doi:10.1093/brain/awh287 *Brain* (2004), **127,** 2572–2583

REVIEW ARTICLE

Neuropathological findings in autism

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Summary

Autism is currently viewed as a largely genetically determined neurodevelopmental disorder, although its underlying biological causes remain to be established. In this review, we examine the available neuropathological literature on autism and discuss the findings that have emerged. Classic neuropathological observations are rather consistent with respect to the limbic system (nine of 14 studied cases showed increased cell packing density and smaller neuronal size), the cerebellum (21 of 29 studied cases showed a decreased number of Purkinje cells, and in all of five cases that were examined for age-related morphological alterations, these changes were found in cerebellar nuclei and inferior olive) and the cerebral cortex (>50% of the studied cases showed features of cortical dysgenesis). However, all reported studies had to contend with the problem of small sample sizes, the use of quantification techniques not free of bias and assumptions, and high percentages of autistic subjects with comorbid mental retardation (at least 70%) or epilepsy (at least 40%). Furthermore, data from the limbic system and on age-related changes lack replication by independent groups. It is anticipated that future neuropathological studies hold great promise, especially as new techniques such as design-based stereology and gene expression are increasingly implemented and combined, larger samples are analysed, and younger subjects free of comorbidities are investigated.

Keywords: autism; neuropathology; cerebral cortex; cerebellum; limbic system

Abbreviations: GAD = glutamic acid decarboxylase

Received May 15, 2004. Revised July 1, 2004. Accepted July 6, 2004. Advanced Access publication August 25, 2004

Introduction

Autism is an oligogenic neurodevelopmental disorder with a heritability of >90% (Bailey *et al*., 1996). It is defined by the presence of marked social deficits, specific language abnormalities and stereotyped, repetitive behaviours (American Psychiatric Association, 1994). Kanner, the first to report on autism, noticed the presence of enlarged heads in some of the children with autism (Kanner, 1943). Several subsequent studies have replicated this finding. Macrocephaly, defined as head circumference above the 97th percentile, was found in ~20% of subjects with autism (Bailey *et al*., 1993; Davidovitch *et al*., 1996; Woodhouse *et al*., 1996; Lainhart *et al*., 1997; Stevenson *et al*., 1997; Fidler *et al*., 2000; Fombonne, 2000; Miles *et al*., 2000; Naqvi *et al*., 2000; Aylward *et al*., 2002; van Karnebeek *et al*., 2002; Courchesne *et al*., 2003). Macrocephaly is not present until the first year of life, however (Lainhart *et al*., 1997; Stevenson *et al*., 1997; Courchesne *et al*., 2003). Consistent with these clinical findings, neuropathological studies have reported increased

brain weight in autistic individuals (Bailey *et al*., 1998; Kemper and Bauman, 1998; Courchesne *et al*., 1999; Casanova *et al*., 2002*a*). Likewise, neuroimaging studies have shown increased brain size in autistic children (Filipek *et al*., 1992; Courchesne *et al*., 2001; Aylward *et al*., 2002; Carper *et al*., 2002; Sparks *et al*., 2002; Herbert *et al*., 2003). However, in autistic adolescents and adults, compared with control subjects, contradictory results have been reported either of increased brain volume (Piven *et al*., 1995, 1996; Hardan *et al*., 2001*a*, *b*) or of no difference in brain volume (Aylward *et al*., 1999; Haznedar *et al*., 2000; Courchesne *et al*., 2001; Townsend *et al*., 2001; Aylward *et al*., 2002; Carper *et al*., 2002; Rojas *et al*., 2002).

Thus, although abundant evidence of increased head circumference, brain weight and brain volume in autism, especially in children, exists, the underlying biological mechanisms of brain enlargement remain to be determined and could involve increased neurogenesis, increased gliogenesis, increased synaptogenesis, disturbed migration of neurons, decreased apoptosis, decreased pruning or complex combinations of these events. This review discusses the existing neuropathological literature on autism (see Table 1) and elaborates on the possibilities for future research, especially in the fields of genetics and neuropathology.

Neuropathological alterations in distinct brain regions

Alterations in the limbic system

Bauman and Kemper were the first to investigate the limbic system in autistic cases. Several case reports (e.g. Bauman and Kemper, 1985, 1987, 1990) and an earlier review (Bauman, 1991) were brought together in one final review by these authors (Kemper and Bauman, 1993). By surveying whole-brain serial sections, six autistic cases (five males, five with mental retardation and four with epilepsy, 9, 10, 12, 22, 28 and 29 years old) were compared with six age- and sex-matched controls. All autistic cases showed increased cell packing density and reduced cell size in hippocampus, subiculum and amygdala, and, although to a lesser extent, in entorhinal cortex, mammillary bodies and septal nuclei. This pattern of small, closely packed neurons, with limited dendritic arbors, resembles that typically seen during earlier stages of brain maturation and may, therefore, reflect features of an immature brain (LeRoy Conel, 1939; Jacobson, 1991). A case report of a 16-year-old female with autism and severe mental retardation showed macroscopically low brain weight (1000 g), ventricular dilation and a thin corpus callosum (Guerin *et al*., 1996). Microscopically, however, no abnormalities were observed in the limbic structures, the cerebral cortex and the cerebellum. Based on an earlier hypothesis by Lyon (1990), Guerin *et al*. (1996) proposed that these findings indicate a reduced density of axons and dendrites in the autistic brain. However, this hypothesis has not been tested in a larger sample size of autistics thus far. Using the Golgi method, the hippocampus of two autistic subjects (a 7-year-old female and a 9-year-old male, both with mental retardation, but without epilepsy) and two control subjects (8 and 13 years old) was examined (Raymond *et al*., 1996). Only one autistic case had adequate CA4 neuronal staining, showing smaller neurons in the CA4 field compared with an age-matched control case. Both autistic cases showed less extensive dendritic branching in the CA1 and CA4 fields. These findings of reduced cell size and simplified dendritic pattern, without dysmorphic features, were consistent with a curtailment of maturation, as suggested previously by Kemper and Bauman (1993). Bailey *et al*. (1998) investigated six autistic cases (all with mental retardation and three with epilepsy) and seven age- and sex-matched controls. In only one of the five examined cases, increased cell packing density was observed in all CA subfields of the hippocampus.

