Brain Research Bulletin, Vol. 55, No. 5, pp. 611–618, 2001

0361-9230/01/$–see front matter

PII S0361-9230(01)00529-9

A quantitative immunohistochemical study of astrocytes in the entorhinal cortex in schizophrenia, bipolar disorder and major depression: Absence of significant astrocytosis

Ruslan Damadzic,[1] Llewellyn B. Bigelow,[1] Leonid S. Krimer,[2] David A. Goldenson,[1†] Richard C. Saunders,[1] Joel E. Kleinman[1] and Mary M. Herman[1*]

[1]Clinical Brain Disorders Branch, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, Bethesda, MD, USA; and [2]Section of Neurobiology, Yale University School of Medicine, New Haven, CT, USA

ABSTRACT: A number of macroscopic changes have been reported in the temporal lobe in schizophrenia. We have evaluated the density of glial fibrillary acidic protein (GFAP)-positive astrocytes in cortical layers 2 through 6 in the intermediate subarea of entorhinal cortex in two cohorts: the first, 15 cases, made up of schizophrenic ($n = 7$) and normal nonpsychiatric control subjects ($n = 8$), and the second, 56 cases, composed of schizophrenic ($n = 14$), bipolar disorder ($n = 13$), major depressive ($n = 14$) and normal control subjects ($n = 15$). No significant difference in density of GFAP-positive astrocytes was detected between the psychiatric diagnostic groups and the normal controls in either of the two cohorts. In both cohorts there was a positive correlation between increasing age and astrocytic density which reached statistical significance in only the larger cohort ($r = 0.38$, $p = 0.004$). Our results find no evidence for astrocytosis in the entorhinal cortex in several mental illnesses. Although other studies have reported macroscopic and other structural abnormalities in this region, we have not detected astrocytic proliferation, which is a typical hallmark of atrophy and/or progressive neuronal loss. © 2001 Elsevier Science Inc.

KEY WORDS: Mesial temporal cortex, GFAP, Mental illness.

INTRODUCTION

In the past two decades, a number of macroscopic changes have been described in the schizophrenic brain, suggesting an involvement of the medial temporal cortex [7,45,53]. Morphometric studies in cases at postmortem have shown enlargement of the cerebral ventricles [16,42], particularly the temporal horn [16]. Also, a reduction in the area or volume of several limbic structures has been reported, notably the left posterior superior temporal gyrus [29], the entorhinal cortex [23], parahippocampal gyrus [14,16], and the hippocampus and amygdala [14,29].

Histologic studies using Holzer's stain for astrocytic fibrils have suggested the presence of increased gliosis in the periventricular structures of the diencephalon and in the basal forebrain [47] in schizophrenic subjects as compared with controls. The entorhinal cortex (ERC) has also been examined for possible cortical laminar disorganization related to schizophrenia [3,4,6,12, 28,33–35,38]. The presence or absence of astrocytosis, as identified immunohistochemically, has been evaluated in multiple sites in the temporal lobe, using computer-assisted quantification of glial fibrillary acidic protein (GFAP)-containing cells and of GFAP optical density [5,43,44]. No increase in GFAP-positive astrocytes or GFAP optical density has been detected in comparing brain regions of schizophrenic and control subjects in the temporal and parahippocampal cortex [5,43,44]. Nor was an increase noted in GFAP-positive astrocytes in the ERC region of seven nondemented schizophrenics [5] or in the ERC or cerebral white matter of chronic schizophrenics [24].

In the present study we have focused our efforts on the intermediate subarea of the ERC, using the nomenclature of Krimer et al. [37], because this area contains neurons which are normally clustered in cortical layer 2, where a neuronal migrational disorder has been proposed in schizophrenia [4,33–35]. We have studied two cohorts, with a combined total of 21 schizophrenic and 23 control subjects. The presence or absence of accompanying astrocytosis is crucial to an understanding of the mechanism of neuropathologic changes reported in the mesial temporal lobe in schizophrenia. Prior studies of the astrocytes in this brain region have suffered from the use of elderly schizophrenics and a minimum number of controls. Toward that end we have examined for the presence of astroglial changes in two separate cohorts of younger schizophrenics, as well as in controls, using tissue sections of the ERC.

MATERIALS AND METHODS

The study was done on two separate cohorts of subjects. The first consisted of one group of 15 postmortem human brains

* Address for correspondence: Dr. Mary M. Herman, Neuropathology Section, Clinical Brain Disorders Branch, National Institute of Mental Health, Intramural Research Program, National Institutes of Health, 36/3A24, 36 Convent Drive, MSC 4091, Bethesda, MD 20892-4091, USA. Fax: +1-(301)-480-0023; E-mail: mh230t@nih.gov
† Deceased.

obtained from the Clinical Brain Disorders Branch (CBDB) at the National Institute of Mental Health. The second was a set of 56 postmortem brains obtained from the Stanley Foundation Neuropathology Consortium (SFNC).

