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A clinicopathological study of autism

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Summary

A neuropathological study of autism was established and brain tissue examined from six mentally handicapped subjects with autism. Clinical and educational records were obtained and standardized diagnostic interviews conducted with the parents of cases not seen before death. Four of the six brains were megalencephalic, and areas of cortical abnormality were identified in four cases. There were also developmental abnormalities of the brainstem, particularly of the inferior olives. Purkinje cell number was reduced in all the adult cases, and this reduction was sometimes accompanied by gliosis. The findings do not support previous claims of localized neurodevelopmental abnormalities. They do point to the likely involvement of the cerebral cortex in autism.

Keywords: autism; neuropathology; megalencephaly; cortical dysgenesis

Abbreviations: ADI = Autism Diagnostic Interview; GFAP = glial fibrillary acidic protein

Introduction

Autism is a severe developmental disorder characterized by impairments in reciprocal social interaction and communication, restricted and stereotyped patterns of behaviour and interests, and an onset before 3 years of age (World Health Organization, 1992). The core disorder affects approximately four in 10 000 children, and is much commoner in males, in a ratio of ~4 : 1. The syndrome was first described by Leo Kanner (1943); he assumed that affected children were of normal intelligence and for several decades the disorder was thought to be psychogenic. An organic basis was first suggested by the finding that three-quarters of sufferers are mentally handicapped (Lockyer and Rutter, 1969) and that at least one-quarter develop epilepsy (Rutter, 1970; Gillberg and Steffenburg, 1987). Subsequently autism was often assumed to be an unusual consequence of brain damage, caused either by medical disorders or obstetric hazards. More recently it has been appreciated that only a minority of cases of autism are associated with medical causes of mental handicap, then most commonly with tuberous sclerosis or Fragile X (Rutter *et al*., 1994). The findings from twin and family studies suggest that the vast majority of idiopathic cases arise on the basis of strong specific genetic influences (Bailey *et al*., 1996). Thus, autism usually appears to represent a severe expression of a specific disease process.

Many regions of the brain have been implicated in the genesis of autism, but the neurobiological basis of the disorder remains unknown. Autism is a rare disorder which was described relatively recently and there have been only a few post-mortem studies. Darby (1976) reviewed 33 diverse cases and found no consistent abnormalities. Two of the four cases reported by Williams *et al*. (1980) had associated disorders (phenylketonuria and probable Rett's syndrome); in one of the two idiopathic cases pyramidal cell dendritic spine density was reduced in the mid-frontal gyrus and the number of cerebellar Purkinje cells was also diminished. Coleman *et al*. (1985) undertook cell counts in several cortical regions from a single case and two control subjects. Consistent differences were not found, although the glia : neuron ratio was smaller in the autistic brain than in the two control subjects. Recent examination of the brainstem of this case (Rodier *et al*., 1996) revealed a hypoplastic facial nucleus and superior olive. Ritvo *et al*. (1986) measured Purkinje cell density in the brains of four autistic and four control subjects and reported significantly lower counts in the autistic brains (the

histopathological findings in the cerebral hemispheres and brainstem have not been reported). There have been two case reports of extremely retarded individuals who were also considered to have autism (Hof *et al*., 1991; Guerin *et al*., 1996).

Bauman and Kemper's study of six brains is the most comprehensive post-mortem study of autism to-date (Kemper and Bauman, 1993). In all cases there was a reduction in Purkinje cell density, but this varied in severity (Bauman, 1991). In four of the cases Purkinje cell density was decreased by 50–95% in some areas (Arin *et al.*, 1991); three of these individuals had a history of epilepsy (Kemper and Bauman, 1993) which could be relevant. In two brains there was also a qualitative decrease in cerebellar granule cell density (Kemper and Bauman, 1993). The neurons of the cerebellar nuclei were enlarged in the brains of two children and decreased in both size and number in those of three adults (Kemper and Bauman, 1993). The dentate nucleus was distorted in one brain (Bauman and Kemper, 1985). Inferior olivary neurons were preserved; they were enlarged in the younger individuals and unusually small in the adults. In five brains the inferior olivary neurons tended to cluster at the periphery of the convolutions.

In the forebrain, abnormally small, densely packed neurons were noted in all areas of the hippocampus, subiculum, mamillary body, septal nuclei and amygdala (Kemper and Bauman, 1993). Hippocampal neuronal counts have been published on only the first of these six cases (Bauman and Kemper, 1985). The size of hippocampal pyramidal cells has been measured in areas CA1 and CA4 of two cases; only the neurons in CA4 were significantly smaller than in the control brains (Raymond *et al*., 1989). The only consistent cerebral cortical abnormalities were in the anterior cingulate region.

Because neuropathological abnormalities have been largely confined to the cerebellum and medial temporal structures, their possible involvement in autism has been the subject of much interest. Bauman and Kemper (1985) argued that decreased Purkinje cell density, in the absence of either glial cell hyperplasia or retrograde olivary cell loss, suggested that the cerebellar abnormality developed at or before 30 weeks gestation. An MRI study of autistic individuals and control subjects (Courchesne *et al*., 1988) found hypoplasia of cerebellar vermal lobules VI–VII, but not of vermal lobules I–V. On the basis of the post-mortem and neuroimaging findings, Courchesne's group have argued that developmental cerebellar abnormalities are the most consistent neuroanatomical lesion in autism, and that such abnormalities can lead to the characteristic symptomatology by several different routes (Courchesne *et al*., 1994). Nevertheless the localized vermal abnormality has not been replicated using similar imaging protocols (see Bailey and Cox, 1996). Temporal horn dilatation, visualized by pneumoencephalography (Hauser *et al.*, 1975), has been cited in support of the hypothesis that medial temporal abnormalities underlie the autistic syndrome (Bauman and Kemper, 1985; DeLong, 1992; Kemper and Bauman, 1993; Bachevalier, 1994). The finding of temporal horn dilatation has not been replicated, and the only quantitative MRI study of the posterior hippocampus did not find any differences between autistic and control subjects (Saitoh *et al.*, 1995).

The focus upon the cerebellum and medial temporal lobe structures arose largely because of the limited post-mortem evidence of abnormalities in other areas. Nevertheless, because autism is associated with epilepsy, EEG abnormalities and mental handicap, the possibility of neocortical involvement in autism has been raised (Minshew, 1991). The main goals of the present study were to determine whether neuropathological abnormalities are more extensive than previously supposed, and to evaluate the previous observations. The brain weights of the first four cases in this study have been previously reported (Bailey *et al*., 1993).

Methods

Case material

Post-mortem brain tissue was obtained from six individuals with autism. Contact was made with clinical colleagues specializing in the diagnosis and treatment of autism and advertisements were placed in the publications of several of the national and international autistic societies. UK pathologists were also informed of the study. By these means post-mortem brain tissue from two cases (1 and 3) and whole brains from four individuals who died since 1991 were obtained. In addition, post-mortem findings, but no tissue, were available from a further 14 cases.

Diagnostic assessment

The parents of five of the six cases included in the study were interviewed using the Autism Diagnostic Interview (ADI), an investigator based instrument of known reliability and validity (Le Couteur *et al.*, 1989). A diagnostic algorithm for autism, based upon ICD-10 criteria, was completed using the information gathered with the ADI. Case 3 was reviewed by one of the authors (M.R.) in adulthood; because he died in the 1970s the parents were not recontacted. The available clinical and educational notes of all cases were reviewed.

Clinical details

The six patients were all male and diagnosed as showing autism in life. Cases 1, 2, 4, 5 and 6 all met ADI algorithm criteria for autism. Relevant medical information and any psychometric findings are noted below. Case histories are provided in the Appendix. To maintain confidentiality details of the circumstances of death have been omitted.

Case 1, age 4 years

Born at 41 weeks gestation weighing 8 lb. No history of peri- or neonatal brain damage. Head circumference just

above the 10th percentile at birth, above the 25th percentile by 9 months and above the 50th percentile by 22 months. Left convergent squint noticed from birth. Advice was sought for head lag and hypotonia at 9 months. He sat unaided at 9 months, cruised at 24 months and walked slowly by 29 months. Developmental ages (months) on the Griffiths scales at 24 months were: locomotor 11; personal–social 18.5; hearing and speech 11.5; eye–hand co-ordination 13.5 and performance 16. At 30.5 months the scores were: locomotor 16.5; personal–social 20.5; hearing and speech 16.5; eye–hand co-ordination 19 and performance 17.5. Extensive biochemical investigations, chromosomal analysis and skull X-ray were normal. He was seen a few weeks before death by his paediatrician; he could run well but flapped his arms and was socially aloof.

