

# Development of the hippocampal formation from 2 to 42 years

## MRI evidence of smaller area dentata in autism

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### Summary

Autism, a neuropsychiatric disorder that severely impairs social, language and cognitive development, has a clinical onset in the first years of life. Because components of the limbic system mediate memory, social and affective functions that are typically disturbed in autism, a developmental defect in the limbic system has been hypothesized to underlie different autistic symptoms, but no developmental study has been performed. To obtain neuroanatomical evidence of limbic system abnormality in autism, we measured the cross-sectional area of the area dentata (AD; dentate gyrus + CA4) and combined area of the subiculum and CA1–CA3 (CAS) using *in vivo* MRI.

Autistic patients aged 29 months to 42 years ( $n = 59$ ) and healthy normal controls ( $n = 51$ ) participated. The cross-sectional area of the AD was significantly smaller than normal in autism, the largest deviation from normal size (–13.5%) being found in autistic children aged 29 months to 4 years. Strong age-related increases were seen in the cross-sectional area of CAS, but autistic and normal subjects were not significantly different. This is the first direct evidence that anatomical abnormality within the limbic system exists from the earliest years of the disorder, and persists throughout development and to middle age.

**Keywords:** autism; MRI; neuroanatomy; development; hippocampus

**Abbreviations:** AD = area dentata; ANOVA = analysis of variance; CAS = combined area of subiculum and CA1–CA3; WISC = Wechsler Intelligence Scale for Children

### Introduction

Autism is a developmental neuropsychiatric disorder characterized by pervasive and persistent deficits in social interaction, communication and cognition (Rapin and Katzman, 1998). Mental retardation is commonly present in affected patients. Given the complexity of behavioural symptoms seen in autism, it is not unexpected that multiple sites of anatomical defect have been hypothesized to underlie different symptoms in this disorder (Bauman and Kemper, 1994; Courchesne, 1997; Kemper and Bauman, 1998; Courchesne *et al.*, 1999).

Historically, developmental defect of the limbic system was among the first such hypotheses posed because components of this system mediate memory, social and affective functions that are typically disturbed in autism (Hauser *et al.*, 1975; Bauman and Kemper, 1985; Bachevalier, 1994; DeLong,

1992; DeLong and Heinz, 1997). One laboratory reported increased neurone packing density in limbic structures in nine post-mortem autism patients, eight of whom were adolescents or adults (Bauman and Kemper, 1994; Kemper and Bauman, 1998), but another group found this defect in only two out of six adult post-mortem autistic cases (Bailey *et al.*, 1998). *In vivo* MRI studies failed to find hippocampal anatomical abnormality in autistic older children, adolescents and adults (Saitoh *et al.*, 1995; Piven *et al.*, 1998; Aylward *et al.*, 1999), but in a small group of 14 autistic adolescents and adults amygdala volume derived from MRI was smaller than normal (Aylward *et al.*, 1999).

Post-mortem and *in vivo* evidence of developmental anatomical abnormality in limbic structures in autism has been slow to emerge. In the literature there are only six

post-mortem cases of autism in people under 19 years of age, and only one of these was younger than 9 years of age, namely a 4-year-old patient. In this patient the septum was completely absent posteriorly, a pathology not present in any other published case. Increased pyramidal cell packing density has been reported in three post-mortem cases of autistic children aged 9, 10 and 12 years (Bauman and Kemper, 1994), but the granule cells and other hilar cells of the dentate gyrus were not examined. Given that individual variability in autism is likely to be significant, these young post-mortem cases are far too few to provide a clear picture of age-related changes in the limbic system. Although post-mortem data have provided rich information on brain structure in autism, the small number of cases limits the amount of information available on brain growth and development. In contrast, MRI affords the ability to image large numbers of patients and chart brain growth more directly. For the study of brain development in autism, post-mortem studies have the severe limitations of few cases available, incomplete age sampling (especially the youngest developmental ages) and uncertain diagnosis in the youngest cases. Although these limitations do not apply to quantitative *in vivo* MRI studies of early limbic anatomical development in autism, there have been no MRI studies of the development of limbic structures. Therefore, there is at present no direct evidence of anatomical defects in the limbic system in autism during early life.

