

Gaze-Fixation, Brain Activation, and Amygdala Volume in Unaffected Siblings of Individuals with Autism

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Background: The broad autism phenotype includes subclinical autistic characteristics found to have a higher prevalence in unaffected family members of individuals with autism. These characteristics primarily affect the social aspects of language, communication, and human interaction. The current research focuses on possible neurobehavioral characteristics associated with the broad autism phenotype.

Methods: We used a face-processing task associated with atypical patterns of gaze fixation and brain function in autism while collecting brain functional magnetic resonance imaging (fMRI) and eye tracking in unaffected siblings of individuals with autism.

Results: We found robust differences in gaze fixation and brain function in response to images of human faces in unaffected siblings compared with typically developing control individuals. The siblings' gaze fixations and brain activation patterns during the face processing task were similar to that of the autism group and showed decreased gaze fixation along with diminished fusiform activation compared with the control group. Furthermore, amygdala volume in the siblings was similar to the autism group and was significantly reduced compared with the control group.

Conclusions: Together, these findings provide compelling evidence for differences in social/emotional processing and underlying neural circuitry in siblings of individuals with autism, supporting the notion of unique endophenotypes associated with the broad autism phenotype.

Key Words: Amygdala, autism, child/adolescent psychiatry, cognitive neuroscience, functional imaging, structural imaging

Autism is a pervasive neurodevelopmental disorder associated with a complex oligogenic etiology (Risch et al 1999). It is estimated that as much as 90% of the variance in the etiology of autism is genetic (Bailey et al 1995). Converging evidence suggests that family members of autistic probands share in some of the behavioral and cognitive features of autism (Bailey et al 1995; Koczat et al 2002; Piven 1999). These characteristics tend to be qualitatively similar but less severely expressed in family members of autistic probands compared with the affected individuals (Constantino and Todd 2003; Lainhart et al 2002). This profile of a constellation of subclinical, mild autistic characteristics has been found to have a higher prevalence in family members of autistic probands compared with the general population and has been termed the "broad autism phenotype" (Le Couteur et al 1996; Piven 2001; Piven et al 1997).

Features of the core symptomatology of autism highlight deficits in social and emotional behavior and include diminished gaze fixation, lack of social or emotional reciprocity, and failure to develop age-appropriate peer relationships (Klin et al 2002; Lord et al 1994). Less severe manifestations of these characteristics are defined in the broad autism phenotype as aloofness, rigidity, and diminished social relationships (Piven et al 1997). More recent studies have found that social deficits characteristic of autism are present in the general population, albeit with lower but continuously distributed severity, and that higher scores on these characteristics are associated with higher scores on neuroticism and lower scores on extraversion and agreeableness

(Austin 2005; Constantino and Todd 2003). These autistic-like traits tend to be more prevalent in boys versus girls in the general population and more common in brothers and fathers versus sisters and mothers of autistic probands.

Recent studies have focused on other neurocognitive phenotypes associated with autistic probands and their family members as potential endophenotypes more proximal to the genetic etiology of autism (Koczat et al 2002). Many studies have investigated language and communication deficits in family members of autistic probands with inconsistent and even contradictory results, especially in regard to siblings of autistic probands (Folstein et al 1999; Pilowsky et al 2003). Other studies have found consistent deficits in spatial working memory in both mothers and fathers of autistic probands (Koczat et al 2002). Finally, there is evidence for weak "central coherence" along with a local processing bias in parents (particularly fathers) of autistic probands (Happé et al 2001); parents of autistic probands show real-life preferences in line with these characteristics (Briskman et al 2001) along with lower empathizing and high systematizing as a characteristic of the broader autism phenotype (Baron-Cohen et al 2005). However, these findings were primarily restricted to the parents and did not extend to the siblings of the autistic probands, suggesting a possible developmental component in the neurobehavioral profile of the broader autism phenotype.

More consistent familial effects have been reported in regard to specific deficits in social and communicative behavior. Studies of recognition of emotional facial expressions in nonautistic parents and siblings of individuals with autism show poorer performance in families with multiple affected individuals (Bishop et al 2004; Bolte and Poustka 2003; Constantino et al 2006). Bishop et al (2004) reported deficits in parents of individuals with autism using the Autism-Spectrum Quotient; these deficits were specific to social skills and communicative behavior. Constantino et al (2006) identified deficits in nonautistic siblings of individuals with autism, particularly those from multiplex families, using the Social Responsiveness Scale. These findings suggest that the broad autism phenotype, while sparing cognitive and language abilities, primarily affects the social aspects of language, communication, and human interaction.

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A number of studies of brain function have revealed atypical patterns of brain activation in individuals with autism when processing faces. The most consistently reported and largest effect is in the fusiform gyrus (FG), an area that is strongly activated during face processing in typically developing individuals (Puce et al 1995) but is hypoactivated during these tasks in individuals with autism spectrum disorders (Critchley et al 2000; Ogaï et al 2003; Pierce et al 2001; Schultz et al 2000). We also have found fusiform hypoactivation in autism compared with a control group during two independent functional magnetic resonance imaging (fMRI) studies using unique face processing tasks (Dalton et al 2005). In addition, we found increased activation in a small region of amygdala in the autism compared with the control group during the face processing tasks. Importantly, activation in both the fusiform gyrus and amygdala was strongly positively correlated with the amount of time spent fixating the eyes (measured with online eye tracking in the scanner) in the autism 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 autism individuals was strongly and positively associated with amygdala activation across both studies, suggesting a heightened emotional response associated with gaze fixation in autism.

