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Gaze fixation and the neural circuitry of face processing in autism

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Diminished gaze fixation is one of the core features of autism and has been proposed to be associated with abnormalities in the neural circuitry of affect. We tested this hypothesis in two separate studies using eye tracking while measuring functional brain activity during facial discrimination tasks in individuals with autism and in typically developing individuals. Activation in the fusiform gyrus and amygdala was strongly and positively correlated with the time spent fixating the eyes in the autistic group in both studies, suggesting that diminished gaze fixation may account for the fusiform hypoactivation to faces commonly reported in autism. In addition, variation in eye fixation within autistic individuals was strongly and positively associated with amygdala activation across both studies, suggesting a heightened emotional response associated with gaze fixation in autism.

Autism is a pervasive developmental disorder associated with a unique profile of social and emotional behavior. The core symptomatology of autism highlights these deficits and includes diminished gaze fixation, lack of social or emotional reciprocity, and failure to develop age-appropriate peer relationships[1,2]. Recent studies have focused on attention to faces and face processing abilities in children with autism, because of the crucial importance of faces as a medium of social communication among humans[3–8]. These studies demonstrate that inattention to faces is an early developmental sign of autism that is apparent as early as 1 year of age[9,10]. In addition, many children with autism are delayed in early, face-related social milestones, such as looking to another person's face to reference that person's reactions or to share their own experience of objects and events[11,12]. These findings raise the possibility that abnormalities in the perception of faces and their communicative signals may contribute to the social impairment that characterizes autism.

Studies on brain function also show atypical patterns of brain activation in individuals with autism when processing faces. The most consistently reported and largest effect is in the fusiform gyrus, an area that is activated strongly during face processing in typically developing individuals but much less activated during these tasks in individuals with autism-spectrum disorders[13–16]. Relatively little attention has been devoted to circuitry that is more highly activated in individuals with autism than in typically developing controls when processing faces. We predicted that individuals with autism would show hyperactivation in brain regions responsible for processing threatening social and emotional cues. We thus expected the autistic individuals to show hypoactivation in the fusiform gyrus along with hyperactivation in the amygdala in response to faces. For both of these brain regions, we also predicted that time spent fixating the eye region of the face would predict the magnitude of activation for the individuals with autism.

Notably, to date, no study has yet reported on the relation between gaze fixation and brain activation patterns during processing of human faces in individuals with autism. We hypothesized that diminished gaze fixation is the proximal cause of the fusiform gyrus hypoactivation commonly reported for individuals with autism when processing faces. We thus predicted that fusiform gyrus hypoactivation would be associated with the lesser time spent in fixating on the eye region of faces by autistic individuals as compared with typically developing controls. Moreover, we also predicted that variations in time spent fixating on the eye region of the face would strongly predict amygdala activation in individuals with autism. To test these hypotheses, we conducted two separate studies, which tested emotion discrimination (Study I) and facial recognition (Study II). In each of these studies, we presented photographs of human faces to individuals with autism and typically developing controls while they were in a magnetic resonance imaging (MRI) scanner, using both standardized and naturalistic unfamiliar faces and naturalistic familiar faces (**Fig. 1**). In both studies, activation in the fusiform gyrus was strongly and positively correlated with the amount of time spent fixating the eye region in the autistic group, suggesting that diminished gaze fixation may account for the fusiform hypoactivation in response to faces commonly reported in autism. In addition, variation in gaze fixation among autistic individuals was strongly and positively associated with amygdala activation across both studies. This suggests that gaze fixation is associated with a heightened emotional response in autism.

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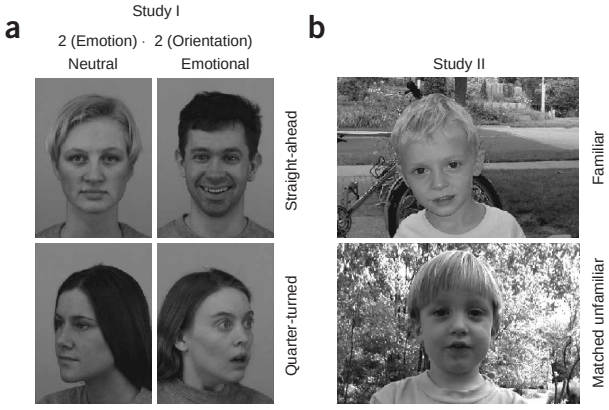

Figure 1 Study designs and exemplar stimuli. (**a**) Study I: 2 (Emotion) × 2 (Orientation) repeated-measures design. Twenty-four emotional faces and 16 neutral faces were used, half with eyes and face oriented straight ahead, and half with face and eyes averted 45 degrees (equal toward the right and left). (**b**) Study II: examples of matched familiar versus unfamiliar photographs. Photographs of participants' family and friends were matched for gender, age, facial expression and orientation as closely as possible to photographs taken by other study participants.

RESULTS

Task accuracy and judgment time: Study I

Three of the individuals with autism performed the emotion discrimination task at below chance level and therefore were not included in any analyses. The control group performed the emotion discrimination task at a near-perfect level and gave significantly more correct responses (M (mean) = 39.4 out of a total possible correct of 40, s.d. = 0.79) than the autistic group (M = 34.1, s.d. = 7.52; $t_{1,10}$ = 2.34, P = 0.04). The group difference in accuracy was not a function of a speed-accuracy tradeoff; individuals in the autistic group were marginally slower than controls ($F_{1,21}$ = 4.27, P = 0.051). The autistic group took significantly longer than the control group to decide whether the face was emotional or neutral when the face was emotional (control M = 1,110.9 ms, s.d. = 182.3; autism M = 1,329.8 ms, s.d. = 206.6; $t_{1,22}$ = 2.75, P = 0.01) and when it was oriented with eyes straight ahead toward the viewer (control M = 1,194.1 ms, s.d. = 243.7; autism M = 1,413.1 ms, s.d. = 247.3; $t_{1,22}$ = 2.18, P = 0.04). There were no group differences in judgment time for the neutral faces or for faces quarter-turned with eyes away from the viewer. These findings suggest that although the task was more difficult for the autistic group, they were still able to perform the task with 85% accuracy. Furthermore, the fact that groups differed in judgment time for the emotional and directed-gaze faces but not for the neutral or averted-gaze faces suggests that these differences are associated with deficits in processing emotional cues from faces and in processing socially engaging faces, rather than deficits in face processing in general.