Case 2, age 23 years

Induced at 42 weeks gestation, weighed 7 lb 2 oz; no history of peri- or neonatal brain damage. Head circumference at 2 years 7 months was 51.5 cm (above 75th percentile). At 3 years 6 months, head circumference was 54 cm (above 97th percentile); a lumbar puncture and EEG were normal. Severe self-injury was a significant management problem and included autoamputation of part of a digit and anal gouging. Medication, used in an effort to control his overactive and difficult behaviour, included: flupenthixol, chlorpromazine, chlorpheniramine, amitriptyline, lithium, carbamazepine and benzodiazepines. There was no definite evidence of epilepsy but, ~6 months prior to death, the subject had two falls accompanied by diminished awareness.

Case 3, age 27 years

Born at 38 weeks gestation weighing 5 lb 12 oz; no history of peri- or neonatal brain damage. He sat at 11 months, stood at 18 months and did not walk until 25 months. At the age of 6 years he sustained a depressed right frontal fracture. He had two seizures at the age of 13 years which involved head deviation to the right, and at the age of 18 years developed generalized seizures which occurred approximately once a month. He had received phenytoin, phenobarbital and carbamazepine. He completed several performance IQ tests at the age of 16 years and performed best on spatial items; his estimated IQ was 43. Using the Vineland scale his social age was estimated as 1.35 years.

Case 4, age 24 years

Born at term weighing 8 lb 7 oz; no history of peri- or neonatal brain damage. His head circumference at 6 years and 10 months was 57 cm (above 97th percentile). An EEG at the age of 6 years was dysrhythmic and slow. His first grand mal seizure occurred at 19 years of age; an EEG showed only minor diffuse abnormalities. His seizures continued, were difficult to control and were preceded by aggressive behaviour. He had been treated with sodium valproate, primidone, lamotrigine and clobazam.

Case 5, age 20 years

Born at term weighing 7 lb 11 oz; mother took a progesterone drug during the first 16 weeks of pregnancy. No history of peri- or neonatal brain damage. The first grand mal seizure occurred at the age of 11 years. These seizures occurred approximately weekly, mainly at night or on waking. An EEG recording consisted mainly of slow wave activity; short bursts of generalized spike and wave activity were also seen. He was initially treated with sodium valproate. This was changed to carbamazepine and then vigabritim added. At the age of 10 years his mental age scores on the Griffiths Scales were (in months): locomotor 46, personal social 38, hearing and speech 22, eye–hand co-ordination 31.5 and performance 52.

Case 6, age 24 years

Delivered by forceps at 39 weeks gestation because of a broad head (head circumference not recorded); he weighed 7 lb 15 oz. No history of peri- or neonatal brain damage. In childhood there were four febrile convulsions and subsequently four afebrile seizures. A convulsion at the age of 22 years may have been a drop attack. He was prescribed ritalin between the ages of 7 and 8 years but he never received anticonvulsants.

Control material

For the morphometric studies, identically processed, individual male control subjects for Cases 2, 4, 5 and 6 were chosen from the cases available at the Institute of Psychiatry. Potential control subjects were not included when intercurrent disease could have affected neuronal counts. An identically processed male control for Case 3 was obtained from the Institute of Neurology, but no cerebellum was available. No suitable, identically processed age-matched male control subjects were available at the Institute of Child Health for Case 1, and tissue from two age-matched females was used. The weight of the brain was not recorded for two control cases.

Tissue processing of whole brains (Cases 2, 4, 5 and 6)

Brains were weighed intact, and the brainstem and cerebellum was separated and weighed. A cerebral hemisphere (left or right, chosen at random) was fixed intact and has not yet been examined histologically. The other hemisphere was freshly sliced and blocks removed for short fixation, cryoprotection and electron microscopy when tissue preservation was adequate. The remaining tissue was fixed in 10% buffered formol saline, and blocks were subsequently taken for paraffin

Table 1 *Brain weight of six mentally handicapped autistic males*

Case	Age (years)	Brain weight (kg)	Normal range* (kg)	Weight of brainstem and cerebellum (kg)	Ratio of total brain to brainstem and cerebellar weights
1	4	1.53	1.25–1.35	0.15	10.2 : 1
2	23	1.60	1.39–1.49	0.19	7.6 : 1
3	27	1.45	1.39–1.49	0.21	7.6 : 1
4	24	1.81	1.39–1.49	0.22	8.2 : 1
5	20	1.41	1.39–1.49	0.21	6.6 : 1
6	24	1.82	1.39–1.49	0.23	7.9 : 1

*Normal ranges given as mean ± 2.5 SD (from Dekaban and Sadowsky, 1978).

embedding, routine staining and examination. Where possible, the cerebral cortex, hippocampus and cerebellum were compared histologically with material from identically processed age- and sex-matched control subjects.

Immunohistochemistry for glial fibrillary acidic protein (GFAP; DAKO, 1 : 1600) and phosphorylated neurofilaments (RT97; Courtesy of BH Anderton, 1 : 100) was performed using the avidin–biotin complex method (DAKO) with diaminobenzidine as the chromogen.

Morphometric studies

As a supplement to subjective assessment, limited morphometry was undertaken. Neuronal counts were performed on sections from three areas: (i) the medial aspect of the superior frontal gyrus at the level of the corpus callosum; (ii) the CA1, CA3 and CA4 sub-fields of the hippocampus (as close as possible to the lateral geniculate body); and (iii) the Purkinje cell layer of the superior aspect of the cerebellar hemisphere.

Sections cut at ~14 μm thickness were stained with cresyl violet and examined with a ×40 objective (×10 eyepiece). Neurons were identified on the basis of classical morphological criteria. For neocortical counts, successive fields, as defined by a rectangular eyepiece graticule were counted from pia to white matter. Neurons whose nucleus lay on either of two adjacent borders of the field boundary (forbidden lines) were excluded. Neurons were considered to lie within the thickness of the section if the mid-point of the nucleus, as defined by a sharply focussed nuclear outline, was present (Everall *et al*., 1991). All section thicknesses were measured by focusing from the top to the bottom of the section with a ×100 oil immersion objective and measuring the distance the microscope stage travelled with a microcator. The product of field size and section thickness provided the reference volume.

In the hippocampus, a single ×40 field of CA1, CA3 and CA4 was counted as described above. The number of nucleolated Purkinje cells was counted and the length of the counted Purkinje cell layer was measured using an IBAS 2000 Kontron image analyser. The product of this length and section thickness yielded a reference area. Purkinje cell counts were therefore expressed as the number per unit area of Purkinje cell layer.

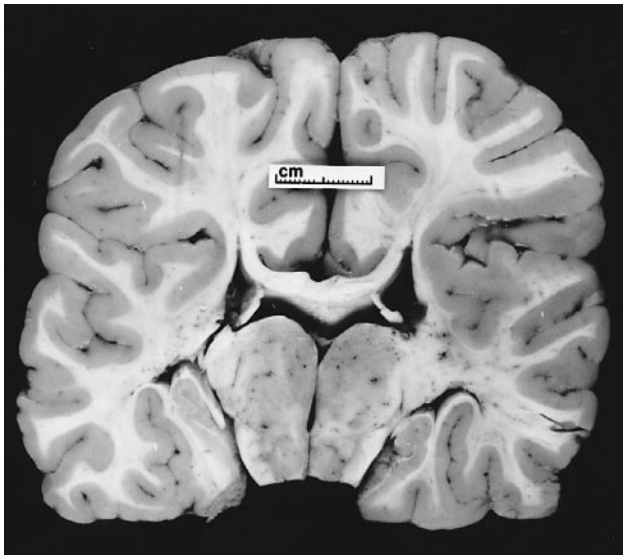

Fig. 1 Case 1. Coronal slice showing large, hyperconvoluted temporal lobes with upturned hippocampi and abnormal lateral ventricles (see text).

White matter neurons were counted deep to the superior frontal sulcus at the level of the head of the caudate nucleus and deep to the superior temporal sulcus at the level of the lateral geniculate nucleus. Sixteen non-overlapping ×400 magnification fields of 7-μm thick haematoxylin and eosin stained sections were counted. Again neurons were identified on the basis of classical morphological criteria.