Complementing this corpus of literature on behavioral and functional imaging differences in individuals with autism is a growing catalog of differences in brain structure from both postmortem (Bauman and Kemper 1985; Casanova et al 2002) and in vivo imaging studies (Brambilla et al 2003). Evidence from lesion and imaging reports points to abnormal development of the amygdala in autism (Amaral et al 2003; Baron-Cohen et al 2000). While two studies have shown enlarged amygdala in young boys with autism (Schumann et al 2004; Sparks et al 2002), findings in adults with autism are converging on the finding of decreased amygdala volume relative to typically developing (Aylward et al 1999; Pierce et al 2001; Rojas et al 2004) or less affected individuals with Asperger syndrome (Haznedar et al 2000); one study reports enlarged amygdala volume in adults identified as having high-functioning autism (Howard et al 2000). Only one published study has evaluated amygdala volume exclusively in adolescents, with no significant difference from control subjects (Schumann et al 2004). Previous work from our laboratory has shown normal amygdala volume in less affected adolescents and young adults with autism but smaller amygdalae in individuals with more severe social deficits (Nacewicz et al, in press). In summary, individuals with very mild autistic deficits tend to exhibit normal to enlarged amygdalae during adolescence and young adulthood, while those with more substantial impairment in social behavior have smaller amygdalae than do typically developing control subjects.

It is the goal of this research to investigate possible behavioral and brain functional and anatomical differences in unaffected siblings of autistic probands using a face processing task associated with robust and atypical patterns of gaze fixation and brain function in autism as described above. It is predicted that siblings may share key characteristics of their autistic probands and display a similar behavioral profile during a face-processing task, such as diminished gaze fixation, consistent with the mild social deficits associated with the broad autism phenotype. Furthermore, to the extent that the siblings show similar behavioral patterns during the face-processing task, we predict similar, albeit more moderate differences in brain activation in the siblings compared with the typically developing control group.

Methods and Materials

Subjects

Subsets of participants were drawn from larger groups of control ($n = 19$), autism ($n = 21$), and sibling ($n = 12$) groups for the behavioral and brain functional analyses and for the brain structural analyses based on methodological restriction (e.g., male subjects only for the anatomical analyses) and/or quality of the data (e.g., good functional and/or structural images) and matched as closely as possible on age and intelligence quotient (IQ) across groups. Participants in these subsets overlapped by 78% for the control and sibling groups and 44% for the autism group. Results from a subset of the control and autism groups have been previously published (Dalton et al 2005), so results presented here focus on comparisons with the sibling group. All participants were screened for physical and psychological disorders via parental and/or self-report by explicitly asking if the participant had any physical disabilities, involuntary motor disorders, psychological diagnoses/disorders, or heightened sensitivity to touch or noise. All participants were also asked if they were currently on any prescription or over-the-counter medications. None of the control participants reported any physical or psychological disorders nor did they report being on any psychotropic medications. None of the unaffected siblings reported any physical or clinically diagnosed autism spectrum disorders. Two of the unaffected siblings reported being on prescription medication (one on Celexa and the other on Depakote). None of the other unaffected siblings reported being on any psychotropic medications. All analyses were performed twice, with one analysis including the two siblings on prescription medication and one analysis excluding these two individuals. All group results remained statistically significant and in the same direction both with and without the two siblings on prescription medication included in the analyses; in fact, a number of results became stronger with these individuals excluded.

Behavioral and Brain Functional Analyses. Eight male subjects and four female subjects with a current diagnosis of autism ($n = 4$) or Asperger syndrome ($n = 8$) based on DSM-IV criteria were recruited for this study via newsletters distributed by Wisconsin area autism societies. Diagnoses were confirmed with the Autism Diagnostic Interview-Revised (ADI-R) ($n = 10$) (Lord et al 1994) and/or clinical interview administered by a trained and certified psychologist at the Waisman Center ($n = 2$). Four of the individuals that had the ADI-R met both lifetime (i.e., ever) and current criteria for autism. Six individuals met lifetime criteria for autism on the ADI-R, but at the time of the scan, they met DSM-IV (current) criteria for Asperger syndrome. The remaining two individuals met DSM-IV criteria for Asperger syndrome at the time of the scan. Ten male and two female healthy, typically developing individuals with no current or past psychological diagnoses served as comparison individuals. Finally, seven male and three female unaffected siblings of the autism group also participated as a sibling comparison group. None of the siblings had a diagnosis of autism or autism spectrum disorder. Verbal, nonverbal, and general IQ were assessed for all groups using the Wide Range Intelligence Test (WRIT). None of the groups differed significantly in age or verbal, visual, or general IQ (Table 1). Parents of all participants filled out the Social Communication Questionnaire (SCQ), a prescreen for the ADI-R, with higher scores indexing a greater probability of autism (Table 1). The autism group scored significantly higher than both the control group [$t(1,22) = 7.54, p < .00001$] and the sibling group [$t(1,20) = 6.02, p = .000007$]. No individual in either the control group or