Task accuracy and judgment time: Study II

Response time and accuracy were not recorded for one of the control individuals and two of the individuals with autism because of mechanical error, so data from these individuals were not included in this analysis. These individuals were retained in all other analyses. Two of the individuals with autism performed the facial recognition task at or below chance level for the photos of familiar versus unfamiliar people but performed with 90% and 100% accuracy for the photos of familiar versus unfamiliar objects, suggesting that they understood the task; therefore, they were retained in this and all other analyses. We performed all subsequent analyses a second time with these two individuals removed, and the results remained statistically significant in the same direction. The autistic group performed the task with 84% accuracy for the images of people and with 95% accuracy for the images of objects, whereas the control group performed the task at near the highest possible score for both images of people (95% accuracy) and objects (98% accuracy). The group difference in accuracy was small but significant for images of people ($t_{1,27}$ = 2.14, P = 0.04) but not significant for images of objects ($t_{1,27}$ = 1.90, P = 0.06). There were no group differences in judgment time for images of either people or objects.

Gaze fixation: Study I

We calculated the time each group spent fixating on the face in general and the eyes and mouth specifically. As predicted, the autistic group spent significantly less time per trial fixating on the eyes than did the control group ($t_{1,19}$ = 1.82, P = 0.04, one-tailed). There were no significant group differences in amount of time spent fixating on the mouth region or the face in general (**Fig. 2a**).

Gaze fixation: Study II

We performed a similar fixation analysis on the eye-tracking data for Study II. A group × familiarity mixed-factors ANOVA was performed on the average amount of time spent fixating on the eyes, mouth and face of the familiar and unfamiliar faces. Again, the autistic group spent significantly less time per trial fixating on the eyes than did the control group (group main effect, $F_{1,28}$ = 5.005, P = 0.03), but we did not find group differences for the mouth or face in general (**Fig. 2b**). Neither the main effect for familiarity nor the group × familiarity interaction were significant for either the mouth or eyes or for the face in general. Notably, the pattern of fixations was nearly identical across the two studies; we found diminished gaze fixation in the autistic group, with no group differences in amount of time fixating on the mouth region or face in general.

Brain activation maps: Study I

Maps plotting the activation in controls minus the activation in autistic subjects were derived across all of the facial photographs to test the hypothesis that individuals with autism show a unique pattern of brain activation while processing standard emotional facial photographs. As predicted, the control group showed significantly greater activation in response to the facial photographs than did the autistic group in large clusters of activation in the bilateral fusiform gyrus (right: $t_{1,23}$ = 6.03, P = 0.00005; left: $t_{1,23}$ = 4.67, P = 0.0001) and the occipital gyrus (right: $t_{1,23}$ = 4.27, P = 0.0003; left: $t_{1,23}$ = 4.87, P = 0.00008), areas

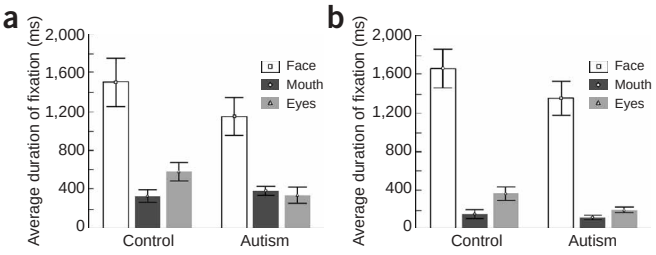

Figure 2 Average fixation durations. (**a**) Study I, average duration of fixation on the mouth and eye region and face in general broken down by group. (**b**) Study II, average duration of fixation on the mouth and eye region and face in general broken down by group. Error bars index the s.e.m.

