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Neuropsychologia 44 (2006) 610–621

NEUROPSYCHOLOGIA

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Neural mechanisms of imitation and 'mirror neuron' functioning in autistic spectrum disorder

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Received 21 July 2004; received in revised form 8 June 2005; accepted 23 June 2005
Available online 2 September 2005

Abstract

An association between autistic spectrum disorder and imitative impairment might result from dysfunction in mirror neurons (MNs) that serve to relate observed actions to motor codings. To explore this hypothesis, we employed a functional magnetic resonance imaging (fMRI) protocol previously used to identify the neural substrate of imitation, and human MN function, to compare 16 adolescent males of normal intelligence with autistic spectrum disorder (ASD) and age, sex and IQ matched controls. In the control group, in accord with previous findings, we identified activity attributable to MNs in areas of the right parietal lobe. Activity in this area was less extensive in the ASD group and was absent during non-imitative action execution. Broca's area was minimally active during imitation in controls. Differential patterns of activity during imitation and action observation in ASD and controls were most evident in an area at the right temporo-parietal junction also associated with a 'theory of mind' (ToM) function. ASD participants also failed to show modulation of left amygdala activity during imitation that was evident in the controls. This may have implications for understanding the imitation of emotional stimuli in ASD. Overall, we suggest that ASD is associated with altered patterns of brain activity during imitation, which could stem from poor integration between areas serving visual, motor, proprioceptive and emotional functions. Such poor integration is likely to adversely affect the development of ToM through imitation as well as other aspects of social cognitive function in ASD.

Keywords: fMRI; Autistic disorder; Asperger's syndrome; Social cognition; 'Theory of mind'; Parietal cortex

1. Introduction

An association between autistic spectrum disorder and impairment of imitative ability is now well established (Rogers, 1999; Smith & Bryson, 1994; Williams, Whiten, & Singh, 2004). This is important because imitation may be a core cognitive process required for the development of social cognitive ability (Meltzoff & Decety, 2003; Meltzoff & Prinz, 2002). Furthermore, it has been suggested that impaired imitative skills in infancy may reflect a neurological deficit that could account for autistic syndromes. Rogers and Pennington (1991) built upon Stern's (1985) model of intersubjective development and suggested that a neurological deficit in mechanisms relating observed behaviour to codings by the self for the same behaviour might lead to poor 'self–other' co-ordination and consequently to the development of autism. Whiten and Brown (1999) built on this theory in exploring the commonalities between imitation and 'theory of mind' (ToM) mechanisms from both developmental and evolutionary perspectives, particularly because both processes may involve the observing individual 're-representing' another individual's cognitive perspective, through the observation of their actions. Williams, Whiten, Suddendorf, and Perrett (2001) in

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doi:10.1016/j.neuropsychologia.2005.06.010

turn, suggested that both ToM and imitation might be affected by a dysfunction of the mirror neuron (MN) system, producing the constellation of symptoms that characterise autistic spectrum disorder.

Mirror neurons, activated both by the perception of an action and, on other occasions, when the same action is executed, have been identified in ventral premotor cortex and anterior parietal regions through the use of single cell recordings in macaque monkeys (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996, 2002; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996; Rizzolatti, Fogassi, & Gallese, 2001). There is now increasing evidence that MNs function in humans much as they appear to do in non-human primates, serving a role in understanding the actions of others (Binkofski et al., 1999; Buccino et al., 2001; Fadiga et al., 1999; Hamzei et al., 2003; Hari et al., 1998).

MNs have also been proposed to be part of a system serving the imitation of actions (Arbib, 2002; Iacoboni et al., 1999; Rizzolatti, Fadiga, Fogassi, & Gallese, 2002), though their role in this function is less clear. Gallese and Goldman (1998) suggest that MNs could utilise their action-understanding function to attribute intention, and thus serve a ToM function. However, macaque monkeys that have an MN capacity, neither show a ToM ability or evidence of imitation (Visalberghi & Fragaaszy, 1990). Therefore, for the MN system to serve an imitation function in humans, either further cognitive abilities are required, or the system itself must have undergone evolutionary modification in some way. Meltzoff and Decety (2003) also suggest that MNs serve imitation, and that the practice of imitation begets ToM, as imitation requires more than resonance between neural codings for action observation and execution. Imitation is also influenced by the attribution of goals and intentions, and a means for representing self–other relations. When imitation is intended, actions need to be observed with this goal in mind. Thus, action is observed differently, consequent to a top-down strategy associated with frontal lobe function.

The neural substrate of imitation in people affected by autistic spectrum disorder (ASD) is therefore of much interest. There is a need to ascertain whether individuals with ASD utilise the MN system less or in a different way during imitation, or whether other neural structures required for imitation are affected. If imitation is critical in the development of normal social cognitive functioning, such investigations may tell us much about the nature of the social cognitive deficit in autism.

Accordingly, the aim of the present study was to test the hypothesis that neural systems serving imitation function abnormally in individuals with ASD and in particular that this will be reflected in abnormal function within a MN system. We adopted the paradigm developed by Iacoboni et al. (2001, 1999), who incorporated an experimental design devised by Brass, Bekkering, Wohlschlager, and Prinz (2000) into a functional magnetic resonance imaging (fMRI) scanning protocol, to compare brain activation during imitation with conditional visuomotor learning (described more fully below). Testing normal subjects, Iacoboni et al. (1999) reported activation in the regions of right anterior parietal cortex and left Broca's area during either performance or observation of a finger movement. Activation was augmented when the movement was performed as an imitation. The authors argued that this provided evidence that humans possess MNs, active in action imitation. The present fMRI study is the first focusing on imitation in ASD, others having targeted other aspects of social cognition in ASD, notably aspects of ToM (Baron-Cohen et al., 1999; Castelli, Frith, Happe, & Frith, 2002; Happe et al., 1996) or object perception (Ring et al., 1999).