Table 1. Participants' Age; Verbal, Visual, and General IQ; and Score on the Social Communication Questionnaire

	Group					
	Control (<i>n</i> = 12)		Autism (<i>n</i> = 12)		Sibling (<i>n</i> = 10)	
	M	SD	M	SD	M	SD
Age (years)	14.16	3.62	14.40	4.77	13.10	3.03
Range	10–21		8–25		8–18	
Verbal IQ	112.75	10.2	103.9	17.68	115.5	16.43
Range	93–130		82–131		93–135	
Visual IQ	115.75	10.2	113.7	14.13	122.3	12.05
Range	101–136		88–139		96–141	
General IQ	115.8	8.28	110.0	15.7	121.7	14.9
Range	98–128		88–132		94–143	
SCQ	.72	.64	20.16	8.93	2.3	2.98
Range	0–2		10–36		0–9	

IQ, intelligence quotient; SCQ, Social Communication Questionnaire.

the sibling group scored above the autism cutoff of 15 on the SCQ. A breakdown of subclinical scores in the three domains of the SCQ (Social/Emotional, Communication/Language, and Restricted/Repetitive Behaviors) for the unaffected siblings is presented in Table 2 for descriptive purposes.

Brain Anatomical Analyses. Previous studies of large samples of typically developing children and adolescents have shown sex differences in amygdala volume out of proportion to differences in brain volume (Giedd et al 1997), and similar discrepancies have been found in children with autism (Sparks et al 2002). Volumetric analyses were, therefore, limited to only male subjects. Nine male, nonautistic siblings with no known psychiatric disorders or prescription medications for autism (see note above) (mean + SD: age 12.9 + 3.2 years, IQ 119.2 + 14) were strictly matched on age and IQ to nine typically developing control subjects (age 12.7 + 3.9 years, IQ 119.6 + 18) and matched on age to nine male subjects with autism spectrum disorders (age 12.9 + 3.0 years, IQ 94.9 + 27; autism *n* = 5, Asperger syndrome *n* = 4).

Procedure

Participants first read and signed a consent form that covered all aspects of the study and magnetic resonance imaging (MRI) procedures. Consent was obtained from parents or legal guardians of all participants younger than 18 years of age. Additional adolescent consent or child assent was obtained from participants under 18 years of age. All participants and parents were prescreened for MRI compatibility prior to 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 recognition task and were shown examples of the stimuli. All scans started with approximately 20 minutes of anatomical scans followed by the 7-minute functional scan, during which the facial recognition task was performed. The total time in the scanner was approximately 30 to 35 minutes and the total time for the full session was approximately 1.5 hours. All participants received \$20 for the simulation session and \$30 for the actual scans for a total of \$50. Some of the participants also received an additional \$30 for an optional diffusion tensor imaging (DTI) scan at the end of the session.

Facial Recognition Task

Participants were asked to perform a recognition task while functional brain images were being acquired. For the task, the participants were asked to decide if a photograph was familiar to them by pressing a button. Ten of the photographs were of the participant's family members or friends, which were taken by the participant prior to the session (familiar faces). For the unfamiliar condition, 10 photographs were presented of other participants' family members and friends, which were matched as closely as possible to the participant's photographs on gender, age, facial expression, and orientation (Dalton et al 2005 has exemplar photographs). Participants were also asked to decide if an additional 20 nonface photographs were familiar (10 familiar, 10 unfamiliar), but results from these conditions are not discussed here due to the heterogeneous content of the nonface photographs. Written consent for the use of personal photographs as matched "unfamiliar" photos for other participants was obtained from participants prior to incorporating their photographs into the unfamiliar stimulus set. All photographs were isoluminant gray scale 800 x 600 pixel arrays centered on an 800 x 600 screen. The photographs were presented using E-Prime software (version 1.1, Psychology Software Tools, Inc., Pittsburgh, Pennsylvania).

Eye Movements

Eye movements, fixations, and pupil diameter were acquired using an iView system that uses a small liquid crystal display (LCD) camera with an infrared light for remote eye tracking (SensoMotoric Instruments, Boston, Massachusetts). The acquired eye data were analyzed using the iView software. This system allows us to display 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 milliseconds) spent looking within a 20-pixel diameter region. The total amount of time spent fixating 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 Parameters

Brain MRI images were acquired with a GE Signa 3 Tesla scanner equipped with high-speed gradients and a whole-head transmit-receive quadrature birdcage headcoil (GE Medical Systems, Waukesha, Wisconsin). Structural brain images were acquired for anatomical localization of functional activity. A three-

Table 2. Breakdown of Subdomain Scores on the Social Communication Questionnaire for the Unaffected Siblings

