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A Microscopic Study of Language-Related Cortex in Autism

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Abstract: Impaired language function is a principle criterion for the diagnosis of autism. The present study of brain from age-matched autistic and control subjects compared brain regions associated with the production and processing of speech. Wernicke's area (Brodmann 22, speech recognition), Broca's area (Brodmann 44, speech production) andthe gyrus angularis (Brodmann 39, reading) from autistic subjects (7-44 years of age) and control subjects (8-56 years of age) were examined microscopically. Striking differences in the density of glial cells, the density of neurons andthe number of lipofuscin-containing neurons were observed in the autistic group compared with the control group. The mean density of glial cells was greater in the autistic cohort than controls in area 22 ($p<0.001$), area 39 ($p<0.01$) andarea 44 ($p<0.05$). The density of neurons was lesser in autism in area 22 ($p<0.01$) and area 39 ($p<0.01$). The autistic group exhibited significantly greater numbers of lipofuscin-containing cells in area 22 ($p<0.001$) and area 39 ($p<0.01$). The results are consistent with accelerated neuronal death in association with gliosis and lipofuscin accumulation in autism after age seven. Production of lipofuscin (a matrix of oxidized lipid and cross-linked protein more commonly associated with neurodegenerative disease) is accelerated under conditions of oxidative stress. Area 22 in autism evidenced the greatest glial increase, the greatest neuronal decrease andthe greatest increase of non-specific cells containing lipofuscin, which itself may contribute to greater free-radical generation in brain.

Key words: Autism, cerebral cortex, gliosis, lipofuscin, oxidative stress

INTRODUCTION

Autism, a developmental neuropsychiatric disease, is diagnosed in early childhood and features social and communication deficits, restricted and repetitive behaviors and interests anda characteristic course[1]. Relatives of autistic subjects are more likely to manifest social, cognitive andpsychiatric deficits[2-4] anda milder form of autism is unassociated with mental retardation[5]. It is strongly suggested that outside the formal diagnosis of autism, there exists an unexpectedly large population with milder, but significant, social and communication deficits[6].

While functional alterations in cortical auditory and language processing in autism are reported[7], language-related cortex of autistic subjects has not been subjected to systematic microscopic examination. Language is a complex brain function, involving numerous subcortical and cortical areas, including areas for primary and secondary auditory processing. For this study we analyzed three higher-order cortical areas involved in the processing of language and production of speech: Wernicke's area, Broca's area and the angular gyrus in the brains from autistic and control subjects.

Wernicke's area (Brodmann area 22) is a supplementary-auditory section of neocortex in the left temporal lobe which mediates the understanding of auditory words. Wernicke's aphasia, an inability to decipher the meanings of the speech sounds, results from damage to area 22. Broca's area is a premotor region of the neocortex located in the left frontal lobe (Brodmann areas 44 and 45) which mediates the production and control of human speech. Lesions to this area (Broca's aphasia) produce speech problems, but do not affect semantic or basic syntactic abilities. Wernicke's and Broca's areas are connected by the arcuate fasciculus; conscious language output originating in Wernicke's area is carried via the arcuate fasciculus to Broca's area, where a detailed and coordinated program for vocalization is compiled and sent to the facial zone of the motor cortex. The angular gyrus (Brodmann area 39) is a small region behind Wernicke's area which contains regions of the occipital, parietal andtemporal lobes. Area 39 facilitates

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interaction of visual and auditory brain cortex andhas been implicated in alexia, dyslexia[8] and agraphia.

Hypothetically, structural alteration of one or more of these cortical areas might contribute to the communication impairment found in autism. The purpose of this study was to assess the microscopic anatomy of Wernicke's area, Broca's area andthe angular gyrus in preserved brain of accurately-diagnosed autistic subjects in comparison to age-matched controls. The pattern of cortical layering, relative thickness of cortical layers andneuronal and glial densities for each layer was determined. Glial density, neuronal density andnumber of nonspecific lipofuscin-containing cells was determined for each subject. The data allowed assessment of each parameter as a function of subject age.

MATERIALS AND METHODS

Human subjects: The tissue required for this study was obtained from the Harvard Brain Tissue Resource Center, the Human Tissue Bank at the Universitiy of Miami andthe NICHD Brain and Tissue Bank for Neurodevelopmental Disorders at the University of Maryland, as facilitated by the Autism Tissue Program. Medical history and documentation of autism by Autism Diagnostic Interview (ADI-R) was provided for each subject. Formalin-preserved blocks from the left-hemisphere were from eight autistic and seven control subjects:

Autism			Control		
Age	Sex	Identifier	Age	Sex	Identifier
7	male	UMB-797	8	male	UMB-662
9	male	B-4925	17	male	B-2234
10	male	BTB-3714	22	male	B-4981
14	male	B-4323	30	male	B-4211
25	male	BTB-3711	46	male	B-4192
26	male	B-5000	52	male	BTB-3692
31	male	B-4871	56	male	B-4503
44	male	B-4541			

Tissue preparation: Formalin-fixed postmortem tissue blocks from areas 22, 39 and 44, were stored in 10% phosphate-buffered formalin. Portions of each block were immersed in 20% sucrose cryoprotectant, then serially sectioned in perpendicular orientation to the pial surface with a sliding microtome, at 100 µm. Adjacent series of sections were stored at -20°C in a solution made of 3% glycerol, 3% ethylene glycol, 1% phosphate buffer (PB) anddistilled water; alternate sections were stained with cresyl violet (0.1%) in order to visualize laminar boundaries. For lipofuscin study, sections were coverslipped with Fluoromount G (Aname 17984-25). All sections were coded and randomized to conform with strict double-blind methodology.

Sample preparation for cell-counts involved several steps. Blocks were divided to provide a portion for microdissection and post-fixation with 2% OsO_4 in 0.9% NaCl containing 1.5% potassium ferrycianure (Sigma, St Louis, MO), for 1 h. Tissue was dehydrated with propylene oxide and flat-embedded in Epon; 1 µm-thick sections were produced with a diamond knife, then dried on glass slides andstained with basic toluidine blue (borate buffer, pH 11).

Immunohistological staining: Astrocytes were stained with polyclonal antibody to glial fibrillary acidic protein (GFAP; DAKO, Denmark). The sections were rinsed initially with TBS (0.05 M Tris and0.15 M NaCl), pH 7.5, then pre-treated with a solution of 50% ethanol and 1% hydrogen peroxide (Sigma, St Louis, MO) in phosphate-buffered saline (PBS) for 30 min in order to remove endogenous peroxidase activity, then rinsed in PBS andsubsequently incubated in 1:5 normal goat serum for 30 min in order to block non-specific staining. Then primary anti-GFAP antibody was applied at 1:200 dilution for 2 h at 4°C, followed by rinse with PBS and incubation with biotinylated goat anti-mouse antibody 1:200 in PBS for 1 h at room temperature andsubsequent processing by the avidin-biotin-peroxidase method (Vectastain ABC immunoperoxidase kit: Vector, Burlingame, CA). DAB (3.3'-diaminobenzidine tetrahydrochloride: Sigma, St Louis, MO) was used as the chromogen. Sections were mounted on glass slides, dehydrated, cleared in xylene and coverslipped.

Morphometric analyses: An optical fractionation method was employed to estimate cell density. This procedure is based on a random systematic choice of cortical tissue samples andperformed by means of a regular, previously-designed grid of counting frames (100×100 µm). An average of 4 fields per cortical layer was counted in each section, resulting in an average of 24 fields for each sample in every cortical area studied. The cells were counted only if their somata fell entirely within the frame area. Cell density was calculated by dividing the total number of cells in each layer by the surface area of the frame (or in the examination of cortical colums, division by the area of the cortical column). These data were expressed as number of cells per mm^2.

