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The role of the medial temporal lobe in autistic spectrum disorders

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Abstract

The neural basis of autistic spectrum disorders (ASDs) is poorly understood. Studies of mnemonic function in ASD suggest a profile of impaired episodic memory with relative preservation of semantic memory (at least in high-functioning individuals). Such a pattern is consistent with developmental hippocampal abnormality. However, imaging evidence for abnormality of the hippocampal formation in ASD is inconsistent. These inconsistencies led us to examine the memory profile of children with ASD and the relationship to structural abnormalities. A cohort of high-functioning individuals with ASD and matched controls completed a comprehensive neuropsychological memory battery and underwent magnetic resonance imaging for the purpose of voxel-based morphometric analyses. Correlations between cognitive/behavioural test scores and quantified results of brain scans were also carried out to further examine the role of the medial temporal lobe in ASD. A selective deficit in episodic memory with relative preservation of semantic memory was found. Voxel-based morphometry revealed bilateral abnormalities in several areas implicated in ASD including the hippocampal formation. A significant correlation was found between parental ratings reflecting autistic symptomatology and the measure of grey matter density in the junction area involving the amygdala, hippocampus and entorhinal cortex. The data reveal a pattern of impaired and relatively preserved mnemonic function that is consistent with a hippocampal abnormality of developmental origin. The structural imaging data highlight abnormalities in several brain regions previously implicated in ASD, including the medial temporal lobes.

Introduction

Autistic spectrum disorders (ASD) are characterized by impairments in social interaction and communication and restricted or repetitive behaviours and interests. The degree of impairment varies enormously. For example, low-functioning children with ASD may be mute or have abnormal language, whilst other children with high-functioning ASD may have good language but nevertheless suffer from social communication problems including inappropriate use of gestures. The underlying neural abnormalities associated with ASD are not entirely clear.

In addition to investigations of social communication problems, language difficulties and face-processing abnormalities, a number of studies have examined mnemonic functions in ASD. These have revealed an impairment in episodic memory (i.e. memory for events and episodes; Tulving, 1972) (Russell, 1996; Bowler *et al.*, 2000; Millward *et al.*, 2000; Gardiner *et al.*, 2003) but not semantic memory (i.e. memory for facts or world knowledge; Tulving, 1972) (at least in high-functioning individuals) (e.g. Ameli *et al.*, 1988; Minshew *et al.*, 1992; Bennetto *et al.*, 1996; Siegel *et al.*, 1996; Farrant *et al.*, 1998).

Previous reports of patients with developmental amnesia have revealed a similar dissociation between episodic memory, which is selectively impaired, and factual memory, which is relatively preserved (e.g. Vargha-Khadem *et al.*, 1997, 2003; Gadian *et al.*, 2000). To date, however, comprehensive memory profiles comparing semantic and episodic memory in individuals with ASD have not been reported.

The neuropathology underlying the dissociation in mnemonic ability in developmental amnesia has been well documented (i.e. severe bilateral hippocampal atrophy in the presence of relatively preserved parahippocampal cortices; Mishkin *et al.*, 1997; Vargha-Khadem *et al.*, 1997; Isaacs *et al.*, 2003). In contrast, the neural abnormalities underlying the mnemonic and other cognitive deficits associated with ASD are not entirely clear.

A considerable amount of research has focused on the role of medial temporal lobe pathology in ASD. Despite this, results of imaging studies examining the integrity of the hippocampal formation in ASD are inconsistent. For example, some have reported reductions in volume (e.g. Aylward *et al.*, 1999; Saitoh *et al.*, 2001), whilst others have found no abnormalities (e.g. Piven *et al.*, 1998; Haznedar *et al.*, 2000; Howard *et al.*, 2000). At postmortem, however, studies have confirmed increased cell density and abnormally small cells in the hippocampal formation (Raymond *et al.*, 1996).

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This study sought to: (i) examine dissociations between episodic and semantic components of cognitive memory in children and adolescents with ASD and (ii) determine whether deficits in any of the memory components are related to underlying medial temporal lobe abnormality as detected by structural magnetic resonance imaging. In addition, the role of the medial temporal lobes in the symptomatology of ASD was further probed using correlation analysis between structural imaging and cognitive and behavioural results.

Materials and methods

Participants

Children with ASD (aged between 8 and 18 years; see Table 1) were recruited through parental support groups (including the National Autistic Society) and from schools specializing in the education of children with ASD. All of the children had been diagnosed by independent psychiatrists/psychologists as cases of Asperger's syndrome ($n = 11$) or high-functioning autism ($n = 3$). This diagnosis was then confirmed by parental interviews carried out by a consultant clinical neuropsychologist (F.V.-K.) using the Australian Scale for Asperger's Syndrome (Garnett & Attwood, 1997). The ratings for all of the children in the ASD group were unambiguously in the range for Asperger's syndrome. The parental responses consistently showed high incidence rates of problem behaviours in the domains of social and emotional abilities, communication skills, cognitive skills and specific interests (see Table 2). Two individuals in the ASD group were rated within the normal range for movement on the Australian Scale for Asperger's Syndrome.