Alterations in the cerebellum

Williams *et al*. (1980) were the first to perform a detailed neuropathological analysis on four individuals with autistic behaviour (three males, 12, 27 and 33 years of age and one female, 3 years of age, all presenting with mental retardation and two with seizures). Cortical and subcortical structures and the cerebellum were examined. Nerve cell loss and replacement gliosis were found in atrophic orbitofrontal and temporal regions in two cases, which were probably due to cerebral trauma that occurred some time after the development of autistic symptoms. The only abnormality likely to be associated with autism was reduced Purkinje cell density in one case, with concomitant epilepsy and profound mental retardation. Thus, no clues as to the cause or the anatomic–pathological substrate of autistic behaviour could be obtained from these cases. Ritvo *et al*. (1986) counted Purkinje cells in the cerebellum of four autistic cases (all males, three with mental retardation, none with seizures) and three male controls. Autistic cases showed a decreased number of Purkinje cells in the cerebellar hemisphere and vermis. Apart from reports on limbic alterations in six autistic cases, Kemper and Bauman (1993) also reported on alterations in the cerebellum. All six autistic cases showed decreased numbers of Purkinje cells (see also Fig. 1). As there was no evidence of glial cell hyperplasia or of retrograde olivary cell loss, both characteristic of a postnatal cerebellar insult (Holms and Stewart, 1908; Rakic and Sidman, 1970), a lesion acquired early in development was suggested. Furthermore, in the two young autistic cases, the neurons in the deep cerebellar nuclei and the inferior olive were large, whereas in the autistic cases, older than 22 years, these neurons were small and pale. It should be mentioned that in normal development, projections from the inferior olive to the Purkinje cells are preceded by projections from the inferior olive to the cerebellar nuclei (Flechsig, 1920). Accordingly, a decreased number of Purkinje cells (which are the final target of the inferior olive projections) may result in an abnormal development of these fetal projections from the inferior olive to the cerebellar nuclei. However, as this fetal circuit was meant to function only for a short period of time, it was postulated that this circuit would eventually fail, resulting in

Table 1 *Neuropathological findings in autism*

	Author and year	Journal	Sample size and characteristics	Region of interest	Results
1	Williams *et al.* (1980)	*Arch Neurol*	4A, 3M; 4, 14, 27, 33 years; 4MR; 2E	Whole brain	Nerve cell loss and replacement gliosis in atrophic orbitofrontal and temporal regions in cases 1 and 3; smaller neurons in CA4; ↓Purkinje cell density in case 1
2	Bauman and Kemper (1985)	*Neurology*	1A, 1M; 29 years; 1MR; 1E. 1C; 1M; 25 years	Whole brain	↑cell packing density and ↓cell size in HIP, subiculum, entorhinal cortex, septal nuclei, mammillary body and selected nuclei of the AMY. Atrophy of neocerebellar cortex, with marked ↓of Purkinje cells and cerebellar nuclei contained ↓numbers of neurons, with the remaining neurons being small and pale
3	Coleman *et al.* (1985)	*J Autism Dev Disord*	1A, 0M; 21 years; 1MR; 0E. 2C; 0M; 18 and 25 years	Auditory cortex and Broca's area	No differences, except for ↓glia in left auditory cortex and ↓numbers of pyramidal neurons in right auditory association cortex
4	Ritvo *et al.* (1986)	*Am J Psychiatry*	4A, 4M; 10, 19, 19, 22 years; 3MR; 0E, 3C; 3M; 3, 10, 13 years	CB	↓Purkinje cell counts in both CB hemisphere and vermis
5	Bauman and Kemper (1987)	*Neurology*	1A, 0M; 11 years; ?MR; 0E. 2C; ?M; age-matched	AMY and HIP	↑cell packing density in HIP and AMY
6	Bauman and Kemper (1990)	*Neurology*	1A, 1M; 12 years; 0MR; ?E. 2C; 2M; age-matched	Limbic system and CB	↑cell packing density of smaller neurons in limbic system; ↓numbers of Purkinje cells; enlarged neurons in deep cerebellar nuclei and inferior olive
7	Bauman (1991)	*Pediatrics*	5A, 4M; 9 (new), 11, 22 (new), 28 (new), 29 years (including study numbers 2 and 5 from the table); 4MR; 4E (3 new)	Limbic system in 4 cases and CB in all 5 cases	Review of earlier findings of ↑cell packing density in limbic system (4/5) and ↓Purkinje cell numbers in CB (5/5)
8	Hof *et al.* (1991)	*Acta Neuropathol*	1A, 0M; 24 years; 1MR; 0E	Detection of neurofibrillary tangles in cerebral cortex and limbic system	Microcephaly (773 g). Neurofibrillary tangles, especially in layer II and III of temporal cortex, probably due to severe head banging
9	Fehlow *et al.* (1993)	*Pediatr Grenzgeb*	1A, 1M; 19 years; 1MR; 0E	CB	Purkinje cell loss in lobules VI and VII
10	Kemper and Bauman (1993)	*Neurol Clin*	6A (including all subjects of study number 7 from the table), 5M; 9, 10, 12 (new), 22, 28, 29 years; 5MR (1 new); 4E (0 new). 6C; age- and sex-matched	Limbic system and CB	Small and densely packed neurons in limbic system (6/6); ACC coarse and poorly laminated in 5/6; ↓Purkinje cell numbers in CB (6/6)
11	Guerin *et al.* (1996)	*Dev Med Child Neurol*	1A, 0M; 16 years; 1MR; 1E	Whole brain	Macroscopic: microcephaly, ↑VENT; thin CC. Microscopic: no abnormalities
12	Raymond *et al.* (1996)	*Acta Neuropathol*	2A, 1M; 7 and 9 years; 2MR; 0E. 2C; ?M; 8 and 13 years	HIP	Smaller neurons in CA4; less dendritic branching in CA1 and CA4
13	Rodier *et al.* (1996)	*J Comp Neurol*	1A, 0M; 21 years; 1MR; 1E, 1C; 1M; 80 years	Pons, medulla and CB	Near-complete absence of the facial nucleus and superior olive along with shortening of the brainstem
14	Bailey *et al.* (1998)	*Brain*	6A, 6M; 4 and 20–27 years; 6MR; 3E, 7C; 5M; age-matched	Whole brain with neuronal counts in SFG, CB, HIP	Megaencephaly in 4; abnormalities in inferior olives in 4; ↓Purkinje cells in all adults; cortical dysgenesis in at least 50%
15	Blatt *et al.* (2001)	*J Autism Dev Disord*	4A, 4M;19, 19, 20, 22 years; 4MR; 2E. 3C; 3M; 16, 19, 24 years	GABAergic, serotonergic, cholinergic, glutamatergic system in HIP	↓GABAergic receptor system