The randomized and codified sections were observed with a 100× oil immersion lens on a Leica DMRB light microscope. 200 µm-wide columns, spanning pia to white matter, were examined and plotted on paper with respect to cortical layers. Neurons and glial cells were distinguished carefully. Neurons-usually, but not always larger than glial cells were distinguishable by their non-spherical shape, stained cytoplasm andvisible nucleolus. In contrast, glial cells were identified by the absence of cytoplasmic staining, small size andround shape.

Thickness of the cortical mantle was assessed with a Leica PL FLUOTAR lens, A.N. 0.12, which was used to represent a column through the entire extent of the cortical mantle on graph paper. The total cortical thickness was noted to vary noticeably among different regions of a given cortical area, thus precluding direct comparisons of absolute values. Hence, cortical thickness was reported as percentage of total cortical thickness.

Lipofuscin identification and quantification: Lipofuscin-containing cells are readily identifiable by autofluorescence. A Zeiss III RS fluorescence microscope with an HBO 50W AC super pressure mercury lamp was utilized for determination of number of cells containing lipofuscin. Both 365 and 450 nm excitation filters were employed andcell counts and cell-density calculations were performed as previously described for glial and neuronal cells.

Reproduceable photographs of intracytoplasmic lipofuscin aggregates were obtained using a confocal laser-scanning microscope (Leica TCS SL) equipped with a Hellion/Neon laser and a 60×oil objective (Leica Plan Apochromat). The tissue sections were excited at 488 and 568 nm wavelengths andthe image was reconstructed from a stack of 10 consecutive confocal planes.

Quantitative analysis of the size of the intracytoplasmic lipofuscin aggregates was performed on single optical images for each brain section. By employing a 60×oil lens in a random-systematic manner, a region of interest (ROI) was selected by computer andaligned over layer I. The sampling ROI was moved vertically through all cortical layers andevery deposit of lipofuscin contained within the select ROI was outlined with a mouse cursor. The quantification of the lipofuscin area was accomplished with an Image Analysis System (Soft Imaging System GmbH, Münster, Germany) program.

Statistical analysis: Cell counts across hexalaminar cortical columns were obtained in an uninterrupted series of counting boxes that spanned the entire depth of cortex from the pial surface to the underlying white matter. Four distinct cortical probes were obtained for each brain area. The cell density for each case was determined by dividing the number of cells counted in each sample by the total area of the chosen region. The data were expressed as the Mean±SD of the sample.

The statistical Student's t-test was used to compare the mean values from individual morphometric parameters between autistic and control subjects of matching ages. The confounding potential influence of postmortem delay andfixation interval were evaluated using the analysis of covariance, which demonstrated no significant relationship.

RESULTS

Analysis of cortical laminar thickness: The thickness of cortical layers in relation to total cortical thickness in the three brain areas examined were not significantly different between autistic and control cohorts (Table 1).

Longitudinal analysis of control data:

Density of glial cells: In controls, the density of glial cells in full-depth cortical sections increased in all three brain areas as a function of age (Fig. 1a-c). Thus, in each brain area, glial density was approximately twice as high in sixth-decade subjects, as compared to the youngest subject, age 8. A steep rise in glial cells was measured in area 39 at age 30 ($p = 0.0212$) and in area 44 at age 30 ($p = 0.0007$) (Fig. 2a-c). Generally, the decrement in glial density was most pronounced in layers III, V and IV (Table 2).

Neuronal density: In controls, a significant decline in neuronal density was evident in full-depth cortical sections of area 22 (by 40% from age 8 to age 56, $p = 0.0187$), but not in area 39 or 44 (Fig. 2d,e.f) Laminar analysis (Table 3) demonstrated age-related declines in all cortical layers of area 22, particularly in layers II (reduced 40% from age 8 to age 56, $p = 0.0025$) and IV (reduced 35% from age 8 to 56, $p = 0.0058$). In area 39, a pronounced decrement was observed in layer V (50% from age 8 to 56, $p = 0.0057$) and in area 44, there was a moderate decline in neuronal density in layers II (reduced 21% from age 8 to age 56, $p = 0.0363$).

Lipofuscin-containing cells: The lipofuscin cell-count (Table 4) did not differentiate between neuronal and

Table 1:. Percentage of total cortical thickness. %: percentage. SD: standard deviation

		Control							Autism						
		8	17	22	30	46	52	56	7	9	10	14	25	31	44
I	%	8.66	6.57	7.57	8.35	6.02	7.67	7.52	9.93	10.51	7.77	10.65	7.06	7.14	7.02
	SD	2.42	1.22	0.35	0.85	1.32	1.15	0.67	3.92	1.35	1.22	1.53	0.74	1.39	1.91
II	%	5.73	7.19	7.31	6.44	7.38	7.48	6.62	7.29	7.67	6.63	8.33	6.10	7.25	6.23
	SD	1.45	1.55	0.59	1.19	1.88	0.58	0.48	2.10	2.04	0.97	1.96	1.30	1.58	0.93
III	%	25.71	25.14	31.28	27.35	28.10	40.89	32.64	41.65	40.34	38.47	37.99	31.25	37.01	29.36
	SD	5.66	2.11	0.97	8.68	6.74	2.03	3.36	9.61	2.26	0.76	5.50	8.00	1.83	5.42
IV	%	9.56	10.19	10.35	8.30	7.31	10.76	9.29	8.18	7.85	7.35	8.58	8.10	7.17	8.60
	SD	1.33	1.54	2.46	1.75	0.92	3.50	1.12	1.44	1.08	1.21	1.71	0.80	1.07	0.91
V	%	27.49	22.50	21.19	23.22	26.16	16.52	19.31	15.90	18.41	22.11	18.74	23.66	23.07	27.69
	SD	3.71	3.40	3.96	5.96	5.37	4.22	2.23	4.75	2.78	2.72	2.15	3.19	5.37	7.27
VI	%	22.86	28.41	22.29	26.34	25.03	16.68	24.63	17.04	15.23	17.68	15.70	23.84	18.37	21.11
	SD	6.07	9.11	5.43	6.55	2.96	2.35	2.93	8.56	2.50	2.93	5.37	7.01	3.22	2.87
I	%	9.59	10.45	10.20	5.48		10.70	11.29	8.81	12.16	7.32	11.44	6.23	8.45	8.20
	SD	1.23	2.01	1.56	0.97		1.07	1.93	1.26	1.63	0.84	1.22	0.36	1.11	0.75
II	%	8.22	10.45	14.58	6.85		6.73	11.29	6.85	10.81	6.10	9.80	6.23	8.45	6.56
	SD	0.46	0.29	1.98	0.45		0.85	1.65	0.45	1.01	0.85	0.22	0.48	0.33	0.18
III	%	36.99	26.87	34.99	30.14		33.64	38.71	33.86	37.84	29.27	37.58	28.35	36.62	31.15
	SD	3.56	1.23	2.63	7.60		2.33	4.95	1.27	2.00	5.49	3.27	2.10	3.89	1.25
IV	%	9.59	8.96	9.62	5.48		9.79	8.06	9.78	9.46	9.76	10.46	7.79	8.45	9.84
	SD	0.99	1.00	0.49	0.13		0.36	1.08	0.85	0.44	0.34	1.22	0.17	0.75	0.61
V	%	20.55	25.37	21.87	38.36		23.85	17.74	27.40	18.92	34.15	24.18	37.38	28.17	29.51
	SD	2.34	2.45	1.12	5.47		2.37	1.20	5.11	2.10	5.22	3.11	1.27	2.33	1.52
VI	%	15.07	17.91	8.75	13.70		15.29	12.90	13.31	10.81	13.41	6.54	14.02	9.86	14.75
	SD	5.77	6.21	2.50	2.36		1.57	3.98	1.87	1.99	2.56	1.32	2.87	2.33	3.22
I	%	9.00		10.69	10.29	6.47	7.62	8.02	5.59	8.88	6.27	7.33	7.69	8.49	8.14
	SD	2.99		4.42	1.12	1.29	2.33	1.10	1.11	2.29	1.57	0.48	0.70	1.85	1.25
II	%	7.16		6.07	7.23	4.63	4.88	5.41	4.70	4.77	6.42	4.99	5.07	5.18	3.90
	SD	0.88		3.33	1.21	0.51	0.46	1.09	0.93	0.70	0.57	1.83	1.00	1.01	0.51
III	%	28.21		30.47	21.76	25.62	29.50	33.13	24.37	21.71	23.87	21.51	26.33	31.94	29.48
	SD	8.72		5.04	4.83	2.58	4.87	4.36	3.53	4.68	1.43	2.35	3.44	4.08	3.19
IV	%	6.12		5.98	7.75	6.44	11.86	6.16	6.48	7.13	7.61	6.88	8.16	7.41	5.43
	SD	0.90		1.34	1.89	1.07	8.10	0.49	0.73	0.96	0.83	1.99	1.44	1.47	1.76
V	%	21.60		19.00	25.79	25.62	24.96	22.47	28.25	27.19	33.51	28.97	28.47	22.60	24.50
	SD	6.12		9.11	7.91	3.26	7.81	4.43	1.94	1.75	3.45	5.09	3.79	2.71	3.49
VI	%	27.90		27.79	27.19	31.22	21.18	24.81	30.62	30.32	22.32	30.31	24.27	24.37	28.55
	SD	6.22		8.12	6.54	0.92	14.84	1.84	2.40	5.06	3.06	9.49	1.46	4.52	8.79

