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fMRI of parents of children with Asperger Syndrome: A pilot study

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Abstract

Background: People with autism or Asperger Syndrome (AS) show altered patterns of brain activity during visual search and emotion recognition tasks. Autism and AS are genetic conditions and parents may show the 'broader autism phenotype.'

Aims: (1) To test if parents of children with AS show atypical brain activity during a visual search and an empathy task; (2) to test for sex differences during these tasks at the neural level; (3) to test if parents of children with autism are hyper-masculinized, as might be predicted by the 'extreme male brain' theory.

Method: We used fMRI during a visual search task (the Embedded Figures Test (EFT)) and an emotion recognition test (the 'Reading the Mind in the Eyes' (or Eyes) test).

Sample: Twelve parents of children with AS, vs. 12 sex-matched controls.

Design: Factorial analysis was used to map main effects of sex, group (parents vs. controls), and sex × group interaction on brain function. An ordinal ANOVA also tested for regions of brain activity where females > males > fathers = mothers, to test for parental hyper-masculinization.

Results on EFT task: Female controls showed more activity in extrastriate cortex than male controls, and both mothers and fathers showed even less activity in this area than sex-matched controls. There were no differences in group activation between mothers and fathers of children with AS. The ordinal ANOVA identified two specific regions in visual cortex (right and left, respectively) that showed the pattern Females > Males > Fathers = Mothers, both in BA 19.

Results on Eyes task: Male controls showed more activity in the left inferior frontal gyrus than female controls, and both mothers and fathers showed even more activity in this area compared to sex-matched controls. Female controls showed greater bilateral inferior frontal activation than males. This was not seen when comparing mothers to males, or mothers to fathers. The ordinal ANOVA identified two specific regions that showed the pattern Females > Males > Mothers = Fathers: left medial temporal gyrus (BA 21) and left dorsolateral prefrontal cortex (BA 44).

Conclusions: Parents of children with AS show atypical brain function during both visual search and emotion recognition, in the direction of hyper-masculinization of the brain. Because of the small sample size, and lack of age-matching between parents and controls, such results constitute a pilot study that needs replicating with larger samples.

Keywords: Autism; Asperger Syndrome; fMRI parents; Sex differences

1. Introduction

The genetic theory of autism (Bailey, Bolton, & Rutter, 1998; Bailey et al., 1995) proposes that autism is strongly heritable, and that first-degree relatives of children with

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autism possess the 'broader autism phenotype' (BAP). Heritability of autism has been estimated to be above 90%, and the BAP has been confirmed among parents of children with autism. For example, parents of children with autism score higher on some of the subscales of the Autism Spectrum Quotient (AQ), compared to controls (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Bishop et al., 2004).

To date there have been no fMRI studies of parents of children with autism or AS to test for the BAP at the neural level. Since people with autism and AS show atypical brain function during fMRI, using tasks such as the Embedded Figures Test (EFT) (a visual search task) (Ring et al., 1999) and the 'Reading the Mind in the Eyes' (or Eyes) task (an advanced emotion recognition task) (Baron-Cohen et al., 1999), it is important to see if on the same tasks, first-degree relatives of children with autism or AS also show atypical brain function. Such a result would implicate these measures as assays for neurocognitive endophenotypes.

The use of the EFT and Eyes tests during fMRI is also relevant to the question of sex differences in brain function, since males in the general population often score higher than females on the EFT (Jolliffe & Baron-Cohen, 1997; Witkin, Dyk, Faterson, Goodenough, & Karp, 1962) and females in the general population often score higher than males on the Eyes test (Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). The study below is a preliminary exploration testing for sex differences in brain function during these two tasks. It is preliminary because only six males are tested, against six females.

1.1. The extreme male brain theory of autism

If sex differences are found, then these tasks also allow a preliminary test of the 'extreme male brain' (EMB) theory of autism (Baron-Cohen, 2002). It has been shown that people with AS are faster than sex-matched controls on the EFT (Jolliffe & Baron-Cohen, 1997), and score below average on the Eyes test. In the study below, we also test if parents of people with AS show such hyper-masculinization. The fact that parents score in a hyper-masculinized direction on both of these tasks in terms of performance is consistent with the EMB theory. Thus, parents of children with autism spectrum conditions (ASC) are faster than sex-matched controls on the EFT, and show lower scores on the Eyes test (Baron-Cohen & Hammer, 1997). In the study reported here we test if parents of children with AS show atypical brain function whilst performing these two tests. A preliminary clue that hyper-masculinization might be found in such parents is that mothers of children with ASC show a masculinized second-to-fourth digit (2D:4D) ratio (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001). Here, we report a pilot test of the EMB theory at the level of the brain. Again, this is a pilot study simply because only six mothers and six fathers are included.

