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Density of Cerebellar Basket and Stellate Cells in Autism: Evidence for a Late Developmental Loss of Purkinje Cells

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Alterations in the cerebellum have been described as a neuropathological feature of autism. Although numerous studies have focused on the Purkinje cell (PC), the projection neuron of the cerebellar cortex, PC function is critically dependent on their innervation by the GABAergic basket cells (BCs) and stellate cells (SCs) in the cerebellar molecular layer. The present study was designed to determine whether there are differences in the packing density of these inhibitory interneurons or whether the ratio of these interneurons to PCs differs in autistic and age-matched control brains. The GABAergic interneurons were identified by using immunohistochemistry for parvalbumin (PV) in serial sections from the posterior cerebellar lobe of six autistic and four control brains and counted using stereological principles. Prior PC counts in the same area on adjacent sections (Whitney et al., 2008) were available and were used to calculate the number of BCs and SCs per PC. In this sample of brains, no statistically significant difference was detected between the autistic and the control groups in the density of BCs or SCs ($P = 0.44$ and $P = 0.84$, respectively) or in the number of BCs or SCs per PC ($P = 0.47$ and $P = 0.44$, respectively). The preservation of BCs and SCs, in the presence of the reduced PC numbers as found in at least two, and possibly three, of these six autistic cases (Whitney et al., 2008) suggests that PCs were generated, migrated to their proper location in the PC layer, and subsequently died in the autistic cases that showed a reduction in PCs.

Key words: cerebellum; basket cells; stellate cells; autism

Autism is a neurodevelopmental disorder characterized by deficits in reciprocal social interactions and communication as well as repetitive or stereotypic behaviors or interests (DMS-IV, 1995). Evidence that genetic factors play an important role in autism has been shown in twin studies (Folstein and Rutter, 1977; Steffenburg et al., 1989; Bailey et al., 1995) and in family studies in which the risk of having a second child with autism is reported to be 50–150 times greater than that of the general population (Smalley et al., 1988; Folstein and Piven, 1991). It has been suggested that as many as two to fifteen genes (Pickles et al., 1995; Risch et al., 1999), and not necessarily the same genes or same combinations (Folstein and Rosen-Sheidley, 2001), contribute to the expression of autism. Although a single candidate gene has not been identified, several genes that code for γ-aminobutyric acid (GABA) receptor subunits have been implicated (Cook et al., 1998; Wolpert et al., 2000; Folstein and Rosen-Sheidley, 2001; Buxbaum et al., 2002; Shao et al., 2003; Ma et al., 2005; Collins et al., 2006). In line with the genetic findings, alterations in components of the GABA system have been identified (Blatt et al., 2001; Fatemi et al., 2002a; Guptill et al., 2007; Yip et al., 2007, 2008). Further evidence of involvement in the GABA system in autism is provided by reports of reduced numbers of GABAergic Purkinje cells (PCs), the sole output neuron of the cerebellar cortex. A recent review by Palmen et al. (2004) indicated that 72% of cases reported in the literature have a decreased number of PCs. In our laboratory, a recent study of six autistic and four control brains revealed that two or three of the autistic cases (33–50%) had a reduced density of PCs (Whitney et al., 2008; see also Table I, column 3). In addition to the GABAergic PCs, there are GABAergic interneurons in the cerebellar cortex that innervate PCs, the basket cell (BC) and the stellate cell (SC), but these

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TABLE I. Case Information on Autistic and Control Brains, Including Age, Sex, Hemisphere, Post-Mortem Interval (PMI), Years in Formalin, No. PC/mm, and Cause of Death*

Group	Case No.	No. PCs/mm	Age (years)	Sex	Hemisphere	PMI (hr)	Years in formalin	Cause of death
Control	Duke-495	5.4	17	F	Unknown	Unknown	Unknown	Unknown
Control	4104	5.3	24	M	Left	5	Unknown	Gunshot
Control	4334	4.0	53	M	Right	23.75	3	Cancer
Control	BCH-13	4.0	30	M	Left	Unknown	6	Unknown
Autism	4414[a,b]	0.5	26	M	Left	47.68	2.5	Seizure
Autism	3845[a,b]	2.6	32	M	Left	Unknown	4	Pancreatitis
Autism	4099	3.3	19	M	Left	3	4.5	CHF
Autism	2431	4.3	54	M	Left	4	8.5	GI bleed
Autism	4259[a,c]	4.3	13	F	Left	18.36	4	Unknown
Autism	3511[a,d]	5.8	27	M	Right	16	7.5	Trauma

*GI, gastrointestinal; CHF, congestive heart failure.
[a]Seizure disorder.
[b]History of phenytoin (Dilantin) use.
[c]History of carbamazepine (Tegretol) use.
[d]History of phenobarbital use.

have not been quantitatively examined in the autistic brain. Although there is some controversy about the classification of cerebellar GABAergic molecular layer interneurons (MLIs), they are most commonly described based on their depth within the molecular layer and their contribution to an axonal plexus (basket) that surrounds the PC soma (Sultan and Bower, 1998) or their innervation of PC cell apical dendrites. Thus, BCs are typically said to reside in the lower one-third of the molecular layer and contribute the axonal plexus surrounding the PC soma (Sultan and Bower, 1998), whereas SCs are typically considered to reside in the upper two-thirds of the molecular layer and synapse with the PC cell dendrite. Both BCs and SCs receive excitatory input from granule cell parallel fibers as well as GABAergic inhibitory input from other BCs and SCs (Palay and Chan-Palay, 1974) and from the Lugaro cells in the granule cell layer (Laine and Axelrad, 1998). Likewise, efferent projections from BCs and SCs include other BCs, SCs, and PCs (Palay and Chan-Palay, 1974).

The establishment of functional synaptic contacts between PCs and MLIs is of critical importance for the prolonged survival of BCs and SCs (Sotelo and Triller, 1979; Feddersen et al., 1992). Furthermore, PCs are necessary for the proper cellular organization of the developing cerebellar cortex (Sotelo, 1990; Feddersen et al., 1992; Smeyne et al., 1995; Soha et al., 1997). This published work, together with the data obtained in the current study regarding BC and SC density and our prior finding of PC density in these same cases (Whitney et al., 2008; see also Table I, column 3), offers the opportunity to formulate a hypothesis for the timing of the reduced density of PCs in the autistic brain, when present. This hypothesis, which is outlined in the Discussion section, is based on work in mutant mice and studies of normal cerebellar development.

Given the reported involvement of the GABA system in autism and the functional relationships between PCs and MLIs, we set out to determine the numerical density of these interneurons by using stereological methods in sections immunolabeled with parvalbumin (PV). With these data, the relationship of the number of PCs to the number of BCs and SCs could be examined. Based on these data, a hypothesis can be proposed regarding the timing of the reduced number of cerebellar PCs in the autistic brain.