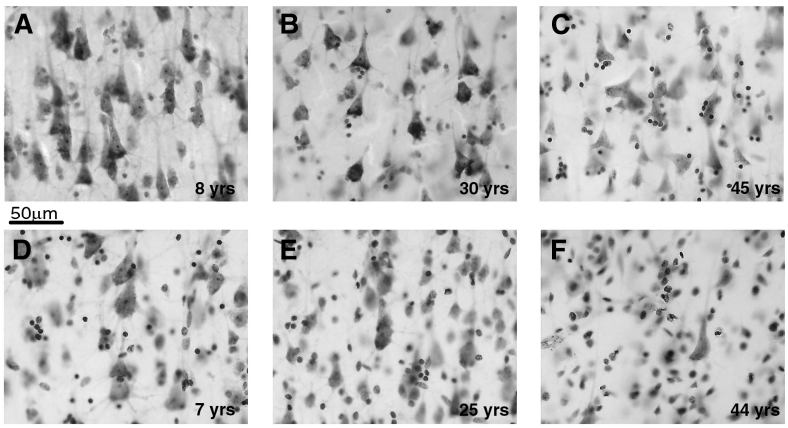

Fig. 1: Photomicrographs illustrating neurons and glial cells using Nissl staining from Wernicke´s (area 22). Control cases (A, B and C) contrast with autistic cases (D, E and F). As expected with aging, the older control subject (C) demonstrated increased density of glial cells and decreased density of neurons. The changes are accelerated in autism, including the cohort's youngest subject, age 7 (D)

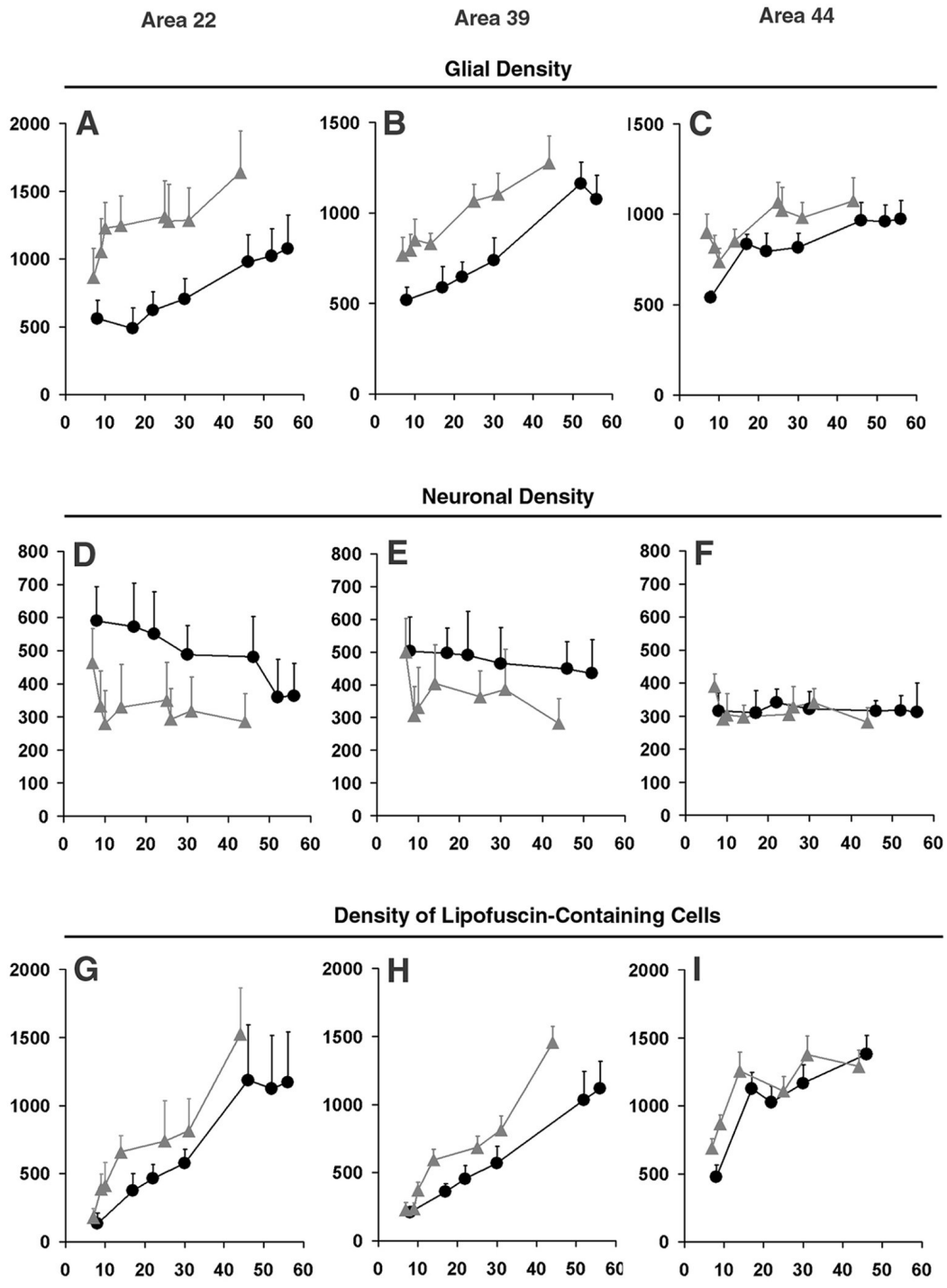

Fig. 2: Density of glial cells, density of neurons, and density of lipofuscin-containing cells in brain areas 22, 39 and 44. Glial cells (A, B and C), neurons (D, E and F), and cells with lipofuscin (G, H and I) were counted along complete cortical columns. Values for controls subjects are graphed in black, for autistic subjects in gray. The horizontal axes correspond to age in years of the subjects, and the vertical axes represent the number of cells mm^{-2}