In the earlier behavioural studies, sex differences were detected when the sample size comprised 25 males and 25 females (Baron-Cohen & Hammer, 1997). It is unclear if sex differences in brain activity would be revealed in a relatively small sample as reported below. It is, however, possible that, even if at the behavioural level sex differences in performance are not apparent with just six males and six females, it may be at the neural level that sex differences will be seen. This is because in behavioural studies what is being measured is performance (speed or accuracy) and the effect size may be quite small. At the neural level, sex differences may exist in how the brain approaches the task. If the two sexes (on average) are employing very different neural strategies to solve the tasks, this may be detectable even with small samples. On this view, the power to detect sex differences may be greater using fMRI than when using behavioural measures. The same arguments apply to comparing parents of children with AS with sex-matched controls. Even if performance differences are not seen at the level of behaviour, differences may be detectable at the level of brain function.

1.2. Predictions

In the present study, we tested three predictions: (1) parents of children with autism would show atypical brain activity during both the EFT and the Eyes tasks. (2) Male and female controls would show different patterns of brain activity on each of these tasks. (3) On both of these tasks, parents of children with AS would show hyper-masculinized brain activation.

2. The experiment

2.1. Sample

Twelve adult controls (six males, six females) from the general population and 12 parents (six mothers, six fathers) of children with AS took part. The latter were recruited from the volunteer database held by the Autism Research Centre in Cambridge University. All participants were right-handed and came from a mix of occupations and social classes. All were free of any medication use, and had no history of neurological or psychiatric conditions. All gave written consent to participate in scanning. Exclusion criteria for subject selection were myopia (the tasks involved being able to see a screen without the option of wearing spectacles in the scanner), claustrophobia (the procedure required staying still in the scanner for up to 1 h), and metal implants in the body (due to the force of the magnetic field in MRI). The two groups were equated for socio-economic status (ascertained by years in education and occupation), and IQ (as measured by the Weschsler Abbreviated Scale of Intelligence [WASI]). They were not equated for age, which is why this study can only be considered preliminary.

2.2. Method

We used the Adult EFT (Witkin et al., 1962). This entails computing how a target shape can fit into a larger spatial display (see Fig. 1A). Previous fMRI studies using this test show that whereas the normal brain activates frontal and parietal areas during this task (Manjaly et al., 2003; Ring et al., 1999), the autistic brain shows significantly less frontal activity and significantly more occipital (visual cortical) activity (Ring et al., 1999). The control condition simply involves visual fixation of a blank screen, as used in the Ring et al. (1999) procedure. This is because the process of interest (disembedding a target shape) could occur if any complex shape was presented, even if not instructed to search for a target.

We also used the 'Eyes' task (Baron-Cohen et al., 1997, 2001). This entails judging which of two mental state words best describes what the person in the photo is thinking or feeling, where stimuli only include the eye region of the face (see Fig. 1B for an example). Previous fMRI studies using this test show that whereas the normal brain activates three areas (superior temporal sulcus, left inferior frontal cortex, and amygdala), the autistic brain shows significantly less activity in the inferior frontal cortex and in the amygdala (Baron-Cohen et al., 1999). The control condition in this test entails judging the person's sex from the eyes alone.

For presentation in the context of fMRI, both tests were prepared as blocked periodic activation paradigms.

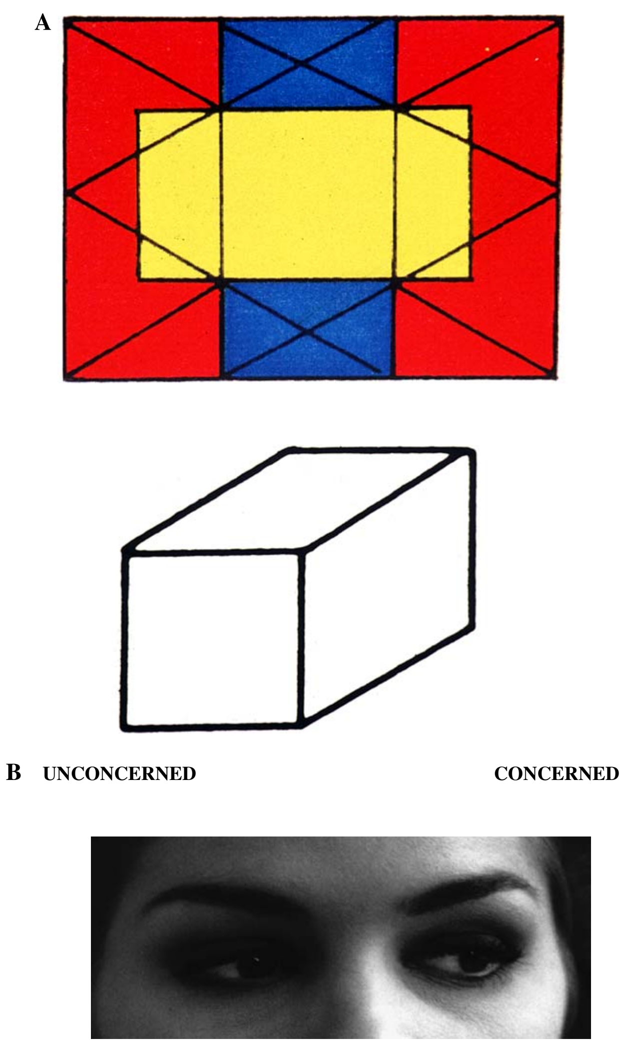

Fig. 1. An example of an item in the (A) EFT and (B) Eyes test.