Subject	SCQ Score on Subdomain			Total
	Social/Emotional	Communication/Language	Restricted/Repetitive	
1	0	0	1	1
2	6	3	0	9
3	0	0	0	0
4	1	0	2	3
5	0	0	0	0
6	3	0	3	6
7	0	0	0	0
8	0	0	1	1
9	1	0	1	2
10	0	0	1	1

dimensional (3-D) T1-weighted, inversion-recovery prepped, fast gradient echo volume was acquired (inversion time [TI] = 600 milliseconds, repetition time [TR] = 9.0 milliseconds, echo time [TE] = 1.2 milliseconds, field of view [FOV] = 240 x 240 mm, flip angle = 10°, number of excitations [NEX] = 1, 256 x 192 matrix, 124 axial slices, slice thickness = 1.1–1.2 mm). After the anatomical images were collected, functional data were collected, using whole-brain echoplanar imaging (EPI). Sagittal acquisition was used to acquire 30 slices per functional volume, with an image thickness of 4 mm and 1-mm gap. Four hundred nine functional images were acquired (TE = 30 milliseconds, TR = 2 seconds, FOV = 240 x 240 mm, 64 x 64 matrix). The resulting voxel size was 3.75 x 3.75 x 5 mm. Images of the faces were presented in three different timing conditions, such that some images were presented synchronously with the TR (TR = 2 seconds) and others were asynchronous with the TR (i.e., jittered). These different timing conditions provided an effective time resolution of 1 second.

Functional Image Analyses

Differential brain activation maps were generated by comparing activation in the autism, control, and sibling groups in a voxel-wise manner using Analysis of Functional Neural Images (AFNI) version 2.31 software (Medical College of Wisconsin, Milwaukee, Wisconsin) (Cox 1996). Data processing steps included image reconstruction in conjunction with smoothing in Fourier space via 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 .033 Hz. The time series was modeled with a least-squares general linear model (GLM) fit to an ideal hemodynamic response function (gamma variate), and the resultant beta 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 seconds; the same lag was used for both the familiar and unfamiliar face conditions. This variable onset allows for sensitivity to the varying blood perfusion rates across the brain, while 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 via identification of anatomical landmarks on the high-resolution inversion recovery (IR) scan (Talairach and Tournoux 1988).

For the within-subjects 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 on the trial 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 faces. Additional whole-brain group by familiarity (familiar, unfamiliar) mixed measures analyses of variance (ANOVAs) were performed. An individual *p*-value threshold of *p* = .005 and a minimum cluster size of 50 contiguous voxels were used to control for multiple comparisons. A less conservative threshold of *p* = .05 was used for the *a priori* region of interest in the amygdala. 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 traditional simple effects analyses to determine the source of the significant effect. To test laterality in regions that were significant in either the right or left hemisphere, significant clusters of interest were dilated out 20% and used to identify the homologous cluster in the opposite hemisphere. Average percentage signal change values from the dilated cluster on the same and homologous sides were extracted, and the values were entered into hemisphere by stimulus by valence ANOVAs.

Structural Image Analyses

Anatomical T1- and T2-weighted images were reconstructed and hand registered to anterior commissure-posterior commissure (AC-PC) space and both entered into a multispectral segmentation/bias correction algorithm (FSL, <http://www.fmrib.ox.ac.uk/fsl/>) (Smith et al 2004) to smooth inhomogeneities in the IR-prepped images. All amygdala tracing was carried out by blind raters using a technique described in detail elsewhere (Nacewicz et al, in press). Briefly, the procedure involved inspection of all three cardinal planes, simultaneously displayed in Spmalyze (http://brainimaging.waisman.wisc.edu/~oakes/spam/spam_frames.htm). Brain images were oriented to the "pathological plane" (Convit et al 1999) and contrast adjusted according to intensity histograms. The optic tract, optic radiations, hippocampus, and inferior horn of the lateral ventricle were used to define the posterior border; temporal lobe white matter, cerebrospinal fluid (CSF), the anterior commissure, and entorhinal cortex were used to define the anterior boundaries. Whole-brain masks were estimated with an automated, threshold-based connected pixel search. Skull, eyes, brainstem, and cerebellum were removed by hand. This technique was found to be highly reliable (Nacewicz et al, in press), with an interrater intraclass correlation of .95 for volume and a high spatial reliability (mean intersection/union = .84). Amygdala data for each group met criteria for normality by the Kolmogorov-Smirnov and Lilliefors tests.

Results

Task Accuracy and Judgment Time

Response time and accuracy were not recorded for one of the control individuals and two of the individuals with autism due to mechanical error and, therefore, data from these individuals are not included in this analysis. These individuals were retained in all other analyses. The autism group performed the task with 74% accuracy for the facial photographs and 91% accuracy for the nonfacial photographs, while the control and sibling groups performed the task at near ceiling for both the facial photographs (control = 97%; sibling = 94%) and nonfacial photographs (control = 98%; sibling = 97%). The autism group's accuracy for the facial photographs was significantly below that for the control [$t(1,29) = 2.88, p = .009$] and sibling [$t(1,16) = 2.15, p = .04$] groups. However, there were no group differences in accuracy for the nonfacial photographs. There were no group differences in judgment time to either the people or the objects. There were no significant correlations between accuracy or judgment time and age, IQ, or SCQ within any of the groups.