Table 2:. Number of glial cells mm^{-2}. M: Mean. SD: standard deviation. T: total cell density

		Control								Autism							
		8	17	22	30	46	52	56	7	9	10	14	25	26	31	44
BA22																	
I	M	106.00	94.00	75.00	19.00	56.00	6.00	6.00	94.00	25.00	26.00	63.00	13.00	38.00	6.00	0.00
	SD	48.01	36.98	43.30	12.93	10.83	5.12	4.38	20.73	12.74	8.80	19.86	6.82	12.50	4.18	0.00
II	M	969.00	944.00	794.00	525.00	825.00	606.00	594.00	400.00	350.00	331.00	431.00	450.00	619.00	375.00	369.00
	SD	108.07	151.43	133.90	139.94	101.55	92.49	105.14	98.43	98.43	71.53	100.58	93.54	94.17	123.74	75.78
III	M	419.00	431.00	406.00	350.00	463.00	475.00	394.00	338.00	250.00	188.00	356.00	275.00	244.00	281.00	206.00
	DS	122.95	83.62	103.64	113.65	45.07	131.10	99.02	115.24	98.43	67.31	125.47	84.78	48.01	94.17	71.53
IV	M	988.00	1031.00	888.00	588.00	1000.00	781.00	644.00	619.00	575.00	538.00	550.00	538.00	475.00	513.00	575.00
	SD	108.25	103.64	150.52	131.50	177.06	144.02	124.22	103.64	107.53	83.85	138.07	96.01	81.01	103.83	101.55
V	M	500.00	619.00	544.00	344.00	506.00	469.00	388.00	350.00	325.00	363.00	413.00	331.00	244.00	363.00	319.00
	SD	140.31	165.24	121.67	106.80	113.71	103.64	112.50	110.40	106.07	112.50	147.37	125.47	20.73	73.95	106.62
VI	M	406.00	369.00	350.00	250.00	317.00	319.00	281.00	338.00	294.00	250.00	269.00	306.00	288.00	256.00	188.00
	SD	145.10	83.62	101.55	104.08	56.98	83.62	87.28	97.31	77.81	63.74	81.73	77.81	27.95	48.01	73.95
T	M	590.00	572.00	550.00	488.00	481.00	359.00	364.00	465.00	333.00	279.00	330.00	350.00	293.00	318.00	286.00
	SD	103.00	132.00	127.30	87.98	123.20	114.30	97.00	102.30	105.80	99.87	129.60	114.43	93.72	102.34	84.37
BA39																	
I	M	56.00	63.00	50.00	56.00		0.00	0.00	50.00	56.00	44.00	38.00	13.00		19.00	6.00
	SD	20.73	27.95	17.68	32.48		0.00	0.00	17.68	27.24	20.73	21.65	8.68		10.83	2.50
II	M	907.00	975.00	943.00	821.00		721.00	693.00	893.00	407.00	421.00	557.00	514.00		636.00	429.00
	SD	210.93	166.77	155.12	115.07		144.02	135.06	183.62	108.07	107.02	180.04	107.53		137.17	97.90
III	M	513.00	431.00	425.00	438.00		325.00	331.00	544.00	306.00	350.00	444.00	400.00		394.00	225.00
	SD	123.11	170.82	138.07	100.78		107.53	120.38	99.02	110.93	118.59	149.35	91.86		99.02	39.53
IV	M	1050.00	994.00	1013.00	1019.00		994.00	956.00	756.00	500.00	581.00	756.00	663.00		813.00	606.00
	SD	131.10	130.35	162.50	75.78		136.22	119.08	116.42	101.55	139.61	110.93	83.85		117.76	125.47
V	M	531.00	531.00	481.00	425.00		250.00	238.00	431.00	331.00	325.00	338.00	325.00		313.00	294.00
	SD	103.64	146.18	146.18	91.86		64.58	93.83	87.34	109.51	136.93	132.88	88.07		143.07	75.78
VI	M	378.00	361.00	350.00	294.00		261.00	269.00	438.00	294.00	238.00	269.00	313.00		306.00	300.00
	SD	87.40	72.93	108.07	99.02		127.93	54.13	97.63	89.05	73.95	83.62	73.95		99.02	93.54
T	M	503.00	497.00	491.00	465.00		448.00	435.00	500.00	307.00	331.00	404.00	363.00		386.00	283.00
	SD	104.10	76.23	132.47	109.98		83.79	103.81	103.30	88.79	122.37	118.92	79.87		122.20	75.40
BA44																	
I	M	144.00	106.00	25.00	113.00	38.00	44.00	81.00	156.00	63.00	69.00	75.00	13.00	38.00	58.00	56.00
	SD	55.43	30.73	17.68	21.65	7.50	10.73	10.98	47.24	27.95	24.13	25.00	9.65	8.00	27.24	20.73
II	M	806.00	606.00	644.00	713.00	550.00	731.00	638.00	800.00	581.00	688.00	681.00	669.00	531.00	563.00	463.00
	SD	89.85	140.73	90.79	67.31	30.62	83.62	87.50	146.84	83.62	94.37	74.13	44.63	66.98	76.03	82.50
III	M	350.00	294.00	306.00	350.00	306.00	300.00	338.00	306.00	263.00	225.00	388.00	300.00	256.00	306.00	238.00
	SD	82.87	72.24	84.01	71.68	80.73	93.53	72.95	71.53	37.50	71.68	80.04	58.63	56.94	63.98	51.54
IV	M	638.00	719.00	575.00	619.00	638.00	544.00	569.00	731.00	638.00	606.00	719.00	581.00	550.00	600.00	525.00
	SD	110.87	127.93	68.47	63.98	62.50	56.94	63.98	76.94	67.31	62.19	72.24	73.69	77.06	84.78	88.39
V	M	344.00	313.00	356.00	288.00	338.00	244.00	288.00	419.00	281.00	344.00	356.00	306.00	275.00	394.00	269.00
	SD	82.60	57.28	48.01	62.50	54.07	73.69	72.95	72.24	60.42	110.93	77.81	73.69	55.90	63.98	44.63
VI	M	319.00	281.00	394.00	219.00	175.00	288.00	244.00	269.00	238.00	265.00	225.00	188.00	231.00	219.00	188.00
	SD	73.94	36.98	71.53	56.94	46.77	27.95	59.62	36.98	54.49	20.73	53.03	45.07	54.13	48.01	62.50
T	M	316.00	310.00	341.00	321.00	315.00	317.00	313.00	390.00	290.00	303.00	298.00	305.00	327.00	340.00	281.00
	SD	55.24	67.50	40.85	53.45	32.14	44.26	87.60	37.92	22.15	65.89	35.20	32.23	63.58	43.56	43.12

glial cells. In controls, the number of lipofuscin-containing cells in full-depth cortical samples increased in all three brain areas from age 8 to age 56 in area 22 by 770% ($p = 0.0015$); area 39 by 430% ($p = 0.0001$); and from age 8 to age 46 in area 44 by 189% ($p = 0.0001$). At corresponding ages, the lipofuscin cell-count was consistently higher in area 44 than in areas 22 or 39 (Fig. 2g-i). Laminar analysis demonstrated greatest gross number of cells containing lipofuscin in layers II and IV, layers with high cell density. Qualitatively, the size of individual lipofuscin aggregates appeared greatest in layers III and V, also the site of largest pyramidal cells (Fig. 3).