To solve the problem of obtaining neurodevelopmental information on the very young autistic child, we produced MRIs of young children suspected to have autism, and re-diagnosed them when they were at least 5 years old. Those who were confirmed as autistic were included in the present study with autistic older children and adults and normal controls. Because of the importance of the hippocampal dentate gyrus in memory development and the new evidence of its lifelong potential for learning-dependent neurogenesis (Kempermann *et al.*, 1997; Gould *et al.*, 1999), we produced MRIs of and measured this structure as well as the hippocampus and subiculum, which, with the dentate, form a trisynaptic circuit that subserves human declarative memory (Andersen *et al.*, 1971; Amaral, 1987, 1993). Because of the very small size of the dentate gyrus and its convoluted anatomical relationship with the hippocampal combined area fields in the anterior pes region, imaging and measurement focused on the cross-sectional area of the area dentata (AD) in its posterior region, where it is possible to visualize it optimally and measure it separately from the hippocampal CA1–CA3 fields and subiculum (CAS). Here the AD, which was designated by Blackstad (Blackstad, 1956), consists of the dentate gyrus and hippocampal field CA4, located within the hilus of the dentate gyrus (Rosene and Van Hoesen, 1987; Duvernoy, 1998).

## Methods

### Subjects

All participants were recruited from community advertisements and referrals. Prior to testing, the nature of

the study and the procedures were explained to each subject and informed consent was obtained. When participants were younger than 18 years of age, informed consent was obtained from their parents. The experimental procedures were approved by the Institutional Review Board of San Diego Children's Hospital Research Center. All patients and control subjects were paid for their participation.

A total of 59 autistic patients, aged 29 months to 42 years, participated (52 males, seven females; mean age 11.2 years, SD 9.2). Table 1 details the cognitive and medical characteristics of the patients. Valid non-verbal IQ scores using either the Wechsler Intelligence Scale for Children (WISC) III, revised WISC (WISC-R), Leiter or Stanford Binet were obtained from 50 of the autistic subjects and ranged from 36 to 85. Valid verbal IQ scores, using WISC-III, WISC-R, Stanford Binet or the Peabody Picture Vocabulary test, were obtained from 40 of the autistic subjects and ranged from 41 to 135. Inclusionary criteria were the diagnosis of autism based on the Autism Diagnostic Interview—Revised (Rutter *et al.*, 1995), the Autism Diagnostic Observation Schedule—Generic (Lord *et al.*, 1998) and the Childhood Autism Rating Scale (Schopler *et al.*, 1980). Of the total 59 autistic subjects, 19 were 29 months to 5 years of age and had been diagnosed with autism using a prospective MRI and longitudinal diagnosis procedure detailed in a companion paper (Courchesne *et al.*, 2001). In this procedure we produce MRIs of children aged 29 months to 5 years with a suspected diagnosis of autism and the diagnosis is confirmed or altered at a later age. Only children confirmed to be autistic were included in the present study; children diagnosed with PDD-NOS (Pervasive Developmental Disorder—Not Otherwise Specified), Asperger's syndrome or any other non-autistic disorder were excluded. In this way, we obtained and analysed neuroanatomical data from a total of 11 children aged 29 months to 4 years whose diagnosis was confirmed as autism (71% of our original sample). Additionally, 32 autistic children aged 5 to 12 years and 16 autistic adolescents and adults aged 13 to 42 years were examined by MRI.

A total of 51 healthy normal people aged 28 months to 43 years participated (40 males and 11 females; mean age 11.4 years, SD 8.0). Based on medical, family and educational history questionnaires, these subjects showed no evidence of developmental, educational, medical, psychological or psychiatric abnormalities or deficiencies on a pre-MRI screening. IQ scores ranged from 88 to 150 for verbal IQ and from 90 to 150 for non-verbal IQ. Thirty-six were children aged 28 months to 12 years and 15 were adolescents and adults aged 13 to 42 years.