Children were excluded from the study if they had additional neurological or psychiatric diagnoses (such as fragile X, epilepsy and attention deficit hyperactivity disorder) or if they were taking medication or had a history consistent with a diagnosis of secondary autism (such as rubella).

A group of normally developing children (aged between 8 and 18 years) were recruited from local London schools to act as controls. These children met the same exclusionary criteria as the children with ASD with the additional requirement that there was no family history of ASD. The control children were selected from a screened cohort to match the children with ASD as closely as possible on age and verbal intelligence quotient (IQ). In addition, the control group that underwent magnetic resonance imaging examinations (scan controls) was also sex matched. Not all of the children in the scan control group were able to complete the full neuropsychological assessment protocol (due to time constraints, etc.) and so additional children (not necessarily sex-matched) were included in the neuropsychological control group (see Table 1).

Ethical approval for this study was obtained from the Institute of Child Health and Great Ormond Street Hospital Ethics Committee and the National Autistic Society. Parental consent and child assent were obtained for each participant in the study in accordance with the declaration of Helsinki.

Neuropsychological assessment

The children completed a series of neuropsychological tests. The results from the memory and attention tests and details of the parental checklist are reported here. Other results from this study have been published elsewhere (Salmond *et al.*, 2003).

Memory

The children completed a series of tests designed to tap different aspects of memory; these are summarized in Table 3. The memory domains are listed in order of increasing dependence on episodic memory (Vargha-Khadem *et al.*, 1997; Gadian *et al.*, 2000; Baddeley *et al.*, 2001). Episodic memory is thought to require the integrity of the hippocampal formation to subserve the full contextual (i.e. temporal and spatial) characteristics of trial-unique stimuli, whilst other domains of memory that are less context dependent (such as recognition and semantic memory) are thought to be subserved by the surrounding cortex (Mishkin *et al.*, 1997).

The Rivermead Behavioural Memory Test was used as an index of episodic memory (Wilson *et al.*, 1991). This test yields a maximum score of 22 and consists of a series of subtests. The subtests include

TABLE 1. Group characteristics

Group	Size	Age (years)	Sex (M/F)	Verbal IQ
Autistic	14	12.9 ± 0.7	13/1	102 ± 4
Control	18	12.6 ± 0.7	6/12	104 ± 2
Scan controls	13	12.1 ± 0.7	13/0	111 ± 4

Data are presented as means ± SEM. IQ, intelligence quotient.

TABLE 2. Australian Scale for Asperger's Syndrome (ASAS) scores for control and autistic spectrum disorder groups

Section of ASAS	Controls	Autism
Social and emotional abilities	0.43 ± 0.21	4.54 ± 0.18
Communication skills	0.22 ± 0.13	4.29 ± 0.33
Cognitive skills	0.52 ± 0.15	4.41 ± 0.33
Specific interests	0.33 ± 0.22	4.41 ± 0.32
Movement skills	0.39 ± 0.33	3.81 ± 0.58

Data are presented as means ± SEM. Mean score per section: 0, no abnormality; 6, maximum score.

TABLE 3. Memory protocol details

Memory domain and assessment battery*
Recognition
Word-list recognition (CMS)
Word-pair recognition (CMS)
Stories recognition (CMS)
Semantic memory
Pyramids and Palmtrees Test
Information (WISC-III/WAIS-III)
Vocabulary (WISC-III/WAIS-III)
Similarities (WISC-III/WAIS-III)
Recall for material presented multiple times
Word-list (CMS)
Word-pair (CMS)
Recall for material presented once
Family pictures (CMS)
Stories (CMS)
Episodic memory
Rivermead Behavioural Memory Test

*For the tests see: CMS, Children's Memory Scale (Cohen, 1997); Pyramids and Palmtrees Test (Howard & Patterson, 1992); WISC-III/WAIS-III (Wechsler, 1991, 1997); Rivermead Behavioural Memory Test (Wilson *et al.*, 1991).

remembering a name, an appointment, a belonging, a route around the room, remembering to deliver a message, orienting to time and place, recognition of simple line drawings of objects and faces and recalling short stories. Additional measures of recall and recognition were obtained using subtests from the Children's Memory Scale (Cohen, 1997) (children aged 17 years and over completed the equivalent subtests in the Wechsler Memory Scale; Wechsler, 1998). Standard scores were obtained for each subtest (with a mean of 10 and SD of 3). In addition to measures of semantic memory obtained from the intelligence scales (e.g. subtests of Vocabulary, Information and Comprehension), another measure was also included, the Pyramids and Palmtrees Test (Howard & Patterson, 1992). This test samples accumulated factual knowledge using pictorial cues and provides an index of semantic memory independent of IQ.