Results

Post-mortem and histopathological findings in the nervous system of Cases 1–6

Case 1, age 4 years

The brain was large and weighed 1525 g (normal range 1250–1350 g). The weight of the brainstem and cerebellum was disproportionately low at 145 g (Table 1). The convolutional pattern of the cerebral cortex was abnormal, with overlarge hyperconvoluted temporal lobes and upwardly rotated hippocampi (Fig. 1). Anteriorly there was a cavum

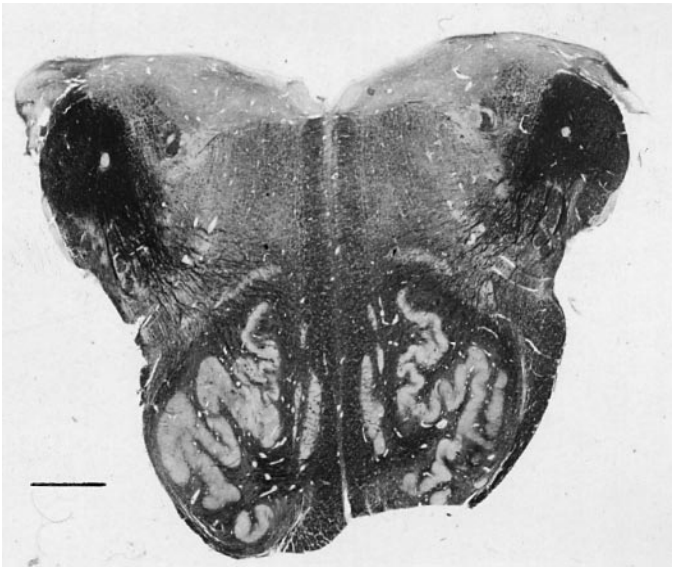

Fig. 2 Case 1. Medulla oblongata. The inferior olives show an abnormal outline; the band of neurons is irregular and broken up. (Luxol fast blue and cresyl violet; bar represents 2.5 mm.)

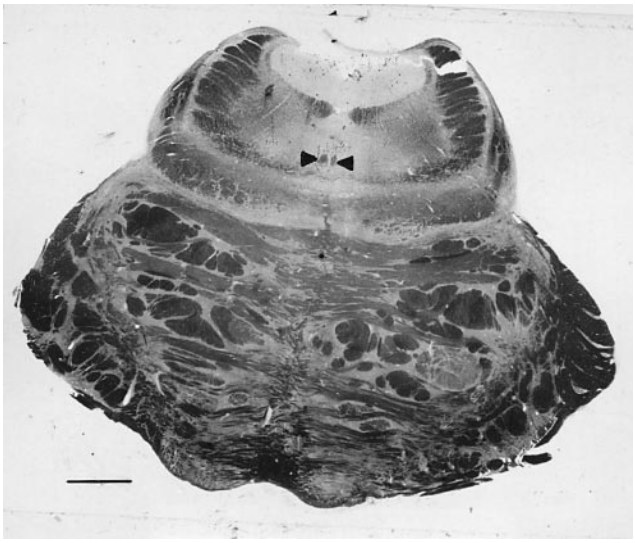

Fig. 3 Case 1. Pons. An aberrant tract, (arrow heads) is seen on both sides adjacent to the midline in the pontine tegmentum. (Luxol fast blue and cresyl violet; bar represents 3.0 mm.)

septi pellucidi but the septum was completely absent posteriorly. The outer angles of the lateral ventricles were abnormally acute. The medulla oblongata was large but the pyramids were relatively small. There was mild widening of the sulci of the superior cerebellar vermis. The inferior olives did not form a continuous ribbon (Fig. 2). There was apparent reduplication of the medial accessory olive, and multiple small bilateral groups of ectopic neurons lay lateral to the olives and in the inferior cerebellar peduncles. An aberrant tract was present in both sides of the pontine tegmentum (Fig. 3). The midbrain was unusually small and the periaqueductal grey matter and raphe nuclei appeared disproportionately large.

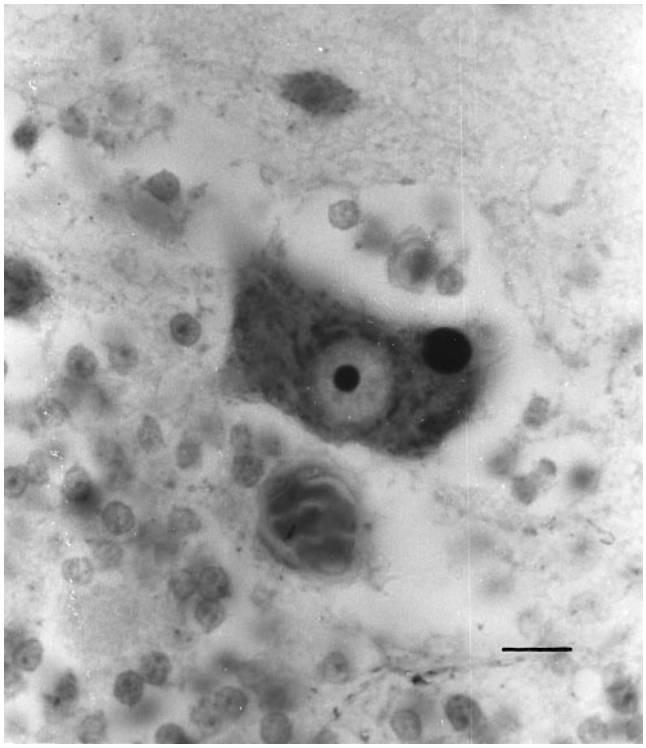

Fig. 4 Case 1. Cerebellum. The Purkinje cells contain round, darkly stained cytoplasmic inclusions (Luxol fast blue and cresyl violet; bar represents 10 μm.)

The perikaryon and proximal dendrites of 30–40% of Purkinje cells in the vermis contained well-defined, round or oval eosinophilic inclusions (mean number two but ranging up to six per cell) that stained homogeneously deep blue with Luxol fast blue and cresyl violet stain and measured up to 7 μm across (Fig. 4). The inclusions were less frequent in the cerebellar hemispheres and were not observed elsewhere. Electron microscopy confirmed that the inclusions were oval or circular and had a uniform intermediate electron density (Fig. 5). No limiting membrane was identified. The dentate ribbon was discontinuous and there were patches of subcortical ectopic grey matter consisting of large mature neurons.

The laminar pattern in the superior frontal gyrus was slightly irregular with clusters of abnormally orientated pyramidal cells, particularly in layer 5 (Fig. 6). There were no neurons that were unusual in size, configuration or neurofilament expression. In some areas in the frontal lobe there was blurred grey–white matter differentiation, and numerous patches of ectopic grey matter and many solitary mature neurons in the white matter. There was a little gliosis subpially and in the white matter of the temporal and frontal lobes. The hippocampal formation and the deep grey matter were unremarkable.

Case 2, age 23 years

The brain weighed 1600 g and was large and slightly swollen, but there was no herniation. There was no atrophy of the

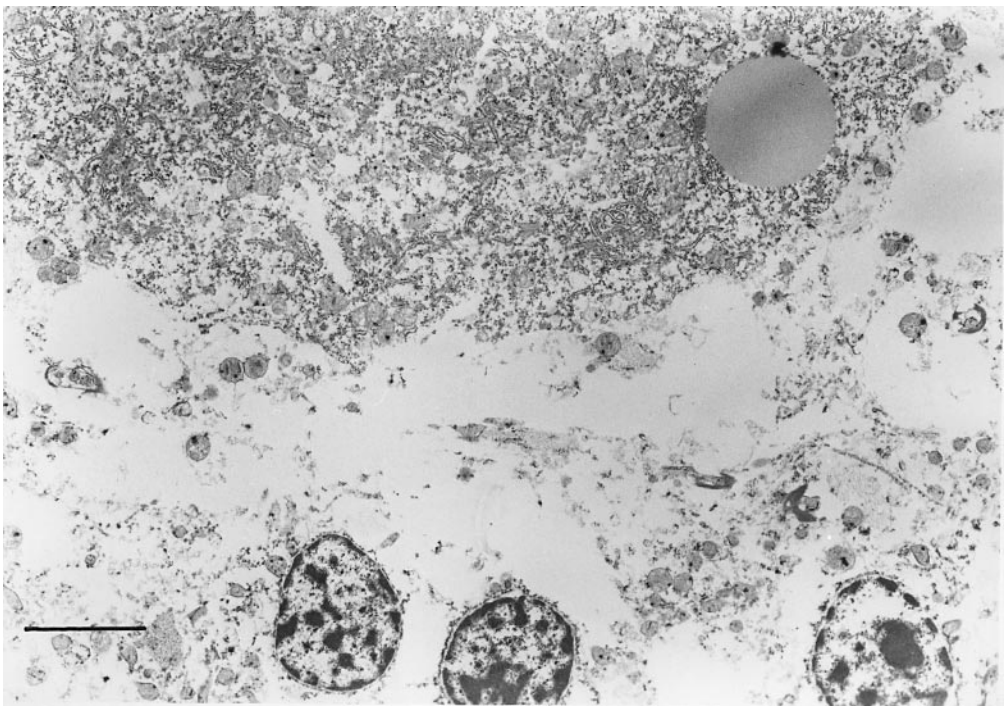

Fig. 5 Case 1. Cerebellum. The Purkinje cell at the top of the figure contains, on the right, a well-defined, homogeneous, moderately electron-lucent inclusion. Note three granule cell neurons at the bottom of the figure. (Electron micrograph; bar represents 5 μm.)