### Neuroanatomical measurements of MRI

Oblique–coronal MRIs of the hippocampal formation perpendicular to its long axis were obtained using an inversion recovery sequence with 5 mm interleaved sections [no gaps; TR (repetition time) = 1500 ms, TE (echo time) = 25 ms,

**Table 1** Cognitive and medical characteristics of autistic subjects ( $n = 59$ ).

	Age (years)	CARS	ADI-R			History of seizures	Co-occurring medical conditions	
			Social	Restricted and repetitive	Communication			
					Verbal autistic individuals* ( $n = 37$ )			Non-verbal autistic individuals† ( $n = 22$ )
Mean $\pm$ SD	11.2 $\pm$ 9.4	40.4 $\pm$ 4.9	25.2 $\pm$ 3.2	7.9 $\pm$ 2.5	17.9 $\pm$ 4.1	13.0 $\pm$ 1.5	25.4%	6.8%‡
Range	2.26–42.2	30–50	18–30	3–12	8–24	9–14		

Autism Diagnostic Interview (ADI-R) and Childhood Autism Rating Scale scores are reported together with medical conditions for autistic subjects. ADI communication scores are reported separately for verbal and non-verbal autistic subjects (verbal subjects: sum of verbal and non-verbal communication scores; non-verbal subjects: non-verbal communication score only). Ranges for ADI subscales for a diagnosis of autism: Social Impairment (10–30), Restricted and Repetitive Behaviours (3–12), Communication Impairment; verbal subjects (8–26), non-verbal subjects (7–14). \*Verbal + non-verbal scores; †non-verbal scores only; ‡four autistic subjects had concurrent diagnosable conditions based on medical examinations and testing; quantitative analyses for amino acids, organic acids and urine analysis revealed that all four patients had elevated levels of plasma lactic acid (lactic acidemia). All autistic subjects were negative for fragile X chromosome as determined by DNA or chromosomal analysis.

TI (inversion time) = 708 ms, number of excitations 2, FOV (field of view) = 16 cm, matrix = 256  $\times$  192), as described in a previous study (Saitoh *et al.*, 1995). For all subjects, the MRI technician positioned the oblique–coronal image slice acquisition coordinates such that one slice was always located at the caudal end of the posterior segment of the uncus, i.e. the intralimbic gyrus. In this way, three contiguous slices posterior to this slice were positioned at locations along the body of the hippocampal formation that were comparable across subjects, as previously described and shown (Fig. 1) (Saitoh *et al.*, 1995). This 15 mm segment of the body of the hippocampal formation is known to have a consistent and simple C-shape across these three cross-sectional slice positions within an individual subject (Amaral and Insausti, 1990).

Images were enlarged digitally ( $\times 5$ ) from 256  $\times$  256 to 1280  $\times$  1280 by replicating individual pixels, and a two-dimensional convolution with an 11  $\times$  11 triangular filter kernel was applied. Outlines of anatomical structures were traced by hand using software (Area) developed in our laboratory, which provided a tracing with a line thickness of 0.125 mm on the enlarged MRIs.

As described and shown previously (Saitoh *et al.*, 1995), the three contiguous 5 mm cross-sectional MRIs of the hippocampal body provided sufficient signal information to show fine details of anatomical structure, i.e. pyramidal cell fields of the subiculum and hippocampus, the fimbria, the alveus, portions of the perforant path and the stratum lacunosum moleculare, which extends to the ‘superficial medullary lamina’, the well-myelinated plexiform or molecular layer in the subicular complex region (Fig. 1). The region of high signal intensity superficial to the pyramidal cell layer marked the stratum lacunosum moleculare and indicated the exact location of the hippocampal fissure. It was therefore possible to demarcate, on each of the three contiguous slices, the area dentata in the body of the hippocampal formation with high reproducibility.