Table 1 *Continued*

	Author and year	Journal	Sample size and characteristics	Region of interest	Results
16	Fatemi *et al.* (2001)	*Synapse*	5A, 5M; 22 years; at least 3MR; ?E. 4C; 4M; 24 years	Bcl-2 and p53 in parietal cortex	32% ↓Bcl-2; 130% ↑p53
17	Fatemi *et al.* (2001*b*)	*Neuroreport*	5A, 5M; 25 years; ?MR; ?E. 8C; 8m; 24y	CB cortex	34–51% ↓Bcl-2
18	Fatemi *et al.* (2001*a*)	*J Autism Dev Disord*	5A (same subjects as in study number 17 from the table), 5M; 25 years; ?MR; ?E. 8C; 8M; 24 years	Reelin and Bcl-2 in CB cortex	>40% ↓reelin and 34–51% ↓Bcl-2
19	Perry *et al.* (2001)	*Am J Psychiatry*	7A, 6M; 24 years; probably 7MR; at least 50% E. 10C, 8M; 32 years, 9MR; 5M; 32 years	Frontal and parietal cortex and basal forebrain	30% ↓M1 receptor binding in parietal cortex; 65–73% ↓α4 nicotinic receptor binding in frontal and parietal cortex; ↑BDNF in forebrain
20	Casanova *et al.* (2002*a*)	*J Child Neurol*	2AS, 2M; 22 and 79 years; 0MR; ?E. 18C; 18M; 9–98 years	Layer III of prefrontal and temporal cortex	Cell columns were more numerous, smaller, and less compact
21	Casanova *et al.* (2002*b*)	*Neurology*	9A, 7M; 12 years; 7MR; 5E. 9C; ?M; 15 years	Layer III of prefrontal and temporal cortex	Cell columns were more numerous, smaller and less compact
22	Fatemi *et al.* (2002*b*)	*Biol Psychiatry*	5A, 8C (CB), 4C (parietal cortex), (same subjects as in study 16 and 17 from the table)	GAD 65 and 67 kDa proteins in CB and parietal cortex	↓65 kDA 48% in parietal cortex and 50% in CB; ↓67 kDA 61% in parietal cortex and 51% in CB
23	Fatemi *et al.* (2002*a*)	*Cell Mol Neurobiol*	5A (same subjects as in study number 17 from the table), 5M; 25 years; ?MR; ?E. 5C; at least 4M; 24 years	CB	24% smaller Purkinje cells; no differences in density
24	Lee *et al.* (2002)	*Brain*	8A (7 overlapping with study 19 from the table) 7M; 25 years; 7MR; 5E. 10C; 6M; 28 years, 11MR; 7M; 33 years	CB	↓α3 and α4 nicotinic receptor binding in granule cell, Purkinje and molecular layers; ↑α7 nicotinic receptor binding in granule cell layer
25	Araghi-Niknam and Fatemi (2003)	*Cell Mol Neurobiol*	5A, 5M; 24 years; ?MR; ?E. 4C; 4M; 24 years	Cerebellar and superior frontal cortex	↓Bcl-2 and ↑p53 both in cerebellar (36 and 38%, respectively) and superior frontal cortex (38 and 68%, respectively)

A = autistic subjects; ACC = anterior cingulate cortex; AMY = amygdala; AS = subjects with Asperger's syndrome; BDNF = brain-derived neurotrophic factor; C = control subjects; CB = cerebellum; CC = corpus callosum; E = epilepsy; GAD = glutamic acid decarboxylase; HIP = hippocampus; M = male; MR = mental retardation; SFG = superior frontal gyrus; VENT = ventricles;. ↓ = decreased; ↑ = increased; ? = not mentioned. Example of how to read the column 'sample sizes and characteristics': in the first study (Williams *et al*., 1980), four autistic subjects were studied, three of them were male, ages were 4, 14, 27 and 33 years (either separate ages, or mean age, or age range is mentioned, dependent on the information given in the article), all four were mentally retarded, two had epilepsy as well. No controls were included.