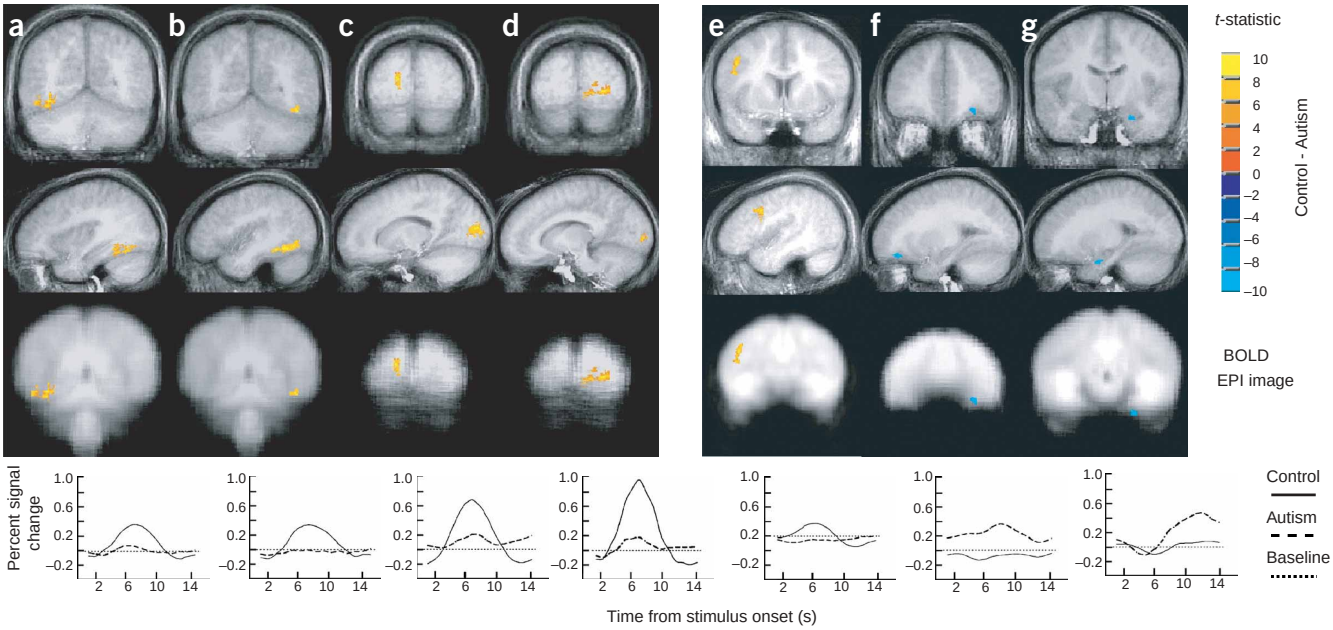

Figure 3 Brain clusters with significant group differences in brain activation across all faces in Study I. (**a**) Right fusiform gyrus: $x = 32$, $y = -57$, $z = -3$; 2,142 voxels. (**b**) Left fusiform gyrus: $x = -39$, $y = -57$, $z = -6$; 508 voxels. (**c**) Right occipital gyrus: $x = 14$, $y = -85$, $z = 13$; 904 voxels. (**d**) Left occipital gyrus: $x = -7$, $y = -91$, $z = 6$; 670 voxels. (**e**) Right middle frontal: $x = 42$, $y = 7$, $z = 32$; 463 voxels. (**f**) Left orbitofrontal gyrus: $x = -24$, $y = 36$, $z = -10$; 119 voxels. (**g**) Left amygdala: $x = -22$, $y = -8$, $z = -18$; 122 voxels. All images are presented in radiological convention such that the right hemisphere is at left of each coronal image. Clusters are color-coded based on the control-minus-autism *t*-statistic values (positive values indicate control values greater than autism). The clusters are also superimposed on an averaged echo-planar blood oxygenation level–dependent imaging (EPI BOLD) signal illustrating adequate signal coverage for each cluster. Averaged MR time series are presented below each cluster for 14 s post-stimulus onset.

associated with face and visual processes, respectively, and in the middle frontal gyrus ($t_{1,23} = 4.30$, $P = 0.0003$; **Fig. 3a–e**). The autistic group showed significantly greater activation than the control group in response to the facial photographs in only two regions: the left amygdala ($t_{1,23} = -2.51$, $P = 0.02$) and orbitofrontal gyrus ($t_{1,23} = -2.96$, $P = 0.007$), areas associated with emotional processes (**Fig. 3f–g**). All 11 individuals with autism showed less activation in the right and left fusiform and occipital gyri and in the right middle frontal region than the average control response. Ten of the 11 individuals with autism showed greater activation in the left orbitofrontal gyrus and amygdala than the average control response. Later analyses showed that these group differences were not mediated by interactions with the emotional expression or orientation of the facial photographs, suggesting robust group differences in the response to facial photographs.

Brain activation maps: Study II

Similar control-minus-autism activation maps were derived across all of the facial photographs to test the hypothesis that individuals with autism also show a unique pattern of brain activation while processing naturalistic facial photographs. As was found in Study I, the control group showed significantly greater activation than the autistic group in response to the facial photographs in the bilateral fusiform gyrus (right: $t_{1,30} = 4.38$, $P = 0.0001$; broken down by IQ: control versus high IQ autism, $t_{1,22} = 3.32$, $P = 0.003$; control versus low IQ autism, $t_{1,22} = 2.91$, $P = 0.008$; high versus low IQ autism, $t_{1,14} = -1.17$, $P = 0.26$; left anterior medial: $t_{1,30} = 4.43$, $P = 0.0001$; broken down by IQ: control versus high IQ autism, $t_{1,22} = 2.83$, $P = 0.009$; control versus low IQ autism, $t_{1,22} = 3.88$, $P = 0.0007$; high versus low IQ autism, $t_{1,14} = 0.97$, $P = 0.35$; left posterior lateral: $t_{1,30} = 4.23$, $P = 0.0002$; broken down by IQ: control versus high IQ autism, $t_{1,22} = 3.16$, $P = 0.004$; control versus low IQ autism, $t_{1,22} = 2.98$, $P = 0.006$; high versus low IQ autism, $t_{1,14} = -0.36$, $P = 0.72$) and right occipital cortex ($t_{1,30} = 4.71$, $P = 0.00005$; broken down by IQ: control versus high IQ autism, $t_{1,22} = 3.46$, $P = 0.002$; control versus low IQ autism, $t_{1,22} = 3.61$, $P = 0.002$; high versus low IQ autism, $t_{1,14} = 0.35$, $P = 0.73$) (**Fig. 4a–d**).

We also performed *a priori* group *t*-tests using a less conservative α value focusing on the region of the amygdala. A cluster in the right amygdala was associated with greater activation in the autistic group than in the control group ($t_{1,30} = -2.36$, $P = 0.025$; broken down by IQ: control versus high IQ autism, $t_{1,22} = -3.16$, $P = 0.004$; control versus low IQ autism, $t_{1,22} = -1.22$, $P = 0.11$; high versus low IQ autism, $t_{1,14} = 0.25$, $P = 0.80$; **Fig. 4e**). All 16 individuals with autism showed less activation than the average control response in the right fusiform, left posterior lateral fusiform and right occipital gyri. Fifteen of the 16 individuals with autism showed less activation than the average control response in the left anterior medial fusiform. Eleven of the 16 individuals with autism showed greater activation than the average control response in the right amygdala. In contrast to Study I, we did not find significant group differences in the middle frontal or orbitofrontal gyri.

An ANOVA was also performed to test the group × familiar interaction. We found significant group × familiar interactions in clusters in the right occipital ($F_{1,30} = 31.41$, $P = 0.000004$) and right posterior fusiform gyrus ($F_{1,30} = 25.34$, $P = 0.00002$). The control group had significantly greater activation for the familiar faces in the right occipital and fusiform gyrus than for the unfamiliar faces, and it had significantly greater activation than did the autistic group for the familiar faces (**Fig. 5**).