2.3. The Eyes task

The 'Eyes' test comprised two conditions (mental state judgement vs. sex judgement) and was a blocked AB paradigm. Thirty-second epochs of the Eyes stimuli were alternated across five cycles. The response involved a forced choice between the two words offered (pressing one of two buttons with the right hand to select the right or left word). Correct words were counterbalanced to left and right side.

2.3.1. Sex judgement

Subjects were visually presented with a series of photographs of eyes and asked to indicate by right-handed button press whether each stimulus was a man or a woman. In this first task (A: gender recognition), instructions to subjects were to decide for each stimulus which of two simultaneously presented words ("male" or "female") best described the face. Each stimulus was presented for 5 s and was followed by a 0.75-s interval in which the screen was blank. Stimuli were drawn from 30 faces of women or men. Stimuli were presented 3.5 m from the subject, subtending visual angles of 10° horizontally and 8° vertically.

2.3.2. Mental state judgement

Subjects were presented with exactly the same stimuli but were asked to indicate by button press which of two simultaneously presented words best described the mental state of the photographed person. Thus, the key difference between the two tasks was the type of judgement the subject had to make when viewing the eyes. Subjects were presented with an example of the stimuli before scanning. For this second task, instruction to subjects was to decide for each stimulus which of two simultaneously presented words best described what the person in the photograph was feeling or thinking. This is exactly as described previously (Baron-Cohen et al., 1999).

2.4. Embedded Figures Task

The EFT test also comprised two conditions and again follows exactly the methodology reported elsewhere (Ring et al., 1999). This also used a blocked periodic ABA … design, where 30-s epochs of the EFT stimuli were presented and there were five cycles of AB alternation in total. The experimental task employed the standard adult EFT that has two alternative versions (Form A and B) which do not differ in terms of number of stimuli, or difficulty. Form A was therefore chosen. This consists of a set of 12 test cards, each depicting a different complex design. For each complex design there is a simple shape hidden somewhere within it. In fact, there are just eight different simple shapes, because some of these are common to several complex designs. In the current study, the administration of the EFT was adapted for use in the scanner. Ten complex designs were chosen at random for testing in the scanner. Before subjects went into the scanner, they were given an additional practice item to ensure that they fully understood the task.

The task was introduced with the following instructions: "You will see a colourful design with a simple shape next to it. You have to look for the simple shape in the larger design. The shape you find in the colourful design will always be exactly the same as the simple shape, so it will be the same size and proportions and the same way up." The practice item was then shown. After the practice item, the subject was settled in to the scanner and was reminded of the task using the same instructions with this addition: "As soon as you've found the simple shape, press the button with your right hand index finger. After you have found one example of the simple shape, keep looking in case you can find any other examples, but you don't have to press the button again until the next design comes up. Go as quickly and as accurately as you can."

We used a blocked periodic ABA… design involving repeated contrasts between a baseline condition and an activation condition. Each condition was visually presented for an epoch of 30 s, and the cycle of alternation between epochs was repeated five times in the course of each 5-min experiment. During each activation epoch, subjects were presented with two randomly selected designs from form A of the EFT task. Each complex design was shown for 15 s simultaneously and side by side with a simple shape that the subject was asked to identify in the complex design. Stimuli were presented on a video monitor approximately 8 ft from the subjects' head. During each baseline epoch, the subjects were asked simply to fixate on a blank screen.

The Eyes and EFT tasks were presented in a counterbalanced order, half the subjects taking the Eyes test first and half the subjects taking the EFT test first. There was a short break between tasks but the subjects did not leave the scanner or move position between tasks.

2.5. fMRI data acquisition

Gradient echo echoplanar imaging (EPI) data were acquired on a GE Signa 1.5 T system (General Electric, Milwaukee, WI, USA). A quadrature birdcage headcoil was used for RF transmission and reception. One hundred T2*-weighted images depicting BOLD contrast (Ogawa, Lee, Kay, & Tank, 1990) were acquired over 5 min (for each task) at each of 14 near-axial non-contiguous 7 mm thick planes parallel to the inter-commissural (AC-PC) line: TE 40 ms, TR 3 s, in-plane resolution 7 mm, inter-slice gap 0.7 mm. This EPI dataset provided almost complete brain coverage.