the atrophy of the involved cells. A case report documented a 19-year-old man, presenting with Ehlers–Danlos syndrome and concomitant mental retardation and autism, who died of a mechanical ileus due to excessive aerophagia (Fehlow *et al*., 1993). This case also exhibited a marked decrease in the number of Purkinje cells in cerebellar lobules VI and VII. However, another case report, of a 16-year-old female with autism and severe mental retardation (Guerin *et al*., 1996), showed no abnormalities in the cerebellum. Bailey *et al*. (1998), in their study of six autistic cases (all with mental retardation and three with epilepsy) and seven age- and sex-matched controls, reported low Purkinje cell counts in all five adult autistic cases, but not in the cerebellum of the 4-year-old autistic boy. Harding and Copp (1997) stated that, considering the normal development of the cerebellar cortex, it would be unlikely that the reported decreased number of Purkinje cells occurred only before 30 weeks of gestation, as was suggested by Kemper and Bauman (1993). A substantially decreased number of Purkinje cells before 32 weeks of gestation would be associated with hypoplastic folia (Harding and Copp, 1997), which was not the case in the brains investigated by Bailey and colleagues. In addition, the reported modest glial hyperplasia would have been another indication of a postnatal decrease in the number of Purkinje cells. Lee *et al*. (2002) examined two autistic cases (both with mental retardation, one with

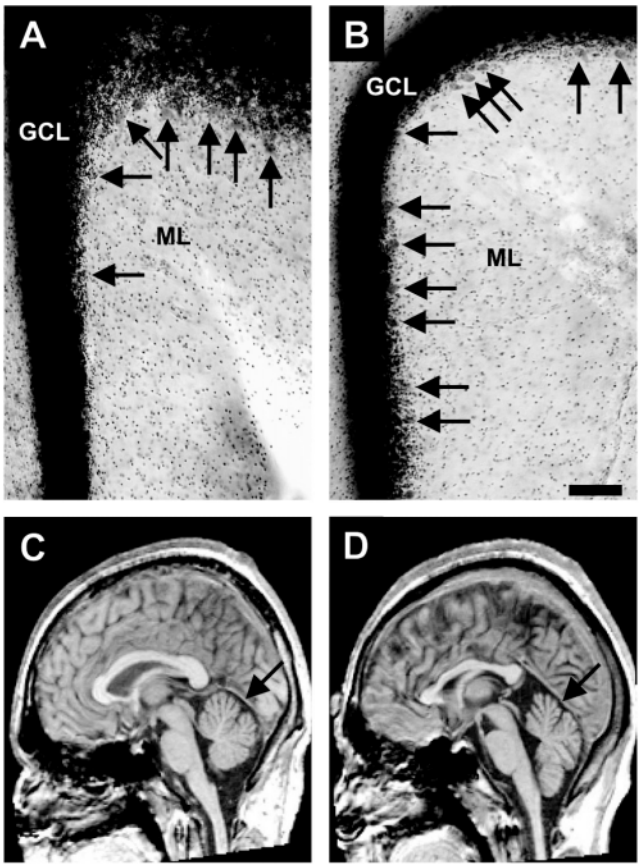

Fig. 1 (**A** and **B**) Representative photomicrographs from 200 μm thick frontal sections of post-mortem brains from a 13-year-old male suffering from autism (**A**) and from a 14-year-old male control (**B**). These pictures show a part of the cerebellum (GCL = granule cell layer; ML = molecular layer). Note the smaller number of Purkinje cells in the brain from the autistic patient compared with the control (arrows). These photomicrographs were produced using a video camera (Hitachi HV-C20A; Hitachi, Japan) attached to an Olympus BX 50 microscope and the Stereo Investigator software (MicroBrightField, Williston, VT). Twelve separate images each were needed to cover the parts of the cerebellum shown. For each separate image, the microscope was focused on the Purkinje cell layer. These images were then assembled into one montage using the Virtual Slice module of the Stereo Investigator software. Final images were constructed using Corel Draw v. 11. Only minor adjustments of contrast and brightness were made, which in no case altered the appearance of the original materials. Bar = 400 μm. (**C** and **D**) Representative MRI scans of the cerebellar midsagittal areas from a 16-year-old male suffering from autism (**C**) and from a 16-year-old male control (**D**) (arrows). Note the somewhat smaller cerebellar midsagittal area in the brain from the autistic patient compared with the control.

epilepsy) and observed a decreased number of Purkinje cells in both cases, whereas cerebellar white matter thinning and demyelination was found in one case. As a result of the consistently reported decreased numbers of Purkinje cells in autism, Fatemi *et al*. (2002*a*) were the first to examine the size of the cerebellar Purkinje cells. Blocks of the cerebella of five adult male autistic subjects (same subjects as in Fatemi *et al*., 2001*a*) were compared with those of five age- and sex-matched controls. A 24% decrease in mean Purkinje cell size was found in the autistic group.

Alterations in the brainstem

As already mentioned in the previous section, Kemper and Bauman (1993) reported alterations in the inferior olive. In the three young autistic cases (9, 10 and 12 years of age), the neurons in the inferior olive were large, whereas in the autistic cases (older than 22 years) these neurons were small and pale, but adequate in number. Furthermore, in all six autistic cases, some of the olivary neurons tended to cluster at the periphery of the nuclear complex. Although the significance of these findings remains to be elucidated, this pattern has been described earlier in some syndromes of prenatal origin that are associated with mental retardation (Sumi, 1970; DeBassio *et al*., 1985). The brainstem of a 21-year-old autistic woman with mental retardation and epilepsy (Rodier *et al*., 1996) showed a near-complete absence of the facial nucleus and superior olive along with shortening of the brainstem between the trapezoid body and the inferior olive, when compared with an 80-year-old male control case. Bailey *et al*. (1998) reported olivary dysplasia in three of the five autistic cases, as well as ectopic neurons related to the olivary complex in another two cases.