Brain activation and gaze fixation: Study I

Although the autistic group averaged less time fixating the eye region than did the control group, there was marked variability in fixation

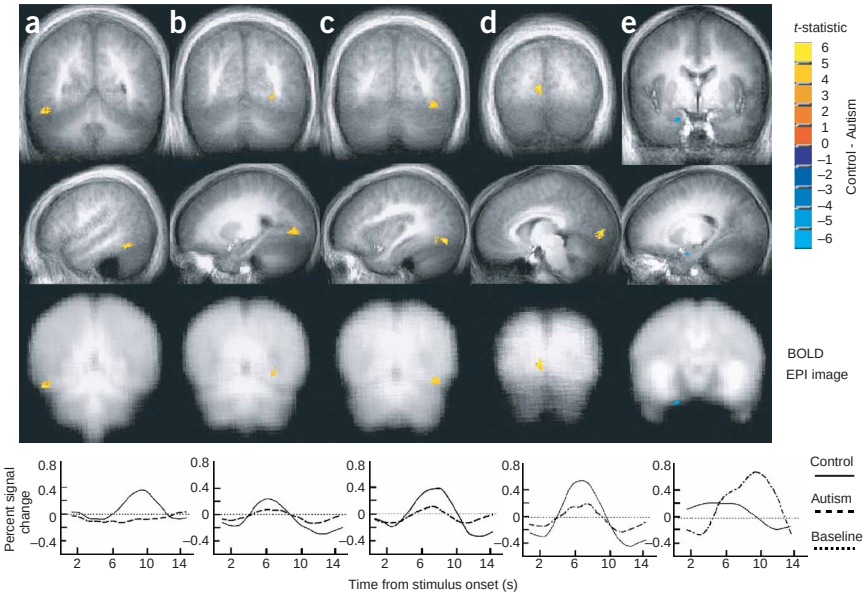

Figure 4 Brain clusters with significant group differences in brain activation across all faces in Study II. (**a**) Right fusiform gyrus: $x = 48$, $y = -53$, $z = -11$; 125 voxels. (**b**) Left anterior medial fusiform gyrus: $x = -25$, $y = -65$, $z = -1$; 322 voxels. (**c**) Left posterior lateral fusiform gyrus: $x = -33$, $y = -73$, $z = -6$; 292 voxels. (**d**) Right occipital gyrus: $x = 6$, $y = -78$, $z = -1$; 304 voxels. (**e**) Right amygdala: $x = 21$, $y = -2$, $z = -20$; 31 voxels. The clusters are color-coded based on the control-minus-autism *t*-statistic values (positive values indicate control greater than autism). The clusters are also superimposed on an averaged EPI BOLD signal illustrating adequate signal coverage for each cluster. Averaged MR time series are presented below each cluster for 14 s post-stimulus onset.

time within the group. We took advantage of this variability by examining across subjects whether time spent fixating on the eye region of the face predicted activation in fusiform gyrus, amygdalae and orbitofrontal cortex, the three key regions identified in the between-group analyses. We regressed the amount of time spent fixating on the eyes on brain activation for each group voxel-wise. Significant clusters of activation were extracted using a conservative threshold method ($\alpha = 0.001$). Brain activation was strongly and positively associated with the amount of time spent fixating on the eyes for the autistic group but not the control group in clusters in the left amygdala (autism, $r = 0.72$, $P = 0.02$; control, $r = -0.18$, $P = 0.58$; group $Z_{1,20} = 2.11$, $P = 0.03$) and the right anterior fusiform gyrus (autism, $r = 0.75$, $P = 0.02$; control, $r = 0.42$, $P = 0.20$; **Fig. 6**). The amount of variance in activation accounted for by eye fixations in the autistic group was 52% in the left amygdala (< 3% in the control group) and 56% in the right anterior fusiform (22% in the control group). IQ, but not performance, was marginally correlated with activation in the right anterior fusiform cluster for the autistic group ($r = 0.65$, $P = 0.06$). However, average duration of eye fixation predicted a significant proportion of variance in this cluster even after variance accounted for by IQ was removed ($F_{1,5} = 9.29$, $P = 0.038$). Neither IQ nor performance was significantly correlated with activation in the left amygdala cluster for the autistic group (IQ, $r = 0.37$, $P = 0.32$; percentage correct, $r = 0.04$, $P > 0.50$).

Brain activation and gaze fixation: Study II

Similar regressions predicting brain activation using the amount of time spent fixating on the eyes were performed on the data from Study II. As in Study I, a significant proportion of the variance in brain activation was associated with the time spent fixating on the eyes for the autistic group, but not for the control group, in a cluster in the right amygdala (autism, $r = 0.55$, $P = 0.03$; control, $r = 0.06$, $P = 0.83$) and right anterior fusiform gyrus (autism, $r = 0.76$, $P = 0.003$; control, $r = 0.14$, $P = 0.64$; group $Z_{1,28} = 2.05$, $P = 0.04$; **Fig. 7**). The amount of variance account for by eye fixations in the autistic group was 30% in the right amygdala (2% in the control group) and 58% in the right anterior fusiform (< 1% in the control group). Neither IQ nor performance accounted for a significant proportion of variance in either the right amygdala (IQ, $r = 0.22$, $P > 0.50$;