2. Methods and materials

2.1. Participants

Ethical permission for the study was granted by the local medical ethics committee and all participants gave informed, signed consent. Right handed adolescent or young adult men with ASD were recruited through clinical services and local agencies. The Autism Diagnostic Interview—Revised (2000) (Lord, Rutter, & Le Couteur, 1994) was completed with the main carer, and the Autism Diagnostic Observation Schedule—Generic (ADOS-G) (Lord et al., 2000) with the participant. If there was any uncertainty about whether participants met diagnostic criteria, clinical records were reviewed and a consensus rating of the ADOS-G videotape was made by a group of experienced clinicians. Normal control participants were recruited through local youth groups. IQ assessments were carried out using the WISC-III R for participants under 16 years and WAIS-IV for 16 years or over. The handedness inventory (Oldfield, 1971) was also administered, and participants were asked for details of any current psychotropic medication. Participants were excluded if they had a neurological disability, full scale IQ < 70, or were not able to be exposed to MRI (e.g. possessing metal implants). Participant details are shown in Table 1. The groups were

Table 1
Participant characteristics: mean (S.D.)

	ASD group ($N = 16$)	Control group ($N = 15$)
Age (years)	15.4 (2.24)	15.5 (1.60)
Verbal IQ	100.9 (23.4)	101.3 (21.1)
Performance IQ	98.4 (19.0)	97.6 (13.9)
Full scale IQ	100.4 (21.7)	99.7 (18.3)
Autism Diagnostic Interview—Revised (2000) algorithm items, mean scores		
Reciprocal social interaction	20.4 (threshold = 10)	N/A
Social communication	15.8 (threshold = 8)	N/A
Repetitive behaviour	5.6 (threshold = 3)	N/A

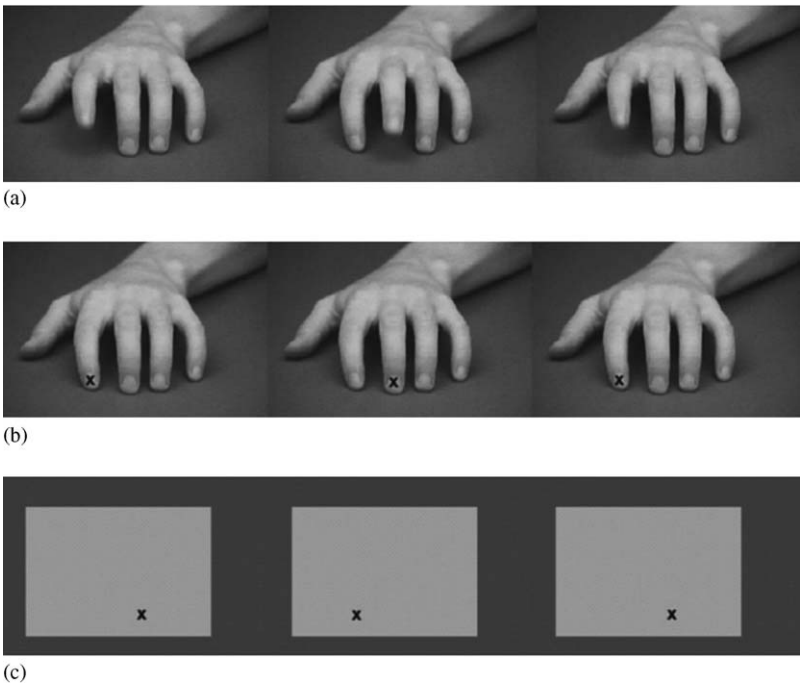

Fig. 1. The three stimulus types: (a) animation, in which either index or middle finger was raised up (five images shown successively for 0.6 s each); (b) symbolic cue, in which either the index or middle finger was marked with a cross; (c) spatial cue, in which a cross was shown on either left or right (see text for experimental conditions).

closely matched on age and IQ. Three ASD participants (but no controls) were prescribed psychotropic medication at the time of imaging. Two were taking methylphenidate and the other was taking clonidine, dexamphetamine and melatonin. All ASD participants had an unambiguous clinical diagnosis of autistic disorder or Asperger's syndrome according to DSM-IV criteria, and scored above threshold on the ADI-R diagnostic algorithm. All but one also scored above threshold for ASD on the ADOS-G diagnostic algorithm. None had identifiable medical conditions underlying their ASD. Whilst all participants were right handed, the mean handedness quotient was significantly reduced in the ASD group (ASD = 74.7, S.D. = 20.0; control = 93.1, S.D. = 7.5; ANOVA: $F = 9.8$, $p = 0.004$) consistent with previous reports (Hauck & Dewey, 2001; McManus, Murray, Doyle, & Baron-Cohen, 1992).

2.2. Experimental protocol

The protocol used was closely based on that reported by Iacoboni et al. (1999) and used the same stimuli, kindly provided by these authors. The three stimulus-types used are shown in Fig. 1. The first consisted of an animation of a left hand at rest on a plain background with either the index finger or middle finger being lifted. The second consisted of a photograph of the hand at rest, with a black cross marking either the index or middle finger. The third consisted of a plain background, with a cross on the left or right side of the screen. Each stimulus type was used in both execution and observation conditions, so there were six experimental conditions. In the first three 'execution' conditions, the participants were instructed to raise the index or middle finger of their right hand according to whether they saw: (a) the animation of an index or middle finger being lifted (imitation condition), (b) whether the cross marked the index or middle finger (symbolic cue execute condition) or (c) the cross was on the left or right of the screen (spatial cue execute condition). In the other three 'observation' conditions (animated finger—observe, symbolic cue—observe and spatial cue—observe), subjects were simply asked to observe the three types of stimuli and not act.

Each stimulus was displayed for 3 s and was shown eight times successively, randomising between index and middle finger. Six stimulus blocks, each lasting 24 s (8×3 s), were alternated with seven rest blocks also lasting 24 s, excepting the final rest block, which lasted 36 s. This resulted in a scan lasting 5 min 20 s, constituting a 'session'. Each session was repeated four times per participant. Before display of each group of eight stimuli, an instruction was presented on the screen as to whether the participant should watch (an image of an eye appeared) or execute a movement (the words 'move finger' appeared). The order of tasks was counterbalanced as to whether observation tasks were followed by execution tasks or vice versa. To monitor whether task performance by individuals was accurate, a camera was installed in the scanner room and connected to a closed circuit TV and video recorder.