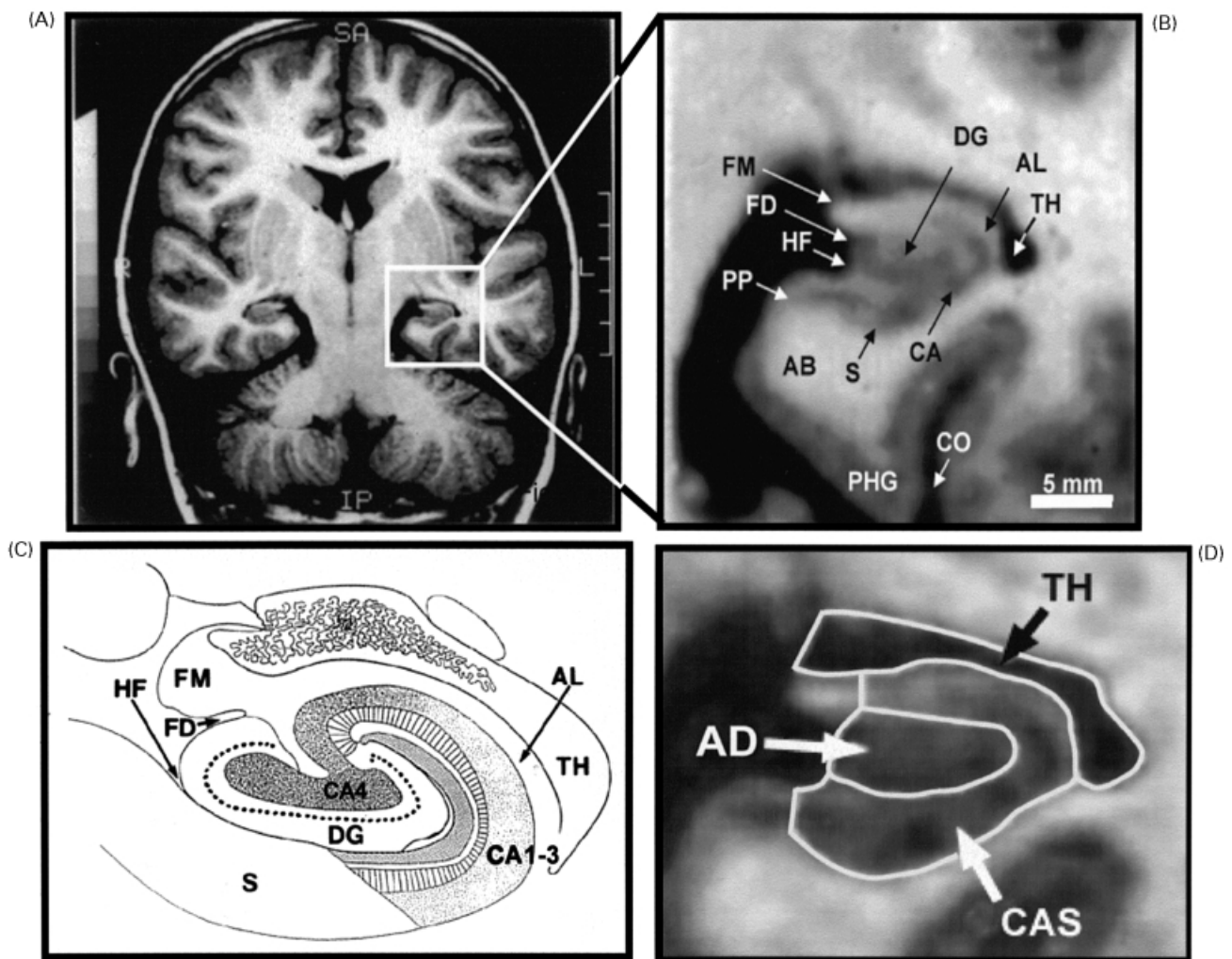
Using anatomical landmarking and tracing procedures described previously (Saitoh *et al.*, 1995), on each of the three contiguous slices, the cross-sectional size of the AD (dentate gyrus + CA4) was measured, as was the CAS. All measurements were performed three times by the same investigator (O.S.), who was blind to the identities and group membership of the subjects through coding of the images. Intraclass correlations, which were calculated to ascertain the intertrial reproducibility of repeated measurements for each area (using reliability analysis from SPSS Base 10.0, 1999), were 0.977–0.985 (mean 0.981) for the total hippocampal formation (AD + CAS) and 0.963–0.972 (mean 0.966) for AD. For each subject, the mean cross-sectional areas of AD and CAS were calculated from the areas measured three times on three contiguous slices on the right and left sides of the brain; the mean from 18 measures (3 measures  $\times$  3 slices  $\times$  2 sides) was used for statistical analysis.

### Statistical analysis

Statistical analysis was carried out using BMDP statistical software (Biomedical Statistical Package; SPSS, Berkeley, Calif., USA). For analysis of variance (ANOVA), study groups were divided into age bins that were narrower at younger ages, when age-related changes were expected to be greatest; the age bins were 2–4, 5–6, 7–12, 13–19 and 20–43 years. A group (2) by age bin (5) ANOVA was performed for AD and CAS measures. A *t* test was performed within the autistic group between patients with and without epilepsy for AD and CAS measures.