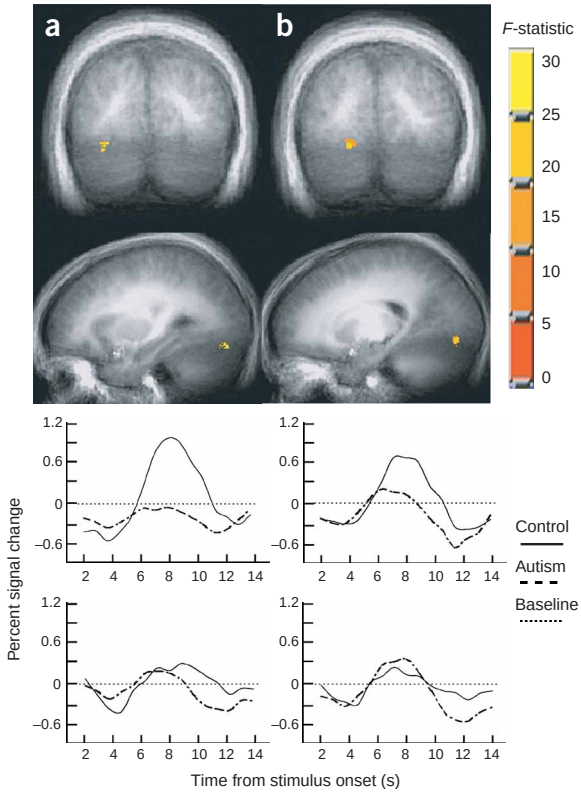

Figure 5 Brain clusters associated with significant Group × Familiarity interactions in brain activation in Study II. (**a**) Right fusiform gyrus: $x = 28$, $y = -76$, $z = -10$; 226 voxels. (**b**) Right occipital gyrus: $x = 18$, $y = -78$, $z = -7$; 318 voxels. Clusters are color-coded based on the Group × Familiar *F*-statistic values. The clusters are also superimposed on an averaged EPI BOLD signal illustrating adequate signal coverage for each cluster. Averaged MR time series are presented below each cluster for 14 s post-stimulus onset. Data for familiar stimuli are in the top graphs and unfamiliar stimuli in the bottom graphs.

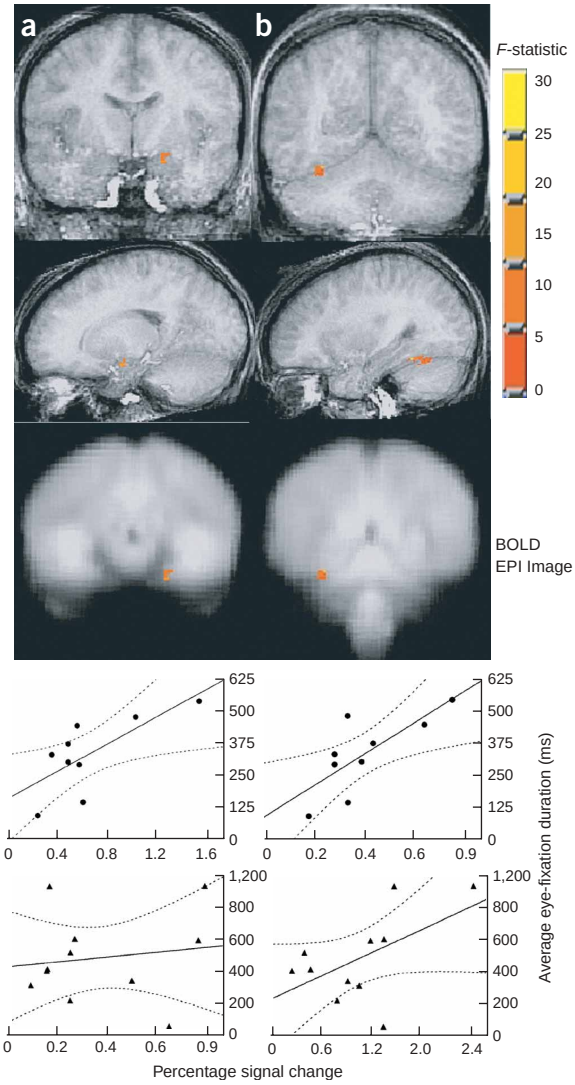

Figure 6 Brain activation clusters associated with average eye fixation time for the autistic and control groups, Study I. (**a**) Left amygdala: $x = -19$, $y = -6$, $z = -13$; 106 voxels. (**b**) Right anterior fusiform gyrus: $x = 28$, $y = -50$, $z = -11$; 76 voxels. The clusters are also superimposed on an averaged EPI BOLD signal illustrating adequate signal coverage for each cluster. Scatter plots showing the relationship between brain activation and average eye fixation are given for each group (autistics, top graphs; controls, bottom graphs) below each cluster. The regression line and 90% confidence bands are superimposed on each scatter plot.

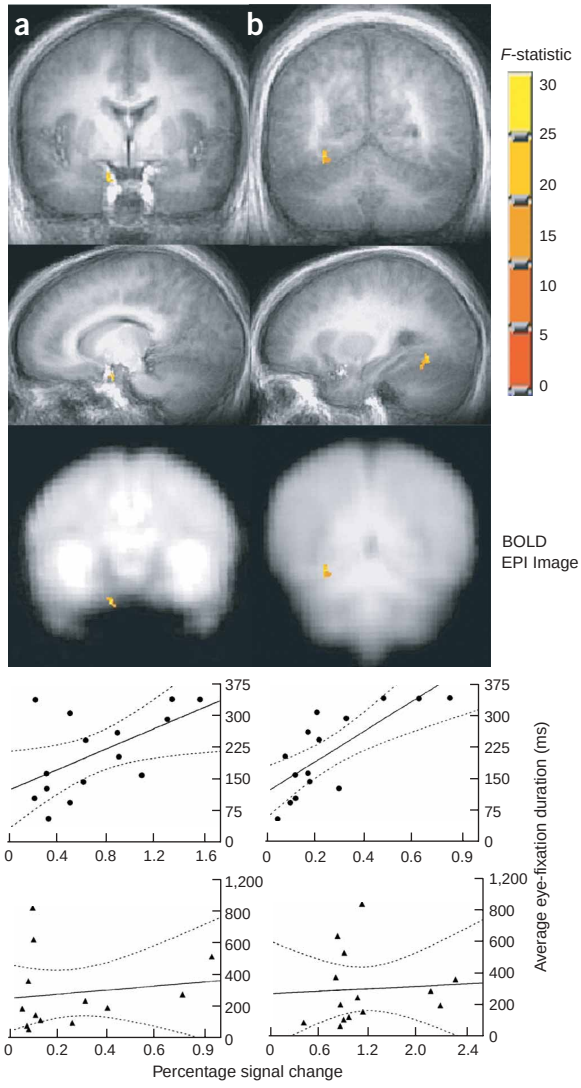

Figure 7 Brain activation clusters associated with average eye fixation time for the autistic and control groups, Study II. (**a**) Right amygdala: $x = 11$, $y = -7$, $z = -20$; 63 voxels. (**b**) Right anterior fusiform gyrus: $x = 27$, $y = -57$, $z = -6$; 113 voxels. The clusters are also superimposed on an averaged EPI BOLD signal illustrating adequate signal coverage for each cluster. Scatter plots showing the relationship between brain activation and average eye fixation are given for each group (autistics, top graphs; controls, bottom graphs) below each cluster. The regression line and 90% confidence bands are superimposed on each scatter plot.