2.3. Imaging procedures

Functional MRI imaging was performed using a 1.5 T scanner (NVi, General Electric Medical Systems, Milwaukee, WI). A quadrature head coil was used to obtain high-resolution gradient echo 3D volumetric images and four sets of functional images using blood oxygenation level dependent contrast. The high-resolution images were collected using a T1 weighted sequence with the following parameters: field of view, 24 cm; 20/6, TR/TE; flip angle, 35°; slices, 124; slice thickness, 1.6 mm; matrix, 256 × 256. Functional MR images were acquired in axial planes with a $T2^*$-weighted single shot, gradient-echo, echo-planar pulse sequence with the following parameters: field of view, 24 cm; 3000/33, TR/TE; flip angle, 90°, slices, 24; slice thickness, 5 mm; matrix, 128 × 128. The head was firmly stabilised between two foam pads.

2.4. Task performance

Task performance during scans was very close to 100%, with just two subjects making minor errors on two occasions. Of 128 scan sessions processed (4 per subject), 54 from controls and 52 from ASD subjects were free from movement artefact and therefore suitable for analysis.

2.5. Postprocessing

Postprocessing was performed off-line on a workstation using SPM 99 (http://www.fil.ion.ucl.ac.uk/spm/), a suite of programs run within the MATLAB environment. Intrasubject registration was performed by aligning all volumes of each session to the first volume of that session and then aligning all sessions to the first volume of the first session using a six parameter rigid body linear registration algorithm. Any session with more than 5 mm or 5° movement was discarded. Intersubject registration was performed using a 12 parameter affine transformation, warping each subject's images into a standard template with a voxel size of 2 mm × 2 mm × 2 mm. Finally, Gaussian smoothing was applied, at 8 mm FWHM. Data analysis was performed in a two-stage mixed-effects analysis (equivalent to a random effects analysis) in which BOLD responses for each subject were first modelled using a standard haemodynamic function in the context of the fixed-effects general linear model. Subject-specific linear contrasts on the parameter estimates were then entered into a second-level analysis to perform within-group analysis using a one-sample *t*-test and between-group analysis using a two-sample *t*-test, resulting in a *t*-statistic for each voxel. For within-group analyses, contrast weights were used to identify clusters of activation. The term "activation" is defined as voxels showing significantly better fit of the haemodynamic model in one condition compared to another. For direct group comparison, two different weight combinations were used to pursue two kinds of contrast: autism group + 1/control group − 1 (to identify voxels showing significantly better fit of the haemodynamic model [i.e. "greater activation"] in the ASD than in the control group); ASD group − 1/control group + 1 (to identify voxels showing better fit for the control group). The resulting Z maps were thresholded with a significance level of $p = 0.001$, not corrected for multiple comparisons. We considered that this choice of threshold was appropriate, within the already robust design of the random effects model, for a study of this nature.

3. Analytic strategy

The protocol of Iacoboni et al. (2001, 1999) employs an imitation condition and five control conditions against which imitation can be contrasted. This affords numerous potential contrasts for analysis. As an initial analytic step, we examined whether the act of imitation resulted in broadly different patterns of activation between the groups in a whole brain analysis, to identify any specific regions of interest that might be particularly relevant. We then moved onto our principal objective, which was to compare 'mirror neuron' activity in individuals with ASD and controls, by repeating the analysis performed by Iacoboni et al. (1999) using SPM. This required us to mask out occipital, temporal and sub-cortical structures, thus confining our analysis to frontal and parietal areas. We then proceeded to compare the imitation condition with action execution (the average of the spatial cue and symbolic cue execution conditions), in the ASD and control groups separately, before exploring whether group status interacted with this comparison. We then focussed on regions of interest (ROIs) identified by these analyses. To ascertain whether group differences in visual cortex activation during imitation were accounted for by differences in activity due to observation, we performed a further masked analysis confined to occipital and temporal lobes. In this analysis, we contrasted imitation with observation of the animated finger in ASD and control groups separately, and then explored where group status interacted with this comparison.

4. Results

4.1. Group comparison of imitation versus rest (whole brain)

Tables 2 and 3 show how the two groups differed in patterns of brain activation that occurred during imitation compared to rest. In comparison to the ASD group, the control group showed greater activation of the right fusiform cortex and an area of right middle occipital gyrus/lingual gyrus as well as smaller areas of left inferior parietal cortex, right lingual gyrus and right middle temporal gyrus. There was also a cluster in the region of the right anterior intraparietal sulcus with coordinates (46, −36, 59) close to those reported by Iacoboni et al. (37, −40, 57). In con-

Table 2
Direct group status interaction with contrast of imitation vs. rest, whole brain analysis (controls > ASD), co-ordinates are in standard Talairach and Tournoux space

X	*Y*	*Z*	Region	BA	*T*	*Z*	Extent
Right							
38	−51	−11	Fusiform gyrus	37	4.96	4.21	326
24	−84	−4	Middle occipital gyrus	18	3.86	3.45	206
46	−36	59	Inferior parietal lobule	40	3.22	2.96	16
28	−66	−2	Lingual gyrus	19	3.09	2.86	29
50	−62	9	Middle temporal gyrus	37	2.79	2.61	24
Left							
−53	−56	40	Inferior parietal lobule	40	3.64	3.28	48

trast, the ASD group showed three small clusters where activity was greater than controls. One of these was in the left amygdala, and the other two were bilateral symmetrical activations of a sub-gyral region of superior temporal gyrus. There was no group difference in activation in Broca's area.