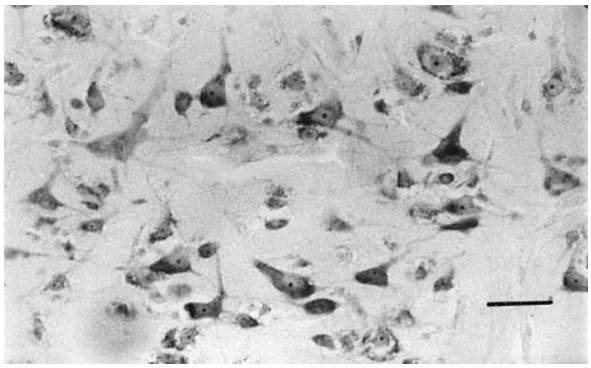

Fig. 6 Case 1. Lamina 5 of the superior frontal gyrus showing irregular orientation of pyramidal neurons. The top of the figure is closest to the pia. (Luxol fast blue and cresyl violet; bar represents 40 μm.)

cerebellar vermis or hemisphere. The inferior olivary ribbon was normally formed, but there were groups of ectopic neurons lateral to both olives. There was also a group of ectopic neurons in an inferior cerebellar peduncle adjacent to the brainstem. The cross-sectional area of the lower end of the aqueduct at the midbrain–pons junction was unusually small (0.2 mm^2). In the cerebellum the number of Purkinje cells was reduced, more so in the hemisphere than vermis, but no empty baskets were seen. There was an increase in GFAP in Bergmann glia. The parietal, frontal and cingulate cortices were thickened in the right cerebral hemisphere, as was the cortex of the superior and middle temporal gyri. The cortex was also unusually cellular. There was an increased

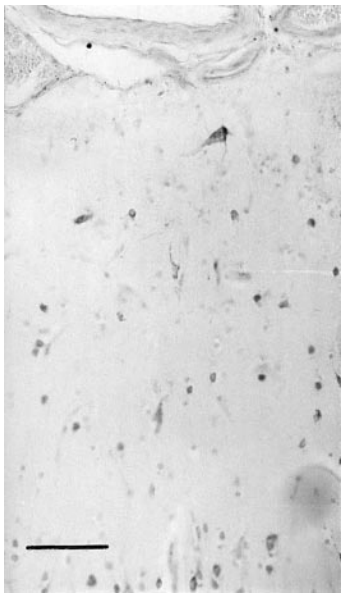

Fig. 7 Case 2. Layer 1 of frontal cortex. Note the increased number of neurons and, in particular, the misorientated pyramidal cell just beneath the pial surface (top). The bar represents 100 μm and lies just above the junction between layers I and II. (Luxol fast blue and cresyl violet.)

number of small neurons in layer 1 of frontal cortex including some inverted pyramidal cells (Fig. 7); these were normal in configuration and neurofilament expression. The corpus callosum was thin, measuring 2.5 mm in thickness just posterior to the genu. Neuronal density appeared to be

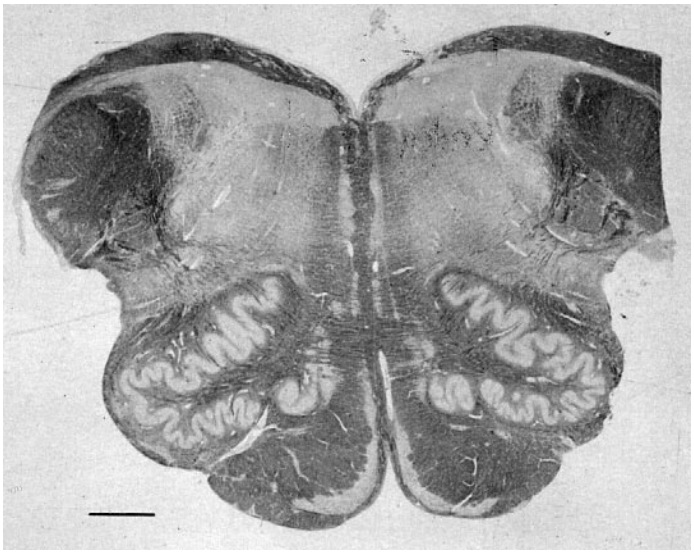

Fig. 8 Case 3. Medulla oblongata. The striae medullares are hypertrophied and the arcuate nuclei appear larger than usual. Compare the regular configuration of the inferior olives with the abnormal outline in Fig. 2. (Luxol fast blue and cresyl violet; bar represents 2 mm.)

increased in the hippocampus. The deep grey nuclei were unremarkable. Electron microscopy of the frontal cortex revealed moderately well-preserved tissue. No abnormalities of synapses, mitochondria, lysosomes or other organelles were identified.

Case 3, age 27 years

The brain weighed 1450 g. The substantia nigra looked pale. In the medulla oblongata the striae medullares were hypertrophied and the arcuate nuclei appeared larger than usual (Fig. 8). The substantia nigra was adequately populated with neurons. There was a bilateral break in the inferior end of the inferomedial olivary ribbon, and small groups of band-like ectopic neurons lay peripheral to the olives. In the cerebellum there was a widespread patchy decrease in the number of Purkinje cells (hemisphere greater than vermis) with an excess of Bergmann glia; the dentate ribbon was discontinuous.

In the cerebral cortex, neuronal density appeared high and there was mild focal disturbance of the laminar pattern in frontal cortex. A group of nerve cells lay deep in the white matter of anterior frontal cortex. There was a small focus of gliosis in the molecular layer of one orbitofrontal gyrus. In the occipital lobe a larger lesion, that involved the whole thickness of the cortex and some of the underlying white matter, extended patchily over two convolutions of the lingual gyrus; the nerve cells had been replaced by large reactive astrocytes. Both lesions were consistent with an old head injury. Neuronal density in the hippocampus appeared increased in all CA areas.

Case 4, age 24

The brain was very large and weighed 1805 g; there was no swelling or herniation. The gyral pattern was normal. There

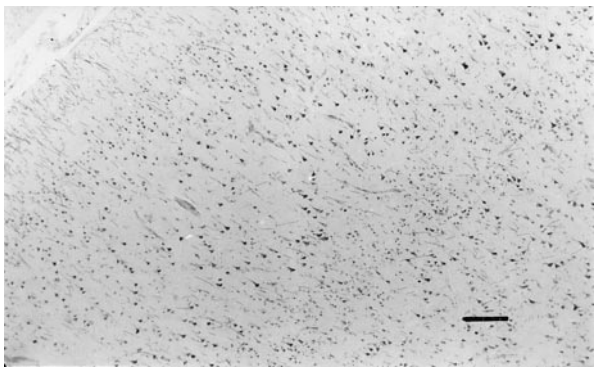

Fig. 9 Case 4. Superior temporal gyrus. The neuronal arrangement is irregular, particularly in lamina 3. Note the pial surface in the top left hand corner. (Luxol fast blue and cresyl violet; bar represents 250 μm.)

was no atrophy of the cerebellar vermis or hemisphere. The brain was soft to touch and contained numerous bacteria that had proliferated post-mortem, but neuronal morphology was generally well preserved. There were small groups of neurons in the inferior cerebellar peduncles. The number of Purkinje cells in the cerebellum appeared reduced in all areas, but there were no empty baskets. There was subpial gliosis in the right cerebral hemisphere. The cortex appeared to be thickened in the main cortical regions and neuronal density looked increased in the frontal and cingulate gyri. In contrast, neuronal density was decreased in the thickened superior temporal gyrus where the laminar pattern was disorganized (Fig. 9). No abnormalities were detected in sections from the hippocampus and deep grey matter.

Case 5, age 20 years

The brain weighed 1405 g and the gyral pattern was normal. The medulla was slightly flattened and the pyramids were

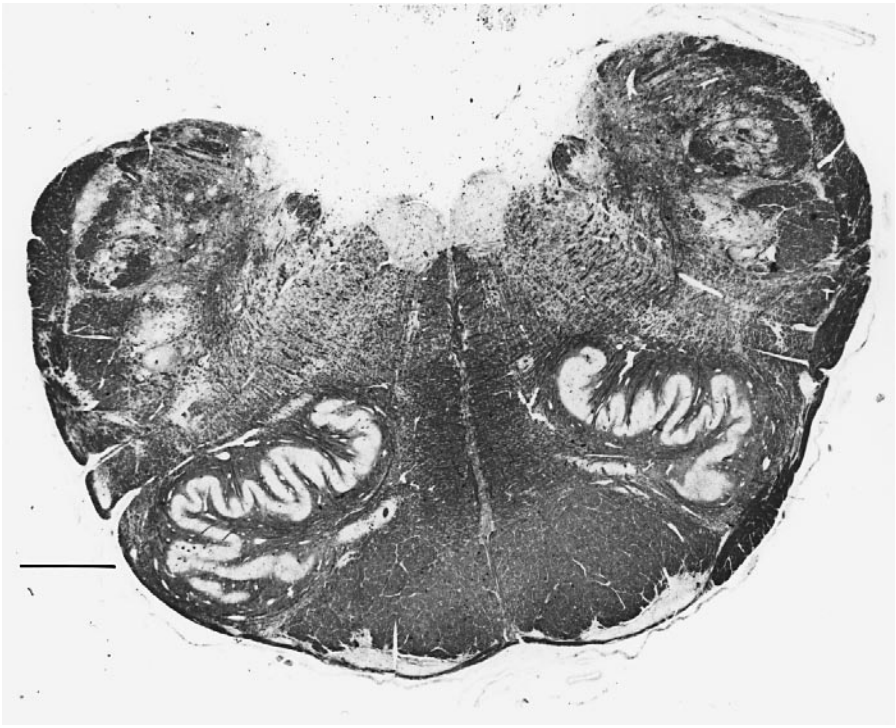

Fig. 10 Case 5. Medulla Oblongata. The medulla is slightly flattened and the pyramids poorly demarcated from each other (Luxol fast blue and cresyl violet, magnification bar represents 2 mm.)