percentage correct, $r = 0.26$, $P > 0.50$) or the right anterior fusiform (IQ, $r = 0.17$, $P > 0.50$; percentage correct, $r = 0.18$, $P > 0.50$) for the autistic group.

Amygdala activation as a function of gaze fixation

We performed *post hoc* analyses within subjects, testing whether the amount of gaze fixation within a given trial predicted amygdala activation during that trial by extracting brain function using a stick function of relative eye fixation time per trial for each individual. Group *t*-tests were then performed focusing on the region of the amygdala, using clustering techniques similar to those described above but with a less conservative *a priori* α of 0.05. Amygdala activation was strongly and positively predicted by amount of eye fixation among the autistic individuals for both Study I ($M = 2.68$, s.d. $= 3.18$; single-sample $t_{1,10} = 3.00$, $P = 0.008$) and Study II ($M = 4.46$, s.d. $= 5.94$; single-sample $t_{1,14} = 2.53$, $P = 0.03$), but not among the control individuals in either Study I ($M = -1.31$, s.d. $= 2.18$; single-sample $t_{1,11} = -1.17$, $P = 0.26$) or Study II ($M = -1.64$, s.d. $= 5.21$; single-sample $t_{1,14} = -1.80$, $P = 0.10$). The group difference in eye fixation related to amygdala activation was significant for both Study I ($t_{1,20} = 3.10$, $P = 0.006$; **Fig. 8a**) and Study II ($t_{1,28} = 2.96$, $P = 0.006$; **Fig. 8b**). We did not find group differences in any other regions using a more conservative whole-brain α of 0.001.

DISCUSSION

These findings show clear differences in how individuals with autism scan and process facial images and suggest that these differences may

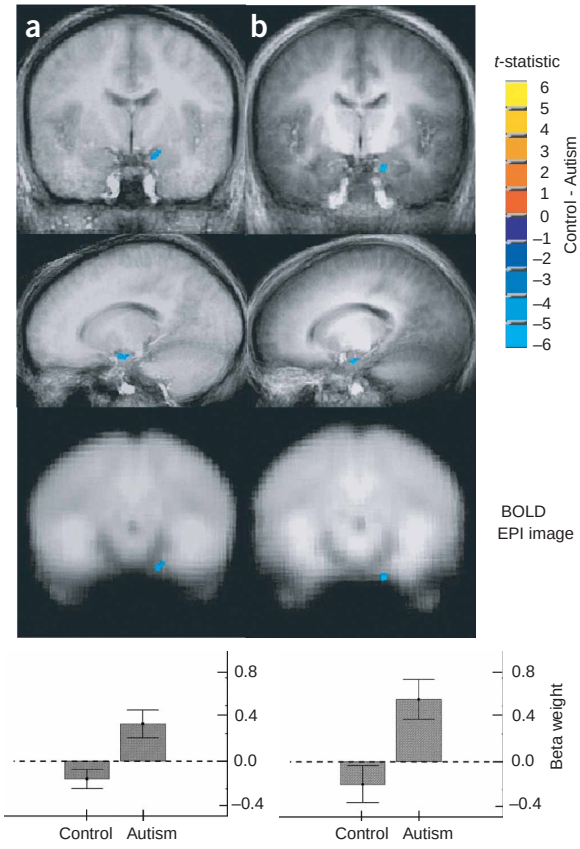

Figure 8 Clusters in the left amygdala associated with group differences in activation as a function of amount of eye fixation within subjects. (**a**) Study I: right amygdala: $x = 18$, $y = -3$, $z = -12$, 110 voxels; control $M = -1.31$, s.d. $= 2.18$, autism $M = 2.68$, s.d. $= 3.18$, group $t_{1,16} = -3.11$, $P = 0.006$. (**b**) Study II: right amygdala: $x = 15$, $y = -8$, $z = -18$, 46 voxels; control $M = -1.64$, s.d. $= 5.21$, $M = 4.46$, s.d. $= 5.94$, group $t_{1,28} = -2.96$, $P = 0.006$. The clusters are also superimposed on an averaged EPI BOLD signal illustrating adequate signal coverage for each cluster. Bar graphs showing the β-weight derived for each group using average eye fixation to predict brain activation are given below each cluster. Error bars index the s.e.m.

be the proximal cause of the commonly reported fusiform gyrus hypoactivation in autism during face processing. Furthermore, they suggest that diminished gaze fixation and fusiform gyrus hypoactivation in autism is pervasive and does not occur merely in response to unfamiliar or standardized faces.

Other fMRI studies using typically developing individuals implicate the right anterior fusiform gyrus in face recognition, with greater activation associated with more familiar faces[17,18]. We have found a region in the right fusiform to occipital cortex associated with significantly greater activation for familiar faces than for unfamiliar faces, but only for the control group. Together, these findings suggest that these regions are associated with facial identification and face processing in general, rather than emotional processes specifically.

Notably, the autistic group showed greater activation in the left amygdala and orbitofrontal gyrus than did the control group in response to the standardized emotional facial photographs, and greater right amygdala activation in response to the familiar and unfamiliar facial photographs. These are areas associated with emotional processes, and these results suggest that processing of faces is associated with a heightened activation in affective central circuitry in autism. Together, these findings suggest that the increased activation in subjects with autism in the orbitofrontal cortex occurs specifically in response to the emotional content of the faces and not to faces in general, whereas activation in the amygdala is not specific to the emotional content of the faces but a response to faces in general.