CBDB Cases

Fifteen subjects were evaluated from this collection [17]; 7 carried the diagnosis of schizophrenia and 8 were controls without psychiatric diagnosis. Table 1 summarizes the demographics. The primary cause of death for both groups was suicide, homicide, or somatic diseases not affecting the brain. Patients with schizophrenia ranged in age from 25 to 80 years, with a mean age of 49 years and a mean postmortem interval of 35 h. Control subjects ranged from age 19 to 85 years, with a mean age of 47 years and a mean postmortem interval of 26 h. Brains were collected mainly from the Office of the Chief Medical Examiner in Washington, DC, USA. Time in formalin fixative for both groups ranged from 2 months to 1 year. Autopsy records were reviewed to exclude cases with hepatic or renal disease (which may cause increases in astroglia). Macro- and microscopic examination of the brain (M.M.H.), including Bielschowsky's silver stain (adapted for paraffin sections) on multiple cortical areas, was used to exclude pathologic conditions, such as Alzheimer's disease, cerebrovascular disease, etc. The clinical diagnosis was established by at least two consensus independent reviews of the available medical records, applying criteria from the third edition, revised, of the *Diagnostic and Statistical Manual of Mental Disorders*.

SFNC Cases

This was a group of 56 postmortem human brains obtained from the SFNC. Table 2 summarizes their demographics. The 56 cases were divided into four groups based on psychiatric diagnosis: schizophrenia ($n = 14$), bipolar disorder ($n = 13$), major depression with and without psychotic features ($n = 14$), and nonpsychiatric controls ($n = 15$). Patients with schizophrenia ranged in age from 25 to 62 years, mean age 46 years, and a mean postmortem interval of 32 h. Subjects from the bipolar group ranged in age from 25 to 61 years, with a mean age of 44 years, and a mean postmortem interval of 34 h. The unipolar depressive group ranged from age 30 to 65, with a mean of 46 years, and a mean postmortem interval of 29 h. Control subjects ranged from age 29 to 68, with a mean age of 48 years, and a mean postmortem interval of 24 h. Brains were collected from several designated medical examiner's offices. Average time in formalin fixative ranged from 4 to 11 months. Review of autopsy records and the pathology examination were performed as above (M.M.H.). The clinical diagnosis was established by at least two consensus independent reviews of the available medical records, applying criteria from the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders*.

Tissue Processing

Brains were collected at autopsy over a period of 15 months (CBDB) or 34 months (SFNC). For CBDB cases, the left cerebral hemisphere was fixed by immersion in 10% phosphate-buffered formalin solution and stored at room temperature for a period of 2 months to 1 year. The ERC and immediately adjacent temporal cortex were cut into three blocks, each block being 1 cm in thickness, and trimmed in a plane approximating the 'stereotactic' coronal plane [37]. The blocks were then embedded in paraffin and sectioned at a thickness of 10 μm. Every 100th and 101st section were stained with cresyl violet for the identification of cell types and Gallyas' silver stain [26] for nerve fibers, respectively. Sections were matched at the level of the intermediate subarea, nomenclature as in Krimer et al. [37] Nearly adjacent sections at 500-μm intervals were stained for GFAP. For SFNC cases, the left or right cerebral hemispheres were fixed by immersion in similar formalin fixative and stored at room temperature for an average period of 4 to 11 months. The blocks were similarly trimmed and processed according to the procedure followed for the CBDB cases. Sections were also matched at the level of the intermediate subarea of the ERC.

Immunohistochemical Analysis for GFAP

Sections were deparaffinized in xylene and rehydrated through graded ethanols to water; endogenous peroxidase activity was blocked with hydrogen peroxide (0.3% in methanol for 30 min). Before application of the primary polyvalent antiserum, sections were saturated in 10% normal goat serum (blocking serum) (Vector Laboratories, Inc., Burlingame, CA, USA) for 10 min. They were then incubated for 40 min with the primary antiserum, i.e., rabbit anti-cow GFAP (Dako Corp., Carpinteria, CA, USA) (1:400 dilution). The avidin-biotin method [30] was performed, using the Vectastain ABC Elite Kit (Vector Laboratories Inc.) and by applying the diluted biotinylated secondary antiserum solution (anti-rabbit IgG) for 10 min. All reagents were diluted in phosphate-buffered saline (pH 7.2). The immunohistochemical reaction was developed in a freshly prepared solution of 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO, USA) for 2–4 min. As positive controls for GFAP, similarly fixed sections from adult human cerebellum were stained in parallel. Negative controls were obtained by incubation with normal rabbit serum substituted for the primary antiserum. All steps were performed at room temperature. Slides were lightly counterstained with hematoxylin, dehydrated, cleared, and mounted.

Method of Counting

Sections of ERC were immunostained for GFAP. Maps were drawn by projection from the Nissl- and Gallyas'-stained tissue sections onto a surface at a total magnification of 17.5×. Two observers confirmed the boundaries of the maps (R.D., R.C.S.). Areas to be counted were outlined on the maps, as were the counting grid squares, in order to confirm the location of the region sampled; each grid area was 0.63 mm^2. Cortical layers 2 through 6 were evaluated. Only astrocytes with GFAP-positive cytoplasm and clearly identifiable pale nuclei were counted. Astrocytes with immunopositive nuclei were not tabulated, even if the cytoplasm was positive, as these were interpreted as overstained cells. GFAP-positive astrocytes were evaluated at a total magnification of 200× by two observers (R.D., M.M.H.).