Gaze Fixations

We calculated the duration of time each group spent fixating the face in general and the eyes and mouth specifically. As predicted, the autism group spent significantly less time on average per trial fixating the eyes (mean [M] = 222.39 milliseconds, SD = 116.98) compared with the control group [M = 348.97 milliseconds, SD = 161.17; $t(1,22) = 2.20, p = .038$].

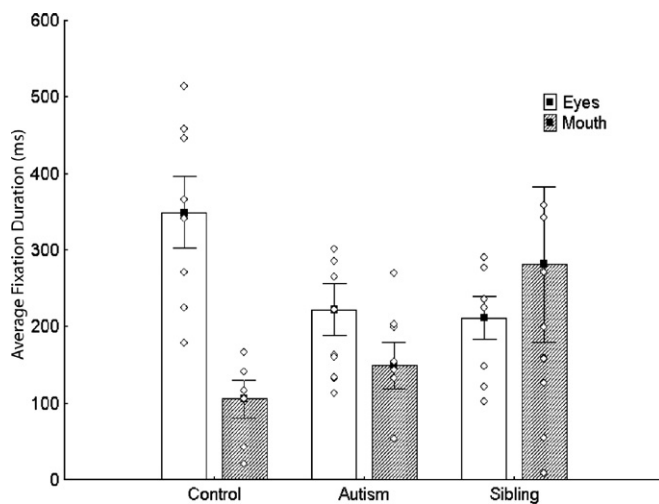


Figure 1. Average duration of fixation (milliseconds) on the mouth and eye region broken down by group. Error bars index the SEM. Raw data points (open diamonds) are plotted for each individual within a group.

Interestingly, the sibling group also showed a significantly less amount of eye fixation time ($M = 211.26$ milliseconds, $SD = 88.15$) compared with the control group [$t(1,20) = 2.41, p = .025$] (Figure 1). There was no significant group difference in eye fixation time between the autism and sibling groups. While there was a tendency for the sibling group to spend more time fixating the mouth ($M = 281.5$ milliseconds, $SD = 321.22$) compared with both the control group ($M = 105.2$ milliseconds, $SD = 84.47$) and the autism group ($M = 149.8$ milliseconds, $SD = 105.3$), these differences were not significant [sibling-control, $t(1,20) = 1.83, p = .08$; sibling-autism, $t(1,20) = 1.34, p = .19$] (Figure 1). Finally, there were no significant group differences in amount of time spent fixating the face in general (control, $M = 1725.3$ milliseconds, $SD = 609.37$; autism, $M = 1508.6$ milliseconds, $SD = 744.14$; sibling, $M = 1631.1$ milliseconds, $SD = 587.04$).

Neither the main effect for familiarity nor the group \times familiarity interaction was significant for the amount of time fixating either the mouth or eyes or the face in general. Again, there were no significant correlations between gaze fixation time and age, IQ, or SCQ within any of the groups.

Brain Activation Maps

Control minus sibling and autism minus sibling activation maps were derived across all of the facial photographs. Group main effects were found in right posterior fusiform [$F(2,31) = 33.72, p < .000001$], left fusiform [$F(2,31) = 5.57, p = .008$], and right amygdala [$F(2,30) = 4.25, p = .02$]. The control group evidenced significantly greater activation to the facial photographs in the right posterior fusiform gyrus compared with the sibling group [$t(1,20) = 6.31, p = .000004$] and autism group [$t(1,22) = 7.08, p < .000001$] (Figure 2A). In contrast, there was no significant difference in left fusiform activation between the control and sibling groups [$t(1,20) = 1.21, p = .23$]. However, the autism group showed significantly less activation in the left fusiform gyrus compared with both the control group [$t(1,22) = 3.22, p = .003$] and sibling group [$t(1,20) = 2.57, p = .02$] (Figure 2B). Conversely, a region in the right amygdala was associated with significantly greater activation in the autism group compared with the control group [$t(1,21) = 2.73, p = .01$] (Figure 2C). While the autism group showed a tendency toward greater activation in this region compared with the sibling group, it did

not reach significance [$t(1,20) = 1.86, p = .07$]. None of the within-subjects familiar main effects or group \times familiar interactions reached significance in any of these clusters. Activation in these clusters was not significantly associated with age, IQ, or SCQ within any of the groups.

Brain Activation and Gaze Fixation

While both the sibling and autism groups averaged less time fixating the eyes compared with the control group, there was marked variability in the amount of looking time on the eyes in these groups. We took advantage of this variability by examining across subjects whether time spent fixating the eye region of the face predicted activation in bilateral fusiform gyrus and amygdala, the three key regions identified in the between-group analyses. We regressed the amount of time spent fixating the eyes on brain activation for the sibling group in a voxel-wise fashion (Dalton et al 2005 has control and autism regressions). Significant clusters of activation were extracted using a conservative threshold ($\alpha = .01$). Activation in clusters in the right