2.6. fMRI data analysis

The data were first realigned to minimize motion-related artefacts and smoothed using a Gaussian filter (FWHM 7.2 mm). Responses to the experimental paradigms were then estimated by general linear modelling using two Poisson kernels ($\lambda = 4$ and 8 s) to model a locally variable hemodynamic response function. At each intracerebral voxel, a goodness-of-fit statistic was computed from the ratio of the sum of squares of deviations of image intensity (from the mean over all time points) due to the model divided by the sum of squares of deviations due to the residuals. This ratio (subsequently called the SSQ ratio) can easily be shown to be functionally equivalent to F under permutation testing as the conversion to F would assume constant factors (degrees of freedom) in the numerator and denominator. Individual SSQ ratio maps were coregistered in the standard space of Talairach and Tournoux (1988) prior to second-level analysis of variance using permutation tests on spatially informed cluster-level statistics for inference (Bullmore, Brammer, et al., 1999; Bullmore, Suckling, et al., 1999; Suckling & Bullmore, 2004).

We tested main effects of group, sex, and group × sex in a two-way ANOVA model and then tested hypothetically predicted rank orders of activation among the four groups by one-way ANOVA with an ordinal factor. For all second-level analyses, the cluster-level threshold for statistical significance $p < .005$ was specified so that the expected number of false positive clusters was less than one per map.

3. Results

3.1. Participants and task performance

The age and IQ of the participants together with the results of their task performance are given in Table 1. Inspection of age of the two groups showed that the parents were older than controls, so in the MRI data analysis age was entered as a covariate. For the EFT, a 2 × 2 ANOVA was performed with group and sex as the between subject factors, and IQ as a covariate. The dependent measure for both tasks was the percent correct score. In the case of the EFT, participants pushed the

Table 1
Group characteristics and task performance (percent scores): Means (*SD*)

Group	Age	IQ	Eyes task (control)	Eyes task (emotions)	EFT
Fathers	39.1 (6.0)	114.8 (4.0)	85.6 (4.6)	80.6 (4.9)	66.7 (26.6)
Mothers	37.3 (5.9)	115.3 (5.5)	84.4 (6.2)	82.2 (6.2)	66.7 (27.3)
Male controls	23.1 (0.6)	120.0 (1.6)	86.7 (3.0)	83.3 (7.6)	76.7 (22.5)
Female controls	21.6 (0.8)	118.0 (4.2)	86.7 (3.0)	87.2 (7.7)	76.7 (19.7)

button whilst in the scanner when they had identified the shape, and this was checked after the scanning session by asking them to trace it out. Both the main effects and interaction were non-significant. Although the parents' score appears lower than controls, it is important to note that this was not a significant difference so they can be regarded as equal to controls. For the Eyes test, a repeated measures ANOVA was conducted with judgement type (sex recognition vs. emotion judgement) as the within subject factor, group (parents vs. controls) and sex (males vs. females) as the between subject factors, and IQ as the covariate. There were no significant main effects or interactions. In a sample size of this magnitude, sex differences in performance would not necessarily be expected. As discussed earlier, absence of significant sex differences in performance in such small samples does not preclude detecting sex differences at the neural level, examined next.

3.2. fMRI results

3.2.1. Factorial analysis

On each task, we used an ANOVA design. This design mirrors the analysis of the performance data. On both tasks, there was a significant main effect of Group, of Sex, and a significant Group by Sex interaction. All comparisons were thresholded at less than one false positive per analysis. All between-group analyses were two-tailed tests. The coordinates quoted are in 'Talairach space' (Talairach & Tournoux, 1988), and in each case represents the 3D cluster centre.

3.2.2. EFT

There was a main effect of Group, with two regions showing greater activity in controls compared to parents. These were both in visual cortex (middle occipital and lingual gyrii) (BA 18). There was also a main effect of Sex, with two regions activated more strongly in females compared to males. These were also in V2 and V3 (BA 18). Finally, there was a significant Group by Sex interaction, again in the region of the middle occipital gyrus (BA 19). These results are shown in Table 2.

3.2.3. Eyes task

There was a main effect of Group, controls activating three regions more than parents, in the mid temporal gyrus (BA 21), and the inferior frontal gyrus (BA 44 and 45). There was also a main effect of Sex, males activating two regions more than females, in the angular gyrus (BA 39) and the dorsolateral prefrontal cortex (BA 9), and females activating two regions more than males, in the inferior frontal gyrus (BA 44, bilaterally). There was also a significant Group by Sex interaction in two regions: The superior temporal gyrus (BA 22) and the inferior frontal gyrus (BA 44). These results are shown in Table 3.