4.1.1. Imitation versus action execution (frontal and parietal regions only) in separate groups

We contrasted imitation with the average of spatial cue and symbolic cue execute conditions in frontal and parietal regions, as performed by Iacoboni et al., in control and ASD groups separately. Control group results are shown in Fig. 2a and Table 4. This analysis generated two clusters (59, −26, 27 and 34, −36, 57) in similar locations to those reported by Iacoboni et al. (1999) in the region of the right parietal cortex (58, −24, 32 and 37, −40, 57). ASD group results are shown in Fig. 2b and Table 5. Only one small cluster of activation was found in this area of somatosensory cortex (61, −18, 38). In both groups there were other areas of significant frontal activation. In the ASD group, this included two small clusters of activity in ventral premotor cortex, which were

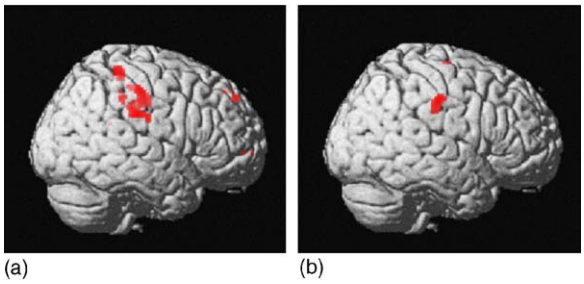

Fig. 2. Imitation vs. [average of spatial cue execution + symbolic cue execution], masked to include frontal and parietal regions only. Random effects analysis, threshold = $p < 0.001$ uncorrected: (a) controls and (b) ASD.

absent in the control group. We were unable to find evidence of greater activation during imitation than other execution conditions in Broca's area in either group, even when we reduced the threshold to $p = 0.05$ uncorrected. In summary, activity in posterior somatosensory cortex was significantly greater during imitation compared to other action execution

Table 4
Locations of clusters shown in Fig. 2a

X	*Y*	*Z*	Region	BA	*T*	*Z*	Extent
Right							
59	−26	27	Inferior parietal lobule	40	7.40	4.73	69
34	−36	57	Postcentral gyrus	40	5.60	4.05	60
59	−19	43	Precentral gyrus	4	5.23	3.89	76
18	50	34	Superior frontal gyrus	9	4.17	3.35	11
36	−29	47	Postcentral gyrus	3	4.14	3.33	7
55	−33	38	Inferior parietal lobule	40	4.13	3.32	11
Left							
−22	43	40	Superior frontal gyrus	8	4.05	3.28	5

Clusters < 5 voxels not reported. Co-ordinates are in standard Talairach and Tournoux space.

Table 3
Direct group status interaction with contrast of imitation vs. rest, whole brain analysis (ASD > controls), co-ordinates are in standard Talairach and Tournoux space

X	*Y*	*Z*	Region	BA	*T*	*Z*	Extent
Right							
44	3	−15	Superior temporal gyrus	38	3.77	3.38	100
20	−18	−18	Parahippocampal gyrus	28	2.81	2.62	20
20	26	21	Cingulate gyrus	32	2.79	2.61	12
Left							
−20	−1	−22	Uncus	Amygdala	4.37	3.81	37
−40	−1	−10	Superior temporal gyrus	21	3.98	3.53	70
−20	−22	64	Precentral gyrus	4	3.33	3.04	57
−28	7	16	Claustrum	–	2.95	2.75	35
−30	8	53	Middle frontal gyrus	6	2.93	2.73	61
−44	−77	8	Middle occipital gyrus	19	2.71	2.54	10

Table 5
Locations of clusters shown in Fig. 2b

X	Y	Z	Region	BA	T	Z	Extent
Right							
61	−18	38	Precentral gyrus (somatosensory cortex)	4	5.16	3.85	48
16	−10	63	Superior frontal gyrus	6	4.45	3.50	5
Left							
−28	31	35	Middle frontal gyrus	9	4.63	3.59	23
−44	41	11	Middle frontal gyrus	10	3.99	3.24	17

Clusters < 5 voxels not reported.

in both control and ASD groups but appeared less extensive in the ASD group.

4.1.2. Group comparison of imitation versus execution

We then subjected the output from the previous analysis (Section 4.1.1) to a group comparison to identify areas where the contrast in activity levels between imitation and execution interacted with group status. No such interaction was found at the threshold of $p < 0.001$ in somatosensory cortex as illustrated in Fig. 3a and b and Tables 6 and 7. However, we found a significant interaction (control > ASD) in the posterior aspect of the inferior parietal lobe on the left side in an area associated with imagination of action (e.g. Grafton, Arbib, Fadiga, & Rizzolatti, 1996). There was also no interaction of group status with the imitation versus other execution contrast in Broca's area. ASD showed greater activity than controls in dorsal premotor cortex during imitation compared with other action execution.

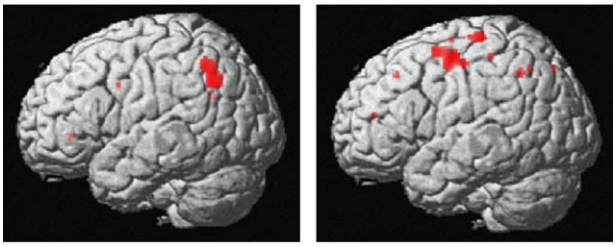

Fig. 3. Interaction of group status with contrast of imitation vs. [average of spatial cue execution + symbolic cue execution], masked to include frontal and parietal regions only. Random effects analysis, threshold = $p < 0.001$ uncorrected: (a) controls > ASD and (b) ASD > controls.

Table 6
Locations of clusters shown in Fig. 3a

X	Y	Z	Region	BA	T	Z	Extent
Right							
18	−55	34	Precuneus	31	2.93	2.73	7
Left							
−53	−56	40	Inferior parietal lobule	40	3.41	3.11	89
−8	35	−2	Anterior cingulate	32	2.79	2.61	6

Control > ASD. Clusters < 10 voxels not reported.