Attention

Three subtests of the Test of Everyday Attention in Children (Manly *et al.*, 1999) were used to obtain standardized measures of selective attention, sustained attention and divided attention.

Parental checklist

A number of checklists were completed by the parents in order to characterize the profile of their children. These included the Autism Behavioural Checklist (Krug *et al.*, 1993) and Sunderland Parental Questionnaire (Sunderland *et al.*, 1983, providing a rating of the frequency of everyday memory problems).

Statistical analysis of neuropsychological data

In order to minimize the number of comparisons carried out (and so reduce the possibility of a Type I error), composite scores were calculated for each of the memory domains in Table 3 (by calculating each child's mean score for the tests included in that domain). Statistical analyses were carried out using Student's *t*-tests with appropriate checks for the normal distribution of the residuals. As five Student's *t*-tests were carried out, the Bonferroni correction sets the significance α level at 0.01 (0.05/5).

The scores from the subtests assessing different aspects of attention were used to determine whether there were significant correlations between the children's attentional abilities and their memory scores. Where appropriate, analysis of covariance was carried out to determine whether group differences in memory scores could be accounted for by attentional differences. The parental questionnaire data were examined to determine whether they provided qualitative support for the neuropsychological test results obtained by the children.

Magnetic resonance imaging acquisition

The children were scanned, unsedated, on a 1.5-T Siemens Vision scanner using a T1-weighted 3D FLASH sequence with the following parameters: TR, 16.8 ms; TE, 5.7 ms; flip angle, 21° and voxel size, $0.8 \times 0.8 \times 1.0$ mm.

Magnetic resonance imaging analysis

The scans were analysed using voxel-based morphometry (VBM) implemented in SPM99 software (Wellcome Department of Imaging Neuroscience, London, UK) searching explicitly for bilateral abnormalities using a conjunction analysis (see Salmond *et al.*, 2000). This methodology was chosen as bilateral abnormality is consistent with the nature of ASD, a neurodevelopmental disorder with a strong genetic component (see Shallice, 1988; Warrington & Warrington, 1990; Vargha-Khadem *et al.*, 1994).

Briefly, the scans were normalized using linear and non-linear transformations (with $4 \times 5 \times 4$ basis functions) to a symmetric template (created by averaging the T1 template supplied with SPM99 with itself flipped) and then segmented using a symmetric probabilistic template. The segmented images were duplicated and the duplicate copy was flipped in the transverse plane prior to smoothing at 12 mm. This smoothing transforms the data into a local measure of grey (or white) matter density [proportion of tissue volume ascribed to grey (white) matter]. No modulation was carried out.

The data were then examined for bilaterally symmetric abnormalities by means of a conjunction analysis using flipped and unflipped datasets to test for the conjoint expression of differences between the ASD and control groups in both hemispheres. This can be conceptualized as searching for homologous areas of abnormality in the two hemispheres. There were no significant differences in global grey matter volume following spatial normalization. Global grey matter and age were included as confounds in the statistical model. As all of the children scanned were male, sex was not included as a confound. Grey and white matter segments were analysed separately.

Significant abnormalities were identified using the false discovery rate correction for multiple comparisons. In addition, given our particular interest in the medial temporal lobe, a small volume correction was carried out. The volume was chosen to encompass the amygdala and much of the hippocampal formation (sphere radius of 15 mm, centred at $\pm 27, -15, -18$) and with a total volume far greater than the volume of these structures. As such, the small volume correction for the medial temporal lobe was highly conservative.

Finally, correlations were carried out between grey matter density and the ASD individuals' cognitive and behavioural scores. The cognitive indices were chosen on the basis of the neuropsychological analyses (see below) and consisted of the scores obtained from the Rivermead Behavioural Memory Test, Information and Vocabulary from the Wechsler Intelligence Scale for Children (WISC-III) and the Autism Behavioural Checklist. Intragroup, bilateral correlations were entered into the design matrix with age as a confound.

All abnormalities reported were significant at $P < 0.05$ with correction for whole brain (false discovery rate see Genovese *et al.*, 2002) or small volume correction comparisons. All images are displayed at a threshold of uncorrected $P < 0.005$. All peaks that survived correction for multiple comparisons are reported but effects that traverse the midline were discounted as these do not represent a conjunction of bilateral effects (see Salmond *et al.*, 2000).

Results

Neuropsychological assessment

There were no significant differences between the groups in age ($t = -0.3$, $P > 0.7$) or verbal IQ ($t = 0.6$, $P > 0.5$) (see Table 1).