### Results

Results are shown in Fig. 2. ANOVA showed that the cross-sectional area of AD was significantly smaller than normal in autism [main effect of study group:  $F(1,108) =$

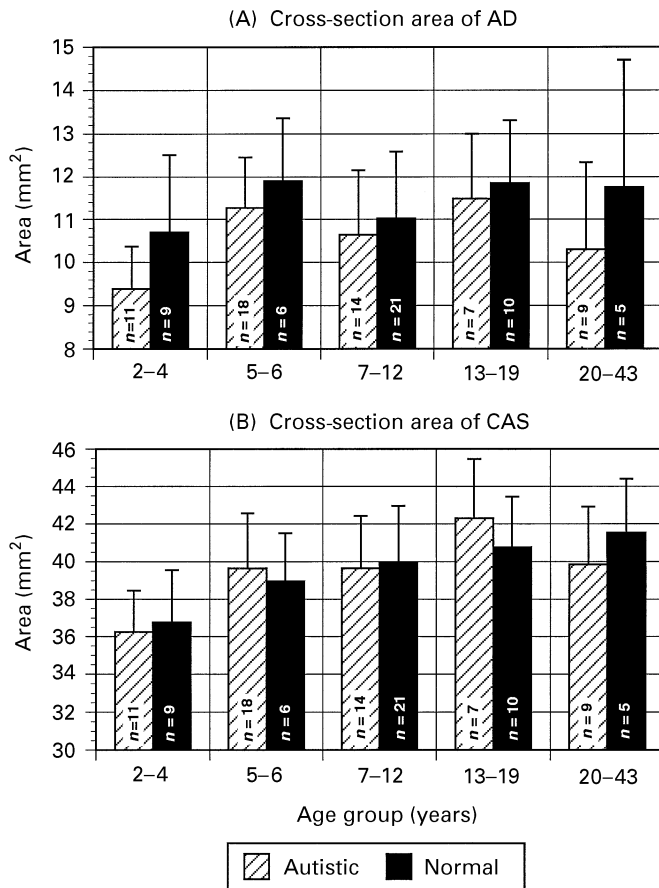


**Fig. 1** The hippocampal formation in the coronal plane. (A) Representative T<sub>1</sub>-weighted coronal image. The left hippocampal formation is indicated with a box. The image is from a 6-year-old autistic boy. (B) Magnified coronal image of left hippocampal formation. AB = angular bundle; AL = alveus; CA = cornu ammonis; CO = collateral sulcus; DG = dentate gyrus; FM = fimbria; FD = fimbriodentate sulcus; HF = hippocampal fissure; PHG = parahippocampal gyrus; PP = perforant path; S = subiculum; TH = temporal horn of the lateral ventricle. (C) Drawing of the hippocampal formation adapted from Duvernoy (Duvernoy, 1988). The cross-sectional areas of the area dentata (AD; dentate gyrus + CA4) and combined subiculum and CA1–CA3 (CAS) were measured as shown in D. Abbreviations as in B. (D) Magnified coronal image of left hippocampal formation with lines depicting anatomical boundaries of AD and CAS. Line thickness was increased for illustrative purposes. Abbreviations as in B and C.

5.32,  $P < 0.05$ ]. This significant difference remained after expressing AD as a proportion of CAS cross-sectional area [ $F(1,108) = 8.69$ ,  $P < 0.01$ ] as well as when this AD to CAS ratio was examined using total brain volume as a covariate [ $F(1,88) = 4.55$ ,  $P < 0.05$ ]. Significant age-related changes in the cross-sectional area of AD were seen in both study groups [main effect of age:  $F(4,108) = 3.18$ ,  $P < 0.02$ ], but there was no group  $\times$  age interaction ( $P > 0.10$ ). For autistic and normal subjects, children aged 5–6 years had the largest AD, and the largest percentage deviation from normal size (–13.5%) was among the youngest autistic children, aged 29 months to 4 years (Fig. 2).

For both study groups, strong age-related increases were seen in the cross-sectional area of CAS, with the maximum size in both groups not reached until adulthood [main effect of age:  $F(4,108) = 9.81$ ,  $P < 0.0001$ ]. However, for the cross-sectional area of CAS there was no significant difference between autistic and normal subjects [ $F(1,108) = 2.10$ ,  $P > 0.10$ ] and no study group  $\times$  age interaction ( $P > 0.10$ ). Absence of group differences in CAS area remained unchanged after covarying for total brain volume ( $P > 0.10$ ).