3.2.4. Group comparisons

The results of the group comparisons are shown in Figs. 2 and 3, and in Tables 4 and 5. On the EFT (Fig. 2, rows 1 and 2), typical females showed greater activity than typical males in the left fusiform gyrus, and bilaterally in the extrastriate cortices. There were no regions that were more active in males relative to females. The female–male comparison was similar to the female–mother comparison. In both comparisons, the female controls show more activity bilaterally in extrastriate cortex (BA 18 and 19), relative to the group they are being compared to. This suggests that the mothers have been masculinized. From Fig. 2, one can also see that there are *no* differences between the mothers and fathers. From Fig. 2 we can see the mothers show even *less* activity than typical males in the left-sided primary visual cortices, suggesting the mothers have an extreme male pattern. Fathers showed *less* activity than typical males in the right middle occipital gyrus and particularly in the left fusiform gyrus. This suggests they too have an extreme male pattern.

Group comparisons on the Eyes task are shown in Fig. 3: Regarding normal sex differences, females activated left and right inferior frontal regions (BA 44 and 45) more strongly than males did. Males also activated left superior temporal (BA 22) and a superior part of the left inferior frontal gyrus (BA 44) more strongly than females did. As on the EFT, the comparison between mothers and fathers did not show the normal sex difference. Thus, if we regard

Table 2
Factorial analysis of the EFT task

Size	(*x*)	(*y*)	(*z*)	Mass	Probability	Slice	BA	Cerebral region
Main effect of Group: Regions where *Controls* activated more than *Parents*								
161	25.1	−75.1	5.5	4.5	.002	11	18	Right MOG
322	−21.2	−72.2	−0.1	8.0	.001	10	18	Left LG
Main effect of Sex: Regions where *Females* activated more than *Males*								
100	25.0	−78.5	2.0	2.7	.002	11	18	Right MOG
67	−22.7	−80.5	−2.1	1.7	.009	10	18	Left IOG
Regions of significant interaction between *Group* and *Sex*								
102	−20.0	−71.9	29.1	3.0	.008	16	19	Left MOG

Key: MOG, middle occipital gyrus; LG, lingual gyrus; IOG, inferior occipital gyrus.
Here, Females refers to females from both the parent group and the control group, combined. Males refers to males from both the parent group and the control group, combined.

Table 3
Factorial analysis of the Eyes task

Size	Tal (*x*)	Tal (*y*)	Tal (*z*)	Mass	Probability	Slice	BA	Cerebral region
Main effect of Group: Regions where *Controls* activated more than *Parents*								
144	−48.8	−49.7	7.4	0.9	.001	12	21	Left MTG
83	46.5	14.0	17.4	0.4	.007	13	44	Right IFG
89	−43.2	15.5	18.7	0.7	.003	14	45	Left IFG
Main effect of Sex: Regions where *Males* activated more than *Females*								
70	−49.9	−51.1	17.1	0.4	.009	13	39	Left AG
86	−41.6	4.2	33.4	0.9	.002	16	9	Left DLPFC
Regions where *Females* activated more than *Males*								
136	47.7	14.2	11.8	0.9	.003	12	44	Right IFG
129	−42.0	17.0	7.0	0.9	.003	12	44	Left IFG
Regions of significant positive interaction between *Group* and *Sex*								
127	−50.3	−45.1	10.3	0.8	.002	2	22	Left STG
180	−40.5	17.5	15.6	1.5	.001	13	44	Left IFG
Regions of significant negative interaction between *Group* and *Sex*								
47	−45.0	6.0	17.7	0.4	.007	14	44	Left IFG

Key: MTG, middle temporal gyrus; IFG, inferior frontal gyrus; AG, angular gyrus; DLPFC, dorsolateral prefrontal cortex; STG, superior temporal gyrus.
Here, Females refers to females from both the parent group and the control group, combined. Males refers to males from both the parent group and the control group, combined.

the bilateral inferior frontal activation as the typical female pattern, and unilateral left inferior frontal activation as the typical male pattern (see Fig. 3, rows 1 and 2), this was not seen in the comparison between mothers and fathers. Instead, mothers showed a masculinized pattern in the absence of bilateral inferior frontal activation and in showing a clear left-sided pattern of activity in this region with respect to fathers.

3.2.5. Ordinal analysis of variance

The extreme male brain (EMB) theory can be tested further in terms of the predicted rank order of activation among the four groups, using a one-way ANOVA with an ordinal factor. Since on both tasks, brain activation group comparisons above (see Figs. 2 and 3) revealed females showing *more* brain activity in specific regions compared to males, the ordinal ANOVA below focused on testing if there were specific regions where the pattern *Females* > *Males* > *Mothers* = *Fathers* was found. Table 6 and Fig. 4 show that on the EFT, two extrastriate brain regions (left cuneus and right middle occipital gyrus) in BA 19 fitted the predicted trend. Similarly, Table 6 and Fig. 5 show that on the Eyes test, two brain regions (left medial temporal gyrus [BA 21] and left dorsolateral prefrontal cortex [BA 44]) fitted the predicted trend. This again suggests hyper-masculinization of the parents on both tasks in functional patterns of activation in these specific brain areas.