The results from the memory assessments reveal that the performance of the ASD group was significantly poorer than controls in the episodic memory domain, even when corrected for multiple comparisons (see Table 4). In contrast, indices of recognition memory, semantic memory and memory for stimuli presented multiple times were relatively preserved. The results from the tests assessing memory

TABLE 4. Memory domain results

Domain	Autistic score	Control score	*t*-value	*P*-value
Recognition memory	9.6 ± 0.4	10.4 ± 0.4	1.2	0.2
Semantic memory	48.4 ± 0.7	48.2 ± 0.4	−0.2	0.8
Multiple presentation	9.1 ± 0.6	10.0 ± 0.6	0.7	0.3
Single presentation	8.8 ± 0.7	10.7 ± 0.6	2.1	0.04
Episodic memory	16.8 ± 0.8	20.7 ± 0.4	4.6	<0.001*

Scores given as means ± SEM. *Significant at Bonferroni correction $P < 0.01$.

TABLE 5. Rivermead Behavioural Memory Test subtest scores

Rivermead Behavioural Memory Test, Subtests	Control scores	Autism scores	*F*-value	*P*-value
First and last name	1.8 ± 0.1	0.9 ± 0.3	12.4	0.001
Belonging	2.0 ± 0.0	1.3 ± 0.2	13.6	0.001
Appointment	1.9 ± 0.1	1.4 ± 0.2	7.4	0.01
Orientation	1.8 ± 0.1	1.4 ± 0.2	4.6	0.04
Story immediate	1.8 ± 0.1	1.4 ± 0.2	4.6	0.04
Story delay	1.9 ± 0.1	1.5 ± 0.2	3.9	0.06
Route delay	2.0 ± 0.0	1.8 ± 0.2	2.5	0.1
Route immediate	1.9 ± 0.1	1.8 ± 0.1	0.6	0.4
Faces	1.8 ± 0.1	1.9 ± 0.1	0.6	0.4
Pictures	1.8 ± 0.1	1.7 ± 0.1	0.1	0.7
Message	1.9 ± 0.1	1.9 ± 0.1	0.04	0.8

for stimuli presented only once (i.e. trial unique) suggest that the children with ASD were performing more poorly than controls, although the result does not survive the Bonferroni correction. The ASD group's performance in the episodic memory domain was not consistently poor on all subtests, showing variation in subtest scores (see Table 5). The ANOVA scores for each subtest are given in Table 5 for qualitative review. No direct interpretation of these scores was made due to the multiple comparisons involved.

There was a significant correlation between sustained attention scores and the episodic memory scores (Pearson's correlation, $r = 0.6$, $P < 0.001$). However, analysis of covariance revealed that sustained attention scores did not account for the difference between the performance of the two groups in the episodic memory domain (ANCOVA $F_{1,29} = 8.8$, $P = 0.006$). For purposes of qualitative interpretation, correlations between the scores of the subtests of episodic memory test and that of sustained attention were also carried out. Significant correlations were found in the Name, Belonging and Story recall subtests ($P < 0.025$).

Results from the Sunderland Parental Questionnaire (see Fig. 1) revealed that the children with ASD scored higher (indicating greater impairment) than the control children. The results from the Autism Behavioural Checklist also revealed higher scores (again indicating greater impairment) in the ASD group compared with the control group (see Fig. 1).

As different control groups were used for the analysis of magnetic resonance and neuropsychological data, an additional comparison was made for the latter dataset. When the comparison was limited to just the controls who were present in both groups, the pattern of the results reported above remained unchanged.

Magnetic resonance analysis

In the ASD group, significant increases were found bilaterally in grey matter density at a whole brain corrected level in the fusiform gyrus, inferior cerebellum (including lobules VIIIA and VIIIB), dorsolateral prefrontal cortex, peri-hippocampal cortex (partly extending to include the medial anterior hippocampal formation) and lateral occipitotemporal sulcus (see Fig. 2 and Table 6). Decreased white matter was found adjacent to the fusiform gyrus grey matter abnormality (see Table 6). Conventional, unilateral voxel-based morphometric analyses were also carried out and did not reveal any additional areas of abnormality.

In addition to these comparisons between groups, correlations were also carried out within the ASD group between grey matter density and scores obtained on the episodic memory domain, semantic memory domain and Autism Behavioural Checklist. A significant correlation with the Autism Behavioural Checklist ratings was found in the junction area between the amygdala, hippocampus and entorhinal cortex on the medial aspect of the rostral temporal lobe ($P = 0.012$ corrected) (see Fig. 3). In particular, more grey matter was associated with greater behavioural abnormality as detected by the Autism Behavioural Checklist. No other significant correlations were found.