Alterations in the neocortex

By counting pyramidal cells, other neurons and glial cells in the primary auditory cortex, Broca's language area and the auditory association cortex, Coleman *et al*. (1985) failed to find consistent differences between the brain of a 21-year-old autistic female, with probable mental retardation, but without seizure disorder, and two control brains (both from females, 18 and 25 years old, respectively). The differences between the two control cases were larger than those between both of them and the autistic case. Of the 42 comparisons made, only six revealed differences between the autistic case and the control cases (i.e. decreased number of glial cells in the left primary auditory cortex and decreased number of pyramidal neurons in the right auditory association cortex in the autistic case), whereas 20 comparisons showed differences between the two control cases. Numerous neurofibrillary tangles were found in layer II and III of the cerebral cortex, especially in the temporal region, of a 24-year-old woman with autism and mental retardation, and severe self-injury behaviour (Hof *et al*., 1991). A few neurofibrillary tangles were also found in the amygdala. It was suggested that these neurofibrillary tangles may be related to severe and chronic head injury, similar to boxers' encephalopathy, where such abnormalities have also been observed (Corsellis *et al*., 1973; Hof *et al*., 1992). Kemper and Bauman (1993), investigating six autistic cases, reported an unusually coarse and poorly laminated anterior cingulate cortex in five of the six autistic cases. A 16-year-old female with autism and severe mental retardation showed no abnormalities in the cerebral cortex (Guerin *et al*., 1996). Bailey *et al*. (1998) reported no alterations in neuronal

counts of the superior frontal cortex of the six autistic cases compared with seven age-matched control cases.

Observations on cortical dysgenesis and migration abnormalities

Bailey *et al*. (1998) reported cortical dysgenesis in four of six autistic cases, with thickened cortices, high neuronal density, presence of neurons in the molecular layer, irregular laminar patterns and poor grey–white matter boundaries. White matter abnormalities were also found in four cases, including ectopic grey matter in three cases and increased number of white matter neurons in one case. The authors stated that cerebral developmental abnormalities, such as megaencephaly (which was present in four of the six cases), are usually associated with heterotopias (Harding and Copp, 1997), that were also found in the present cases. Fatemi and colleagues pursued the investigation of the neurochemical parallels of decreased Purkinje cell counts that have been consistently reported in autism. In two overlapping studies, levels of reelin (Fatemi *et al*., 2001*a*) and Bcl-2 (Fatemi *et al*., 2001*a*, *b*) were measured in the cerebellar cortex of five adult autistic males (IQ and seizure status unknown) and eight adult controls. Reelin, the product of the *reelin* gene, is a signalling protein that is involved in the control of neuronal migration and correct lamination during the embryonic period, and of synaptic plasticity in adult life (Fatemi, 2002). The Bcl-2 protein governs programmed cell death (apoptosis) in the developing brain. More than 40% reduction in reelin and 34–51% reduction in Bcl-2 were found by Fatemi *et al*. (2001*a*, *b*). Reduction in reelin has been found to be associated with disturbed neuronal migration and lamination of the cerebral and cerebellar cortex in mice (Gonzalez *et al*., 1997; Fatemi *et al*., 1999; Fatemi, 2001) and was suggested to be involved in migrational processes during the early development of the human brain (Piven *et al*., 1990; Persico *et al*., 2001). Moreover, reductions in blood reelin have been associated with severe mental retardation and hypoplastic cerebellum, findings that have both been reported in autism. The reported reduction in Bcl-2 might influence programmed cell death as this protein strongly inhibits apoptosis. Following these reports of reduced levels of the anti-apoptotic protein Bcl-2 in the cerebellum from autistic patients (Fatemi *et al*., 2001*a*, *b*), levels of Bcl-2 and p53 (a key regulator of neuronal apoptosis; Araki *et al*., 2000) were measured in the parietal and superior frontal cortex of five adult autistic males (three with mental retardation, seizures unknown) and four adult male controls (Fatemi and Halt, 2001; Araghi-Niknam and Fatemi, 2003). A reduction of >30% (32% in parietal and 38% in superior frontal cortex) in Bcl-2 expression was reported, comparable with the reduction observed in the cerebellar cortex (Fatemi *et al*., 2001*a*, *b*). In contrast, an increase in p53 expression (130% in parietal and 68% in superior frontal cortex) was found. These abnormalities in Bcl-2 and p53 were correlated with the presence of severe mental retardation (mean IQ of the patients was 25). Both the decrease in the anti-apoptotic Bcl-2 and the increase in apoptosis-controlling p53 were thought to result in a greater propensity for cell death. Indeed, it was suggested previously that increased brain volume in autism may be found only in high-functioning subjects (Akshoomoff *et al*., 2002), whereas autistic subjects with (severe) mental retardation would display normal or even smaller brain volumes compared with controls.

Recently, the configuration of so-called minicolumns was investigated in autism and Asperger syndrome (Casanova *et al*., 2002*a*, *b*). Casanova and colleagues posed that cell minicolumns are supposed to be a basic functional unit of the brain that organizes neurons in cortical space (Mountcastle, 1997). Instead of cell counting, an overall cell density measure was used, estimating the amount of space occupied by cell somas in a certain predefined area. More numerous, smaller and less compact minicolumns were found in nine autistic subjects (seven with mental retardation, five with epilepsy, four with macroencephaly) compared with nine control cases (Casanova *et al*., 2002*b*) and in two adults with Asperger's syndrome compared with 18 control subjects (Casanova *et al*., 2002*c*). However, the functional significance of these minicolumns is still unclear (Hutsler and Galuske, 2003). Several attempts have been made to identify these minicolumns as the anatomical correlate of the smallest processing unit in the cerebral cortex; however, further research will be required to solve this issue unequivocally (Jones, 2000).