Longitudinal analysis of autism data

Density of glial cells: In the autistic cohort, the density of glial cells (Table 2) in full-depth cortical sections increased significantly over time in area 22 (89% from age 7 to age 44, $p = 0.0061$) and in area 39 (66% from age 7 to age 44, $p = 0.0012$), but not in area 44

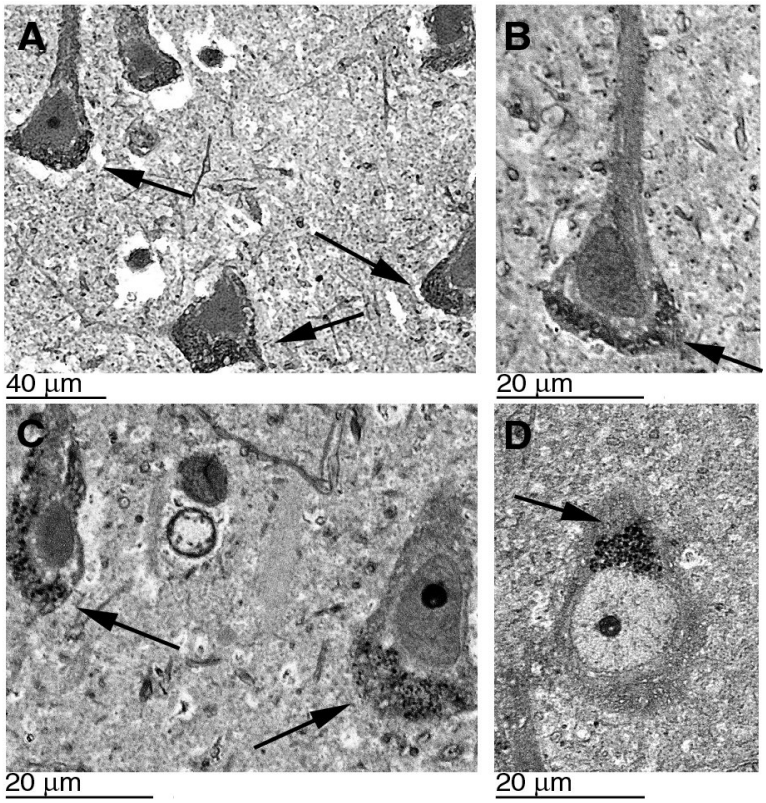

Fig. 3: Photomicrographs illustrating intracytoplasmic deposits of lipofuscin (arrows) in toluidine blue-stained semi-thin sections. The size of individual lipofuscin aggregates in the cerebral cortex of 17-year-old autistic subjects (B and D) are similar to those in the 52-year-old control subject (A and C)

(Fig. 2a-c). A steep rise in density of glial cells in area 22 between age 7 and 10 (42%) was statistically significant ($p = 0.0424$) andfollowed by a plateau from age 10 to age 31.

Laminar analysis (Table 2) demonstrated statistically significant increase in density of glial cells in all layers of area 22; the least increase from age 7 to age 44 was seen in layer V and VI (48%, $p = 0.0080$; 65%, $p = 0.0040$). Although full-depth cortical sections did not demonstrate statistically-significant age-related changes in glial density, significant increases were found in layer III of area 39 (136% from age 7 to age 44, $p = 0.0026$) and layers IV and V of area 44 (97% increase from age 7 to age 44, $p = 0.0041$; 93% increase from age 7 to age 44, $p = 0.0005$).

Neuronal density: In the autistic cohort, decreased density of neurons over time in full-depth cortical samples was evident in all three brain areas, with a steep early decline followed by essential plateau in density of neurons no later than age 10 (Fig. 1d-f). From age 7 to age 44, density of neurons decreased in area 22 by 39% ($p = 0.0324$), in area 39 by 43% ($p = 0.0144$) and in area 44 by 30% ($p = 0.0090$). The most pronounced decrease in neuronal density from age 7 to age 44 in area 22 was layer VI (39%, $p = 0.0351$), in area 39, layer II (57%, $p = 0.0042$) andin area 44, layer II (42%, $p = 0.007$) (Table 3).

Lipofuscin-containing cells: In full-thickness cortical samples from autistic subjects, the number of lipofuscin-containing cells increased over time in all three areas of brain. Pronounced increases were found between age 7 and age 14 in area 22 (69%, $p = 0.014$), area 39 (149%, $p = 0.0002$) and area 44 (45%, $p = 0.002$) (Fig. 2g-i). In this younger subset, the range of lipofuscin-containing cells was 180 cells mm^{-2} in area 22, 226 cells mm^{-2} in area 39 and 690 cell mm^{-2} in area 44 (Table 4).

Transversal analysis: Comparison of autistic versus control subjects.

Table 3: Number of glial cells mm^{-2}. M: Mean. SD: standard deviation. T: total cell density