Discussion

The results from this study suggest that the ASD group have a relatively selective deficit in episodic memory with preservation of semantic memory skills. VBM analysis revealed increased grey matter

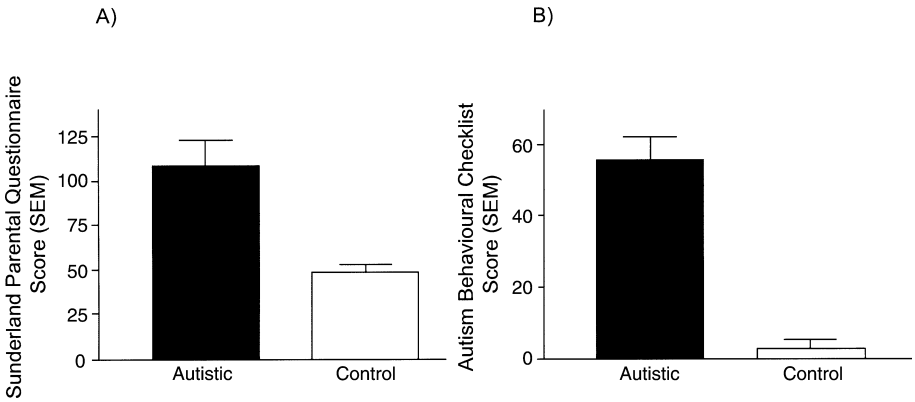

FIG. 1. Results from parental questionnaire. (A) Scores from Sunderland Parental Questionnaire [mean (SEM)]. (B) Scores from Autism Behavioural Checklist [mean (SEM)].

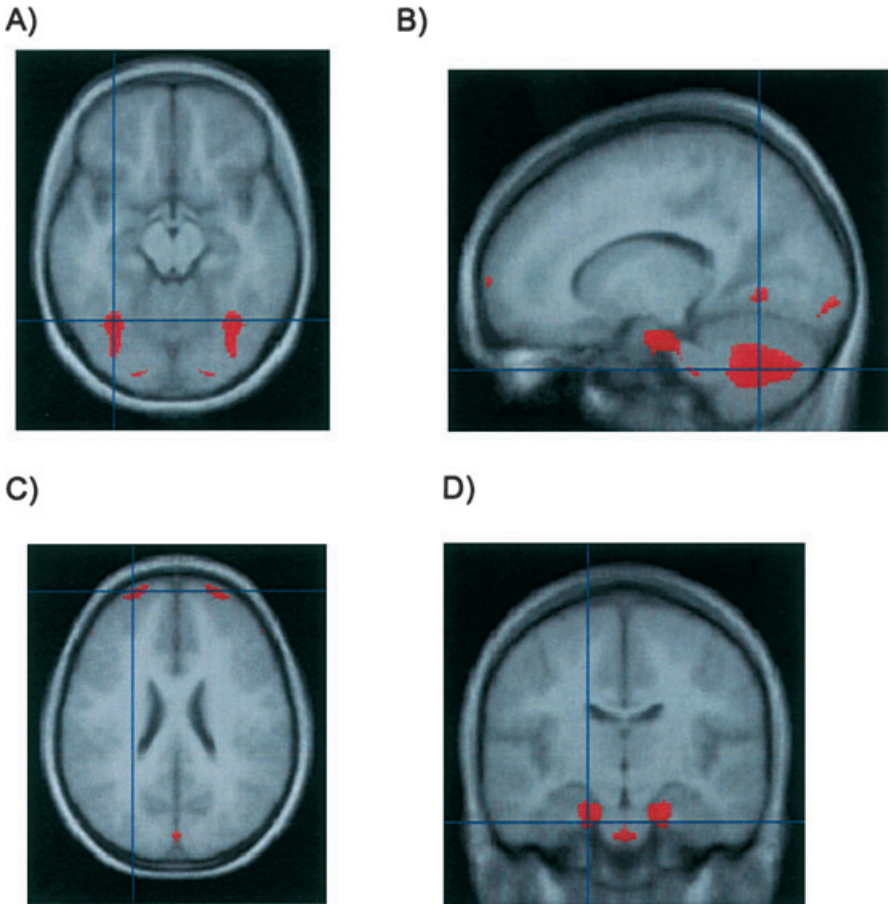

FIG. 2. Statistical parametric maps for increased grey matter density superimposed on the mean normalized image of the group data, at a threshold of uncorrected $P < 0.005$. The cross-hairs on the figures indicate the location of the maximal peak. Figures are displayed in neurological convention (left hemisphere is on the left). (A) ±34, −63, −14 fusiform gyrus; (B) ±15, −62, −40 cerebellum; (C) ±26, 62, 24 dorsolateral PFC; (D) ±18, −16, −30 anterior hippocampal formation and (at location of maximal peak) peri-hippocampal cortex.