4. Discussion

The present report is a pilot study showing data from parents of children with an autism spectrum condition, whilst performing a visual search test (the EFT) and an emotion recognition test (the Eyes test) during fMRI. Results indicate that parents of children with ASC show atypical brain activity, relative to controls. This pattern is assumed to arise from their genetic status as first-degree relatives of people with an ASC, that is, as one aspect of the broader autism phenotype (BAP). However, because the parents were older than controls, the rival hypothesis is that the group differences found here simply reflect age effects. Efforts were made to control for age in the fMRI analysis by treating age as a covariate, and we have no a priori reason to expect age to be producing the pattern of results seen here. As a pilot study, these results are intriguing, and raise the question that a larger, age-matched sample could answer, as to whether these differences are evidence of the BAP.

Beyond establishing atypical brain activity in such parents, we also tested for sex differences among the controls, and for evidence relevant to the 'extreme male brain' (EMB) theory of autism. Again, it is important to introduce the caveat that with only six males and six females in the typical control groups, this study must be considered a pilot. That said, finding significant sex differences in such small samples raises the possibility that sex differences in brain activity during these tasks can be detected with sufficient power even when comparing $n = 6$ males and 6 females. Sex differences on EFT and the Eyes test at the neural level were found. In particular, on the EFT task females showed more activity in visual cortical areas (BA 19). On the Eyes task females showed more activity bilaterally in the inferior frontal gyrus (BA 44). In addition, males showed a stronger left-lateralized inferior frontal gyrus (BA 44 and 45).

Evidence consistent with the hyper-masculinization theory of parents' brain activity on these tasks is as follows: (1) on the EFT, female controls showed more activity in extrastriate

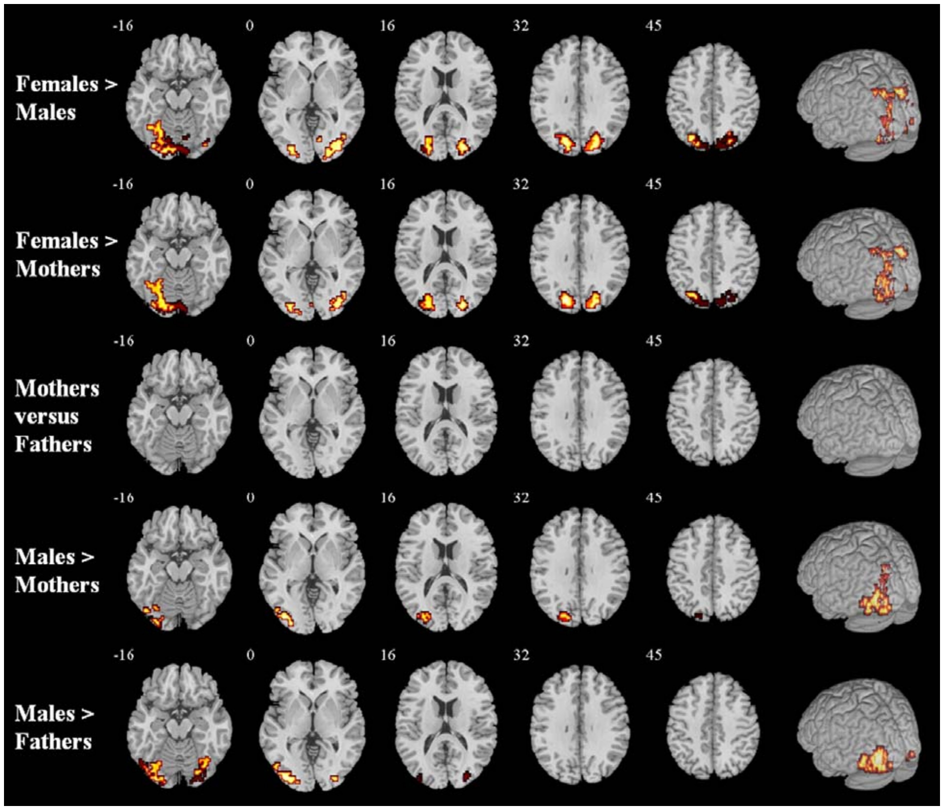

Fig. 2. Group comparison maps in the EFT task. Activation sites have been superimposed onto a series of transaxial brain slices extending from $z = -16$ to $z = 45$ in Talairach coordinate space, and on a 3D rendering of the brain.