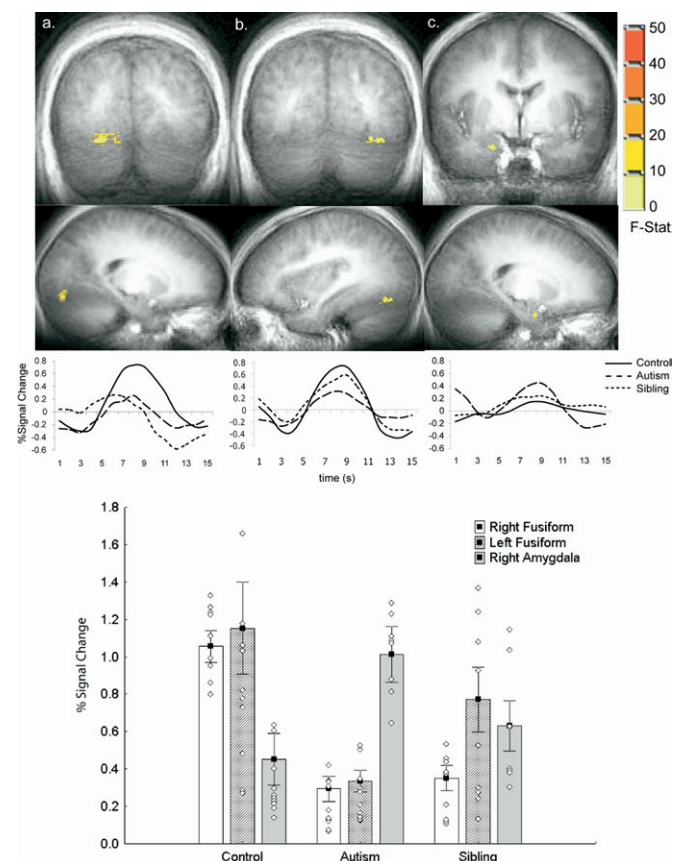


Figure 2. Brain clusters with significant group main effects in brain activation (percent signal change) across all faces images. (A) Right fusiform gyrus: $x = 22, y = -78, z = -9$; 421 voxels (3368 mm³); $F(2,31) = 32.72, p < .000001$; (B) Left fusiform gyrus: $x = -33, y = -65, z = -11$; 254 voxels (2032 mm³); $F(2,31) = 5.57, p = .008$; (C) Right amygdala: $x = 19, y = -2, z = -21$; 78 voxels (624 mm³); $F(2,29) = 4.25, p = .02$. Images are presented in radiological convention such that the right hemisphere is displayed on left of each coronal image. The clusters are color-coded based on the group main effect F -statistic values. Averaged MR time series are presented below each cluster for the 14 seconds poststimulus onset. A bar graph depicting percent signal change means and standard errors for each cluster broken down by group, overlaid with individual raw data points (open diamonds), is presented below the averaged MR time series. MR, magnetic resonance.

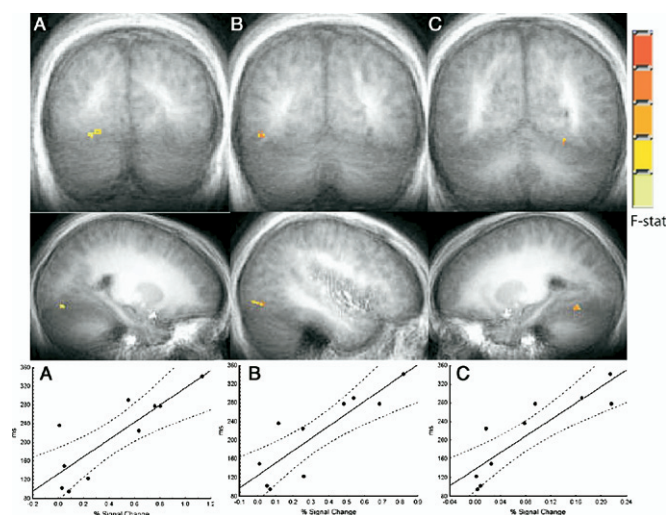


Figure 3. Brain activation clusters associated with average eye fixation time for the sibling group. **(A)** Right posterior fusiform gyrus: $x = 23, y = -77, z = -5$; 66 voxels (528 mm^3); $r = .84, p = .003$. **(B)** Right posterior lateral fusiform gyrus: $x = 41, y = -70, z = -4$; 62 voxels (496 mm^3); $r = .86, p = .002$. **(C)** Left fusiform gyrus: $x = -24, y = -60, z = -10$; 65 voxels (520 mm^3); $r = .88, p = .001$. Images are presented in radiological convention such that the right hemisphere is displayed on left of each coronal image. Scatter plots of the relationship between average eye fixation time (milliseconds) and percent signal change in a given cluster are presented for the sibling group under each cluster.

and left fusiform gyrus was strongly and positively associated with the amount of time spent fixating the eyes for the sibling group (Figure 3A-C). The amount of variance in activation accounted for by eye fixations in the sibling group was 70% in the right posterior fusiform, 74% in the right lateral fusiform, and 77% in the left fusiform. The relationship between eye fixation and fusiform activation in the sibling group mirrors that reported for the autism group in our previously published work (Dalton et al 2005). Furthermore, a similar pattern of a positive relationship between eye fixation and left fusiform activation was found here in the control group ($r = .77, p = .003$) using a less conservative significance threshold ($\alpha = .05$). For both the autism and control groups, approximately 60% of the variance in regions of the fusiform gyrus can be accounted for by eye fixations.