TABLE 6. Significantly increased and decreased grey matter densities

Area	Coordinates (mm)			*Z*-value	*P*-value*
	x	*y*	*z*		
Significantly increased grey matter densities					
Fusiform gyrus	±34	−63	−14	4.44	0.019
Cerebellum	±15	−62	−40	4.27	0.019
Anterior hippocampal formation and peri-hippocampal cortex	±18	−16	−30	4.14	0.019
Frontal cortex (dorsolateral region)	±26	62	24	3.94	0.019
	±38	38	42	3.89	0.019
	±44	50	18	3.54	0.037
Lateral occipitotemporal sulcus	±44	−60	−2	3.34	0.026
Occipital lobe	±20	−99	−8	3.38	0.033
Significantly decreased grey matter densities					
Adjacent to fusiform gyrus	±36	−64	−12	4.75	0.04

*All results reported with false discovery rate (FDR) whole brain correction.

density in the hippocampal formation, peri-hippocampal cortex, fusiform gyrus, dorsolateral prefrontal cortex, cerebellum and inferior occipitotemporal cortex. In addition, grey matter density in the junction area between the amygdala, hippocampus and entorhinal cortex on the medial aspect of the rostral temporal lobe was found to correlate with ratings of autistic behaviour.

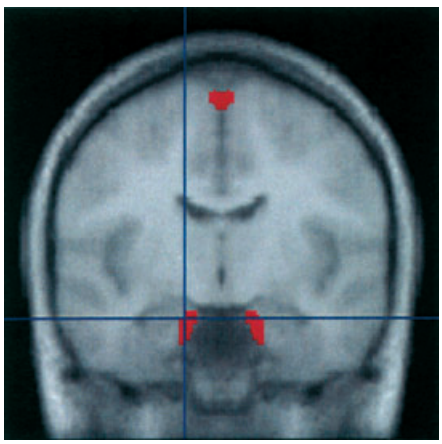

FIG. 3. Correlation in autistic spectrum disorder group between score on Autism Behavioural Checklist and grey matter density at the junction of the amygdala, hippocampal formation and entorhinal cortex (threshold $P < 0.002$ with small volume correction, ±15, −9, −22).

The neuropsychological data are consistent with the pattern of impairment suggested in the literature in high-functioning individuals with ASD. Semantic memory has not previously been explicitly studied in individuals with ASD. However, given the link between

verbal IQ and semantic memory, almost by definition individuals with average verbal IQ are likely to have intact semantic memory. Several studies have reported on cohorts of individuals with ASD who have average or above average verbal IQ (Minshew *et al.*, 1992; Siegel *et al.*, 1996) suggesting that a subgroup of autistic individuals have intact semantic memory. Recall of stimuli presented multiple times has also been shown to be preserved in previous studies of individuals with ASD (e.g. Minshew *et al.*, 1992; Minshew & Goldstein, 1993; Bennetto *et al.*, 1996; Buitelaar *et al.*, 1999).

Good recognition skills have been reported by a number of different groups (e.g. Ameli *et al.*, 1988; Bennetto *et al.*, 1996; Brian & Bryson, 1996; Farrant *et al.*, 1998), although there is some suggestion that there may be some difficulty in visual recognition of faces or stimuli with agency (see Ellis *et al.*, 1994; Blair *et al.*, 2001). Such deficits are not inconsistent with the results of the current study, where the recognition memory assessment was restricted to the auditory verbal domain.

Although previous investigations of episodic memory in ASD have not used measures comparable to those used in this study, the findings are consistent with studies documenting impairments in event memory (e.g. Boucher, 1981; Boucher & Lewis, 1989; Russell, 1996; Millward *et al.*, 2000). Further, Gardiner and colleagues have reported deficits in high-functioning autistic adults in recollection-based recognition (associated with episodic memory) but not familiarity-based recognition (Bowler *et al.*, 2000).

The memory profile of the ASD group appears to indicate a relatively selective episodic memory deficit and comparative preservation of semantic memory. It is interesting to note that there is a trend towards impaired performance in the ASD group relative to controls in the single-presentation memory domain. Deficits in recall of trial-unique stimuli are consistent with episodic memory deficits. The remaining domains wherein there was no difference in performance between the two groups involved recall after repeated presentation or cued recall, both of which reduce the reliance on intact episodic memory for successful task performance (Tulving, 2001).

The finding of increased grey matter density might reflect a lack of appropriate levels of programmed cell death or a failure of neurones to migrate appropriately (such increases have also been noted in other developmental disorders of genetic origin; Watkins *et al.*, 2002). Consistent with such hypotheses, postmortem studies have reported increased cell density in the hippocampus (Raymond *et al.*, 1996). Some structural imaging studies have reported decreased hippocampal volumes (Aylward *et al.*, 1999; Saitoh *et al.*, 2001) whilst others have found no detectable change (e.g. Piven *et al.*, 1998; Haznedar *et al.*, 2000; Howard *et al.*, 2000; Sparks *et al.*, 2002). Decreased hippocampal volumes are not necessarily inconsistent with the current findings; for example, the hippocampal formation may both be smaller in volume and have increased grey matter density (as suggested by the voxel-based morphometry results). It is important to be aware that VBM is a high spatial precision analysis of the relative tissue composition of all brain regions. It is not concerned with different tissue types. For example, a selective loss of white matter or CSF in the hippocampus would be expressed as a decrease in hippocampal volume using classical volumetry and an increase in grey matter density (i.e. the proportion of volume occupied by grey matter). Decreases in volume might also not be detected by voxel-based morphometry due to individual variation in the location of reduced volume of the hippocampal formation. Further studies are clearly needed to help unravel the complexities of the hippocampal abnormalities in ASD.