These findings indicate that the commonly reported hypoactivation in the fusiform gyrus during face processing in autism may be a function of how individuals with autism scan the face rather than a group difference in which area of the brain is used to process faces. The finding that neither face nor eye fixation time is significantly correlated with activation in fusiform gyrus in the control group in either study may be due to ceiling effects for eye fixation in the controls. Additional studies specifically manipulating eye fixation in both typically developing individuals and individuals with autism are needed to more rigorously test this hypothesis.

Eye fixation time is positively associated with activation in the amygdala in the autistic group but not in the control group across both studies, independent of facial emotion expression or gaze orientation and facial familiarity, suggesting that this is a response to eye fixation in general. These findings suggest that amygdala hyperactivation in the autistic group is not a function of generalized overall arousal due to the MRI environment or task-dependent performance anxiety but is specific to the amount of eye fixation. Notably, these findings are not associated with IQ or task performance in the autistic group across both studies, suggesting that they are specific to autism rather than an artifact of confounded cognitive delay. Furthermore, the control and autistic groups were matched for chronological age in both studies, so these group differences cannot be attributed to differences in maturation, although it should be pointed out that a number of the participants in both groups were adolescents.

On the basis of these findings, we suggest that within the autistic group, eye fixation is associated with negatively valenced overarousal mediated by activation in limbic regions such as the amygdala. We propose that diminished gaze fixation within the autistic group may facilitate reduction of overarousal to social stimuli. According to this model, face-processing deficits in autism arise from hyperactivation in the central circuitry of emotion that produces heightened sensitivity to social stimuli, leading to characteristic diminished gaze fixation, which in turn results in atypical activation of the fusiform gyrus. Additional studies including those involving direct measures of arousal (both self-reported and physiological) are warranted to further test this model.

METHODS

Subjects. ***Study I.*** Fourteen males (age $M = 15.9$ years, s.d. $= 4.71$) with autism were recruited for this study from a list of individuals with a diagnosis of autism in the Madison and Milwaukee area maintained for research purposes by the Waisman Center at the University of Wisconsin–Madison. Diagnoses were confirmed with the Autism Diagnostic Interview–Revised (ADI-R)[19] or with a clinical interview administered by a trained and certified psychologist at the Waisman Center. All participants met DSM-IV criteria for autism or Asperger disorder. One participant was non-verbal and two others had minimal functional speech with pronounced echolalia; the remaining 11 individuals were verbally fluent. The three verbally delayed individuals performed the task at or below chance level and were therefore not included in any of the analyses. General IQ was assessed for the remaining 11 autism participants using the Wide Range Intelligence Test (WRIT; $n = 9$; ref. 20) or the Stanford-Binet test ($n = 2$). The average IQ for the autistic group was 94 (s.d. $= 19.47$), which did not differ significantly from the standardized average of 100 (s.d. $= 15$) reported for the normal population ($t_{1,9} = 0.41$, $P = 0.69$). Twelve healthy, typically developing males (age $M = 17.1$ years, s.d. $= 2.78$) with no current or past psychological diagnoses served as comparison individuals. General IQ was not assessed for the control group; therefore, a direct comparison of IQ

between the autism and control groups was not possible. The groups were matched for chronological age. ***Study II.*** Sixteen males (age $M = 14.5$ years, s.d. = 4.60) with autism were recruited for this study through a newsletter distributed by the Autism Society of Wisconsin. Diagnoses were confirmed with the ADI-R or by a clinical interview administered by a trained and certified psychologist at the Waisman Center. All participants met DSM-IV criteria for autism or Asperger disorder. Two of the individuals with autism had minimal functional speech with pronounced echolalia; the remaining 14 individuals were verbally fluent. General IQ was assessed using the WRIT with an autistic group average of 92.1 (s.d. = 27.7). Sixteen healthy, typically developing males (age $M = 14.5$ years, s.d. = 4.56) with no current or past psychological diagnoses served as comparison individuals. General IQ for the control group was 123.1 (s.d. = 12.74). All group analyses were performed two ways: (i) by comparing the two groups without matching on IQ, as reported here, and (ii) by breaking the autistic group into two subgroups based on IQ: high IQ ($n = 8$) and low IQ ($n = 8$). The high-IQ group did not differ from the control group in age or IQ. A similar pattern of results was found under both analyses comparing the control group to both the high and low IQ autistic groups. No differences in brain activation were found between the high and low IQ autistic subgroups for any analyses. The groups were matched for chronological age.

Procedure. Participants first read and signed a consent form that covered all aspects of the study and MRI procedures. Consent was obtained from parents or legal guardians of all participants younger than 18 years. Additional adolescent consent was obtained from participants 13–18 years of age, and a separate child assent was obtained from participants under 12 years of age. All participants and parents were prescreened for MRI compatibility before any exposure to the actual scanner. All sessions began with a simulation session during which participants and parents were acclimated to the MRI environment using a mock-up of an MRI scanner. During the simulation session participants were also given instructions for the facial emotion discrimination task (Study I) or the facial recognition task (Study II) and were shown examples of the appropriate stimuli. All participants and parents were allowed as much time as needed to become comfortable with the mock scanner and were encouraged to ask questions. Once it was clear that the participants were comfortable with the fake scanner and they had indicated that they understood the instructions for the appropriate task, they were escorted to the real scanner for the actual scans. All scans started with approximately 20 min of anatomical scans followed by the 7-min functional scan during which the participants performed either the facial emotion discrimination task or the facial recognition task. The total time in the scanner was approximately 30–35 min and the total time for the full session was approximately 1.5 h. All participants received $20 for the simulation session and $30 for the actual scans, for a total of $50.