Table 7
Locations of clusters shown in Fig. 3b

X	Y	Z	Region	BA	T	Z	Extent
Right							
28	29	37	Middle frontal gyrus	8	2.79	2.61	26
36	8	36	Precentral gyrus	9	2.74	2.57	8
Left							
−22	5	51	Sub-gyral	6	3.54	3.21	47
−20	−22	64	Precentral gyrus	4	3.32	3.04	34
−36	−4	44	Middle frontal gyrus	6	2.93	2.73	71
−44	−13	47	Precentral gyrus	4	2.80	2.62	10
−28	−50	43	Superior parietal lobule	7	2.55	2.40	8

ASD > controls. Clusters < 5 voxels not reported.

4.2. ROI analyses

The previous analyses generated a number of ROI. We were particularly interested in the areas relating to 'mirror neuron' activity identified in the anterior parietal lobe. We identified coordinates of the relevant peaks in our previous analyses and for each set of coordinates we examined activity within the surrounding 27 voxels (1 cm^3) for each condition separately. For the anterior parietal lobe (analysis, Section 4.1.1), peaks of activity in controls and ASD were at slightly different locations. To choose one of these locations would generate a bias towards finding greater activity in one group or the other, and so we examined both. Parameter estimates determined from the SPM model are shown in Fig. 4a and b. In the first location (59, −26, 27), controls showed greater activation than the ASD group in all execution conditions. In the second location (61, −18, 38), imitation was associated with similar levels of activation in both groups but activity related to other execution was absent for the ASD group. Activation in relation to imitation at the peak location for the ASD group was of a similar magnitude to that for controls at both locations. If the results of these plots are considered together with the SPM maps in Fig. 2a and b, it is evident that imitation-related activity is of a similar magnitude but more extensive in somatosensory cortex in controls than ASD. However, activity in somatosensory cortex is also greater for controls than ASD for non-imitative execution. This would explain why no interaction occurs for these areas in the group × condition-contrast analysis of Section 4.1.2.

Secondly, we were interested in activity in the region of the left amygdala identified in the test of group interaction with the imitation versus rest contrast. The results are shown in Fig. 5a and b suggesting marked task-related modulation in controls that was absent in the ASD group. To test for this, we used Bartlett's test to compare the homogeneity of variance in the mean activity time course for observation and execution (including imitation) between the two groups (Table 8). The variances in a 27 voxel region of the left amygdala (centre

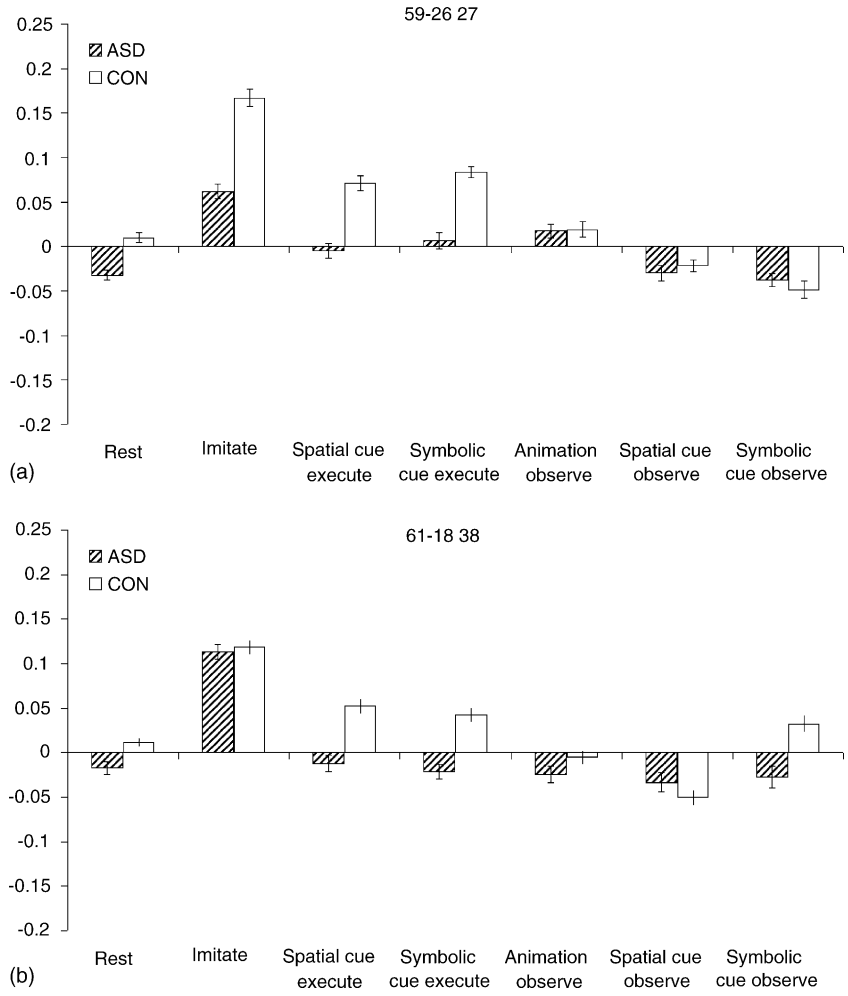

Fig. 4. Parameter estimates determined from SPM fit of model to time course data for two groups of 27 voxels in anterior parietal lobe close to anterior parietal sulcus showing relative contributions of experimental conditions to BOLD signal. Area was a peak of activity in (a) controls and (b) participants with ASD.

−20 −1 −22) and two regions in the anterior parietal lobe (centre 59 −26 27 and 61 −18 38) are shown for the ASD group and control group. During the imitation and other execution conditions, but not during observation only, there was significantly less variance in the ASD group than controls in the amygdala ($p = 0.001$) but not in either of the parietal regions.