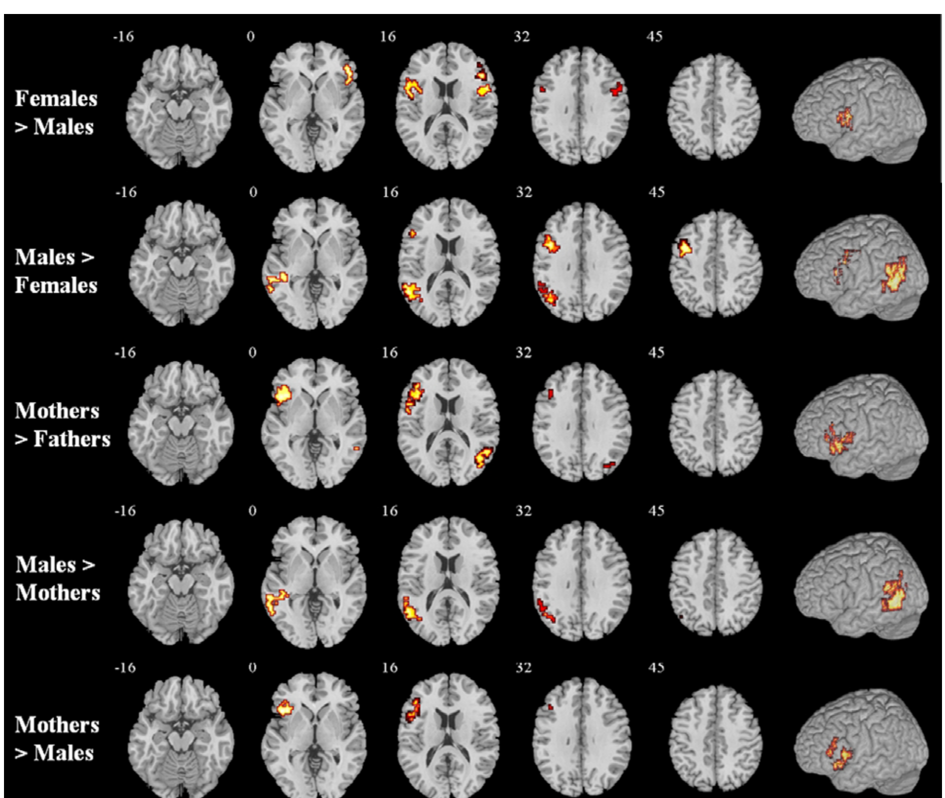

Fig. 3. Group comparison maps on the Eyes task. (Slices and renderings as in Fig. 2.)

Table 4
EFT task: Group comparisons (3D coordinates only)

Size	Tal (*x*)	Tal (*y*)	Tal (*z*)	Mass	Probability	Slice	BA	Cerebral region
Females > Males								
354	−17.1	−72.6	2.4	12.9	.002	11	18	Left LG
210	25.1	−74.6	16.7	11.2	.003	13	19	Right MOG
Females > Mothers								
374	−19.5	−72.3	1.2	13.3	.009	11	18	Left LG
154	25.2	−74.6	16.1	8.2	.004	13	19	Right MOG
Fathers > Mothers								
0	0	0	0	0	0	0	0	
Mothers > Fathers								
0	0	0	0	0	0	0	0	
Males > Mothers								
174	−28.7	−74.5	−1.6	6.6	.006	10	18	Left IOG
Males > Fathers								
102	28.6	−75.4	−9.5	3.3	.009	9	19	Right MOG
168	−32.3	−73.9	−11.3	7.9	.003	8	19	Left FG

Key: LG, lingual gyrus; MOG, middle occipital gyrus; IOG, inferior occipital gyrus; FG, fusiform gyrus.
Here, Females refers to females in the control group alone, and Males refers to males in the control group alone.

Table 5
Eyes task: Group comparisons (3D coordinates only)

Size	Tal (*x*)	Tal (*y*)	Tal (*z*)	Mass	Probability	Slice	BA	Cerebral region
Females > Males								
65	47.3	25.5	4.0	0.6	.006	11	45	Right IFG
65	50.0	6.5	19.3	0.6	.006	14	44	Right IFG
60	−45.1	7.5	15.9	0.5	.007	13	44	Left IFG
Males > Females								
170	−48.4	−46.9	13.2	2.3	.001	13	22	Left STG
96	−40.3	9.0	32.0	1.5	.001	16	44	Left IFG
Mothers > Fathers								
69	45.5	−58.2	12.4	0.6	.006	13	39	Right MTG
166	−41.3	2.1	7.2	2.0	.001	12	45	Left IFG
Fathers > Mothers								
52	−43.0	−0.3	30.5	0.8	.004	16	6	Left PG
Males > Mothers								
148	−49.4	−46.9	10.4	1.9	.001	12	22	Left STG
Mothers > Males								
95	−40.4	20.4	5.6	1.2	.003	11	45	Left IFG
Males > Fathers								
265	−46.9	−46.6	10.5	3.2	.002	12	22	Left STG
117	−42.6	15.5	18.9	1.6	.002	14	45	Left IFG
Mothers > Females								
63	−41.2	17.6	−0.2	1.1	.002	10	47	Left IFG

Key: IFG, inferior frontal gyrus; STG, superior temporal gyrus; MTG, middle temporal gyrus; PG, precentral gyrus.
Here, Females refers to females in the control group alone, and Males refers to males in the control group alone.