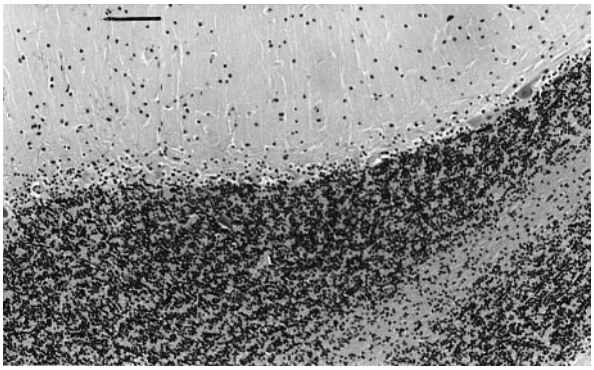

Fig. 11 Case 5. Cerebellar hemisphere. There is a reduced number of Purkinje cells and a mild increase in Bergmann glia. (Haematoxylin and eosin; bar represents 100 μm.)

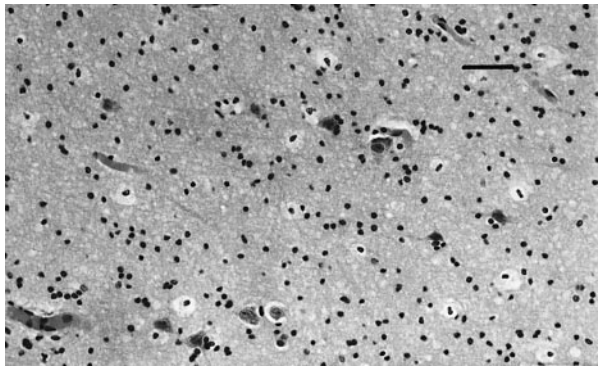

Fig. 12 Case 5. White matter in the superior temporal gyrus. The density of neurons is 40/mm^2 in this field. (Haematoxylin and eosin; bar represents 50 μm.)

poorly demarcated from each other (Fig. 10). There was no atrophy of the cerebellar vermis or hemisphere. Histologically, the external arcuate nuclei and the external arcuate fibres were prominent. The anterior portions of the inferior olivary ribbons were interrupted and asymmetrically bilaterally attenuated. In the cerebellum there was a diffuse decrease in Purkinje cell density in the hemisphere and vermis (Fig. 11). There was a slight increase in Bergmann glia number and a moderate patchy increase in GFAP staining in the molecular layer. In the left cerebral hemisphere the leptomeninges showed sparse perivascular lymphocytic cuffs, and there was widespread capillary engorgement in the grey matter. The white matter of the superior temporal gyrus contained many scattered mature neurons (Fig. 12). There were numerous corpora amylacea within the subpial zone and molecular layer of the insular cortex. The striatum and internal capsule contained a few lymphocytic cuffs. The hippocampus and parahippocampal gyrus were unremarkable.

Case 6, age 24 years

The brain was unusually large, swollen and weighed 1820 g, but there was no herniation. The gyral pattern was normal. The medulla oblongata had not been completely removed. There was no atrophy of the cerebellar vermis or hemisphere. There was no histological evidence of oedema. The neurons of the locus coeruleus were loosely grouped and there was a slight reduction in the number of nigral neurons. In the cerebellum there was a moderate reduction in the number of Purkinje cells in the hemisphere and vermis, and moderate

Table 2 *Pathological findings in cerebral cortex and underlying white matter*

Case	Cortical dysgenesis	White matter	Other
1	Slightly irregular laminar pattern in superior frontal gyrus with clusters of abnormally orientated pyramidal cells. White matter/layer 6 boundary poorly defined.	Numerous patches of ectopic grey matter and increased numbers of single neurons	
2	Thickened cortex and increased neuronal density. Increased numbers of neurons in lamina 1 of frontal cortex, including inverted pyramidal cells.		
3	Increased neuronal density and mild focal disturbance of laminar pattern in frontal cortex	Patch of nerve cells deep in the deep white matter of anterior frontal cortex. Increased number of single neurons.	Small shallow focus of gliosis in frontal cortex. Larger area of gliosis involving full cortical thickness in one occipital lobe.
4	Thickened cortices and increased neuronal density in frontal cortex and cingulate gyri. Laminar pattern of superior temporal gyrus disorganised.	Subpial gliosis.	
5		Increased number of single neurons in superior temporal gyrus.	Numerous corpora amylacea within subpial zone and molecular layer of insular cortex.
6		Increased number of white matter neurons.	

Table 3 *Pathological findings in the brainstem*

Case	Olivary dysplasia	Neuronal ectopia	Other
1	Dysplastic olives with apparent reduplication of medial accessory olive.	Bilateral groups of neurons lateral to olives and in inferior cerebellar peduncle.	Large medulla oblongata with small pyramids. Aberrant pontine tract bilaterally. Small midbrain.
2		Bilateral groups of neurons lateral to olives. Group of neurons in one inferior cerebellar peduncle.	Unusually small aqueduct.
3	Bilateral break in the inferior end of the inferomedial olives.	Bilateral band-like groups of neurons lateral to olives.	Hypertrophy of striae medullaris and enlarged arcuate nuclei.
4		Small groups of neurons in inferior cerebellar peduncles.	Widely dispersed locus coeruleus.
5	Bilateral break in the anterior portions of the inferior olives.		Slight flattening of medulla anteroposterior. Fissure separating pyramids poorly defined. Prominent external arcuate nuclei and external arcuate fibres.
6	Adequate section not available.		Neurons of locus coeruleus loosely grouped.

patchy increase in GFAP in Bergmann glia, without an obvious increase in cell number. In the right cerebral hemisphere, there were neurons in the white matter, and the deep white matter arteries showed scanty lymphocytic cuffing. The basal ganglia were unremarkable. No obvious abnormality was noted in the hippocampus in a section taken from a cryoprotected block.

The neuropathological findings (in the cerebral cortex and underlying white matter, the brainstem and the cerebellum) are summarized in Tables 2–4.

(During revision of this manuscript the brain and spinal cord of a cachectic, handicapped 41-year-old woman with autism became available for study. She met ADI criteria for autism. The brain weighed 1233 g. There was partial reduplication of the inferior olive at one level. Purkinje cells were irregularly aligned with some lying in the deep molecular layer. The Bergman glia appeared excessively numerous and there was an excess of corpora amylacea in the molecular layer. There were excess numbers of multipolar neurons in the molecular layer of the right cerebral hemisphere. The amygdaloid nucleus contained tightly packed round or elongated clusters of mature large neurons, some within bundles of myelinated fibres that appeared out of place.)

Table 4 *Pathological findings in the cerebellum*

Case	Purkinje cell layer	Other
1	Cytoplasmic inclusions in Purkinje cells in the vermis and hemisphere.	Break in dentate ribbon. Patches of subcortical ectopic grey matter.
2	Decreased numbers of Purkinje cells and increase in GFAP in Bergmann glia.	
3	Patchy decrease in numbers of Purkinje cells and proliferation of Bergmann glia.	Break in dentate ribbon.
4	Decreased numbers of Purkinje cells.	
5	Decreased number of Purkinje cells. Areas of Bergmann glia proliferation and moderate patchy increase in GFAP staining.	
6	Decreased numbers of Purkinje cells and moderate patchy increase in GFAP in Bergmann glia.	

Table 5 *Neuronal density (10^{-2} × n/mm^3) in superior frontal gyrus*

Case	Autistic subjects			Control subjects		
	Age (years)	Brain (kg)	Count	Age (years)	Brain (kg)	Count
2	22	1.60	330	20	NA	259
4	24	1.81	194	24	1.50	311
5	20	1.40	144	40	1.47	180
6	24	1.82	216	43	1.52	226
IOP average†			(221)*			(244)
3	27	1.45	365	35	NA	342
1	4	1.53	336	4	1.18‡	373
				6	1.23‡	395

NA = not available. †Figures in brackets are averages for Institute of Psychiatry processed cases. ‡Female. *Exact P = 0.56, Mann–Whitney U, Wilcoxon rank sum test.