Study I: facial emotion discrimination task. Participants were asked to perform a facial emotion discrimination task while functional brain images were acquired. For the task the participants were asked to decide whether a picture of a human face was either emotional (showing any emotion, such as happiness, fear or anger) or neutral (no obvious show of emotion) by pressing one of two buttons. The faces were black-and-white photographs of emotional and neutral faces, taken from the Karolinska Directed Emotional Faces set (KDEF)[21]. All faces were 400 × 543 pixel arrays centered on an 800 × 600 black screen. All of the faces were of college-age students (equal number of male and female faces) trained to produce neutral and exaggerated emotional expressions. Half of the faces were looking straight ahead with eyes on the viewer, the other half were quarter-turned to the right or left with eyes averted from the viewer. Sixteen of the faces had a neutral expression, and 24 had an exaggerated emotional expression (eight each of happiness, fear and anger). Each face was presented for 3 s with 5–7 s between faces. Participants were asked to press the appropriate button as soon as they had decided whether the face was neutral or was showing any emotion, but to reduce performance anxiety they were not explicitly instructed to respond as quickly as possible. The faces were presented using E-Prime software (version 1.1, Psychology Software Tools), which allowed for the acquisition of both accuracy and response time (ms) for each trial.

Study II: facial recognition task. Participants were asked to perform a recognition task while functional brain images were acquired. For the task, the participants were asked to decide whether a photograph was familiar to them by pressing a button. Ten of the photographs were of the participants' family members or friends, and had been taken by the participant before the session (familiar faces). For the unfamiliar condition, ten photographs of other participants' family members and friends were presented, matched as closely as possible to the participant's photographs for gender, age, facial expression and orientation. Participants were also asked to decide whether an additional 20 photographs of objects were familiar (ten familiar, ten unfamiliar), but results from these conditions are not discussed here. All photographs were isoluminant gray scale 800 × 600 pixel arrays centered on an 800 × 600 screen. The photographs were presented using E-Prime software (version 1.1, Psychology Software Tools), which allowed the acquisition of both accuracy and response time (ms) for each trial.

Eye movements. Eye movements, fixations and pupil diameter were acquired for both studies using an iView system with a remote eye-tracking device (SensoMotoric Instruments, 2001). The acquired eye data was analyzed using the iView software. This system allows us to show eye movement as the gaze position of the pupil over a certain length of time (gaze path) along with the amount of time spent on any given fixation point (gaze fixation). Eye fixations were defined as the amount of continuous time (minimum 50 ms) spent looking within a 20-pixel-diameter region. The total amount of time spent fixating on the face in general, each eye and the mouth region was calculated as the sum of fixations within each of those four predefined regions for each face.

Imaging. Brain MRI images were acquired with a GE Signa 3-T scanner equipped with high-speed gradients and a whole-head transmit-receive quadrature birdcage headcoil (GE Medical Systems). Structural brain images were acquired for anatomical localization of functional activity. For Study I, a three-dimensional T1 SPGR volume was acquired (TE = 8 ms, TR = 35 ms, FOV = 240 × 240 mm, flip angle = 30°, NEX = 1; 256 × 192 matrix, 124 axial slices, slice thickness = 1.1–1.2 mm). For Study II, a three-dimensional T1 inversion recovery volume was acquired (TE = 8.0 ms, TR = 21.0 ms, FOV = 240 × 240 mm, flip angle = 30°, NEX = 1; 256 × 256 matrix, 124 axial slices, slice thickness = 1.1–1.2 mm). After the anatomical images were collected, functional data were collected, using whole-brain echo-planar imaging (EPI). Sagittal acquisition was used to acquire 30 slices per functional volume, with an image thickness of 4 mm and gap of 1 mm. Four hundred and nine functional images were acquired (TE = 30 ms, TR = 2 s, FOV = 240 × 240mm, 64 × 64 matrix). The resulting voxel size was 3.75 × 3.65 × 5 mm. Images of the faces were presented in three different timing conditions, such that some images were presented synchronously with the TR and others were asynchronous with the TR (that is, jittered). These different timing conditions provided an effective time resolution of 1 s.

Image analyses. Differential brain activation maps were generated by comparing activation in the autistic and the control groups voxel-wise using National Institutes of Health Analysis of Functional Neural Images (AFNI) software, version 2.31 (ref. 22). Data processing steps included image reconstruction in conjunction with smoothing in Fourier space with a Hanning window (full width at half maximum = 1 voxel), six-parameter rigid-body motion correction, removal of skull and ghost artifacts, and application of a high-pass temporal Fourier filter that removed frequencies slower than 0.016 Hz. The time series was modeled with a least-squares general linear model (GLM) fit to an ideal hemodynamic response function (γ variate), and the resultant β-weights were converted to percentage signal change. During the GLM fit, the time-to-onset of response was allowed to vary independently for each voxel from 0 to 4 s; the same lag was used for each of the four emotion × orientation conditions in Study I and both the familiar and unfamiliar face conditions in Study II. This variable onset allows for sensitivity to the varying blood perfusion rates across the brain, and fixing the time lag as the same for all conditions ensures that the responses are properly separated and estimated. The resultant percentage signal change maps from the GLM were transformed into the standardized Talairach space through identification of anatomical landmarks on the high-resolution anatomical image[23].

For the within-subject analyses of the effect of eye fixation on brain activation, a stick function was created for each subject with the relative eye

fixation time per trial (amount of time spent fixating on the eye region for a given trial, minus the average eye-fixation time across all trials, divided by 100), as the predictor. The resultant time series was then modeled and extracted using similar techniques, as outlined above.

To identify the group differences in brain regions associated with processing faces, a *t*-test was performed between the groups across all the face conditions in both studies. Additional whole-brain group × emotion (emotion, neutral) × orientation (straight, side) mixed-measures ANOVA was performed for Study I, and group × familiarity (familiar, unfamiliar) mixed-measures ANOVA was performed for Study II. An individual *P*-value threshold = 0.001 and a minimum cluster size of 50 contiguous voxels was used to control for multiple comparisons. For clusters meeting the individual *P*-value and cluster-size threshold combination for the interaction and main effects of interest, the average percentage signal change value was extracted for each condition and participant, and the values entered into simple effects analyses to determine the source of the significant effect.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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