For the cross-sectional areas of CAS and AD, there was not a significant difference between the 15 autistic patients with and the 44 without a history of seizure episodes ( $P > 0.10$ ).



**Fig. 2** Mean cross-sectional areas of AD and CAS by age group: (A) area dentata (AD = dentate gyrus + CA4). (B) CAS (CA1–CA3 + subiculum). Error bars represent the standard deviation. AD cross-sectional area was smaller in autistic patients than in normal controls. The largest difference in AD size between autistic and normal individuals occurred in children aged 29 months to 4 years (13.5%). The cross-sectional area of CAS did not differ between autistic patients and normal controls. The mean age of autistic and normal subjects was not significantly different within age bins ( $P > 0.10$ ).

## Discussion

The present MRI evidence shows that autism is characterized by hypoplasia (undergrowth) of the AD, a component of hippocampal memory circuits. CAS area measures in autism were not significantly different from normal. This is the first direct evidence that anatomical abnormality within the limbic system exists from the earliest years of this disorder, and persists throughout development and up to middle age.

At present there are no published post-mortem data for young autistic children near the age of clinical onset. However, post-mortem evidence from older autism patients (9 years old to adult) does document anatomical abnormalities in the limbic system in most (Bauman and Kemper, 1994), though not all (Bailey *et al.*, 1998), cases of autism studied. When such abnormalities are present, there is increased pyramidal cell-packing density (number of neurones per volume) and reduced neuronal cell size in limbic areas (hippocampus proper, subiculum, entorhinal cortex,

amygdala, mammillary body, anterior cingulate cortex and septum) (Bauman and Kemper, 1994). Additionally, a morphometric study of two of these older autistic post-mortem patients showed that pyramidal neurones were smaller than normal in CA4 but not in CA1, while dendritic branching in both regions was decreased (Raymond *et al.*, 1996). Bauman and colleagues interpreted these observations as developmental in origin, but by themselves, none are definitively indicative of any particular age of onset and could be prenatal or post-natal. Direct observations of the youngest autistic brain are lacking.

In the present study, we report significantly smaller AD cross-sectional areas in autism, which could be due to abnormalities in CA4, the dentate gyrus, or both regions. More studies are needed to determine the cellular underpinning of this finding. However, several interesting speculations may be discussed. Increased pyramidal cell-packing density and decreases in dendritic branching in the hippocampus proper, as reported in post-mortem studies (Bauman and Kemper, 1994), might contribute to hypoplasia in CA4. The finding that neurone size reductions were more severe in CA4 than CA1 (Bauman and Kemper, 1994) may explain why hypoplasia was detected in AD but not CAS measurements. Additionally, the present *in vivo* MRI finding that abnormality in AD is present at the time of clinical onset (~2 years of age) lends support to Bauman's interpretation of post-mortem data as developmental in origin, although again the age of onset could be pre- or post-natal.

In addition to CA4 hypoplasia, abnormally small AD measurements in autism might be due to hypoplasia of the dentate gyrus. There is a lack of direct histological examinations of the granule cells and other hilar cells (Amaral, 1978) of the dentate gyrus in autism. One study examined the dentate gyrus of one 19-year-old autistic man as a control for Rett patients but reported only qualitative staining differences between cases, showing only minor staining abnormalities in dentate granule cells compared with severe staining abnormalities in Rett patients (Leontovich *et al.*, 1999). The present *in vivo* MRI evidence of reduced cross-sectional area of AD in autism provides evidence that the dentate gyrus might be abnormal in autism and affirms the need for histological studies of this structure in autism.

The hippocampal formation has a complex series of feed-forward and recurrent connections (Yeckel and Berger, 1990; Amaral, 1993; Jones, 1993). The question arises of why the CAS areas (CA1–3 and subiculum) might be spared if the dentate gyrus and/or CA4 are abnormally small. One explanation was mentioned above: greater pyramidal size reduction in CA4 than in CA1, as reported in post-mortem studies of autism (Bauman and Kemper, 1994). Other studies report differing vulnerabilities among CA fields to ageing, disease and experimentally induced lesions (for review, see Duvernoy, 1998). Developmental differences between CAS and AD might also contribute to the finding of reduced AD measures in autism without significant differences in CAS. In the hippocampal formation of the rhesus monkey, neurones

that will migrate to the hippocampus and subiculum are generated exclusively in the ventricular zone lining the medial wall of the lateral cerebral ventricle, while neurones of the dentate gyrus are also generated in the prospective hilus of the dentate gyrus (Nowakowski and Rakic, 1981). An interesting possibility is that, in autism, neurone proliferation is disturbed in the prospective hilus but is not disturbed in the ventricular zone, leading to hypoplasia in the dentate gyrus but a CAS area of normal size.