Alterations in the cholinergic system

The cholinergic system has been shown to play a significant role in cortical development (Hohmann and Berger-Sweeney, 1998). Cholinergic afferents innervate the cerebral cortex during the most dynamic periods of neuronal differentiation and synapse formation. Disruption of cholinergic innervation during early postnatal development results in delayed cortical neuronal development and permanent changes in cortical architecture and cognitive function (Hohmann and Berger-Sweeney, 1998). Abnormalities have been found in the basal forebrain (septal) cholinergic neurons of autistic cases, such as larger neurons at younger ages and smaller neurons at older age (Bauman and Kemper, 1994). Perry *et al*. (2001) investigated cholinergic biomarkers in the basal forebrain and the (frontal and parietal) cerebral cortex in the brains of seven autistic cases (all with mental retardation, at least 50% with epilepsy), nine mentally retarded but not autistic cases, and 10 controls. In the autistic cases, muscarinic M1 receptor binding was found to be 30% lower in the parietal cortex compared with both the normal comparison cases and the non-autistic mentally retarded cases. In addition, α4 nicotinic receptor binding was reduced by 65–73% in the frontal and parietal cortex in both autistic and non-autistic, mentally retarded cases compared with the controls. In the basal forebrain of autistic subjects, the only abnormality was an increase in brain-derived neurotrophic factor

(BDNF), an increase that had been found previously in neonatal bloodspots of children who later developed autism or mental retardation (Nelson *et al*., 2001). These results indicated normal presynaptic cholinergic activity, but abnormal postsynaptic cholinoceptive function, the M1 receptor being located postsynaptically. Following the report of Perry *et al*. (2001), the same group examined cholinergic activities in the cerebellum of these autistic cases (with an additional one). Eight autistic adults (seven with mental retardation, five with epilepsy), 11 age-matched subjects with mental retardation but no autism, and 10 age-matched controls were included in this study (Lee *et al*., 2002). In the autistic cases, the nicotinic receptor, consisting primarily of α3 and α4 subunits, was reduced by 40–50%, whereas an opposite increase in nicotinic receptor consisting of the α7 subunit was reported. The exact relationship between these receptor abnormalities and autism and mental retardation remains to be determined.

Alterations in the GABAergic system

Like the cholinergic system, the GABAergic system also has an important role in early neuronal development, and has also been suggested to be involved in autism (Cook *et al*., 1998; Schroer *et al*., 1998). During the early neonatal period, GABA provides most of the excitatory drive to developing neurons rather than being an inhibitory neurotransmitter (Cherubini *et al*., 1991; Barker *et al*., 1998). Blatt *et al*. (2001) investigated four neurotransmitter systems (i.e. the GABAergic, serotonergic, cholinergic and glutamatergic system) in the hippocampus of four autistic adult male cases (all with mental retardation and two with epilepsy) and three adult male control cases. The GABAergic system was the only neurotransmitter system found to be significantly reduced in autism. The other three neurotransmitter systems did not show any differences between the autistic and control cases. Although the significance of these findings is not clear, it was suggested that a decrease in the availability of inhibitory GABA receptors could alter receptor activity. As a consequence, the threshold for development of seizures, a frequent co-morbidity of autism (Bailey *et al*., 1998), would be reduced. Fatemi *et al*. (2002*b*) investigated the level of glutamic acid decarboxylase (GAD), the rate-limiting enzyme responsible for the conversion of glutamate to GABA in the brain. The levels of the 65 and the 67 kDa GAD were measured in the cerebellum of five autistic and eight control cases and in the parietal cortex of five autistic cases (three overlapping with the cerebellum cases) and four control cases (all overlapping with the cerebellum cases). The 65 kDa GAD protein was reduced by 50% in the cerebellum and by 48% in the parietal cortex of the autistic cases. The 67 kDa GAD protein was reduced by 51% in the cerebellum and by 61% in the parietal cortex of the autistic cases. These decreases in GAD were thought to subserve a deficit in GABA availability, as reported by Blatt *et al*. (2001). In addition, a deficit in GABA was not only suggested to play a role in the aetiology of seizures, it was also proposed to affect several important biological functions, such as locomotor activity, learning and circadian rhythms (Soghomonian and Martin, 1998). However, as the sample sizes and the number of studies on the GABAergic system in autism have been very small thus far, no definite statement can be made about the exact role of the GABAergic system in the aetiology of autism.

Are MRI findings in autism a structural observable correlate of the neuropathological findings?

Research on the neuropathology of autism has been hampered by the lack of availability of large sample sizes and closely matched control groups. Structural MRI, on the other hand, is uniquely suited to scan (repeatedly) the brains of large groups of (young) patients and matched controls *in vivo* and map neuroanatomic abnormalities. Unfortunately, to date, structural MRI findings cannot be directly correlated to the neuropathological findings in autism, although the repeatedly reported increased brain volume detected with MRI (Filipek *et al*., 1992; Piven *et al*., 1995, 1996; Courchesne *et al*., 2001; Hardan *et al*., 2001*a*, *b*; Aylward *et al*., 2002; Carper *et al*., 2002; Sparks *et al*., 2002; Herbert *et al*., 2003) seems consistent with the frequent observation of an increased brain weight in autism (Bailey *et al*., 1998; Kemper and Bauman, 1998; Courchesne *et al*., 1999; Casanova *et al*., 2002*a*; see also Courchesne *et al*., 2000). Neuropathological studies have consistently reported smaller and more closely packed neurons in the limbic system in autistic patients, whereas MRI findings are rather equivocal. Volumes of limbic structures of autistic subjects have been found either increased (Howard *et al*., 2000; Sparks *et al*., 2002), decreased (Aylward *et al*., 1999; Pierce *et al*., 2001; Saitoh *et al*., 2001; Herbert *et al*., 2003) or unchanged (Saitoh *et al*., 1995; Piven *et al*., 1998; Haznedar *et al*., 2000; Howard *et al*., 2000) compared with those of control subjects. Likewise, the consistent observation of a decrease in Purkinje cell number and density does not have an MRI equivalent. Although early MRI reports consistently showed smaller midsagittal cerebellar hemispheres in autism (Gaffney *et al*., 1987; Murakami *et al*., 1989) or vermis (Courchesne *et al*., 1988; Hashimoto *et al*., 1995; Ciesielski *et al*., 1997) (see also Fig. 1), more recent reports did not (Filipek *et al*., 1992; Garber and Ritvo, 1992; Holttum *et al*., 1992; Kleinmand *et al*., 1992; Nowell *et al*., 1990; Piven *et al*., 1992, 1997). This lack of agreement in cerebellar segmentation among neuroimaging studies might be partially explained by using different MRI systems, as was reported most recently (Lotspeich *et al*., 2004). It is important to keep in mind that generally these studies have not accounted for IQ as a confounding factor (Piven *et al*., 1992). Thus, although both neuropathological and MRI studies investigate brain structures, the two techniques have failed to provide correlated and consistent data.