MATERIALS AND METHODS

Six autistic and four control, formalin-fixed cerebella were obtained from the Harvard Brain Tissue Resource Center, Kathleen Price Bryan Brain Bank at Duke University Medical Center, and University of Maryland Brain Bank. All control brains were free from gross pathology and were obtained from individuals without history of neurological disorders. These same 10 cases were previously used in our laboratory to examine the density of PCs (Whitney et al., 2008). Table I includes the case number, group, age, sex, hemisphere, post-mortem interval (PMI), years in formalin, number of PCs per millimeter, and cause of death of each case and is derived from Whitney et al. (2008).

Tissue Processing

In each case, a 2 cm × 2 cm × 2 cm parasagittal block of tissue, cut perpendicular to the folia, was obtained from cerebellar hemisphere lobule crus II, located inferior to the horizontal fissure (Schmahmann et al., 2000). Prior to tissue processing, each case was coded to ensure that the investigators were blind to the case data throughout the experimental process. All tissue blocks were cryoprotected (Rosene et al., 1986) and embedded in albumin-gelatin prior to freezing in order to hold the folia together after cutting and during subsequent processing (Crane and Goldman, 1979). The blocks were then flash frozen in −75°C 2-methylbutane and placed in a −80°C freezer for at least 48 hr before being serially sectioned on a sliding microtome from medially to laterally. Eighteen series per brain were cut at 30 μm and two series

were cut at 60 μm so that sections within a series were spaced 660 μm apart. Sections were thaw mounted onto gelatin-subbed slides, air dried, and stored in a –20°C freezer until the day they were stained.

From each brain, ten 30-μm on-the-slide sections were immunostained using a batch-processing procedure in four groups (two pairs, two triplets), matched for age and sex, to ensure that conditions were identical for the autistic and control groups. Immunostaining for PV was conducted using specially designed wells (PolyFab Inc., Avon, MA) that minimized antibody volume. The optimal anti-PV antibody concentration (mouse monoclonal) was predetermined to be 1:750 (Swant Laboratories, Belinzona, Switzerland). Sections were pretreated in a 1% hydrogen peroxide solution to quench endogenous peroxidases and a blocking serum to minimize nonspecific binding. Sections were then incubated in the primary antibody for 48 hr at room temperature, followed by a 1-hr incubation in a biotinylated secondary antibody (Vector Laboratories, Burlingame, CA). An avidin-biotinylated peroxidase complex solution (ABC) was then added (Vector Laboratories), and the antigen was visualized with 3,3′-diaminobenzidine as the chromogen by incubating in hydrogen peroxide (1.5%) for 10 min. The reaction was stopped by washing sections in dH_2O. Sections were air dried overnight, lightly counterstained with thionin, and coverslipped with Permount.

Basket Cell and Stellate Cell Counts

The equipment and computer software used in this study were identical for all cases and included a Nikon Eclipse E600 microscope (Nikon Instruments Inc., Melville, NY), Optronics DEI-750 CE camera control unit (Optronics, Goleta, CA), MAC 2002 motor stage control unit (Ludl Electronic Products, Ltd., Hawthorne, NY), and Stereo Investigator software from MircoBrightField Inc. (Williston, VT).

Ten sections from each autistic and control case, equally spaced by 660 μm, were counted for both MLI types, BCs and SCs. The BC and SC counts were performed on sections adjacent to those previously used to obtain total PC counts (Whitney et al., 2008). All sections were cut from tissue blocks taken from lobule crus II, located inferior to the horizontal fissure (Schmahmann et al., 2000). However, because of variability in the tissue samples provided by the brain banks, we were not able to identify definitively the identical folia in all brains that could be counted in its entirety. Consequently, in all brains, the superiormost folium in the most medial section was identified for counting. For that region of interest, stereological sampling principles were maintained across all sections (Gundersen, 1986; West et al., 1991), determining both cell number and the area of molecular layer sampled so that data are presented as cell density measures (No. cells/mm^3). Additionally, with Stereo Investigator software and a ×2 objective lens, the identical length along the PC layer used for PC counts (Whitney et al., 2008) was measured in the PV-immunostained sections. This measurement defined the region of interest from which we were later able to calculate the number of BCs per PC and the number of SCs per PC. Next, with a ×2 objective lens and a transparent ruler superimposed on the computer image, the examiner outlined the lower one-third and upper two-thirds of the molecular layer. These two regions of interest allowed the examiner to obtain two separate density measures, one for BCs (No. BCs/mm^3) and one for SCs (No. SCs/mm^3). Within each region of interest, the interneurons were systematically sampled; the approach used was based on the optical disector method (Gundersen, 1986; West et al., 1991) and ensures that all objects, regardless of size, shape, and orientation, have an equal chance of being counted.

After the two regions of interest were defined, the magnification was increased to a ×60 oil-immersion objective lens. A 600 μm × 450 μm grid with a 100 μm × 75 μm counting frame was used to sample the two ROI randomly and systematically. To avoid double counting in the x or y planes, the lower and left borders (x and y) of the counting frame, as well as their extended edges, were considered forbidden, and BCs and SCs contacting these lines were not counted (Gundersen, 1986). The BCs or SCs within the counting frame and those contacting the upper and right borders of the counting frame were included in the counts (Gundersen, 1986). To avoid double counting in the z-axis, an exclusionary plane at the top of the section was implemented. This z-axis exclusionary plane was used instead of guard volumes because sections with an original thickness of 30 μm, when thaw-mounted onto slides, shrink in the z-axis to 7.5–8.5 μm. At this thickness and with the optics available, it was not possible to implement reliable guard volumes. Although this does introduce problems resulting from lost caps (Hedreen, 1998), the same process was used in all cases, so the relative difference between sections is not affected. After completion of the counting in one frame, the motorized stage shifted to the next counting frame. This process was repeated until all counting frames within the region of interest were viewed. Then, the second region of interest on the same section was examined.

The numerical density (N_v), was calculated as a ratio of the total number of molecular layer interneurons (BCs or SCs) counted (ΣQ) to the total disector volume, $\Sigma v(dis)$; the latter is determined by the number of times (ΣP) the disector hits the ROI reference space multiplied by the disector volume v(dis).

$$N_v = \frac{\sum Q}{\sum v(dis)} = \frac{\sum Q}{\sum P * v(dis)}$$

The estimated total number of BCs or SCs (N) in the region of interest was then calculated as the product of N_v and the reference volume, V(ref). The V(ref) was calculated as the product of the outlined ROI (area) and section thickness.

$$N = N_v * V(ref)$$

Finally, the density of BCs and SCs (N_T) within the region of interest, V(ref), was calculated as follows

$$N_T = \frac{N}{V(ref)}$$

where N_T represents the estimated number of molecular layer interneurons (BCs or SCs) per cubic millimeter.