		Control							Autism							
	age	8	17	22	30	46	52	56	7	9	10	14	25	26	31	44
BA22																
I	M	592	516	708	1000	992	1108	1141	916	975	942	817	1083	1050	842	1150
	SD	103.75	116.67	89.37	247.95	82.92	75.92	86.20	153.66	132.02	134.11	144.34	151.84	158.99	138.19	121.34
II	M	558	608	642	674	825	1209	1155	800	1200	1242	1400	1200	875	1267	1630
	SD	118.73	125.55	147.90	211.48	121.05	123.32	192.03	197.20	154.56	138.19	131.23	158.11	147.90	187.08	174.80
III	M	491	350	430	475	817	967	992	717	758	1033	1258	983	824	1166	1434
	SD	168.94	98.60	131.23	170.78	121.34	224.85	190.58	178.73	173.81	227.30	173.81	186.34	103.75	187.08	217.31
IV	M	567	534	608	776	1133	1234	1283	992	1084	1316	1442	1217	1133	1750	1825
	SD	108.01	147.20	147.90	170.78	124.72	222.36	153.66	176.97	167.50	167.50	125.55	128.02	179.51	222.98	209.99
V	M	467	459	675	917	1119	1108	1192	1058	1040	1163	1175	1059	1108	1225	1575
	SD	110.55	125.55	178.54	189.54	158.77	243.10	216.51	155.23	220.32	222.05	240.80	217.79	116.37	236.14	215.22
VI	M	475	283	475	1025	1167	1140	1217	792	1192	1192	1184	1108	1091	1217	1308
	SD	125.55	98.60	165.62	214.95	139.44	217.79	206.16	144.10	157.01	203.27	180.28	155.23	98.25	184.84	183.14
T	M	560	488	622	704	978	1023	1077	863	1054	1229	1244	1311	1279	1287	1638
	SD	134.63	152.37	136.76	153.81	198.22	201.00	243.20	216.13	243.87	186.56	223.62	265.92	267.88	237.68	306.55
BA39																
I	M	508	467	608	808		1042	1133	658	892	867	667	1050		1158	1142
	SD	64.01	113.04	125.55	111.49		180.08	200.00	95.38	72.17	108.01	40.82	128.02		180.08	144.10
II	M	625	600	725	842		1058	1100	1067	1167	1325	875	1150		958	1017
	SD	138.19	154.56	144.10	147.90		170.58	204.12	198.61	182.57	144.10	212.62	254.41		186.15	144.34
III	M	475	367	575	758		992	1042	458	758	933	792	942		783	1092
	SD	155.23	126.93	208.67	138.19		237.32	134.11	138.19	212.62	179.51	147.90	184.65		192.21	216.51
IV	M	675	625	808	1008		1125	1117	917	1083	1208	1142	1242		1367	1450
	SD	170.58	205.99	219.06	127.75		207.33	186.34	207.50	172.40	222.83	132.02	116.37		149.07	202.07
V	M	650	467	633	992		1092	1058	767	1042	1033	958	1217		1042	1208
	SD	144.34	143.37	184.09	168.94		222.83	217.79	187.08	254.27	181.05	200.52	232.74		249.86	175.40
VI	M	683	442	592	792		1267	1283	892	1192	1275	1008	1142		875	1200
	SD	121.34	116.37	151.61	178.54		190.03	172.40	157.01	200.52	153.43	147.90	212.62		181.62	197.20
T	M	520	590	645	739		1163	1077	767	794	852	830	1066		1102	1277
	SD	67.87	112.78	86.56	123.56		117.98	132.56	99.89	87.56	112.70	56.76	90.87		118.97	147.65
BA44																
I	M	592	525	508	944	887	927	1008	550	705	575	791	683	629	967	1105
	SD	76.93	123.89	53.15	105.46	95.42	139.45	112.61	125.97	82.24	127.77	133.73	53.49	86.86	99.43	171.26
II	M	558	708	685	677	879	750	1040	700	767	541	675	841	830	960	895
	SD	107.90	75.65	66.01	51.00	102.30	92.30	129.79	183.90	114.05	83.42	185.83	121.94	76.93	92.30	100.40
III	M	492	675	653	661	774	919	984	625	616	608	683	825	758	823	790
	SD	66.50	132.02	117.14	95.42	102.01	130.04	133.98	73.47	103.98	69.84	47.71	83.42	117.42	108.20	141.53
IV	M	567	733	710	798	960	968	1105	617	698	843	841	1108	1016	1274	1218
	SD	52.88	144.94	60.35	112.61	160.28	197.54	141.30	103.28	93.51	95.08	129.79	61.94	86.86	123.91	246.07
V	M	467	691	669	766	927	1153	1145	567	733	1008	916	1025	1024	1347	1097
	SD	103.28	60.35	57.59	171.26	143.13	136.93	183.19	45.62	127.00	155.33	66.50	132.30	129.84	176.93	149.57
VI	M	475	891	863	847	1387	1266	1185	1059	1008	1133	1226	1033	1073	1565	1137
	SD	92.30	61.94	74.71	47.71	133.00	183.42	121.50	135.94	189.99	131.03	51.00	92.30	146.72	123.89	185.83
T	M	540	835	796	817	965	960	973	901	819	738	852	1067	1023	981	1075
	SD	35.87	56.29	98.27	78.68	101.27	91.09	104.64	101.09	65.20	74.00	63.91	108.86	125.68	86.49	127.44

Density of glial cells: The density of glial cells in full-thickness cortical samples was greater in autism than age-matched controls andthere was no intersection of plotted curves. Collectively, samples from autistic subjects evidenced significantly greater density of glial cells in area 22 (90%, $p = 0.0002$), area 39 (40%, $p = 0.0032$) andarea 44 (20%, $p = 0.0262$). Greater mean density of glial cells was evident in autism compared to controls in all six cortical layers. Plotted curves for glial density demonstrated an approximate linear increase in control cohorts in all brain areas andin area 39 of the autistic cohort. For subjects of age 7 through 10, area 22 from autistic subjects evidenced a steep linear increase between age 7 and age 10, then plateau through age 26 andarea 44 of the autistic cohort demonstrated a linear decline (Fig. 2a-c). Generally, laminar glial density in autistic subjects was greater than controls of corresponding age, although no difference was found in layer IV (granular) andlayers II and III (supragranular) of area 44. It also was noted that the autism-versus-control difference in glial density gradually diminshed between age 7 and age 31 in layers V and VI of area 44, while it increased in other layers (Table 2).

Table 4: Number of cells containing lipofuscin/ mm^2. M: Mean. SD: standard deviation. T: total cell density

		Control							Autism						
		8	17	22	30	46	52	56	7	9	10	14	25	31	44
BA22															
I	M	105	177	323	556	677	1145	1202	89	460	411	790	702	758	1306
	SD	42.50	63.06	104.52	145.08	150.00	349.19	463.71	50.81	129.84	123.39	96.77	227.42	272.58	516.94
II	M	32	274	411	589	597	996	1105	52	427	435	621	839	702	1347
	SD	17.74	99.55	143.31	207.82	301.61	542.98	439.52	49.19	114.52	108.87	237.90	397.58	244.35	451.61
III	M	97	226	298	516	718	1097	1153	93	435	524	637	742	790	1137
	SD	28.23	90.32	93.71	98.23	276.61	477.42	401.45	34.68	107.26	130.65	175.00	290.32	223.39	360.48
IV	M	121	218	363	524	758	1395	1484	105	556	645	718	847	879	1444
	SD	50.81	50.81	88.81	240.00	340.32	508.95	504.03	91.13	75.81	137.90	246.77	298.39	319.35	540.32
V	M	129	298	419	556	694	1097	1089	153	524	565	589	790	815	1194
	SD	64.52	102.30	129.84	219.35	340.32	446.77	376.29	51.61	115.32	107.26	195.97	260.48	297.58	427.42
VI	M	153	202	379	492	871	984	1115	234	403	653	790	774	734	1226
	SD	52.42	97.50	115.32	187.10	403.06	413.71	421.37	56.45	154.84	135.48	312.90	254.03	293.87	350.81
T	M	134	373	464	576	1184	1124	1171	180	389	413	660	737	815	1525
	SD	77.92	129.56	102.27	103.89	408.94	388.00	368.32	65.78	109.22	168.29	117.82	298.88	233.76	537.22
BA39															
I	M	65	153	290	492		1161	1169	210	355	362	597	863	898	1218
	SD	23.21	63.49	49.70	189.20		234.20	202.56	75.40	67.80	98.60	93.20	125.40	119.20	29.30
II	M	121	129	339	637		1185	1145	153	548	466	758	911	960	1234
	SD	75.12	30.12	55.12	92.09		145.78	216.33	45.21	60.79	98.70	123.6	113.34	117.29	140.25
III	M	153	218	274	677		1065	1121	169	387	492	605	944	935	1089
	SD	69.72	73.29	70.73	156.90		198.89	198.99	34.98	64.33	60.08	86.75	113.34	145.80	121.31
IV	M	145	153	339	718		1002	1387	177	476	540	621	1113	1105	1419
	SD	79.23	53.40	119.56	105.37		249.60	257.30	90.34	98.78	67.89	105.80	123.23	239.06	148.34
V	M	169	250	395	621		1008	1048	129	371	435	573	863	879	1161
	SD	56.5	68.30	79.20	114.50		198.34	265.80	34.70	56.8	67.80	54.12	86.34	70.15	122.23
VI	M	194	234	347	605		952	1032	177	395	371	613	895	911	1121
	SD	67.8	91.10	64.98	107.30		113.20	199.20	33.09	66.08	54.98	78.90	111.21	177.14	109.23
T	M	209	361	455	568		1034	1117	226	236	370	593	686	813	1456
	SD	43.56	55.98	98.23	123.40		208.71	198.47	56.50	43.89	59.93	79.32	81.09	102.95	117.34
BA44															
I	M	234	796	786	1105	926			230	548		826	866	896	1204
	SD	43.15	63.06	98.25	98.64	56.49			45.85	66.00		66.08	34.59	43.12	85.32
II	M	234	577	707	617	956			321	498		518	796	1075	1154
	SD	33.99	51.16	63.23	99.11	85.32			39.44	37.19		43.56	65.12	112.56	109.99
III	M	332	478	478	697	796			388	548		677	806	816	736
	SD	58.45	39.98	69.56	75.23	79.56			62.23	55.74		66.50	90.12	74.10	85.95
IV	M	405	826	647	856	1025			678	587		956	1016	1264	1194
	SD	64.12	67.78	63.94	78.06	99.87			56.98	69.33		87.28	99.20	123.56	132.56
V	M	268	816	776	667	1224			508	558		786	876	1005	796
	SD	68.01	112.32	78.92	56.36	103.56			36.87	55.37		82.71	96.32	112.09	53.94
VI	M	310	856	796	866	1125			440	488		856	866	1006	846
	SD	56.00	73.75	75.28	82.81	136.45			58.79	40.23		97.25	86.34	109.00	66.93
T	M	477	1125	1026	1167	1379			690	866		1256	1108	1377	1288
	SD	87.20	120.38	117.95	132.47	137.74			69.56	65.47		136.59	109.33	137.86	121.03