Morphometry

Amongst the normal control subjects there was considerable variation in neuronal counts in the frontal cortex and hippocampal sub-fields, but more consistency in the Purkinje cell counts. The range of frontal counts in the brains of autistic individuals was similar to that in the control subjects, although the count in Case 5, who had severe epilepsy, fell below the control range (Table 5). There were no consistent differences between autistic and control subjects in neuronal counts across the different hippocampal sub-fields, although Case 3 had elevated counts in all CA fields compared with his control (Table 6). Purkinje cell counts were consistently lower in the adult autistic cases than in control subjects (Table 7). Statistical comparison of neuronal densities between control and autistic cases from the Institute of Psychiatry (Mann–Whitney U, Wilcoxon rank sum test) only revealed significant differences in the density of Purkinje cells (exact $P = 0.02$). White matter neuronal densities in the autistic brains were unremarkable in the areas systematically counted (data not shown).

Post-mortem findings in cases where tissue was unavailable for study

Post-mortem findings, but not tissue, were available for a further 14 individuals with a clinical diagnosis of autism. Brain weights were recorded in four young adult males (1530, 1450, 1400 and 1300 g), one young adult female (1400 g) and one 16-year-old female (1330 g). Eleven brains were reportedly macroscopically normal. The meninges were thickened and adherent in one case with a history of meningitis in infancy. In the brain of the 16-year-old girl the cortical ribbon was narrower than expected and subcortical white matter was reduced in amount. The substantia nigra was rather poorly pigmented. Microscopically, the cortex was described as well populated by nerve cells. No microscopic reports were available on any other brains.

Discussion

The identified neuropathology in this series is already more extensive than previously reported. The findings included increased brain size and developmental abnormalities of the cerebral cortex, brainstem and cerebellum; in some cases there was also secondary pathology. Claims of consistently elevated neuronal density in the hippocampus have not been replicated. It seems unlikely that misdiagnosis is responsible for any discrepancies with previous findings. Although only one individual was seen during life (Case 3), all of the cases had received a clinical diagnosis of autism. In addition case notes were reviewed after death and the parents interviewed using the ADI (Le Couteur *et al*., 1989). Nevertheless, because the cases in this study were all mentally handicapped, the applicability of the findings to more able individuals is uncertain. The heterogeneity of autism is emphasized by Case 1, which stands out because of the severe early motor difficulties, the severity of the malformations and the presence of Purkinje cell inclusions.

Four brains were large with little evidence of significant oedema. In two cases macrocephaly was also noted during childhood. In Case 1 the temporal lobes were enlarged and hyperconvoluted, but there were no gyral abnormalities

Table 6 *Neuronal density (10^{-2} × n/mm^3) in areas CA1, CA3 and CA4 of the hippocampus*

Case	Autistic subjects				Control subjects			
	Age (years)	CA1	CA3	CA4	Age (years)	CA1	CA3	CA4
2	22	231	156	104	20	167	164	92
4	24	239	168	103	24	145	236	99
5	20	189	208	98	26	186	229	149
					40	197	266	145
					43	216	231	93
IOP average[†]		(220)*	(177)*	(102)$^{(*)}$		(182)	(225)	(116)
3	27	334	247	154	35	215	180	124
1	4	306	279	306	4	298	322	279
					6	212	237	162

[†]Figures in brackets are averages for Institute of Psychiatry processed cases. *Exact $P = 0.10$ and $^{(*)}$exact $P = 0.56$, Mann–Whitney *U*, Wilcoxon rank sum test.

Table 7 *Purkinje cell densities (n/mm^2) of Purkinje cell layer of the cerebellum*

Case	Autistic subjects		Control subjects	
	Age (years)	Count (*n*/mm^2)	Age (years)	Count (*n*/mm^2)
2	22	169	20	220
4	24	196	26	241
5	20	128	40	220
6	24	160	43	268
IOP average[†]		(163)*		(237)
3[‡]	27	131		
1	4	251	4	231

[†]Figures in brackets are averages for Institute of Psychiatry processed cases. [‡]No identically processed cerebellar tissue from a young male control was available. *Exact $P = 0.02$, Mann–Whitney *U*, Wilcoxon rank sum test.

in the other cases. There were also several instances of macroscopically abnormal development of the brainstem, and of the corpus callosum in one brain.

There was microscopic pathology in the cerebral hemispheres, cortex and cerebellum. Abnormalities in cortical development were seen in individual cases, including: areas of increased cortical thickness, high neuronal density, neurons in the molecular layer, neuronal disorganization, poor differentiation of the grey–white matter boundary, neuronal heterotopias and focally increased numbers of single neurons in the white matter (Table 2). In the brainstem (Table 3), the inferior olives were malformed in three brains and ectopic neurons related to the olivary complex were seen in a further two. Olivary dysplasia was associated with enlarged arcuate nuclei in two cases. In one brain there was also a subtle abnormality of the locus coeruleus. Purkinje cell density was decreased in all the adult cases, but inclusions were seen only in Case 1. In two cases there were minor developmental cerebellar abnormalities.

Hippocampal neuronal density looked relatively high in two cases, but only in Case 3 (Table 6) was there any evidence of increased density in all CA subfields. There was no evidence of a statistically significant increase in cell density in the cases processed at the Institute of Psychiatry compared with control subjects (Table 6); however, sampling and number of cases were limited. There was no hippocampal sclerosis or other pathology in any case. Examination of the amygdala has been limited by tissue sampling for neurochemistry but, with the exception of the most recent case, no abnormalities were identified.

In several brains there was also evidence of acquired pathology. The number of Bergmann glia was increased in three cases and there was also increased staining for GFAP, but no empty baskets were seen (the presence of groups of basket cell axons in the absence of the Purkinje cell perikaryon that they normally ensheath is generally interpreted as evidence for acquired Purkinje cell loss). Cerebral subpial gliosis was observed in two cases. There were increased numbers of corpora amylacea in the insular cortex of Case 5 (and in the molecular layer of the cerebellum of the most recently identified case). The two areas of cortical gliosis in Case 3 were probably a consequence of the head injury in childhood.

Comparison with previous studies

The most striking contrast with previous findings is that four of the six brains were unusually large and heavy, although megalencephaly was uncommon amongst the cases unavailable for study. Brain weights were not reported by Ritvo *et al*. (1986) or by Kemper and Bauman (1993); however, in this latter series they were apparently 100–200 g heavier than expected in most subjects aged <12 years, but 100–200 g lighter than expected in the majority of adult subjects (Bauman, 1996). One of the cases in Darby's literature review (1976) had a heavy brain, weighing 1550 g at 5 years of age (case 11 in that review); the brain of one of the two idiopathic cases described by Williams *et al.* (1980) weighed 1520 g (their case 1), and the other weighed 1430 g (their case 3); and the female described by Coleman *et al.* (1985) had a brain weight of 1380 g (Rodier *et al*., 1996). There are two

reports of autistic individuals with unusually small brains; one was associated with premature closure of the cerebral sutures and severe self injury (Hof *et al*., 1991); the other was also profoundly handicapped and physically disabled (Guerin *et al*., 1996). The association between autism and increased brain weight contrasts with the finding of microcephaly in many cases of mental handicap unaccompanied by autism (see for instance Cole *et al.*, 1994).

There is some convergent evidence for increased brain size in a proportion of individuals with autism. Three MRI studies have found increased brain volume in child and adult subjects. Filipek *et al*. (1992) reported increased brain volume in autistic children compared with normal subjects, developmental language disorder and non-autistic mentally handicapped control subjects. Piven *et al.* (1995, 1996) found increased total brain volume in adolescents and adults compared with normal control subjects. Increased head circumference in autistic individuals has been noted in several different samples (Hauser *et al*., 1975; Bolton *et al.*, 1994; Bailey *et al.*, 1995, Woodhouse *et al.*, 1996; Lainhart *et al.*, 1997). Together these data suggest that the finding of megalencephaly (brain weight $>$ 2.5 SD above the mean) is in keeping with a tendency towards increased brain size in some adults and children with autism.

Cortical dysgenetic lesions have not been a prominent feature of previous studies. They were not highlighted by Kemper and Bauman (1993), with the exception that anterior cingulate cortex was consistently unusually coarse and poorly laminated, and associated with increased cell packing density in one case (Kemper, 1988). Polymicrogyria has been seen in two post-mortem cases (Ritvo *et al.*, 1986; Kemper, 1988) and several MRI studies have observed developmental cortical abnormalities in a small proportion of patients (Gaffney and Tsai, 1987; Piven *et al*., 1990; Schifter *et al*., 1994).