Amygdala Activation as a Function of Gaze Fixation

Post hoc analyses, testing whether amount of gaze fixation within a given trial predicted amygdala activation during that trial, were performed within subjects by extracting brain function using a stick function of eye fixation time per trial for each individual. Group t tests were then performed focusing on the region of the amygdala using similar clustering techniques as described above with a less conservative a priori α of .05. Consistent with our previously reported results, amygdala activation was strongly and positively predicted by amount of eye fixation within the autism individuals [single-sample t test, $t(1,11) = 4.83, p = .0005$] but not within the control individuals [single-sample t test, $t(1,11) = -.88, p = .40$]. Similar to the control group, amygdala activation was not predicted by amount of eye fixation within the siblings [single-sample t test, $t(1,8) = 1.02, p = .33$]. The autism group showed significantly greater eye fixation related amygdala activation compared with both the comparison group [$t(1,22) = 2.14, p = .04$] and sibling group [$t(1,19) = 2.82, p = .01$] (Figure 4). The difference between eye fixation

related amygdala activation in the control versus sibling groups was not significant [$t(1,19) = -.96, p = .35$]. No group differences were found in any other regions using a more conservative whole-brain α of .001.

Amygdala Volume

For the full sample ($N = 27$), mean volumes (\pm SD) were $1833 \pm 175 \text{ mm}^3$ and $1827 \pm 172 \text{ mm}^3$ for left and right amygdalae, respectively. As we have previously reported (Nacewicz et al, in press), mean amygdala volume for the autism group ($1766 \pm 125 \text{ mm}^3$) was significantly decreased from that of typically developing individuals [$1944 \pm 166 \text{ mm}^3$; $t(1,16) = 2.58, p = .02$]. Although the nonautistic siblings did not express the full spectrum of autistic behavior and had high IQs, amygdala volume in the sibling group showed a near significant decrease compared with control subjects [$1781 \pm 179 \text{ mm}^3$; $t(1,16) = 2.00, p = .06$] and was similar to their fully affected brothers [$t(1,16) = .21, p = .83$]. After statistical correction for age and brain volume by multiple regression, amygdala volume in nonautistic siblings was significantly decreased from control volumes [$t(1,16) = 2.24, p = .04$] (Figure 5).

Discussion

These findings reveal robust differences in gaze fixation and brain function in response to images of human faces in unaffected siblings of individuals with autism. As with eye fixation, unaffected siblings also show abnormalities in amygdala volume that are comparable with individuals with the narrow autism phenotype. Both the sibling and autism groups spent significantly less time than the control group fixating the eye region in response to naturalistic photographs of both familiar and unfamiliar human faces. Both the sibling and autism groups also displayed hypoactivation in areas of the right fusiform gyrus compared with the control group in response to the familiar and unfamiliar faces. While this effect was bilateral for the autism group, it was restricted to the right hemisphere in the sibling group. Both the sibling and control groups showed greater activation in the left fusiform gyrus compared with the autism group. Furthermore, eye fixation time was strongly and positively associated with activation in regions of the right and left fusiform in the sibling group, similar to that found in a larger

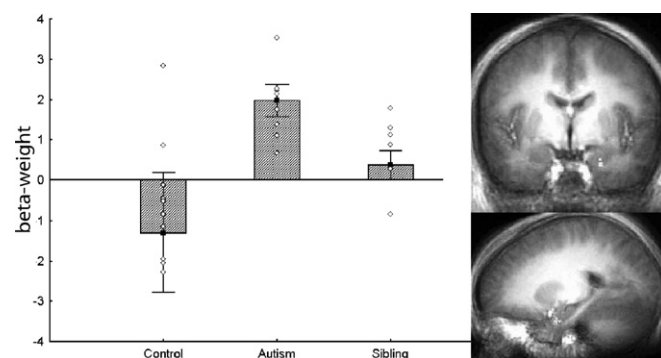


Figure 4. A cluster in the left amygdala associated with group differences in activation as a function of amount of eye fixation within subjects. Left amygdala: $x = 22, y = -3, z = -22$, 55 voxels (440 mm^3). A bar graph depicting the β -weight derived for each group using average eye fixation to predict brain activation is presented to the right of the brain images. Error bars index the SEM. Raw data points (open diamonds) are plotted for each individual within a group.

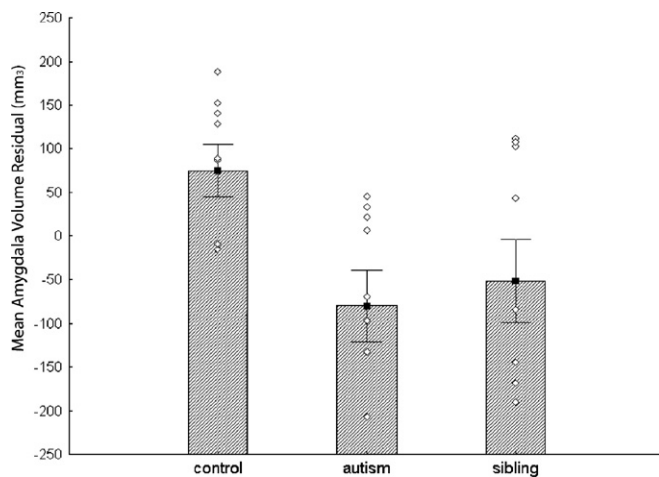


Figure 5. Average amygdala volume (mm^3), corrected for age and total brain volume, across the control, autism, and sibling groups. Error bars index the SEM. Raw data points (open diamonds) are plotted for each individual within a group.

autism sample (Dalton et al 2005). Importantly, this pattern of a positive relationship between eye fixation and fusiform activation was also found (at a lower threshold) for the control subjects.