4.3. Imitation versus animated finger—observe (occipital and temporal regions only)

The control group but not the ASD group did show some activity in visual cortex that was greater during imitation than observation at the $p < 0.001$ threshold, but there was no evidence for any such activity in the ASD group (Fig. 6a and

Table 8
Comparison of the variance (Bartlett's test for homogeneous variance) of the mean activity time course in a 27 voxel region of the left amygdala (centre −20 −1 −22) and two regions in the anterior parietal lobe (centre 59 −26 27 and 61 −18 38) for the ASD group and control group

		Variance			
		ASD	Control	Chi square	p
Amygdala (−20 −1 −22)	Execution	0.084	0.175	13.542	0.001
	Observation	0.081	0.091	0.348	0.840
Parietal region 1 (59 −26 27)	Execution	0.136	0.138	0.006	0.997
	Observation	0.137	0.143	0.047	0.997
Parietal region 2 (61 −18 38)	Execution	0.126	0.110	0.474	0.789
	Observation	0.131	0.111	0.704	0.703

$N = 104$ and mean = 1900 for all comparisons.

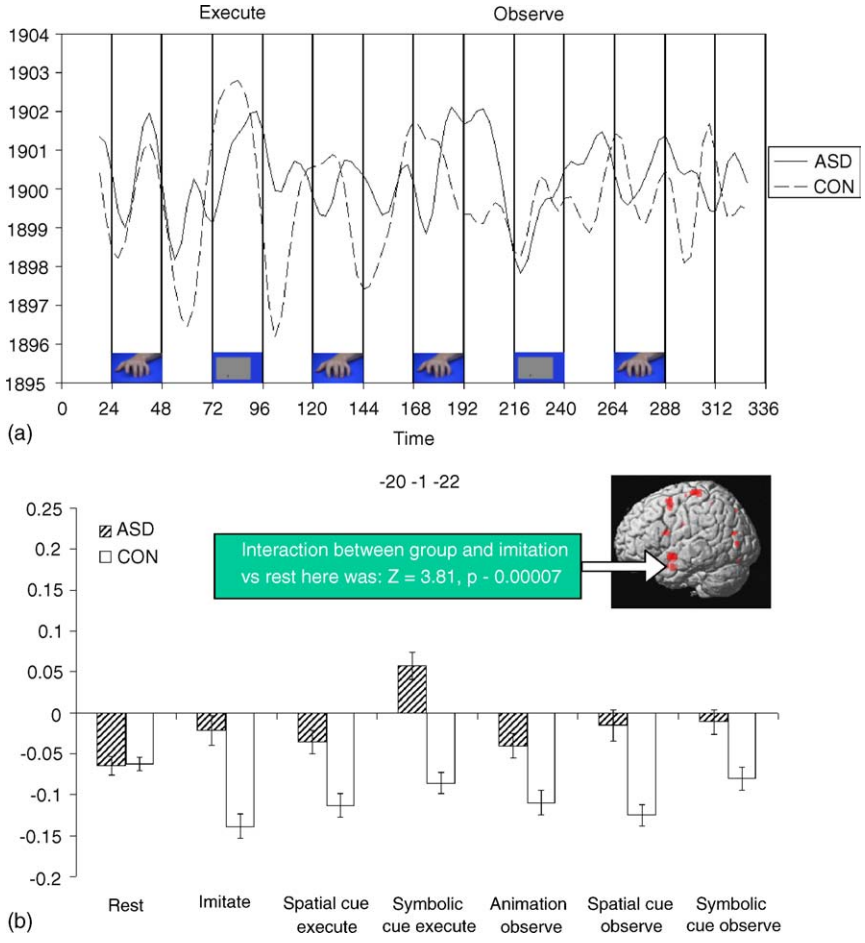

Fig. 5. (a) Mean time course of activity in 27 voxels in left amygdala during task performance showing clear activity induced modulation during task performance that is greatest during imitation. (b) Bar graph shows parameter estimates determined from SPM fit of model to time course data showing relative contributions of experimental conditions to BOLD signal.

b and Table 9). We did not replicate the group differences previously evident in the contrast between imitation and rest, which concerned the right fusiform gyrus and right lingual gyrus. This suggests that differences in these areas were also associated with observation alone. We then sought to identify areas where group status interacted with the imitation versus animation observation contrast (Fig. 7a and Table 10), and identified two clusters of activity that were specific to imitation, in the left lingual gyrus which locates to just outside of V2, and at the right temporo-parietal junction (TPJ). An ROI analysis for this latter area, conducted as above, showed that the controls showed activation during imitation but not observation at this site, whereas in the ASD group the converse was true (Fig. 7b).

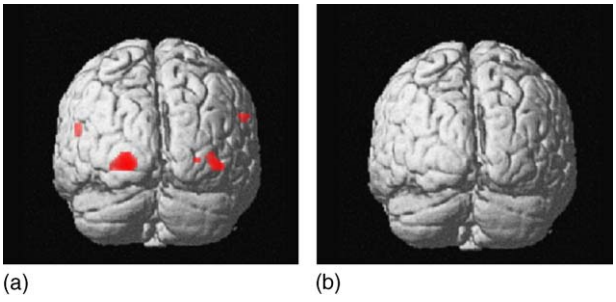

Fig. 6. Imitate vs. animation observation (masked to include only occipital and temporal regions), random effects analysis, threshold = $p < 0.001$ uncorrected: (a) controls and (b) ASD.

Table 9
Locations of clusters shown in Fig. 6a

X	*Y*	*Z*	Region	BA	*T*	*Z*	Extent
Right							
44	−76	−1	Inferior occipital gyrus	19	4.92	3.74	33
59	−32	27	Inferior parietal lobule	40	4.42	3.49	21
38	−87	1	Middle occipital gyrus	18	3.95	3.22	10
Left							
−18	−93	1	Cuneus	17	5.16	3.85	147
−46	−2	4	Insula	13	4.48	3.51	14
−53	−44	19	Superior temporal gyrus	13	4.33	3.43	17

Imitation vs. observation (controls). Masked to include only occipital and temporal regions. Clusters < 10 voxels not reported.