TABLE II. Correlation Matrix*

	Group	No. PCs/mm	No. BCs/mm^3	No. SCs/mm^3
Group	—	r = 0.40; $P = 0.25$	r = 0.28; $P = 0.44$	r = 0.07; $P = 0.84$
No. PCs/mm	r = 0.40; $P = 0.25$	—	r = 0.21; $P = 0.57$	r = 0.35; $P = 0.34$
No. BCs/mm^3	r = 0.28; $P = 0.44$	r = 0.21; $P = 0.57$	—	r = 0.96; $P < 0.0001$
No. SCs/mm^3	r = 0.07; $P = 0.84$	r = 0.35; $P = 0.34$	r = 0.96; $P < 0.0001$	—

*This correlation matrix includes the correlation coefficient (r values) and P values for all of the relationships examined. A significant relationship was demonstrated between the density of BCs and SCs, with r = 0.96 and $P < 0.001$. No significant relationship was observed between the group (autistic or control) and the density of PCs, density of BCs, or density of SCs. Furthermore, no significant relationship was observed between the density of PCs and the density of BCs or density of SCs.

Given the controversy over classification of basket and stellate interneurons, the data, in addition to being expressed as No. BCs/mm^3 and No. SCs/mm^3, are also expressed as the No. MLIs/mm^3. Additionally, because we had previously determined the density of PCs in the identical area and along the identical length of the PC layer on adjacent 30-μm calbindin-immunolabeled sections (Whitney et al., 2008; see also Table I, column 3), data are also expressed as the number of BCs per PC, the number of SCs per PC, and the total number of MLIs per PC. To determine the number of these interneurons per PC, we divided the total estimated number of BCs, SCs, and MLIs by the total number of PCs along the same length.

Statistical Analysis

The difference in the mean density of interneurons (No. BCs/mm^3, No. SCs/mm^3, and No. MLIs/mm^3) between the autistic and control brains was examined using the *t*-test statistic. The Pearson correlation was used to examine the relationship between the density of BCs and the density of SCs (Table II). Pearson correlation was also used to examine the relationship between the density of MLIs (No. BCs/mm^3, No. SCs/mm^3) and the density of PCs (No. PCs/mm; Table II). The relationship between group (autistic, control) and BC and SC density was also explored (Table II). Furthermore, the *t*-test analysis was used for comparison of number of BCs per PC (No. BCs/PC), number of SCs per PC (No. SCs/PC), and number of MLIs per PC (No. MLIs/PC) between the autistic and control brains. Finally, to examine the relationship between No. MLIs/mm^3 and years in formalin as well as No. MLIs/mm^3 and PMI, the Pearson correlation was calculated.

RESULTS

Numbers of Molecular Layer Interneurons

The numerical density of BC interneurons in the lower one-third of the molecular layer is shown in Figure 1. In the control brains, the density ranged from 17,219 to 32,129 BCs/mm^3, with a mean density ± SD of 23,751 ± 7,658 BCs/mm^3. The corresponding densities for the autistic brains were 14,222–28,293/mm^3, with a mean density ± SD of 20,258 ± 5,980 BCs/mm^3. The numerical density of SC interneurons is shown in Figure 2. In the control brains, the density ranged from 9,014 to 20,849 SCs/mm^3, with a mean density ± SD of 14,794 ± 6,072 SCs/mm^3. The corresponding densities for the autistic brains were 8,164–22,586 SCs/mm^3, with a mean density ± SD of 13,990 ± 6,164 SCs/mm^3. Figure 3 represents the density of the combined BC and SC (MLI) interneurons. In the control brains, the density ranged from 13,152 to 26,489 MLIs/mm^3, with a mean density ± SD of 19,272 ± 6,853 MLIs/mm^3. The corresponding densities for the autistic brains were 11,193–25,440 MLIs/mm^3, with a mean density ± SD of 17,124 ± 6,045 MLIs/mm^3. With the available number of cases, statistical analysis by *t*-test did not detect a significant difference in the density of BC, SC, or MLIs between autistic brains compared with the control brains, with $P = 0.44$, $P = 0.84$, and $P = 0.62$, respectively. Additionally, photographs from two autistic and two control cases (see Fig. 7) visually demonstrate the variability in molecular layer interneuron density within the autistic and control groups.

Correlations of Analyses of Molecular Layer Interneurons

Pearson correlation, used to examine the relationship between the No. BCs/mm^3 and the No. SCs/mm^3, returned a value of r = 0.96 ($P < 0.0001$; Table II). Thus, as BC density increased, SC density also increased. Using the Pearson correlation, a statistically significant relationship was not identified when examining the relationship between the densities BCs and PCs or the relationship between the densities of SCs and PCs, with r = 0.21 ($P = 0.57$) and r = 0.35 ($P = 0.34$), respectively (Table II). Analysis of the relationship between group (autistic, control) and the No. BCs/mm^3, using the Pearson correlation, was not statistically significant, with r = 0.28 ($P = 0.44$; Table II). Similarly, analysis of the relationship between group (autistic, control) and the No. SCs/mm^3 was not statistically significant, with r = 0.07 ($P = 0.84$; Table II).

Number of Molecular Layer Interneurons Relative to Purkinje Cells

The number of BCs per PC is shown in Figure 4. In the controls, the number of BCs per PC ranged from 16.0 to 34.9, with a mean ± SD of 22.7 ± 8.5 BCs/PC. In the autistic group, these numbers ranged from 10.6 to 124.5, with a mean ± SD of 39.2 ± 42.7 BCs/PC. Compared with the other cases, autistic case 4414 appears as an outlier, with 124.5 BCs per PC (Fig. 4).

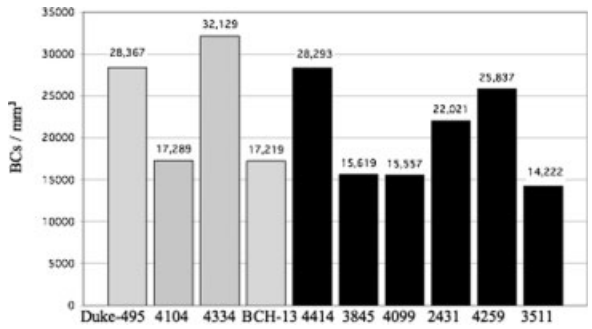

Fig. 1. The No. BCs/mm^3 for each control case (gray bars) and autistic case (black bars) is shown. In the control brains, the density ranged from 17,219 to 32,129/mm^3, with a mean density ± SD of 23,751 ± 7,658/mm^3. In autistic brains, the density ranged from 14,222 to 28,293/mm^3, with 20,258 ± 5,980/mm^3. With this sample, no statistically significant difference was identified between the control and the autistic groups, with $P = 0.44$.

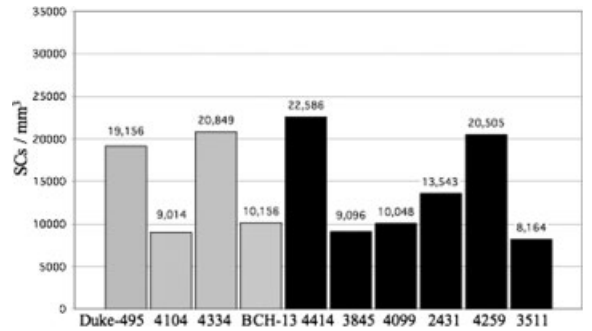

Fig. 2. The No. SCs/mm^3 for each control case (gray bars) and autistic case (black bars) is shown. In the control brains, the density ranged from 9,014 to 20,849/mm^3, with a mean density ± SD of 14,794 ± 6,072/mm^3. The corresponding densities for the autistic brains were 8,164–22,586/mm^3 and 13,990 ± 6,164/mm^3. With this sample, no statistically significant difference was identified between the control and the autistic groups, with $P = 0.84$.