In this study there was no significant cerebellar atrophy or apparent granule cell loss; neuronal size in the dentate nucleus and olive appeared unremarkable; and there was no tendency for olivary cells to cluster at the periphery of the convolutions. The extent of the facial nuclei has not yet been assessed. There was, however, clear evidence of developmental brainstem abnormalities that have not been reported previously (Table 3). The MRI findings in the brainstem in autism are largely contradictory, probably reflecting methodological differences and the small size of the relevant structures.

Possible mechanisms

Primary megalencephaly may be associated with increased cell number and increased cell size. Frontal cortical neuronal density appeared to be increased in three cases—including in some areas of thickened cortex—but this is not evident in the limited morphometry. The wide variation in cortical neuronal density in control subjects, and the possibility of cortical neuronal loss secondary to epilepsy, limit the conclusions that can presently be drawn. Nevertheless, there is no evidence of substantially decreased neuronal density in the megalencephalic brains, suggesting that raised total cell number may contribute to brain enlargement. Increased cell replication and impaired developmental cell death might both lead to an excess of neurons. Programmed cell death is well documented in mammalian postnatal cerebral cortex, but also affects a significant proportion of cells in proliferative and, to a lesser extent, postmitotic regions of murine foetal cerebral cortex (Blaschke *et al*., 1996).

Different cortical dysgenetic lesions occurred either alone or in combination, but there was no evidence of neuronal cytomegaly, abnormal neuronal configuration or abnormal neurofilament expression. Although focal increases in white matter neuronal density were seen (Table 2 and Fig. 12), limited morphometry did not reveal a generalized increase. The co-occurrence of different patterns of dysgenesis is not uncommon (Prayson and Estes, 1995), and the findings suggest that there may be abnormalities in cortical neuronal proliferation, migration and programmed cell death (Rorke, 1994; Mischel *et al*., 1995).

Evidence of abnormal neuronal migration, and possibly abnormal control of cell number, was also found in the brainstem and cerebellum. Whether shared mechanisms underlie the cortical and brainstem findings is unclear. Nevertheless olivary anomalies seldom occur in isolation and heterotopias are usually associated with cortical developmental abnormalities, particularly megalencephaly, pachygyria and lissencephaly (Harding and Copp, 1997). Olivary development involves long distance migration from the primary precerebellar neuropepithelium, which is also the source of cells forming the arcuate nuclei and basis pontis (Essick, 1912); the cells forming the dentate nucleus arise from the superior portion of the rhombic lip. A tendency for inferior olivary neurons to cluster at the periphery of the convolutions was not identified, but Kemper and Bauman's (1993) observation may be related to the abnormalities that were observed in this series. In a case of Coffin–Siris syndrome (DeBassio *et al.*, 1985), peripheral clustering of olivary neurons occurred in association with islands of ectopic olivary neurons, a large medial accessory olive, unusually large arcuate nuclei and ectopic neurons in the white matter of the cerebellum at the level of the dentate nucleus.

There is only limited evidence, so far, that abnormal development also affects more rostral brainstem structures. An aberrant pontine tract was identified in Case 1 and there was mild disorganization of the locus coeruleus in Case 6. Kemper and Bauman (1993) observed disorganization of the nucleus locus coeruleus and the nucleus raphe dorsalis in one brain and, in another case, the centrally placed neurons of the basis pontis were apparently more densely packed and enlarged compared with control subjects (Kemper, 1988).

Decreased Purkinje cell density is a relatively consistent observation across the post-mortem studies, although in several cases in this series many stretches of folia were well populated with Purkinje cells. The frequency of developmental medullary abnormalities indirectly supports the hypo-

thesis that the Purkinje cell findings have a developmental basis. Kemper and Bauman (1993) argued that, in the absence of glial cell hyperplasia or retrograde olivary cell loss, decreased Purkinje cell density pointed to a loss occurring at, or before, 30 weeks gestation. Nevertheless, if substantial Purkinje cell loss occurs only early in development, then the apparently normal development of the cerebellar cortex is slightly puzzling. Purkinje cells play a central role in normal cerebellar development and they control proliferation of cells in the murine external granule layer (Feddersen *et al.*, 1992; Smeyne *et al*., 1995). Postmitotic external granule layer cells migrate to form the internal granule layer (IGL), which increases in thickness in man from the 5–6th prenatal month to the 4–5th postnatal month (Raaf and Kernohan, 1944), being very thin until 32 weeks gestation (Friede, 1973). Consequently, any substantial loss of Purkinje cells prior to 32 weeks gestation could be associated with hypoplastic cerebellar folia. The modest patchy glial cell hyperplasia seen in this series raises the additional possibility of postnatal loss of Purkinje cells, perhaps related to epilepsy (although, as yet, no empty baskets have been identified). Whilst olivary gliosis has not been observed, moderate loss of olivary cells may be difficult to identify, as the cells normally show great variation in density and lie relatively far apart (Brodal, 1940). The Purkinje cell inclusions seen in the only child in this series add a further complication. Such inclusions have not previously been reported and their aetiology is unknown. Purkinje cell density was unremarkable in this case, but we are not aware of any precedent for the complete disappearance of inclusion-bearing cells.

In summary, developmental neuropathology was not localized to the limbic system, cerebellum (Kemper and Bauman, 1993), or derivatives of a single hindbrain rhombomere (Rodier *et al*., 1996). Although there is evidence of abnormal neuronal migration, other factors influencing neuronal number, survival and orientation also seem to be important. There is clearly a need for further study of the pathological basis of increased brain size. Of course, some developmental pathology may be either a consequence of maldevelopment at remote sites, or epiphenomena of more fundamental abnormalities. Identifying the genes predisposing to autism may help to clarify these relationships. With regard to timing, it would be premature to conclude that a single developmental event led to these findings. Nevertheless, abnormal development had sometimes begun by the time of olivary cell migration, which occurs before the end of the 3rd month.

Relationship to symptomatology

Explanations of developmental cognitive and behavioural dysfunction by neuropathology are precarious, as brain function may not be impaired and inferences based upon localization in adults may not be pertinent. As yet, no single pathology common to all cases of autism has been identified. Of course, autism is a complex behavioural disorder and it would be too much to expect such specificity at this level of analysis.

The finding of cortical dysgenetic lesions and megalencephaly suggests that cerebral dysfunction may underlie some cognitive and behavioural abnormalities, and may provide the pathological substrate of epilepsy. The inconsistency of the neuropathological findings indicates, however, that they are probably imperfect markers of abnormal cortical development and organization. Whilst brainstem abnormalities might cause neurological impairments (Rodier *et al*., 1996), they seem unlikely to lead directly to high level cognitive deficits, but possibly both types of impairments can occur in more severely affected individuals. No consistent hippocampal abnormalities were identified in this series and systematic examination of the amygdala, medial septal nuclei, mamillary bodies and related structures has yet to be undertaken. Involvement of the amygdala remains an important possibility, but the evidence of cortical and brainstem maldevelopment has removed the imperative to argue that all autistic symptomatology arises from medial temporal and related structures. The relationship of cerebellar abnormalities to symptomatology (if any) remains uncertain. Although cerebellar maldevelopment may prove to be a marker of the disease process, behavioural consequences remain hypothetical until cerebellar dysfunction has been demonstrated unambiguously. In summary, in this study we have found no evidence for a highly localized pathology that seems likely to underlie autism. Instead, the findings raise the possibility that a combination of diverse, but related, neurodevelopmental abnormalities give rise to the characteristic symptomatology, and the associated mental handicap and epilepsy.

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Appendix

Case histories

Case 1

As a baby he was a poor feeder who disliked being held. A clinical hearing test was failed at 7.5 months but the parents knew that he could hear soft sounds and was sensitive to vibrations. He had persistent difficulties with gross motor control, was clumsy and did not chew. He could be propped to stand at 2 years of age but could not move from this position. He acquired a few sounds but no speech; he screamed frequently, especially if there was an echo. He did not turn to his name or speech, and never followed eye gaze or pointing. He could sometimes follow simple instructions, particularly if context bound. He did not imitate or copy, but would sometimes point to a picture in a book. In infancy he continued to dislike being held and sometimes urinated when picked up. He took no interest in people and would only look at his parents if they jumped and waved their arms. He appeared to focus on parts of people and was more interested in his parents' glasses and earrings than their faces; he was particularly interested in buckles and zips. He could spot small items such as milk bottle tops and paper clips but would ignore large objects in the environment. He would not seek comfort if hurt. He would bite his parents and other children, and appeared to enjoy the chaotic reaction that this provoked. He became increasingly destructive and overactive. He was interested in mechanical things and would spend most of the day in minute examination and manipulation of tiny objects; his fine motor coordination appeared unimpaired, although he acquired few fine motor skills. He liked to fiddle with bunches of keys, and would attempt to put these in locks. He enjoyed watching a spinning top, and would spin wheels for hours; he also liked watching credits at the end of television programmes. He flicked light switches repeatedly. He would often flap his arms and pant, particularly if excited, and this could be accompanied by rocking on his toes. He liked to look at the ceiling and spin, and also enjoyed going on roundabouts. In the 1st year he rubbed his feet together and clenched his hands together in the midline; when older he engaged in hand stereotypies close to his face. He gnawed at his fingers and nails, head-banged and pulled at his penis. He appeared intrigued by pain; he went back repeatedly to an exposed mains socket to get a shock and he cut himself with a razor. He would occasionally cry if he hurt himself but appeared insensitive to temperature. He had marked pica and would drink the water in a paddling pool until sick.

