of sustained looking than their typical peers (A, B) Expected results for (A) the gaze aversion hypothesis and (B) gaze indifference hypothesis. (C) Sustained effects of physical cueing are measured as the time-until-decay of a significant association between physical cueing location and percentage of looking to the cued region. For discrete time periods, plotted in 133.3 ms intervals, the association between cueing target location and percentage of fixation to cued region is plotted as scatter plots with regression lines for typically-developing toddlers (top row, yellow) and for toddlers with ASD (bottom row, green). Regression lines for time periods with significant associations ($p < 0.05$) are plotted with more saturated shades for each group. (D) Measured continuously, the association ($r$ value of correlation) between degree of physical cueing for eye-looking and percentage of eye-looking shows rapid decline in typically-developing toddlers (yellow) and slower decline in toddlers with ASD (green). Shaded regions show bootstrapped 95% confidence intervals. Despite equivalent latencies to first saccade (Figures 1 & 2), toddlers with ASD show a stronger, more persistent association between cueing target location and subsequent fixation location, looking more at the eyes when cued for eye-looking and more at the mouth when cued for mouth-looking—an indicator of relative insensitivity to the content at the cued target location in toddlers with ASD. ASD = autism spectrum disorder; TD = typically developing.
Figure 4. At moments with strongest implicit cueing for increased eye-looking, two-year-olds with ASD neither look fully away from the eyes nor shift gaze to the periphery (A, B) Expected results for eye-looking in response to implicit cueing for eye-looking based on (A) the gaze aversion hypothesis and (B) gaze indifference hypothesis. (C) In typically-developing toddlers, implicit cueing for increased eye-looking, ranked by quartiles, positively predicts increased eye-looking among independent typically-developing children using leave-one-out cross-validation. (D) In toddlers with ASD, implicit cueing for eye-looking does not result in gaze aversion; instead, a significant increase in probability of eye-looking is observed. (E-H) Fixation density in two-year-olds with ASD is unrelated to levels of implicit cueing for increased eye-looking. (E, F) Expected results for fixation density in response to implicit cueing for eye-looking based on (E) gaze aversion hypothesis and (F) gaze indifference hypothesis. (G) In typically-developing toddlers, implicit cueing for increased eye-looking, ranked by quartiles, positively predicts greater fixation density; as implicit cueing increases, typically-developing toddlers are more likely to fixate on the same location at the same time. (H) In toddlers with ASD, fixation density is unrelated to levels of implicit cueing for eye-looking, indicating that they do not avert gaze to peripheral locations during periods of increased implicit cueing for eye-looking. ASD = autism spectrum disorder; TD = typically developing.

\[ F_{3,111} = 34.30, p < 0.001 \]
\[ F_{3,75} = 12.78, \ p < 0.001 \]
\[ F_{3,111} = 23.69, \ p < 0.001 \]
\[ F_{3,75} = 1.60, \ p = 0.20 \]
Table 1
Participant characterization data and percentage of visual fixation time to regions-of-interest

<table>
<thead>
<tr>
<th></th>
<th>ASD Group (N = 26)</th>
<th>TD Group (N = 38)</th>
<th>Test Statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>21/5</td>
<td>26/12</td>
<td>$X^2 = 1.21$</td>
<td>0.27</td>
</tr>
<tr>
<td>Age, months</td>
<td>27.1 (6.5)</td>
<td>24.1 (8.0)</td>
<td>$t_{1,62} = -1.61$</td>
<td>0.11</td>
</tr>
<tr>
<td>Nonverbal function, months</td>
<td>21.6 (9.0)</td>
<td>24.8 (9.8)</td>
<td>$t_{1,62} = 1.33$</td>
<td>0.19</td>
</tr>
<tr>
<td>Verbal function, months</td>
<td>16.3 (12.4)</td>
<td>24.8 (9.2)</td>
<td>$t_{1,62} = 3.15$</td>
<td>0.003</td>
</tr>
<tr>
<td>ADOS Social Score</td>
<td>9.9 (3.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dx, Aut/PDD-NOS</td>
<td>19/7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Eyes, % 28.4 (14.9) 51.5 (19.9) $t_{1,62} = 5.06$ <0.001
Mouth, % 42.7 (21.6) 24.7 (20.0) $t_{1,62} = -3.42$ 0.001
Body, % 14.2 (8.0) 12.3 (8.1) $t_{1,62} = -0.90$ 0.37
Object, % 14.8 (13.3) 11.4 (10.5) $t_{62} = -1.13$ 0.26

1 Clinical characterization data and percentage of visual fixation time provided as mean (s.d.) for children with autism spectrum disorders (ASD) and typically-developing (TD) peers
2 Verbal function, age-equivalence score in months on the Visual Reception subtest of the Mullen Scales of Early Learning
3 Nonverbal function, age-equivalence score in months on the the Receptive and Expressive Language subtests of the Mullen Scales of Early Learning
4 ADOS Social Score, total score on the social algorithm of the Autism Diagnostic Observation Schedule (higher scores denote higher levels of social disability)
5 Dx, diagnosis; Aut, Autistic Disorder; PDD-NOS, Pervasive Developmental Disorder-Not Otherwise Specified