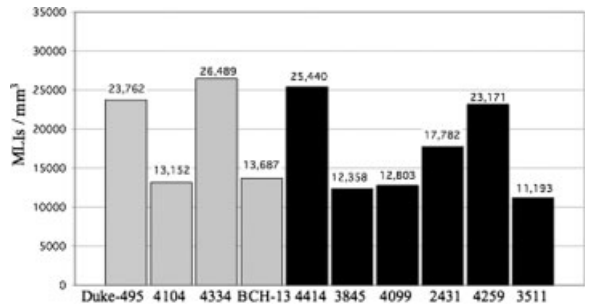

Fig. 3. The No. MLIs/mm^3 for each control case (gray bars) and autistic case (black bars) is shown. In the control brains, the density ranged from 13,152 to 26,489/mm^3, with a mean density ± SD of 19,272 ± 6,853/mm^3. The corresponding densities for the autistic brains were 11,193–25,440/mm^3 and 17,124 ± 6,045/mm^3. With this sample, no statistically significant difference was identified between the control and the autistic groups, with $P = 0.62$.

This high number can be explained by the fact that autistic case 4414 had the lowest density of PCs (0.5 PC/mm; see Table I) but a normal density of BCs that fell within the control range (28,293 BCs/mm^3; see Fig. 1). The reduced PC numbers, coupled with normal BC numbers, yielded a high number of BCs per PC and show that BCs were preserved in this case with decreased PCs. The number of SCs per PC is shown in Figure 5. In the control cases, the number of SCs per PC ranged from 16.7 to 41.9, with a mean ± SD of 27.5 ± 11.1 SCs/PC. In the autistic cases, these numbers ranged from 12.3 to 195.3, with a mean ± SD of 56.3 ± 68.9 SCs/PC. Again, autistic case 4414 appears as an outlier, with 195.3 SCs per PC (Fig. 5), this case having the lowest density of PCs (0.5 PC/mm; see Table I) coupled with a high density of SCs (22,586 BCs/mm^3; see Fig. 2). Thus, the reduced PC numbers, coupled with high SC numbers, yielded a high number of SCs per PC and show that SCs were preserved in this case with decreased PCs. The number of combined BCs and SCs (MLIs) per PC is shown in Figure 6. In the controls, the number of MLIs per PC ranged from 32.7 to 76.8, with a mean ± SD of 50.1 ± 19.5 MLIs/PC. In the autistic group, these numbers ranged from 22.9 to 319.8, with a mean ± SD of 95.5 ± 111.5 MLIs/PC. With the available number of cases, statistical analysis by *t*-test did not detect a significant difference in the number of BCs, SCs, or MLIs per PC between autistic brains and control brains, with $P = 0.47$, $P = 0.44$ and $P = 0.45$, respectively.

Effect of Time in Fixative and Post-Mortem Delay

Pearson correlation, used to examine the relationship between density of MLIs (BCs and SCs) and years in formalin (two control, six autistic), returned a value of $r = 0.58$ ($P = 0.13$). These data, although not statistically significant, suggest that 33% of the variability in the density of MLIs may be accounted for by the number of years in formalin ($r^2 = 0.33$). It is important to note, however, that this is a positive correlation; as years in formalin increases, the No. MLIs/mm^3 also increases. Thus, longer time periods in formalin did not appear to have a detrimental effect on the number of molecular layer interneurons identified with PV immunohistochemistry. Pearson correlation, also used to examine the relationship between density of MLIs and PMI (two control, five autistic), returned a value of $r = 0.71$ ($r^2 = 0.50$; $P = 0.07$). Again, this is a positive correlation; as PMI increases, the No. MLIs/mm^3 also increases. Thus, the longer PMI did not appear to have a detrimental effect on the MLIs.

DISCUSSION

Summary of Results

This study presents the first stereological cell counting data on cerebellar MLIs in the autistic brain. In both the autistic and the control cases, there was a significant correlation in the density of BCs and SCs ($P <$

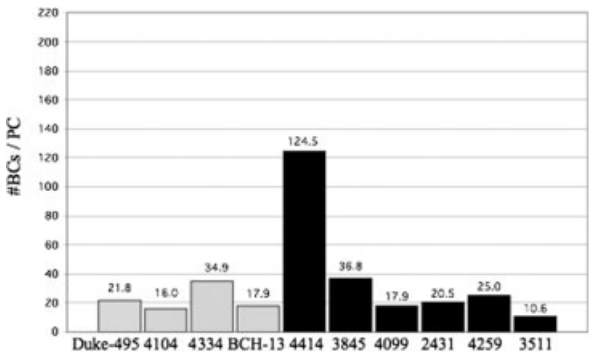

Fig. 4. The No. BCs per PC for each control case (gray bars) and autistic case (black bars) is shown. In the controls, the number of BCs per PC ranged from 16.0 to 34.9, with a mean ± SD of 22.7 ± 8.5 BCs/PC. In the autistic group, these numbers ranged from 10.6 to 124.5, with a mean ± SD of 39.2 ± 42.7 BCs/PC. With this sample, no statistically significant difference was identified between the control and the autistic groups, with $P = 0.47$.

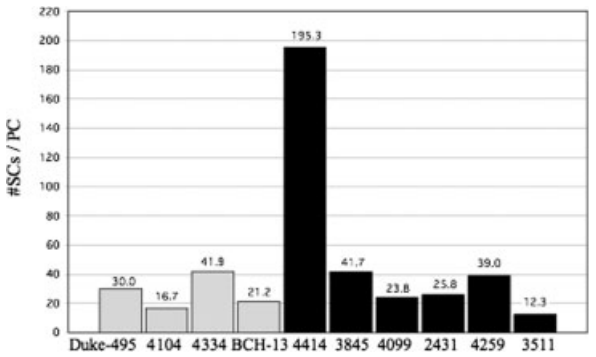

Fig. 5. The No. SCs per PC for each control case (gray bars) and autistic case (black bars) is shown. In the controls, the number of SCs per PC ranged from 16.7 to 41.9, with a mean ± SD of 27.5 ± 11.1 SCs/PC. In the autistic group, these numbers ranged from 12.3 to 195.3, with a mean ± SD of 56.3 ± 68.9 SCs/PC. With this sample, no statistically significant difference was identified between the control and the autistic groups, with $P = 0.44$.