Discussion

In this review, we have attempted to provide an extensive overview of the available neuropathological literature of autism. Although some consistent results emerge, the majority of the neuropathological data remain equivocal. This may be due to lack of statistical power, resulting from small sample sizes and from the heterogeneity of the disorder itself, to the inability to control for potential confounding variables such as

Table 2 *Consistent neuropathological findings in autism*

Finding	*n*	New	MR	E	Results
Increased cell packing density and smaller neurons in the limbic system					
Kemper and Bauman (1993)	6	6	5	4	6 of 6
Guerin *et al*. (1996)	1	1	1	1	0 of 1
Raymond *et al*. (1996)	2	2	2	0	2 of 2 (only HIP measured)
Bailey *et al*. (1998)	6	6	6	3	1 of 5 (only HIP measured)
Total	15	15	14/15	8/15	9 of 14 (64%)
Decreased numbers of/smaller Purkinje cells in CB					
Williams *et al*. (1980)	4	4	4	2	1 of 4
Ritvo *et al*. (1986)	4	4	3	0	4 of 4
Fehlow *et al*. (1993)	1	1	1	0	1 of 1
Kemper and Bauman (1993)	6		5	4	6 of 6
Guerin *et al*. (1996)	1		1	1	0 of 1
Bailey *et al*. (1998)	6		6	3	5 of 6 (only the child unaffected)
Fatemi *et al*. (2002a)	5	5	?	?	2 of 5
Lee *et al*. (2002)	2		2	1	2 of 2
Total	29	14	22/24	11/24	21 of 29 (72%)
Age changes in CB nuclei and inferior olive					
Bauman (1991)	5		5	4	Large neurons in the 2 children, pale and small neurons in the 3 adults
Total	5	0	5	4	5 of 5 (100%)
Brainstem/olivary dysplasia					
Rodier *et al*. (1996)	1	1	1	1	1 of 1
Bailey *et al*. (1998)	5		5	3	3 of 5
Total	6	1	6/6	4/6	4 of 6 (67%)
Alterations in the neocortex					
Coleman *et al*. (1985)	1	1	1	0	0 of 1
Hof *et al*. (1991)	1	1	1	0	1 of 1 ↑NFT
Kemper and Bauman (1993)	6		5	4	5 of 6 poorly laminated ACC
Guerin *et al*. (1996)	1		1	1	0 of 1
Bailey *et al*. (1998)	6		6	3	0 of 6 abnormal FR neuronal count
Total	15	2	14/15	8/15	6/15 (40%)
Cortical dysgenesis					
Bailey *et al*. (1998)	6		6	3	4 of 6
Fatemi *et al*. (2001*b*)	5	5	3 or >	?	As a group ↓Bcl-2 and ↑p53 PA
Fatemi *et al*. (2001*a*)	5		?	?	As a group ↓Bcl-2 and reelin CB
Casanova *et al*. (2002*c*)	2AS	2AS	0	?	1 of 2 had smaller minicolumns
Casanova *et al*. (2002*a*)	9	9	7	5	As a group smaller minicolumns
Araghi-Niknam and Fatemi (2003)	5		?	?	As a group ↓Bcl-2 and ↑p53 FR
Total	32	14 + 2	16/22	8/15	
Abnormalities in cholinergic system					
Perry *et al*. (2001)	7	7	7	3 or >	As a group ↓M1 PA; ↓α4 FR + PA; ↑BDNF forebrain
Lee *et al*. (2002)	8	1	7	5	As a group ↓α3 and α4 and ↑α7 in CB
Total	15	8	14/15	8/15	
Abnormalities in GABAergic system					
Blatt *et al*. (2001)	4	4	4	2	↓GABAergic system in HIP
Fatemi *et al*. (2002b)	5		?	?	↑65 and 67 kDA GAD in CB and parietal cortex
Total	9	4	4/9	2/9	

ACC = anterior cingulate cortex; AS = Asperger's syndrome; BDNF = brain-derived neurotrophic factor; CB = cerebellum; E = epilepsy; FR = frontal cortex; GAD = glutamic acid decarboxylase; HIP = hippocampus; MR = mental retardation; *n* = number of autistic subjects; New = number of brains mentioned for the first time (58 autistic and two Asperger); NFT = neurofibrillary tangles; PA = parietal cortex; ? = not mentioned.

gender, mental retardation, epilepsy and medication status, and, importantly, to the lack of consistent design in histopathological quantitative studies of autism published to date. Furthermore, the investigation of different brain structures could have contributed to the disparate findings. However, when considering the available data, a number of conclusions can be drawn (Table 2). First, a decrease in the number of Purkinje cells throughout the cerebellar hemispheres without significant gliosis and features of cortical dysgenesis has been found consistently by different research groups. Secondly, although not replicated by independent research groups, increased cell packing density of smaller neurons in the limbic system and age-related abnormalities in the cerebellar nuclei and the inferior olive have been reported in the majority of the studied cases. Finally, both the nicotinic and muscarinic cholinergic and the GABAergic system are likely to be impaired in autism.