The grey matter density in the junction area of the amygdala, hippocampal formation and entorhinal cortex was found to correlate with the severity of symptomatology of ASD. This is consistent with the importance of this region in the pathogenesis of ASD, whilst the absence of a group difference in density may reflect individual variation (consistent with that reported in Salmond *et al.*, 2003). Evidence supporting variation in the nature of any amygdala abnormality comes from previous structural imaging studies, where some have reported increased volumes (Howard *et al.*, 2000; Sparks *et al.*, 2002) and others, decreased volumes (Aylward *et al.*, 1999; Pierce *et al.*, 2001). Further work is required to investigate the nature of amygdala abnormality in ASD, especially as the inconsistencies may also reflect the relatively small cohorts often studied in structural imaging research.

Considered together, the structural imaging results from this study show many consistencies but also some inconsistencies with the literature. Previous voxel-based morphometry studies have also reported abnormal grey matter density in the peri-hippocampal cortex, frontal cortex, fusiform gyrus, occipital temporal gyrus and cerebellum (Abell *et al.*, 1999; Waiter *et al.*, 2004). However, there are a number of inconsistencies, including the precise location of the affected region of the structures (e.g. cerebellum, frontal cortex), the nature of the abnormality (increased or decreased grey matter density) (e.g. occipital-temporal junction) and the pattern of neural abnormality. These discrepancies may be explained by a number of methodological differences. The previous two studies did not use the bilateral conjunction analysis. Abell *et al.* (1999) did not use non-linear spatial transformation (which improves the accuracy of the colocalization of structures; see Salmond *et al.*, 2002) and neither study made their statistical inferences at a threshold of $P < 0.05$ corrected for multiple comparisons (raising the likelihood of false positives). Preliminary individual voxel-based morphometric analyses from this study sample found evidence of abnormalities in the hippocampal formation, amygdala, frontal lobes and cerebellum (Salmond *et al.*, 2003). The results from the group analysis in this study are broadly consistent with the preliminary findings, although the amygdala abnormality is not retained. This suggests that there were some divergent patterns of abnormality among the individuals in the group (see above).

The finding of abnormalities in the cerebellum, fusiform gyrus and frontal lobes is consistent with the results of several imaging studies (Courchesne *et al.*, 1988; Minshew *et al.*, 1993; Pierce *et al.*, 2001). In addition, executive function deficits (Hughes *et al.*, 1994) and facial recognition deficits (Ellis *et al.*, 1994) (consistent with frontal and fusiform functions) have been noted in ASD. In relation to the cerebellum, it should be noted that there is little agreement as to which areas of the cerebellum are abnormal, with some reports suggesting selective abnormality of vermal lobules VI and VII (Courchesne *et al.*, 1988) and others suggesting a more diffuse abnormality throughout the cerebellum (Arin *et al.*, 1991). Factors which may account for the inconsistency across studies include the incidence of epilepsy (Honavar & Meldrum, 1997), different subject inclusion criteria and differing levels of verbal skills. Indeed it is interesting to note that there may be two subgroups of ASD, those with hypoplasia and those with hyperplasia of the cerebellum (Courchesne *et al.*, 1994). Perhaps these groups are characterized by different verbal skills.

The imaging results from this study indicate that multiple neural areas are abnormal in ASD, consistent with previous research (see above). However, the mnemonic profile found in this study, particularly the gradation of impairment increasing the more hippocampal-dependent the memory task becomes, implicates the role of the medial temporal lobe in this aspect of cognitive memory in ASD. Previous studies of patient groups with developmentally acquired pathology have reported episodic memory impairments in the presence of relatively preserved semantic memory associated with selective damage to the hippocampal formation (e.g. developmental amnesics and premature infants; Vargha-Khadem *et al.*, 1997; Isaacs *et al.*,

2000, 2003). The memory profile of the ASD group in this study is very similar to that of the preterm children (i.e. no evidence of recognition impairment and a mild, but significant, impairment on the Rivermead Behavioural Memory Test). This might suggest that the episodic memory deficit is linked to the abnormalities in the hippocampal formation found in the magnetic resonance imaging analysis. However, the nature of the hippocampal abnormality in the ASD group (increased grey matter density) is different from that in the preterm group (decreased hippocampal volumes). A further complicating factor in comparing these groups of children is that different regions within the hippocampal formation might be affected in the two groups. Moreover, although the mean score of the preterm group on the Rivermead Behavioural Memory Test is similar to that of the ASD group, the individual subtests which the ASD group found difficult are not the same as those on which the preterm children are impaired (Isaacs *et al.*, 2000, 2003).