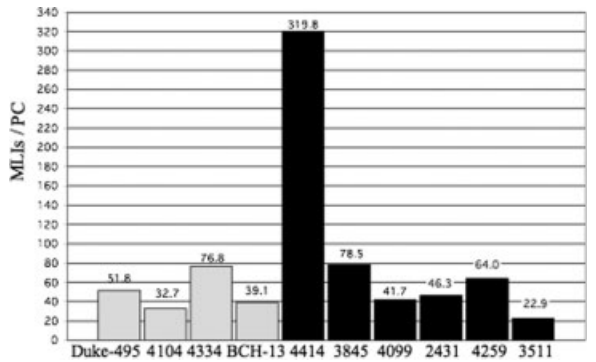

Fig. 6. The No. MLIs per PC for each control case (gray bars) and autistic case (black bars) is shown. In the controls, the number of MLIs per PC ranged from 32.7 to 76.8, with a mean ± SD of 50.1 ± 19.5 MLIs/PC. In the autistic group, these numbers ranged from 22.9 to 319.8, with a mean ± SD of 95.5 ± 111.5 MLIs/PC. With this sample, no statistically significant difference was identified between the control and the autistic groups, with $P = 0.45$.

0.001) without a significant difference in the density of BC and SC interneurons, with $P = 0.44$ and $P = 0.84$, respectively. There was no significant relationship between the density of these interneurons (BCs, SCs) and group (autistic, control; Table II, Figs. 1–3).

Discussion of MLI Counts

Although the sample size in this study was modest, the density and variability of MLIs in both the autistic and control cases in the current study are in close agreement with data from Andersen et al. (1992). In their study of the normal human brain, the density ranged from approximately 8,000 to 32,000 MLIs/mm^3. In the current study, the density of the MLIs in the autistic cases ranged from 11,193 to 25,440 MLIs/mm^3 and in the control cases from 13,152 to 26,489 MLIs/mm^3.

There was no significant relationship between the density of the interneurons (No. BCs/mm^3, No. SCs/mm^3) and the density of PC (No. PCs/mm; $P = 0.57$, $P = 0.34$; Table II). A significant difference in the No. BCs per PC and the No. SCs per PC between the control and the autistic cases was also not identified ($P = 0.47$ and $P = 0.44$, respectively; Figs. 4, 5). The number of MLIs per PC in the control brains (Fig. 6) is in close agreement with data from Andersen et al. (1992). They reported a mean of 50.0 MLIs/PC in the human cerebellum. In the current study, the mean number of MLIs/PC in the control brains was 50.1. In the autistic brains, the mean number of MLIs/PC was higher, at 95.5, because of a decreased number of PCs in at least two, and possibly three, of the six autistic cases (Table I, Figs. 3–6). These data demonstrate that MLIs were preserved in cases with reduced PCs numbers.

The finding that autistic cases with a reduced density of PCs had preservation MLIs was the basis from which we developed our hypothesis for the timing of PC loss in autistic cases with reduced PC numbers. Specifically, we looked to studies of mutant mice, normal rat cerebellar development, and normal human cerebellar development to formulate this hypothesis. Collectively, this work reveals the importance of the early presence of PCs for BC and SC survival.

Contribution of Mutant Mice Studies to Our Hypothesis of the Timing of PC Reduction

To this point in time, many researchers have been careful to indicate that there is reduction in PCs in the autistic brain, because there was no direct evidence that PCs were generated and then died at a later time. However, published work in mutant mice with PC loss at varying developmental times lends evidence that, in autistic brains with reduced PC numbers, PCs were generated during the prenatal period and subsequently died. This research has shown that PCs are critical for the proper cellular organization and development of the cerebellar cortex (Sotelo, 1990; Feddersen et al., 1992; Smeyne et al., 1995; Soha et al., 1997). Specifically, in mice with disruption in PC development, it has been

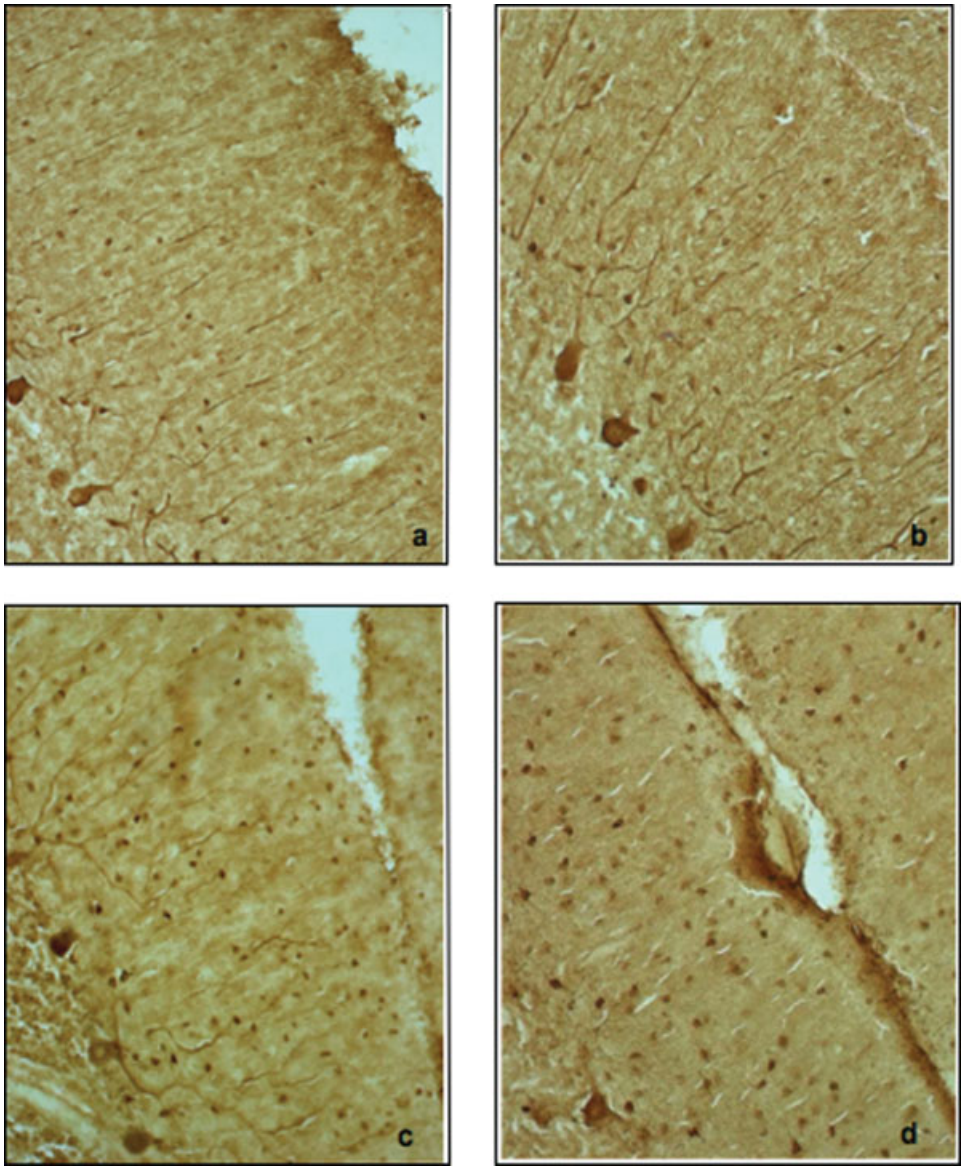

Fig. 7. Molecular layer basket and stellate cells are shown in a–d. Note the variability in molecular layer interneuron density. **a:** Control brain 4104. **b:** Autistic brain 3511. **c:** Control brain Duke-495. **d:** Autistic brain 4414. All photographs taken at ×200. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