Neuronal density: Mean neuronal densities for full-thickness cortical samples were significantly reduced in the autistic cohort as compared to controls in area 22 (38% reduction, $p = 0.0012$) and area 39 (24% reduction, $p = 0.0011$), but no significant difference was found in area 44. In relation to controls, decrements in neuronal density were greatest in area 22. Values in autism were lower at all corresponding ages in area 22 andall but age 7 in area 39. Steep initial declines in neuronal density from age 7 to age 10 and essential plateau thereafter in areas 22 and 39 in autism contrasted with gradual linear declines in controls (Fig. 2d-f).

In area 22, the difference in neuronal density grew from 20% fewer neurons at age 10 ($p = 0.0132$) to 42% fewer neurons in adolescence ($p = 0.0395$) (Fig. 2d). The greatest difference in neuronal cell density in area 22 was evident in layers II and IV (Table 3). The neuronal density in area 39 for the control group declined very little with increasing chronological age. Neuronal density in area 39 was equivalent for the youngest autistic (age 7) and control (age 8) subjects. In area 39, a steep decline in neuronal density was evident between age 7 and ages 9/10 in autism (34%; $p = 0.0282$), but density in the eldest autistic subject (age 44), was not substantially lower than at ages 9/10 (Fig. 2e).

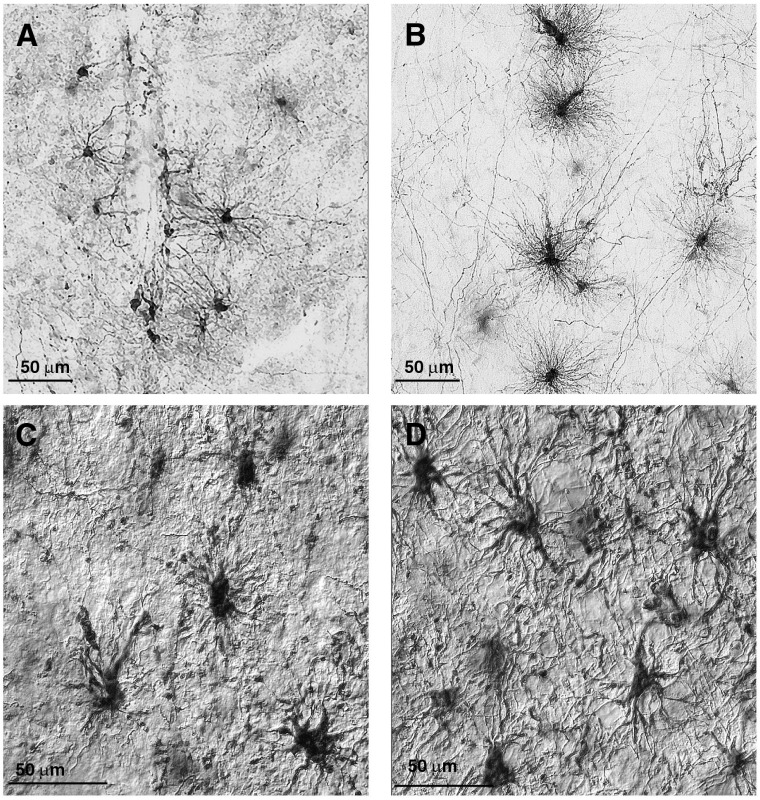

Fig. 4: Photomicrographs illustrating glial cells using immunocytochemical staining methods for the glial fibrillary acidic protein (GFAP). A and B demonstrate protoplasmic astrocytes and C and D demonstrate fibrous astrocytes. Conspicuous alterations of the morphology of the glial cells are evident in autistic patients (B and D), compared to control cases (A and C)

Laminar analysis indicated that the decrement of neurons evident in autism relative to controls at age 7 in area 22 was most pronounced in layers V or VI (the infragranular layer), than in layers II or III (Table 3). By age 10, the neuronal density in layer II of area 22 in autism was 53% lower than corresponding control ($p = 0.0055$). In area 44, lower neuronal density in autism versus controls was found primarily in cortical layers II and III in younger subjects; in older subjects, decrements relative to controls were found in nearly all layers (Table 3).

Lipofuscin-containing cells: The density of lipofusin-containing cells in full-thickness cortical samples was greater in the autistic cohort than controls: area 22 (50% greater, $p = 0.0007$) and area 39 (44% greater, $p = 0.0096$). Other than equivalent values for the youngest of either cohort for areas 22 and 39, autistic subjects showed greater density of lipofuscin-containing cells than controls at all corresponding ages. The youngest (age 7 years) member of the autistic cohort evidenced greater density of lipofuscin-containing cells (46% greater, $p = 0.0086$) in area 44 than the corresponding 8-year-old control.

Relative to controls, the autistic group evidenced steep increases in the density of lipofuscin-containing cells between age 7 and 14 in all three brain areas. (Fig. 2g-i) For instance, the density of cells with lipofuscin in area 22 in autistic subjects of age 9/10 was equivalent to density in the control subject of age 17; by age 14, the density of cells containing lipofuscin in autism were approximately twice the density in control subject of age 17. ($p< 0.0168$) (Fig. 2g). Similar sharp rises were apparent among younger members of the autistic cohort in areas 39 and 44. However, significant increases were not observed in area 44 in autism after age 10 (Table 4).

Laminar analysis demonstrated greater density of lipofuscin-containing cells particularly in layers II and IV in all three areas of brain (Table 4). Microscopic

observation imparted the impression that larger lipofuscin aggregates were more concentrated in layers III and V in areas 22 and 39.

Morphological analysis of glial cells: Variant astrocyte morphology was observed in autism. In comparison to controls, both the subclass of protoplasmic astrocytes (characterized by short, thick, highly-branched processes) and the subclass of fibrous astrocytes (characterized by long, thin, less branched processes) demonstrated earlier hypertrophy of the perikaryon and processes in autism. (Fig. 4a-d) Further, the subclass of protoplasmic astrocytes exhibited larger size, denser ramification of fine processes anda fibrinoid aspect in austim that was not appreciated in control specimens (Fig. 4b).

DISCUSSION

The present autism study constitutes the first neuroanatomical analysis of diverse regions of the cerebral cortex involved in linguistic processing and production of speech, across a broad age-group. Autistic cortex was markedly altered in comparison to age-matched controls, including increased density of glial cells, decreased density of neurons andincreased density of nonspecifc lipofuscin-containing cells.