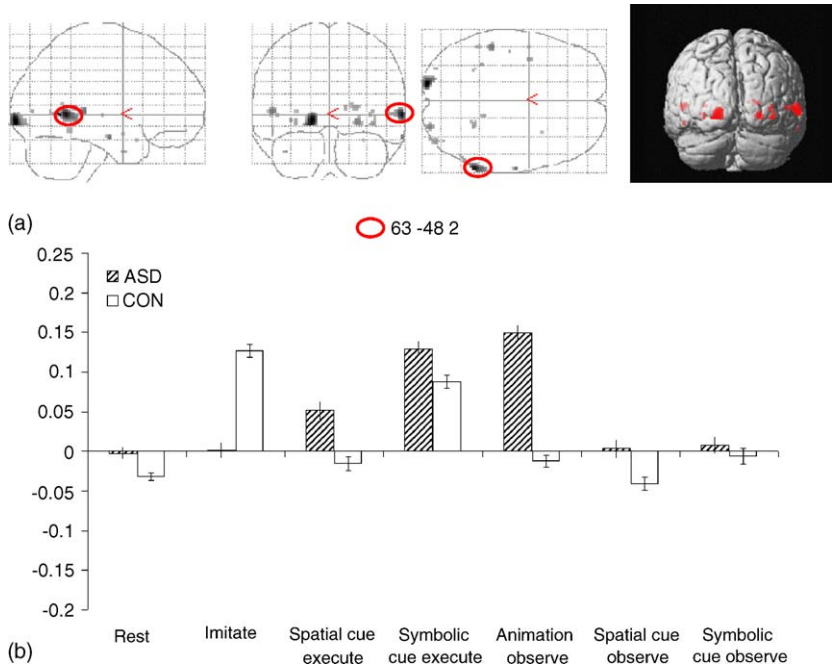

Fig. 7. (a) Areas where group status interacts with the contrast of imitation vs. animation observation (masked to include only occipital and temporal regions), random effects analysis, threshold = $p < 0.001$ uncorrected. Areas shown are where controls were greater than ASD group (there were no significant clusters for the ASD > control contrast). (b) Region of interest analysis for 27 voxels centred on area in posterior temporal cortex circled red.

Table 10
Locations of clusters shown in Fig. 7a

X	*Y*	*Z*	Region	BA	*T*	*Z*	Extent
Right							
63	−48	2	Middle temporal gyrus[a]	22	3.72	3.35	84
24	−89	−1	Lingual gyrus	18	2.88	2.68	21
Left							
−16	−91	0	Lingual gyrus	17	3.91	3.49	97
−50	−33	5	Middle temporal gyrus	22	2.99	2.77	18

Imitation vs. observation. Masked to include only occipital and temporal regions. Controls > ASD group. Clusters < 10 voxels not reported.
[a] Shown in Fig. 7.

5. Discussion

Both ASD and control groups showed activity in the right somatosensory cortex that was greater during imitation than during the other execution conditions. This replicates Iacoboni et al.'s (1999) finding. In the control group, this activation extended upwards to a second cluster also identified by Iacoboni et al. (1999). However, the activation in the ASD group appeared less extensive and did not include this latter area. Examination of the graph plots for the inferior parietal areas (Fig. 4) indicates that activation in the ASD group during imitation at 61, −18, 38, was of a similar level to that in the control group at 59, −26, 27. Interestingly though, the ASD group showed minimal activation of somatosensory cortex during non-imitative action execution. This meant that although imitation caused less extensive activation of MN areas in ASD, so did other types of execution. Consequently, a test of group interaction with the contrast of imitation with other types of execution found no difference, because activation during both imitative and non-imitative action execution was diminished in ASD. Diminished activity secondary to non-imitative action execution also appeared to account for the group difference seen in posterior parietal cortex in Fig. 3a and Table 6. Activity in parietal cortex during any motor activity would be expected secondary to proprioceptive feedback, and this is evident in controls. Tsatsanis et al. (2003) have suggested disconnectivity between thalamus and cortex occurs in ASD. These findings raise the possibility that proprioceptive feedback could be adversely affected in ASD possibly as a result of poor thalamo-cortical connectivity.

In contrast to the control group, the ASD group also showed greater activity specifically during imitation of dorsal premotor cortex and regions of dorsal prefrontal cortex (Fig. 3 and Tables 6 and 7). This raises the possibility that the ASD group were relying more on visuomotor learning (associating previously learnt motor actions to different visual stimuli) to perform the imitative task (Toni & Passingham, 1999).

Unlike Iacoboni et al. (1999), we were unable to identify activity in Broca's area in control participants indicative of MN functioning. Such differences are difficult to explain as resulting from differences in magnetic field strength (1.5 T versus 3.0 T) of the scanners used in our and Iacoboni's studies, or through the use of different statistical models. They remained absent at a very low threshold, and our sensitivity

was sufficient to replicate Iacoboni et al.'s findings at other locations. One difference between Iacoboni et al.'s (1999) and our study may have been the use of instructions. We gave a visual instruction before each block to minimise the likelihood of rehearsal by internal verbalisation. Iacoboni et al. (1999) do not say how instructions were given, but they do show activation of Broca's area in the spatial and symbolic cue observation conditions, indicative of a non-specific source of activation of this area. Grezes, Armony, Rowe, and Passingham (2003) and Makuuchi (2005), have also failed to find activation of Broca's area specific to imitation. Hamzei et al. (2003) were able to detect activity in Broca's area using an action–observation paradigm, which involved action on an object directed to the mouth (drinking from a cup), and Rizzolatti et al. (2001) have found that only actions involving objects activate the MN region in monkeys. As this experimental task did not prove to be associated with activation of MNs in Broca's area in normal subjects, possible MN activity in this area in ASD would be better examined using a task that more strongly and specifically activates MNs in this region. One such task might be oro-facial imitation as employed by Nishitani, Avikainen, and Hari (2004). They found that some of their participants with ASD, unlike controls, did not activate Broca's area during imitation, and also that activation was weaker and delayed in this area. Another useful task might be action upon an object as employed by Avikainen, Kulomaki, and Hari (1999). Although they did not find a statistically significant group difference between ASD and control groups, their numbers were small and appear to indicate a trend towards lower MN activity in the ASD group.