shown that the cerebellum does not possess the normal three-layer cortex and that there are gross abnormalities in cerebellar foliation (Feddersen et al., 1992; Smeyne et al., 1995). In the L7ADT transgenic mouse, for example, PC death occurs early in development and results in a small cerebellum that lacks both normal foliation and cytoarchitecture (Smeyne et al., 1995). Three transgenic mouse strains (SV4, SV5, SV6) examined by Fedderson et al. (1992) provide further evidence that PCs are necessary for normal cerebellar development. Fedderson et al. (1992) found that the SV5 transgenic mouse, which experiences disrupted PC development at a time when these cells are still immature, had an overall reduced cerebellar size, abnormal foliation, and abnormal cerebellar cytoarchitecture. In contrast, death of mature PCs in the SV4 and SV6 mice was associated with normal cerebellar foliation and normal cerebellar cortical architecture. In the autistic brain, although there have been inconsistent reports of atrophy of selective folia (Courchesne et al., 1988, 1994a,b; Murakami et al., 1989; Garber et al., 1989; Garber and Ritvo, 1992; Hashimoto et al., 1992; Holttum et al., 1992; Kleiman et al., 1992; Piven et al., 1997; Hardan et al., 2001; Sparks et al., 2002), there is no evidence of cerebellar cortical malformations (Williams et al., 1980; Bauman and Kemper, 1985; Ritvo et al., 1986; Kemper and Bauman, 1993; Bailey et al., 1998; Lee et al., 2002; Fatemi et al., 2002b; Whitney et al., 2008).

Additionally, the autistic cases in the current study with a reduced density of PCs and concomitant normal density of MLIs provide further evidence for the timing of PC loss in the autistic brain. It is proposed, based on

work in mutant mice, that the survival of cerebellar interneurons is dependent on the establishment of functional synaptic contacts prior to PC death (Sotelo and Triller, 1979; Feddersen et al., 1992). This suggests that the survival of BCs and SCs in brains with reduced PC numbers, as seen in cases 4414, 3845, and possibly 4099, requires that BCs and SCs established functional synaptic contacts prior to PC death. It is proposed that, once these synaptic contacts are established, BCs and SCs are able to persist without the PC target (Sotelo and Triller, 1979). In contrast to the L7ADT and S5V mice that lose PCs prior to synaptogenesis, PCs in the *nervous* mutant mouse develop normally until P23, at a time when major synaptic contacts have been established (Sotelo and Triller, 1979). These mutants then experience marked PC loss (Sotelo and Triller, 1979). Qualitative analysis, more than 1 year later, reveals that the BC and SC populations are ''relatively unaffected'' (Sotelo and Triller, 1979). The *pogo* (Jeong et al., 2000) and *toppler* (Duchala et al., 2004) mutant mice also lose PCs following the period of synaptogenesis. Both of these mutants have preservation of their BC and SC populations (Jeong et al., 2000; Duchala et al., 2004). Thus, we propose, based on these data, that, in autistic brains where a reduction in PC is observed, the PCs are lost following the period when major synaptic contacts have been established. The timing of these synaptic contacts is discussed below.

Cerebellar Synaptogenesis

In the human cerebellum, BCs are first observed in the molecular layer between 16 and 19 weeks of gestation, with SCs observed 5 weeks later (Rakic and Sidman, 1970; Bayer et al., 1993). Bayer et al. (1993) have speculated on the timing of synaptogenesis in the human cerebellum, based on developmental patterns in the rat. They speculate that, in the human brain, BC-parallel fiber synaptogenesis begins in the lower molecular layer at approximately 28 weeks of gestation and the BC-PC synapse begins soon thereafter, by 30 weeks of gestation. Synaptogenesis then proceeds in an inside-out pattern, and, by approximately 32 weeks of gestation, both SC-parallel fiber and SC-PC synapses begin to form in the upper molecular layer. Rakic and Sidman (1970), from direct observation of the developing human cerebellum, place the development of the BC plexus on the Purkinje cell in the human brain at some time after 32 weeks.

Hypothesis: Timing of PC Loss in the Autistic Brain

Observations in mutant mice and timing of the synaptogenesis in the developing human cerebellum, taken together with the preserved density of interneurons in the autistic brain with decreased number of PCs, suggest the earliest timing of the cerebellar pathology is the latter one-fourth of the gestational period or early postnatal period. This gestational timing for the loss of PCs is slightly later than that proposed by Kemper of Bauman (1993). They speculate that the loss of PCs might have occurred at or before about 30 weeks of gestation. This timing was based on two observations. One was that the decreased number of PCs was not accompanied by loss of neurons in the inferior olive, a loss that regularly occurs in the human brain with loss of PCs after 39 weeks of gestation (Holms and Stewart, 1908; Norman, 1940). At this late gestational stage, climbing fibers from the contralateral inferior olive densely innervate the PC dendritic tree. The other was the initiation of the complex pattern of climbing fiber innervation of the PC at about 30 weeks of gestation. According to Marin-Padilla (1985), the olivocerebellar climbing fiber initially forms a pericellular plexus around the Purkinje cell body at 29–31 weeks of gestation and at 34–36 weeks is concentrated above Purkinje cell body and then starts to climb up the Purkinje cell dendrites at about 36 weeks of gestation. The timing of the dependence of the inferior olive could conceivably be any time during this trajectory.

The current study, taken together with prior published work on the preservation of inferior olivary neurons in the autistic brain (Kemper and Bauman, 1993), overwhelmingly favors a late prenatal loss of PCs, during the latter one-fourth of the gestation period. Despite the strong evidence for a prenatal loss of PCs, it remains possible that PC loss, when present in the autistic brain, may extend into the postnatal period. This potential scenario is based on the transient innervation pattern of immature PCs. In the developing rodent cerebellum, a single PC is innervated by multiple climbing fibers (Mariani and Changeux, 1981; Crepel, 1982; Kakizawa et al., 2000). As the rodent cerebellum matures, however, surplus climbing fibers regress and, by the second to third postnatal week, a time that corresponds to the final few weeks of human gestation (Bayer et al., 1993), each PC is innervated by a single climbing fiber (Mariani and Changeux, 1981; Kano et al., 1997; Hashimoto and Kano, 2005). Experimental manipulation of metabotropic glutamate receptors type 1 (mGluR1; Kano et al., 1997) and NMDA receptors (Kakizawa et al., 2000), however, has been shown to halt the regression of the surplus climbing fibers. Consequently, in these experimental animals, mature PCs persist with innervation from multiple olivocerebellar climbing fibers. This alteration interferes with the one-to-one relationship between climbing fibers and PCs and, perhaps, alters the dependence of inferior olivary neurons on PC survival. Although there is currently no evidence to suggest that a similar process occurs in autistic brains with reduced PC numbers, we cannot discount the possibility that some unknown alternative process is responsible for the survival of the inferior olivary neurons in these brains. In this regard, the age-related changes in the inferior olive and deep cerebellar nuclei, as described by Kemper and Bauman (1993), may signify an alternate circuitry and, thus, an alternate process by which inferior olivary neu-

rons are preserved in autistic cases with reduced PC numbers.