Case 2

He sat at 7 months and walked at 1 year. He was dry and clean by 2 years, but 6 months later developed secondary enuresis. Parental concern was aroused at 24 months by speech delay and by his habit of sitting on a table and spinning a record. There was no pre-speech babble and, although he would repeat 'one, two, three' when climbing steps at 18 months of age, he developed no other speech until the age of 7 years. High tone hearing loss was queried, but was thought to be insufficient to interfere with speech acquisition. He did not react to voices or people although he could hear the rustle of sweet papers. He was able to hum tunes and he had favourite records. He did not imitate or point or use gestures to communicate; as a child he would take his mothers hand and lead her to something he wanted. Later in life he acquired a small vocabulary, but would only talk when pressed; most of the time he was mute. He would usually reply with a single word, but had a

few direct phrases such as ‘go away’ or ‘I want my mummy’. His articulation was poor. As a young child he did not understand even simple instructions, but this improved with intensive teaching. There is no history of echolalia. As a young child he avoided eye contact, but this had been taught by the age of 11 years. He took no interest in his parents and was unconcerned by their absence. He avoided other children, made no social overtures and would not seek or respond to comfort when hurt or upset. He did not cry until the age of 6 years, and had a limited range of facial expression which could be inappropriate. As a child he did not engage in pretend play. He would spend many hours on a swing, fiddling with straws (which mother had to carry with her), watching a spinning top or fiddling with pliable objects or pieces of string. He was good at shapes and completed simple jigsaw puzzles upside down. He liked manipulating small objects and was skilled at spotting small objects that people had lost. Certain aspects of his life were routinized. He disliked changes in the furniture at home; he would usually return pieces to their original place. He avoided going to bed unless accompanied by his mother. When older, walks and his mother’s visits (when he was in residential care) had to be conducted in a stereotyped manner. He had many hand mannerisms which were accompanied by swaying, he also frequently looked at his hands and became upset if he was stopped. He liked to spin and bounce and had a dancing routine when music was played; he would rock in stressful situations. He was socially disinhibited and as an adult could attack other residents without provocation. He attended a number of different educational centres until he was 9 years of age when he was transferred to a residential school for autistic children until the age of 14 years. He was then moved to a long-stay hospital where he remained until his death.

Case 3

In infancy, he was inaccessible and detached. He had no language except for the word ‘no’, but he did make some noises and imitated environmental sounds such as a dog barking. He understood simple instructions. His needs were anticipated by his mother whom he would follow around. He was aloof and would not play with other children; he preferred to line up small articles such as pins and needles in rows. As a child he could be indiscriminately affectionate towards strangers but as an adult he resented the company of others. In childhood he had rigid likes and dislikes concerning food, and had to sit at the same spot and use the same spoon; a crooked table cloth distressed him. He collected matches, pins, knives and bus tickets, and enjoyed breaking up razor blades into little pieces and holding them in his mouth. He also enjoyed music. Later he had an obsession for string, thread and shoelaces which he flicked in front of his eyes and sometimes ate. When older, he flicked his face with the empty end of a sleeve which he watched. He had stereotyped postures and mannerisms when excited; he flicked his fingers and flapped his hands from the elbows. He sometimes walked on his toes. When younger he was constantly active, wringing his hands and slapping his head, but became underactive in adulthood. He showed no response to pain and sometimes tore out his own hair. He entered a residential facility at the age of 7 years.

Case 4

He sat at 6 months and walked at 16 months. The parents were concerned at 18 months because of a lack of interest in his mother and her activities, his tendency to wander and the need to wake him for feeding. His first word was yoghurt at 36 months. He subsequently developed a small vocabulary of single words but these were not used between the ages of 5 and 12 years. He did not point or use gestures to communicate and did not attend to speech. He echoed some single words and acquired some stereotyped phrases such as ‘cup of tea please’ and ‘tie my shoelace’; he would say ‘goodbye’ when he wanted others to leave. Most speech was related to food needs and was poorly articulated. He understood single but not double commands. He liked music, and could hum tunes, sing songs and fill in missing words from songs. He ignored people around him, was unconcerned by his parents absence and disliked people coming too close; however, he could enjoy rough and tumble. He did not make social approaches for either physical needs or comfort, but as an adult would sometimes touch his parents. He was socially disinhibited and could also laugh inappropriately. Between the ages of 2 and 3 years he carried a hairbrush and waved a poker in front of his eyes like a windscreen wiper; similarly, he twiddled with sticks and wire. He also liked to put objects in the fire and watch them burn. He lined up items and enjoyed jigsaws. He would touch velvet, petals and leaves. He had complex hand and finger mannerisms in front of his eyes which were accompanied by a rigid facial expression. He bottom hopped until the age of 16 years and rocked violently, breaking four sofas. He was routinized and disliked change; he objected to his mother not having her legs crossed, a door not being fully closed and new clothes. He would notice if objects were moved. As a child he was overactive and had good balance. He was taught to ride a bicycle over the course of 2 years but could never kick a ball. When older, he chewed objects and could not pass cigarette butts without picking them up and eating them. When upset he would bite the back of his fingers and sometimes head-banged; he was insensitive to cold and pain.

Case 5

His parents were concerned at 14 months because of his unusual language development. At 4 months he did not alert to noises and later did not respond to pointing. First words were acquired at 10 months but were used for a few weeks and disappeared, as did subsequent new words. He sat at 8 months, walked at 16 months and was first assessed at 22 months because he walked with his hands raised. He did not usually respond to voice, and deafness was queried. He retained the ability to say a few single words, but did not speak on a daily basis, and any language usually related to food or drink. He did not use his small vocabulary to communicate, neither did he gesture, point or use eye gaze communicatively. Articulation was poor. There was no imaginative or imitative play and little curiosity. As an adult he could say ~100 words, knew a few signs and understood single words. His parents were also concerned in childhood about hyperactive unpredictable behaviour and lack of awareness of danger. Bladder and bowel control were not acquired until the age of 10 years. As a child he did not raise his arms to be lifted, gaze directly at others or check back; his response to other people was unpredictable and he did not greet his parents, show them objects or direct their attention. Facial expression was limited and often inappropriate. He was relatively insensitive to pain and did not offer others comfort. As a young child he was unaffectionate and even in adulthood socially disinhibited. He had

no childhood peer relationships but formed an attachment to a fellow resident in adulthood. He lacked curiosity and as a child played with the doors and wheels of toy cars, and enjoyed the texture of sand and pebbles. He was preoccupied by the Ladybird series of books and knew all the local stockists; he liked to carry a book and if left alone would flick its corners all day. He enjoyed repetitively listening to stories on cassettes. He flapped when running or excited, and in enclosed spaces walked in circles. In childhood chewing was immature, he frequently dribbled and chewed or mouthed most things.

Case 6

The parents sought advice at 24 months because of speech delay. There was no pre-speech babble. First words were acquired at 10 months, numbered <10, were not used communicatively and had disappeared by the age of 4 years. There was no social use of sounds, no gesturing or pointing and no imitation of activities. Usually he did not respond to voice, although he did put his hands over his ears to certain domestic sounds. He learnt a limited number of social gestures, understood single words and in adulthood had five to six signs. In childhood he took little notice of his parents and there was no greeting, showing or sharing. He was aloof and would not come for comfort. Any eye contact was fleeting and facial expressions were sometimes inappropriate and reduced in range. He did not check back and there was no separation anxiety. He could be shy with strangers but also socially disinhibited. He took no interest in other children, but sometimes enjoyed being chased or engaging in rough and tumble with his parents. There was no imaginative play. He was an overactive child. He enjoyed tearing paper and later flicking grass against his ear. He always had to have a 'flicker' of some sort but was not interested in this visually. His parents took efforts to prevent him becoming too routinized. He was obsessed by water and would flush a line of school toilets repeatedly. He would empty anything that was only half full, had to break panes of glass that were cracked and would unravel any clothes with imperfections. He smelt most things and from the age of 14 years compulsively touched objects. He occasionally flapped whilst walking and there was some moderate self injury.