Imitative impairment in ASD could also be the result of impaired processing in areas of visual cortex that are concerned with processing movement. In a contrast between imitation and action observation, we identified bilateral differences in lingual gyrus and middle temporal gyrus at the temporo-parietal junction. This was greatest on the right for the TPJ and on the left for the lingual cortex. The imitation-specific activity at the right TPJ is of particular interest because of the association of this area with ToM function (Castelli et al., 2000; Saxe & Kanwisher, 2003; Saxe, Xiao, Kovacs, Perrett, & Kanwisher, 2004; Vogeley et al., 2001) that is impaired in ASD (Castelli et al., 2002). It seems unlikely that our imitation task required any sort of mentalising function. However, if imitation plays a role in ToM development, a brain area used for imitation during development, it may also come to be utilised to serve ToM. If this process is impaired in ASD, whereby imitation contributes to ToM development (Rogers & Pennington, 1991; Whiten & Brown, 1999; Williams et al., 2001), diminished activation of this area during an imitative task might be expected in an ASD group. In an analysis focussed on the right TPJ, we have found striking group differences. For the controls, this area was active during imitation but not during observation, where the opposite was the case for the ASD group. This raises the possibility that normally motor activity during imitation increases activity in this region, perhaps influencing perception. Support for this hypothesis has recently come from Astafiev, Stanley, Shulman, and Corbetta (2004), who also found that motor activity was associated with increased activity in occipito-temporal cortex and in the lingual gyrus. Alternatively, during imitation, visual processing areas may receive input from parietal areas serving action representation as discussed above, or from frontal areas associated with executive function (Decety et al., 1997). Such input could be deficient in ASD because of a lack of proprioceptive feedback to the parietal lobes as we have indicated occurs, diminished frontal activity or because of impaired feedback connectivity (Hill & Frith, 2003). In any case, our results raise the intriguing possibility that development of ToM function in this region depends upon input related to motor activity altering function of areas that would otherwise only be active secondary to observation.

Another very interesting difference that emerged on the whole brain comparison concerned the left amygdala. On a whole brain comparison, amygdala activity was greater during imitation in ASD than it was in controls. The ROI and time course analysis in Fig. 5 showed deactivation in the control group during tasks that was greater than in the ASD group. Comparing the variance of signal changes in the two groups showed this to be greater in controls during execution but not during observation. One explanation may be that amygdala activity was generally higher in the ASD group but this would not explain why the difference was present in the imitation versus rest comparison at a whole group level, or why variance in time courses was similar in observation conditions. An alternative explanation is that the amygdala is being actively modulated during imitation in controls. As this task was not emotive, it may be associated with a reduction in amygdala activity. The amygdala has been suggested by others to lie at the core of the autistic syndrome (Baron-Cohen et al., 2000) and here we raise the possibility that the amygdala is closely connected with imitation-related action processing systems. This would be expected if the amygdala is a key regulator of social cognitive function, and amygdaloid involvement in action perception-connectivity forms the foundations of social cognitive neural systems. Some authors have raised the possibility that a lack of motivation may account for the imitative impairment in autism. Here, we suggest that it is more likely the lack of emotional connectivity with imitation that is impaired in ASD, so that it is not always experienced in such a self-reinforcing manner.

We consider our research findings to have considerable validity. We were able to complete fMRI scans of substantial duration with a large sample of participants with ASD diagnosed according to stringent research criteria, and to compare them with a control group well matched on age and IQ. Our sample did not show global developmental delay and so our results were not confounded by non-specific effects of learning disability. Our scanning protocol, in contrast with several previous neuroimaging studies of ASD (Baron-Cohen et al., 2000, 1999; Castelli et al., 2002; Happe et al., 1996; Ring et al., 1999), used a simple and unchallenging task,

and so results were not confounded by performance differences. However, because the task was simple, it may not have activated imitative processes as extensively as more challenging tasks might do. Such tasks now merit further research in this area. It is possible as suggested by Müller, Cauich, Rubio, Mizuno, and Courchesne (2004) and Müller, Kleinhans, Kemmotsu, Pierce, and Courchesne (2003) that motor activity in people with ASD may show a different pattern of neurofunctional organisation. Unlike Müller et al., however, we did not find any group differences in the spread of activations in ASD and control groups, making this somewhat less likely to be relevant.

As mentioned in the introduction, neural systems serving imitation may be critical for the development of social cognitive function. The present study was cross-sectional, and differences in the neural basis of imitative functioning in a group of adolescents are not necessarily the same as those that occur at earlier developmental stages in the same children. However, if our findings were replicated in young children with ASD, we anticipate these individuals would use movement-analysis pathways less, and integrate visual information about actions with their existing 'body-knowledge' less. We suggest that this would have a major adverse effect on other aspects of social cognitive development.

6. Conclusions

In conclusion, we found robust evidence for differences between a control and an ASD group in the patterns of brain activation associated with imitation. These affected MN function in anterior parietal areas which were less extensively activated. However, MNs seem to serve an imitation function as a result of being embedded within a broader and hemispherically specialised system of neural components that in more complex imitative situations, could contribute to analysis of perceived movement, emotional content and ToM. Although we found evidence of differential MN activation during imitation, this was apparently part of a larger and more complex set of group differences in the neural substrate of imitation. The ASD group did not show expected activation of somatosensory cortex during non-imitative action and also did not show modulation of left amygdala that was apparent in the control group. Another striking difference was a failure to show activation of right posterior middle temporal gyrus at the temporo-parietal junction during imitation, but instead to show activation of this area during observation, whilst the converse was true for the control group. These findings point towards abnormal patterns of integration of areas serving imitation in ASD, between areas that serve visual analysis, motor action, proprioception and emotional processing. This is likely to have important implications for imitative development in ASD which in turn is likely to have consequences for the development of ToM and other social cognitive development. Further research is required to explore experimental paradigms that are more potent activators of these regions.

Acknowledgements

We gratefully acknowledge the enthusiastic participation of all the young people who took part in this study, and the help given by professional colleagues in recruiting participants with ASD. This study was funded by the Chief Scientist's Office of the Scottish Executive. AW was supported by a Leverhulme Fellowship during the writing of this paper.

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