CONCLUSIONS

From the data of the current study, we conclude that, despite genetic and receptor binding evidence of involvement of the GABAergic system in autism, there is no evidence of an abnormality in the number or distribution of the GABAergic BC and SC interneurons in the cerebellar molecular layer. Additionally, data from the current study, taken together with prior published work on the preservation of inferior olivary neurons in the autistic brain and evidence from studies on mutant mice and normal rat and human cerebellar development, provide the strongest evidence to date that, in autistic brains with PC reduction, PCs were generated, migrated to their proper location in the PC layer, and then died. Although the evidence overwhelmingly supports a loss of PCs in the latter part of the gestational period, we cannot rule out the possibility that PC loss persists into the postnatal period.

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REFERENCES

Andersen BB, Korbo L, Pakkenberg B. 1992. A quantitative study of the human cerebellum with unbiased stereological techniques. J Comp Neurol 326:549–560.

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. 1995. Autism as a strongly genetic disorder: evidence from a British twin study. Psychol Med 25:63–77.

Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P. 1998. A clinicopathological study of autism. Brain 121:889–905.

Bauman ML, Kemper TL. 1985. Histoanatomic observations of the brain in early infantile autism. Neurology 35:866–874.

Bayer SA, Altman J, Russo RJ, Zhang X. 1993. Timetables of neurogenesis in the human brain based on experimental determined patterns in the rat. Neurotoxicology 14:83–144.

Blatt GJ, Fitzgerald CM, Guptill JT, Booker AB, Kemper TL, Bauman ML. 2001. Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. J Autism Dev Disord 31:537–543.

Buxbaum JD, Silverman JM, Smith CJ, Greenberg DA, Kilifarski M, Reichert J, Cook EH Jr, Fang Y, Song CY, Vitale R. 2002. Association between a GABRB3 polymorphism and autism. Mol Psychiatry 7:311–316.

Collins AL, Ma D, Whitehead PL, Martin ER, Wright HH, Abramson RK, Hussman JP, Haines JL, Cuccaro ML, Gilbert JR, Pericak-Vance MA. 2006. Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. Neurogenetics 7:167–174.

Cook EH, Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ, Lincoln A, Nix K, Haas R, Leventhal BL, Courchesne E. 1998. Linkage-disequilibrium mapping of autistic disorder with 15q11–13 marker. Am J Hum Genet 62:1077–1083.

Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL. 1988. Hypoplasia of cerebellar vermal lobules VI and VII in autism. N Engl J Med 318:1349–1354.

Courchesne E, Townsend J, Saitoh O. 1994a. The brain in infantile autism: posterior fossa structures are abnormal. Neurology 44:214–223.

Courchesne E, Saitoh O, Yeung-Courchesne R, Press GA, Lincoln AJ, Haas RH, Schreibman L. 1994b. Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: identification of hypoplastic and hyperplastic subgroups with MR imaging. Am J Roentgenol 162:123–130.

Crane AM, Goldman PS. 1979. An improved method for embedding brain tissue in albumin-gelatin. Stain Technol 54:71–75.

Crepel F. 1982. Regression of functional synapses in the immature mammalian cerebellum. Tends Neurosci 5:266–269.

Diagnostic and statistical manual of mental disorders, 4th ed. (DSM IV). 1994. Washington, DC: American Psychiatric Association.

Duchala CS, Shick HE, Garcia J, Deweese DM, Sun S, Stewart VJ, Macklin WB. 2004. The *toppler* mouse: a novel mutant exhibiting loss of Purkinje cells. J Comp Neurol 476:113–129.

Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. 2002a. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biol Psychiatry 52:805–810.

Fatemi SH, Halt AR, Realmuto G, Earle J, Kist DA, Thuras P, Merz A. 2002b. Purkinje cell size is reduced in cerebellum of patients with autism. Cell Mol Neurobiol. 22:171–175.

Feddersen RM, Ehlenfeldt R, Yunis WS, Clark HB, Orr HT. 1992. Disrupted cerebellar cortical development and progressive degeneration of Purkinje cells in SV40 T antigen transgenic mice. Neuron 9:955–966.

Folstein SE, Piven J. 1991. Etiology of autism: genetic influences. Pediatrics 87:767–773.

Folstein SE, Rosen-Sheidley B. 2001. Genetics of autism: complex aetiology for a heterogeneous disorder. Nat Rev Genet 2:943–955.

Folstein SE, Rutter M. 1977. Infantile autism: a genetic study of 21 twin pairs. J Child Psychol Psychiatry 18:297–321.

Garber HJ, Ritvo ER. 1992. Magnetic resonance imaging of the posterior fossa in autistic adults. Am J Psychiatry 149:245–247.

Garber HJ, Ritvo ER, Chiu LC, Griswold VJ, Kashanian A, Freeman BJ, Oldendorf WH. 1989. A magnetic resonance imaging study of autism: normal fourth ventricle size and absence of pathology. Am J Psychiatry 146:532–534.

Gundersen HJ. 1986. Stereology of arbitrary particles. J Microsc 143:3–45.

Guptill JT, Booker AB, Gibbs TT, Kemper TL, Bauman ML, Blatt GJ. 2007. [^{3}H]flunitrazepam-labeled benzodiazepine binding sites in the hippocampal formation in autism: a multiple concentration autoradiographic study. J Autism Dev Disord 37:911–920.

Hardan AY, Minshew NJ, Harenski K, Keshavan MS. 2001. Posterior fossa magnetic resonance imaging in autism, J Am Acad Child Adolesc Psychiatry. 40:666–672.

Hashimoto T, Murakawa K, Miyazaki M, Tayama M, Kurdoa Y. 1992. Magnetic resonance imaging of the brain structures in the posterior fossa in retarded autistic children. Acta Paediatr 81:1030–1034.

Hedreen JC. 1998. Lost caps in histological counting methods. Anat Rec 250:366–372.

Holms G, Stewart TG. 1908. On the connection of the inferior olives with the cerebellum in man. Brain 31:125–137.

Holttum JR, Minshew NJ, Sanders RS, Phillips NE. 1992. Magnetic resonance imaging of the posterior fossa in autism. Biol Psychiatry 32:1091–1101.

Jeong YG, Hyun BH, Hawker R. 2000. Abnormalities in cerebellar Purkinje cells in the novel ataxic mutant mouse, *pogo*. Brain Res Dev Brain Res 125:61–67.

Kakizawa S, Yamasaki M, Watanabe M, Kano M. 2000. Critical period for activity-dependent synapse elimination in developing cerebellum. J Neurosci 20:4954–4961.