cortex than male controls, and both mothers and fathers showed even less activity in this area than sex-matched controls. (2) Unlike the typical sex difference observed in controls on this task, there were no differences in group activation between mothers and fathers of children with AS. (3) The ordinal ANOVA of the EFT identified two specific extrastri-

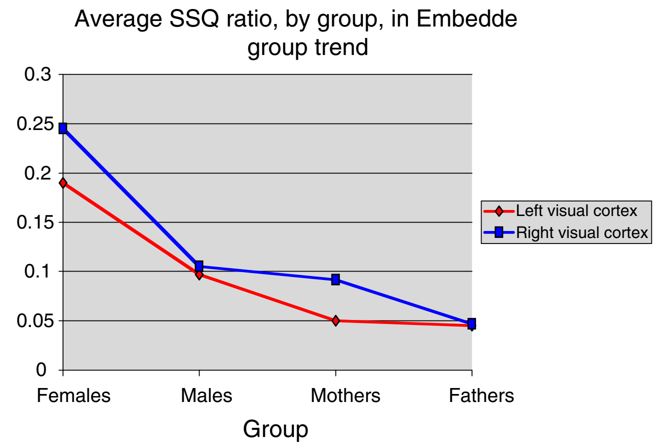

Fig. 4. Ordinal analysis of variance for the EFT: regions fitting the model.

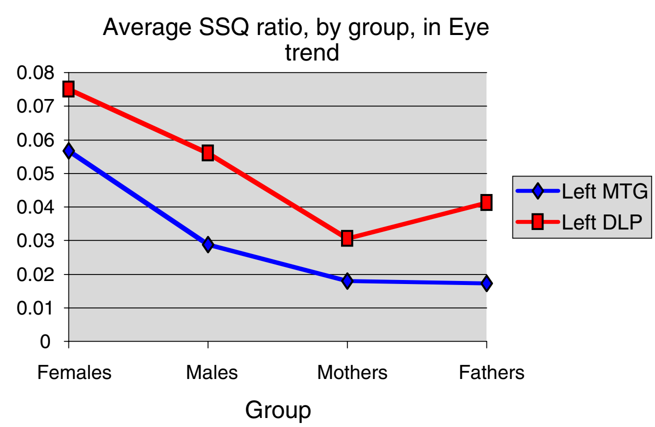

Fig. 5. Ordinal analysis of variance for the Eyes task: regions fitting the model.

ate regions that showed the pattern *Females > Males > Mothers = Fathers*: left and right BA 19. (4) On the Eyes task, female controls showed more activity in the left inferior frontal gyrus (BA 44) than female controls, and this was not seen when comparing mothers to males, or when comparing mothers to fathers. (5) The ordinal ANOVA of the Eyes task identified two specific regions that showed the pattern *Females > Males > Mothers = Fathers*: BA 21 and 44. BA 44 overlaps with a region that previous studies have identified as involved in 'theory of mind' (Frith & Frith, 1999).

We interpret these results as showing that the genetic characteristics of these parents (as carriers of the genes for ASCs) may lead to hyper-masculinization of the brain. As flagged up earlier, the rival explanation of the differences observed between parents and controls is that these are age-effects, since the two groups were not age-matched. Future work needs to disentangle these two interpretations. It would also be important in future studies to include other independent measures of the BAP in the same sample.

We draw the following conclusions: First, parents of children with ASC show atypical brain activity on both the EFT and Eyes tests, which may reflect the BAP, that is, their genetic status as first-degree relatives of such children.

Table 6
Ordinal analysis of variance

Size	Tal (*x*)	Tal (*y*)	Tal (*z*)	Mass	Probability	Slice	BA	Cerebral region
Regions where activation in *Females* > *Males* > *Mothers* = *Fathers* on the EFT								
437	−14.0	−75.0	31.0	13.8	.0003	16	19	Left Cuneus
277	29.0	−78.0	−7.0	9.5	.0009	9	19	Right MOG
Regions where activation in *Females* > *Males* > *Mothers* = *Fathers* on the Eyes task								
117	−55.0	−50.0	5.0	0.8	.005	11	21	Left MTG
88	−40.0	6.0	37.0	1.1	.002	17	44	Left DLPFC

Key: MOG, medial occipital gyrus; MTG, middle temporal gyrus; DLPFC, dorsolateral prefrontal cortex.
Here, Females refers to females in the control group alone, and Males refers to males in the control group alone.

Second, even though a comparison of 6 males and 6 females drawn from the typical population is too small to reveal behavioural or performance differences between the sexes, sex differences at the neural level are clearly evident with such sample sizes. The causes of such neural sexual dimorphism are likely to include differences both in socialization (Baron-Cohen, 2003) as well as innate factors (Connellan, Baron-Cohen, Wheelwright, Ba'tki, & Ahluwalia, 2001). Finally, parents of children with ASC show patterns of brain activity consistent with hyper-masculinization.

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