An alternative explanation might therefore be that the memory profile arises in the context of abnormalities in multiple neural areas. Indeed, the episodic memory impairment (particularly pronounced for prospective memory items pertaining to the 'self') may be a reflection of the social deficits associated with the syndrome of ASD. For example, aspects of episodic memory may have a greater dependency on attending to inherently social aspects of the world than semantic memory. This proposal is supported by inspection of the children's performance on individual subtests of the Rivermead Behavioural Memory Test where particular difficulty is shown with those subtests that have a heavy social demand (e.g. memorizing someone's name, remembering to ask for a belonging, remembering an appointment and orientation to time and place). This is in contrast to performance on other subtests, even those thought to depend heavily on episodic memory (e.g. memorizing a route). Additional evidence supporting this possibility comes from reports of superior abilities for the recall of spatial arrangements in the environment (Caron *et al.*, 2004), a task typically thought to depend on episodic memory. From an anatomical perspective, this memory profile would be consistent with ASD being attributable to a neural abnormality elsewhere in the brain, presumably connected to the hippocampal formation (perhaps the frontal cortex, or potentially multiple areas). The aberrant connections to the hippocampal formation would then potentially lead to the abnormalities in the hippocampal formation itself, as found in this study. Such 'knock on' effects occurring in areas connected to abnormal areas have been noted in animal studies (Hanlon & Sutherland, 2000).

Limitations of the study

There are a number of potential limitations of this study that should be considered. Overlapping but non-identical control groups were used for the imaging and neuropsychological assessments, which may have had some influence on the comparisons performed. However, there are a number of reasons to suggest that this is unlikely to have had a significant effect. There is little evidence to support sex differences in memory (Ionescu, 2000). In addition, limiting the neuropsychological analysis to just the controls who were present in both groups did not change the pattern of the results, despite a reduction in power.

For the imaging analyses, we used the false discovery rate correction for multiple comparisons rather than the more stringent familywise error correction. Given that the abnormal regions that we detect are broadly in line with expectations based on the literature, we regard this correction as appropriate.

We have proposed here that the results represent a selective deficit in episodic memory. However, it could be argued that this may merely be an effect of difficulty, with the tasks assessing the other memory domains being easier and therefore less sensitive. In the absence of a test that directly compares and equates episodic with semantic memory, we have selected standardized tests that provide a gradation in terms of increasing episodic memory demands and hence hippocampal dependency. With this caveat in mind, the data nevertheless indicate that this is unlikely to be an appropriate interpretation, however, as the scores on the Pyramids and Palmtrees Test are below ceiling level (maximum score 52) even in the control group, demonstrating that the children did not find the test to be too easy. Further, there was no evidence of the controls finding the episodic memory assessment difficult.

It is possible that the low scores by the ASD group on the Rivermead Behavioural Memory Test may be a consequence of poor attention. However, the deficit in episodic memory remains even after covarying for the scores in sustained attention. Indeed, inspection of the subtest correlations with sustained attention suggests that several of the impaired scores on the subtests are not associated with deficits in sustained attention. Moreover, such an interpretation would predict a generalized difficulty with all of the subtests, which is inconsistent with the data.

It is important to acknowledge that this study investigated a relatively small cohort of children with ASD. As such, the study may not have sufficient power to detect subtle differences in neuropsychological performance between the control group and the children with ASD. The conclusion that certain aspects of memory (such as recognition memory) are relatively preserved must therefore be interpreted cautiously. Future studies should investigate different aspects of cognitive memory in larger cohorts.

This study investigated a cohort of high-functioning children and adolescents with ASD. It is unclear how applicable these results are to lower-functioning autistic individuals. There is some evidence to suggest that lower-functioning individuals may have a more generalized memory impairment (e.g. Barth *et al.*, 1995) and may show different neuropathology (e.g. Bauman, 1996). It is possible that some (or all) of the impaired functions are a reflection of delayed development and in time they may evolve and potentially resolve. Whilst this may be unlikely in the light of the persistent nature of ASD, an investigation with an adult cohort is necessary to address this issue. However, episodic memory impairments (Bowler *et al.*, 2000; Gardiner *et al.*, 2003) and hippocampal formation abnormalities have also been reported in adult cohorts (Saitoh *et al.*, 2001). Future studies should address these issues.

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Abbreviations

ASD, autistic spectrum disorder; IQ, intelligence quotient; VBM, voxel-based morphometry.

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