Kano M, Hashimoto K, Kurihara H, Watanabe M, Inoue Y, Aiba A, Tonegawa S. 1997. Persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking mGluR1. Neuron 18:71–79.

Kemper TL, Bauman ML. 1993. The contribution of neuropathologic studies to the understanding of autism. Neurol Clin 11:175–187.

Kleiman MD, Neff S, Rosman NP. 1992. The brain in infantile autism: are posterior fossa structures abnormal? Neurology 42:753–760.

Laine J, Axelrad H. 1998. Lugaro cells target basket and stellate cells in the cerebellar cortex. Neuroreport 9:2399–2340.

Lee M, Martin-Ruiz C, Graham A, Court J, Jaros E, Perry R, Iversen P, Bauman M, Perry E. 2002. Nicotinic receptor abnormalities in the cerebellar cortex in autism. Brain 125:1483–1495.

Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, Ritchie MD, Delong GR, Abramson RK, Wright HH, Cuccaro ML, Hussman JP, Gilbert JR, Pericak-Vance MA. 2005. Identification of significant association and gene–gene interaction of GABA receptor subunit genes in autism. Am J Hum Genet 77:377–388.

Mariani J, Changeux J-P. 1981. Ontogenesis of olivocerebellar relationships I. Studies by intracellular recordings of the multiple innervations of Purkinje cells by climbing fibers in the developing rat cerebellum. J Neurosci 1:696–702.

Marin-Padilla M. 1985. Neurogenesis of the climbing fibers in the human cerebellum: a Golgi study. J Comp Neurol 235:82–96.

Murakami JW, Courchesne E, Press GA, Yeung-Courchesne R, Hesselink JR. 1989. Reduced cerebellar hemisphere size and its relationship to vermal hypoplasia in autism. Arch Neurol 46:689–694.

Norman RM. 1940. Cerebellar atrophy associated with etat marbre of the basal ganglia. J Neurol Psychiatry 3:311–318.

Palay SL, Chan-Palay V. 1974. Cerebellar cortex; cytoloty and organization. New York: Springer-Verlag.

Palmen S, van Engeland H, Hof PR, Schmitz C. 2004. Neuropathological findings in autism. Brain 127:2572–2583.

Pickles A, Bolton P, MacDonald H, Bailey A, Le Couteur A, Sim CH, Rutter M. 1995. Latent-class analysis of recurrence risks for complex phenotypes with selection and measurement error: a twin and family history study of autism. Am J Hum Genet 57:717–726.

Piven J, Saliba K, Bailey J, Arndt S. 1997. An MRI study of autism: the cerebellum revisited. Neurology 49:546–551.

Rakic P, Sidman DL. 1970. Histogenesis of the cortical layers in human cerebellum particularly the lamina dissecans. J Comp Neurol 139:473–500.

Risch N, Spiker D, Lotspeich L, Nouri N, Hinds D, Hallmayer J, Kalaydjieva L, McCague P, Dimiceli S, Pitts T, Nguyen L, Yang J, Harper C, Thorpe D, Vermeer S, Young H, Hebert J, Lin A, Ferguson J, Chiotti C, Wiese-Slater S, Rogers T, Salmon B, Nicholas P, Petersen PB, Pingree C, McMahon W, Wong DL, Cavalli-Sforza LL, Kraemer HC, Myers RM. 1999. A genomic screen of autism: evidence for a multilocus etiology. Am J Hum Genet 65:493–507.

Ritvo ER, Freeman BJ, Scheibel AB, Duong T, Robinson H, Guthrie D, Ritvo A. 1986. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC autopsy research report. Am J Psychiatry 146:862–866.

Rosene DL, Roy NJ, Davis BJ. 1986. A cryoprotection method that facilitates cutting frozen sections of whole monkey brains for histological and histochemical processing without freezing artifact. J Histochem Cytochem 34:1301–1315.

Schmahmann JD, Doyon J, Toga AW, Petrides M, Evans AC. 2000. MRI atlas of the human cerebellum. San Diego, CA: Academic Press. p 3–20.

Shao Y, Cuccaro ML, Hauser ER, Raiford KL, Menold MM, Wolpert CM, Ravan SA, Elston L, Decena K, Donnelly SL, Abramson RK, Wright HH, DeLong GR, Gilbert JR, Pericak-Vance MA. 2003. Fine mapping of autistic disorder to chromosome 15q11-q13 by use of phenotypic subtypes. Am J Hum Genet 72:539–548.

Smalley SL, Asarnow RF, Spence MA. 1988. Autism and genetics. A decade of research. Arch Gen Psychiatry 45:285–295.

Smeyne RJ, Chu T, Lewin A, Bian F, Crisman SS, Kunsch C, Lira SA, Oberdick J. 1995. Local control of granule cell generation by cerebellar Purkinje cells. Mol Cell Neurosci 6:230–251.

Soha JM, Kim S, Crandall JE, Vogel MW. 1997. Rapid growth of parallel fibers in the cerebella of normal and *staggerer* mutant mice. J Comp Neurol 389:642–654.

Sotelo C. 1990. Cerebellar synaptogenesis: what we can learn from mutant mice. J Exp Biol 153:225–249.

Sotelo C, Triller A. 1979. Fate of presynaptic afferents to Purkinje cells in the adult nervous mutant mouse: a model to study presynaptic stabilization. Brain Res 175:11–36.

Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, Maravilla KR, Giedd JN, Munson J, Dawson G, Dager SR. 2002. Brain structural abnormalities in young children with autism spectrum disorder. Neurology 59:184–192.

Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G, Bohman M. 1989. A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. J Child Psychol Psychiatry 30:405–416.

Sultan F, Bower JM. 1998. Quantitative Golgi study of the rat cerebellar molecular layer interneurons using principal component analysis. J Comp Neurol 393:353–373.

West MJ, Slomianka L, Gundersen HJ. 1991. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat Rec 231:482–497.

Whitney ER, Kemper TL, Bauman ML, Rosene DL, Blatt GJ. 2008. Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: a stereological experiment using calbindin-D28k. Cerebellum 7:406–416.

Williams RS, Hauser SL, Purpura DP, DeLong GR, Swisher CN. 1980. Autism and mental retardation: neuropathologic studies performed in four retarded persons with autistic behavior. Arch Neurol 37:749–753.

Wolpert CM, Menold MM, Bass MP, Qumsiyeh MB, Donnelly SL, Ravan SA, Vance JM, Gilbert JR, Abramson RK, Wright HH, Cuccaro ML, Pericak-Vance MA. 2000. Three probands with autistic disorder and isodicentric chromosome 15. Am J Med Genet 96:365–372.

Yip J, Soghomonian JJ, Blatt GJ. 2007. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. Acta Neuropathol 112:559–568.

Yip J, Soghomonian JJ, Blatt GJ. 2008. Increased GAD67 mRNA expression in cerebellar interneurons in autism: implications for Purkinje cell dysfunction. J Neurosci Res 86:525–530.