An arrest of normal development has been proposed to explain the findings in the limbic system, whereas the decrease in Purkinje cell numbers is likely to be largely prenatal in origin (Kemper and Bauman, 1998). The features of cortical dysgenesis, such as increased neuronal density, increased cortical thickness, ectopic grey matter and poor differentiation of the grey–white boundary, are suggestive of abnormalities in cortical lamination (i.e. abnormalities in neuronal proliferation and migration) and apoptosis (Rorke, 1994). In support of this possibility are the findings of reductions in reelin (implicated in regulation of layering of the cortex) and Bcl-2 (implicated in the process of apoptosis).

As to the timing of the neuropathological abnormalities in autism, all authors have suggested a prenatal origin, most probably during the first 6 months of gestation (Piven *et al*., 1990; Rorke, 1994; Rodier *et al*., 1996; Bauman *et al*., 1997; Courchesne, 1997; Bailey *et al*., 1998; Gillberg, 1999). It should be mentioned, however, that according to a hypothesis by Gillberg (1999), there could be at least two different pathways to autism, one connected with primary temporofrontal dysfunction (and late prenatal–early postnatal origins) and another linked to primary brainstem dysfunction (and early prenatal origins). Furthermore, a recent report by Kern (2003) has suggested that it is conceivable that some children become autistic from neuronal cell death or brain damage occurring postnatally as a result of injuries, as some cases of autism do not show symptoms until a substantial period after birth. Indeed, Purkinje cells can be selectively vulnerable to certain types of insult such as ischaemia, hypoxia, excitotoxicity, viral infections, heavy metals, and toxins such as ethanol (Welsh *et al*., 2002).

Taken together, there is evidence from neuropathological data for an evolving pathological process in the autistic brain that extends from the fetal period of brain development into adulthood. However, the mechanisms underlying these alterations remain unknown. Likewise, the underlying causes of the reported decreased nicotinic receptor binding in the cholinergic system are not understood, although it is known that nicotine enhances several cognitive and psychomotor behaviours (Granon *et al*., 2003), suggesting the potential for intervention through cholinergic receptor modulation. The same holds true for the apparent reduced function of the GABAergic system.

Thus, besides the ongoing classic neuropathological research, future studies will benefit from focusing on techniques aiming to disentangle the underlying biological mechanisms of autism. Design-based stereological approaches to neuropathology may become a key methodology, as they can provide information about the degree of maturation or health of brain cells and overall brain development (West, 1993; Hof and Schmitz, 2000; Schmitz and Hof, 2004). Design-based stereology permits precise and reliable measurement of number, size and spatial distribution of cells within a given brain region, using standardized protocols. Another relatively new approach, holding great promise in the immediate future of autism research, is the study of gene expression. It is expected that extensive and detailed investigations of gene expression will help to understand the molecular and cellular basis of the neuropathology of autism. Region-specific alterations in gene expression will be reflective of neuroadaptive processes underlying the neuropathological findings of autism reported in the literature. In the field of autism, gene expression is still in its infancy, although some results have already been published. For example, preliminary data have shown a complete absence of αB-crystallin, a small heat-shock protein functioning as a molecular chaperone, in the frontal cortex of brains from autistic patients (Pickett, 2001). Purcell *et al*. (2001*a*) investigated the neural cell adhesion molecule (NCAM), a developmentally regulated protein, in a sample of 10 autistic cerebella and 16 control cerebella. A decrease in the longest of three isoforms (180 kDa) of NCAM was found in the cerebella from the autistic patients. In addition, the mRNA levels of two genes, both members of the glutamate system, were found to be increased in the cerebellum of the same autistic cases (Purcell *et al*., 2001*b*). Identifying changes in gene expression will ultimately be useful to provide molecular diagnostic tests for autism and to identify specific cellular pathways that have been disrupted in this disorder.

In conclusion, classic neuropathological observations in autism show increased cell packing density and smaller neuronal size in the limbic system, decreased number of Purkinje cells in the cerebellum, and features of cortical dysgenesis or migration disturbances. However, the underlying neurobiological basis remains elusive. The implementation of newer techniques, such as design-based stereology and large-scale analysis of gene expression, holds great promise and might eventually result in the elucidation of the aetiology of autism.

Note added in proof

A recent study applying functional MRI during sentence comprehension indicated a lower degree of information integration across large-scale cortical networks involved in language processing as a possible neural basis of disordered language in

autism (Just *et al.*, Brain 2004; 127, 1811–1821). It will be attractive to test this hypothesis in future neuropathological studies of autism.

Acknowledgements

We wish to thank the following institutions and colleagues for the provision of human tissue: the Harvard Brain Tissue Research Center (Belmont, MA), the University of Maryland Brain and Tissue Bank for Developmental Disorders (Baltimore, MD), the US Autism Tissue Program (Princeton, NJ), Dr J. Wegiel (New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY) and NIH grant NO1 HD13138. The work was financially supported by the European Community (Quality of Life and Management of Living Resources, QLK6-CT-2000-60042, QLK6-GH-00-60042-56; to S.J.M.C.P.), the Korczak foundation (to H.v.E.), the US National Alliance for Autism Research (to C.S. and P.R.H.) and by NIH grant MH66392 (to P.R.H.). P.R.H. is the Regenstreif Professor of Neuroscience. We thank Dr D. P. Perl and Dr H. Heinsen for support and helpful discussion.

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