Previous reports suggest neurodevelopmental aberration in autism. Kemper and Bauman (1993) reported a coarse and poorly-laminated cingulate cortex in autism andother authors have presented functional evidence of abnormal connections, as well as a delay in the maturation of some cortical circuits [9-12]. Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) scans of autistic subjects suggest cortical ectopias, a distinctive feature of altered neuronal migration[10, 13-15].

The present study found no deviation from normal cortical layering pattern, evidence of ectopic cells, nor differences in the relative thickness of cortical layers in areas 22, 39 and 44 of brain from autistic subjects. If the cytoarchitectonic criteria of Benes[16] are valid, these findings argue strongly against the hypothesis of altered neuronal migration in the neuropathogenesis of autism.

Cortical gliosis in autism: In all three brain areas (22, 39 and 44), full-thickness cortical samples from autistic subjects of all ages demonstrated greater density of glial cells in comparison to controls (up to more than double). Since thickness of the cortical layers is not altered in autistic subjects, the finding of greater glial density in autism implies a greater gross number of glial cells in each area due to cellular proliferation andnot due to reduction of neuropil volume. Proliferation of glial cells as well as the hypertrophic morphological changes in glia reported in this study are consistent with reactive gliosis in autism.

Proliferation of glial cells occurs in diverse pathologies, including ischemia, trauma, neurodegenerative disorders andsenescence[17-22]. Glial fibrillary acidic protein (GFAP), a major intermediate structural filament protein expressed predominantly in mature astrocytes, is considered a hallmark of gliosis under conditions of neuronal injury[23-25]. A marked increase in the number of neuroglial cells in the middle frontal gyrus, anterior cingulate gyrus andcerebellum of autistic patients, as well as increased inflammatory cytokines in parenchyma and cerebrospinal fluid (CSF) have been reported in autism[26]. Significantly elevated concentrations of GFAP were reported in the CSF, frontal, parietal andcerebellar cortices of children with autism[27,28]. The results of the present study present further evidence for widespread gliosis in autism.

A toxic etiology for autism has not been excluded. Many toxins induce gliosis, including metals such as lead, iron andmercury, which specifically induce glial proliferation, degeneration anddecreased cellular function in some regions of the brain[29-33]. Neurotoxicity of metals is primarily mediated by increased oxidative stress and both increased metals and increased oxidative stress are reported in autism[34,35]. Hypothetically, environmental exposure in sensitive subjects might underlie glial proliferation and neuronal death in the pathogenesis of autism.

Neuronal attrition in autism: Other investigators have reported reduced numbers of neurons (cerebellar Purkinje and granule cells)[36-38,15] in brain of subjects with autism, but there are no prior reports of decreased neurons in cerebral cortex. Coleman[39] examined auditory brain regions, including Broca's area, from a single subject with a presumptive diagnosis of autism, but found no differences in neuronal density relative to control subjects. Magnetic resonance imaging of autistic subjects of 3-4 years of age demonstrated less n-acetyl aspartate (NAA)[40], a metabolite produced by neurons, but not glia.

The present study employed a larger cohort of systematically diagnosed autistic subjects andfound a striking reduction in neuronal density in area 22 and 39 in autism relative to controls, including lower density of neurons in area 22 from the youngest member of the autistic cohort (age 7). Neuronal density in area 39 was equivalent in the youngest autistic subject and youngest control (age 8), suggesting that the reduced neuronal density in older autistic subjects is not explicable on the

basis of failed genesis, but due to attrition. Loss of cortical neurons has been described in other neuropsychiatric pathologies, such as schizophrenia, bipolar disorder andmajor depressive disorder, all of which coexist with different cognitive impairments, some similar to those observed in autism[41-44].

Decrease in density of neurons in autism was most pronounced in cortical layers II and IV. While the present study did not separately tabulate pyramidal cells and smaller neurons, its findings are comparable to decreased pyramidal cells in layers II and III and, less conspicuously, in layers V and VI in frontal and temporal neocortex in Rett syndrome [45], a neurodegenerative disease associated with autism[46-48]. The relationship of increased glial cell density and decreased neuronal cell density varied in autism by brain area. Increased glial density coincided with decreased neuronal numbers in area 22 and are 39, but not area 44. A significant increase in glial density in the youngest member of the autistic cohort (age 7) was associated with decreased neuronal density in area 22, but not in area 39. Determination of glial and neuronal densities in a larger cohort including younger subjects is needed to elucidate the temporal and functional relationship of these two parameters.

Indications of oxidative stress in autism: One of the more interesting findings of the present study is the significant increase of lipofuscin-containing cells in autism. Lipofuscin is classically associated with recognized neurodegenerative diseases andthere is no prior record of previous examination of brain from autistic subjects for lipofuscin. Increased lipofuscin was reported previously in brain from subjects with Rett's syndrome [49-51].

Lipofuscin is an intralysosomal polymeric material originated from autophagocytosed cellular components that cannot be degraded or exocytosed. Biochemical analysis of lipofuscin reveals a complex aggregated by-product composed primarily of oxidatively-modified proteins and lipids[52-55]. In addition, lipofuscin is a depot for metals, including redox-active and heavy metals[56]. Lipofuscin accumulation in cells is accelerated under conditions of oxidative stress[57-59]. Experimentally, lipofuscin itself induces neurotoxicity via generation of free-radicals[52-54].

Several authors have proposed the involvement of free radicals andcross-linking reactions by intralysosomal degradation products, as initial steps in the formation of lipofuscin[58,60,61]. Higher levels of lipofuscin may be viewed as a marker for heightened oxidative processes which are potentially harmful to brain cells[52], or for greater oxidative damage to brain cells.

Extensive research has demonstrated that oxidative stress plays a seminal role in the pathology of several neurological diseases, including Alzheimer's disease[62,63], Down's syndrome[64], Parkinson's disease[65,66], schizophrenia[67,68] andbipolar affective disorder[69]. Interestingly, recent studies have reported increased free-radicals, lower levels of antioxidant proteins andgreater oxidatively modified biomolecules in peripheral samples from autistic children[70-77].

Significantly increased numbers of lipofuscin-containing cells were found in the three brain areas of subjects with autism andwere most pronounced in area 22, which also featured the greatest increase in glial density and greatest decrease in neuronal density. Since the lipofuscin cell-count did not differentiate neurons and glia, the profile of brain cells with increased lipofuscin in autism remains undetermined. As noted earlier, the size of lipofuscin aggregates was judged greatest in layers III and V in areas 22 ad 39, which is noted to be the cite of the largest pyramidal neurons. In one area (44), increased lipofuscin was evident in the youngest of the autistic cohort (age 7), suggesting that greater lipofuscin as well as differential glial and neuronal densities may be detectable in younger autistic subjects in further studies.

The data presented are strictly phenomenological anddo not afford firm conclusions about mechanistic relationships among the three measured parameters. While the study did not determine the extent of glial (versus neuronal) lipofusin content, it is known that lipofusin appears in reactive glial cells[78] andthat oxidative stress in glial cells alters their function[79,80]. Greater lipofuscin in brain of autistic subjects of age 7-44 probably reflects greater oxidative stress in brain, as suggested by peripheral measurements of oxidative stress in autism[70-72,81]. The increased numbers of cells containing lipofuscin may reflect (a) an environmentally-induced reactive glial response injurious to neurons, (b) an environmentally-induced direct oxidative injury to neurons, or (c) both.

In summary, the present study of language-related cortical areas 22, 39 and 44 demonstrated a greater density of glial cells, a lesser density of neurons andan increased density of non-specific lipofuscin-containing brain cells in autism. Changes in these parameters were progressive andvaried in extent by cortical area and layer.

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