These findings suggest that the hypoactivation in the right fusiform gyrus during face processing in the sibling group may be a function of how the siblings scan the face rather than a fundamental abnormality in the fusiform region. This interpretation is also supported by the finding of no difference in left fusiform activation in the sibling group versus typically developing control group, suggesting that the sibling group is using a similar yet less extensive neural network to process faces compared with the typically developing control subjects. Together, these findings suggest an intermediate pattern of reduced right fusiform along with intact left fusiform activation in the siblings during face processing, which may be accounted for by differences in gaze fixation rather than a unique neural circuitry for face processing associated with autism and/or the broad autism spectrum. Interestingly, the sibling group did not show enhanced amygdala activation compared with the control group, as reported here and in a larger autism sample (Dalton et al 2005). Similarly, eye fixations were not predictive of amygdala activation for the sibling group in contrast to that found for the autism group. This suggests that the sibling group is not showing a heightened activation in affective neural circuitry in response to human faces compared with the autism group. Unlike the autism group, it appears that eye fixations within the sibling group are not necessarily associated with negatively valenced overarousal mediated by activation in limbic regions such as the amygdala. This suggests that the neural substrates underlying the diminished gaze fixation in the unaffected siblings are not dependent on amygdala activation. However, the sibling group again evidenced an intermediate pattern of amygdala activation both to the faces in general and specifically as a function of eye fixation compared with the autism and typically developing groups. Larger samples are required to resolve whether the small differences seen here in amygdala activation between the sibling and control groups are significant and reliable.

In contrast to the amygdala functional results, the nonautistic siblings did evidence a significant reduction in amygdala volume

from control values that was nearly identical to that in the autism group (Nacewicz et al, in press). Because the sibling group was matched in age and IQ to the typically developing control group, these differences in amygdala volume cannot be attributed to group developmental differences or mental retardation. This finding is in contrast to that of normal amygdala volume in parents of autistic probands (Rojas et al 2004). One major difference from our study is that more than half the parents in the Rojas et al (2004) study were female. Differences in amygdala volume were less apparent in female subjects with even the narrow phenotype of autism (Sparks et al 2002) and female family members seem to be less affected by the broader phenotype (Constantino and Todd 2003); this could explain these discrepant findings. Future studies may include statistically meaningful groups of both male and female siblings to allow comparisons by sex. Given recent findings of genetic linkages found only in families without affected female members (Cantor et al 2005; Stone et al 2004; Sutcliffe et al 2005), it would also be interesting to compare volumetric differences in families with only affected male members, as in this study, to those with affected female members.

Taken together, the abnormal eye fixation and accompanying amygdala abnormality in nonautistic siblings, combined with the finding that differences in eye fixation are related to differences in amygdala volume between individuals with autism (Nacewicz et al, in press), suggest that eye fixation may be a useful quantitative trait (either behavioral or phenotypic) to determine the genetic and environmental contributions to autistic social deficits.

One of the limitations of the current study is that we did not directly measure characteristics of the broad autism phenotype in our sibling sample (Austin 2005). While none of the siblings met criteria for the narrow autism phenotype using a standardized autism prescreen checklist, they may have evidenced more subtle differences in social and emotional processes associated with the broad autism phenotype (Constantino et al 2006). Follow-up research should focus on the relations between quantitative behavioral traits and brain function and structure on the one hand and severity of autistic-like characteristics in unaffected siblings of autistic probands on the other hand. The generalizability of these results is also potentially limited by the small sample sizes and relatively high IQs of all three subject groups. Furthermore, while the current study demonstrates adequate power to detect significant differences among the control, autism, and sibling groups, the specificity of these findings to autism families in general warrants further investigation.

This study provides evidence for differences in social/emotional processing and underlying neural circuitry in first-degree relatives of individuals with autism. While this study provides support for a broad autism phenotype, particularly in regard to social/emotional characteristics, the underlying genetic progenitor of this phenotype or subphenotypes and if and how they relate to the narrow autism phenotype is unknown (Kates et al 2004; Tager-Flusberg and Joseph 2003). This and most fMRI studies lack the statistical power to model differential genetic versus environmental contributions to the broad versus narrow autism phenotype. However, an understanding of the neuroanatomical and neurofunctional profiles associated with the broad autism phenotype holds tremendous promise in helping to elucidate the relationship between genes, brain, and behavior both from a clinical perspective of autism as well as a more

general epidemiologic perspective of social and emotional development.

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