Recent developmental studies of the dentate gyrus have implications for cognitive impairment in autism in the light of the present finding of AD hypoplasia in autism. The human dentate gyrus is one of the last brain regions to receive its final full developmental complement of neurones (Bayer *et al.*, 1993) and immature dentate granule cells are present at 15 months of age (Seress, 1992). Also immature at birth are the synaptic connections between dentate granule cells and their target neurones. This developmental stage of initial memory circuit connectivity may continue during the first 3 years post-natally (Seress, 1998). The present quantitative MRI findings raise the hypothesis that, in the autistic brain, dentate gyrus development is even more protracted, and this may substantially lengthen the period of formation of the well-characterized hippocampal 'trisynaptic' circuits (Andersen *et al.*, 1971; Amaral, 1993), perhaps delaying or derailing the emergence of normal hippocampal memory function in those with this disorder.

An important question is how early aberrant dentate gyrus development would adversely influence the development of those other hippocampal subregions through activity-dependent molecular mechanisms. An animal model testing the consequences of an early developmental dentate gyrus defect for the structural development of the hippocampal and subicular regions, as well as for later memory function, could employ a recently described method of neonatal X-irradiation that reduces the number of dentate granule cells without affecting the number of hilar neurones and pyramidal cells of Ammon's horn (Czurko *et al.*, 1997).

Differing cytoarchitectonic characteristics of the four CA fields may also contribute to the finding of smaller AD but spared CAS in autism. The pyramidal cell density in CA4 has been reported to be a third that of CA2 and CA3 and half that of CA1 (Mouritzen Dam, 1979; Babb *et al.*, 1984; Mani *et al.*, 1986; Mathern *et al.*, 1997). A cross-sectional measure of the area of CA4 contains fewer pyramidal cell bodies and processes than CAS measures, and mossy axons from the dentate granule cells and interneurons would therefore contribute to the cross-sectional area of CA4 to a greater degree. An abnormality in the granule cells of the dentate gyrus could disproportionately reduce the cross-sectional area of CA4 by reducing the number of mossy fibres traversing CA4.

Recent evidence shows that, in humans as well as in mice and monkeys, the neurogenesis of dentate granule cells is not limited to pre- and perinatal life as previously thought, but occurs even in adult life (Eriksson *et al.*, 1998). The rate

of neurogenesis during life is increased or decreased by factors that may be relevant for autism. Specifically, the rate of neurogenesis increases in an enriched environment in animals (Kempermann *et al.*, 1997). If this happens in humans as well, then early diagnosis of autism and the subsequent exposure to enriched learning conditions might promote enhanced growth of this structure in these children. Conversely, social stress decreases the rate of neurogenesis in primates (Gould *et al.*, 1998) and may also lead to hippocampal atrophy in man (Sapolsky, 2000).

The dentate gyrus is also known to have an important role in temporal lobe epileptogenesis (Jones, 1993; Brooks-Kayal *et al.*, 1998). A higher prevalence of epilepsy and its predominance of complex partial seizures are characteristic of autism, in contrast with non-autistic neuropsychiatric disorders (Steffenburg *et al.*, 1996). In the present study, however, no significant difference in AD cross-sectional area between the presence and absence of epilepsy was found, but additional studies are needed.

No single structure accounts for the pervasive and persistent neurological and behavioural symptoms of autism, and developmental defects in multiple anatomical systems almost certainly will interact (for review, see Bauman and Kemper, 1994; Courchesne and Pierce, 2001). Establishing the neural basis of autistic behaviour will therefore require functional MRI and ERP (event-related potential) neuroimaging studies that directly test correlations between specific anatomical defects (e.g. dentate underdevelopment) and specific functional or behavioural impairments (e.g. contextual memory).

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