Specific Details for Counting

For CBDB cases, sections immunostained for GFAP were obtained from the intermediate subarea of ERC. GFAP-positive astrocytes were counted from each case, in a total area of 40 mm^2 in two slides separated by a 500-μm interval. Counts between two observers varied less then 6%. Inter-rater reliability as expressed as an intraclass correlation coefficient was 0.99. For SFNC cases, sections immunostained for GFAP were obtained from the same layers in the intermediate subarea of ERC. GFAP-positive astrocytes were counted in cortical layers 2 through 6 in an area of 40–70 mm^2; the larger area was counted whenever possible, because only one slide (not two as above) was available for evaluation. Counts between the two observers varied by 5% or less. The intraclass correlation coefficient between the two raters was 0.99.

TABLE 1
DEMOGRAPHICS OF CLINICAL BRAIN DISORDERS BRANCH SUBJECT GROUPS

Groups	Age	Sex	Race	Cause of Death	PMI	Brain Weight (g)	Lat
Schizophrenic							
Br#1	32	M	H	Suicide	24	1600	L
Br#2	64	F	B	Asphyxia	21	1260	L
Br#3	60	F	B	Hypertensive CVD	48	1350	L
Br#4	36	M	W	Suicide	35	1600	L
Br#5	46	F	W	Hyperthermia, enlarged heart	80	1280	L
Br#6	25	M	W	Suicide	24	1400	L
Br#7	80	F	W	Arteriosclerotic CVD	11	1300	L
Mean±SD	49 ± 20	3M / 4F			35 ± 23	1399 ± 145	
Controls							
Br#1	64	F	B	CVD, prev. heart surg. w/bleeding	22	1280	L
Br#2	64	F	B	Cardiac tamponade	16	1050	L
Br#3	34	F	B	Pneumonia	24	1200	L
Br#4	20	M	B	Homicide (GSW)	25	1400	L
Br#5	52	M	W	Arteriosclerotic CVD	26	1480	L
Br#6	85	F	W	Suicide (Fluoxetine poisoning)	48	1300	L
Br#7	19	M	B	Homicide (GSW)	24	1450	L
Br#8	38	F	B	Car accident	21	1200	L
Mean±SD	47 ± 23	3M / 5F			26 ± 10	1295 ± 145	

M, male; F, female; W, White; B, Black; H, Hispanic; CVD, cardiovascular disease; GSW, gunshot wound(s); PMI, postmortem interval in hours; Lat, laterality (side). All brain weights were obtained before fixation. They were fixed by immersion in a phosphate-buffered 10% formalin solution for a period of 2 months–1 year. Time in fixative was similar for the schizophrenic and control groups.

Nuclear Size Measurement (CBDB and SFNC Cases)

Nuclear size was determined by measuring the longest diameter of the nuclei of GFAP-positive cells in the intermediate subarea in cortical layers 2 through 6. Two slides separated by 500 μm were evaluated in each CBDB subject and in one slide per SFNC subject. The average nuclear diameter per subject was computed from 20 measured nuclei in the CBDB cohort and from 10 nuclei in the SFNC cohort. The reliability of this measure was checked by having two persons (R.D., M.M.H.) examine the same four representative cells from each subject (CBDB cohort) or the same two cells per subject (SFNC cohort). The resulting intraclass correlation was 0.96 (CBDB) or 0.92 (SFNC), indicating excellent reliability.

RESULTS

Results for CBDB Cases

The mean number of immunopositive astrocytes in the intermediate subarea for the control group for layers 2 through 6 was 604, with a range of 234–1447; and for the schizophrenic group was 306, with a range of 232–415. Abercrombie formula-corrected cell densities [1,36] are shown in Table 3. Because of the small N and large variance, we used the nonparametric Mann-Whitney U-test and found no significant difference in the cell density between schizophrenic and control groups ($U = 20$, $p = 0.35$) (Table 3). There were no significant differences between the two subject groups with regard to age at death, gender, postmortem interval (PMI), brain weight, or nuclear diameter of the GFAP-positive astrocytes. The left side of the brain was examined in all cases. Gender had no effect on cell density. One-tailed Spearman rank order correlations of cell density with age at death, PMI, age of onset, and duration of illness were likewise not significant.

Results for SFNC Cases

The mean density of GFAP-positive astrocytes for the control group for layers 2 through 6 was 291 ± 189 (SD) with a range of 21–723; for the schizophrenic group, 271±276, with a range of 44–1120; for the bipolar disorders group, 305±236, with a range of 12–794; and for the major depressive group, 243±229, with a range of 49–911 Abercrombie formula- corrected cell densities are shown in Table 4. Because of the large variance, we used the nonparametric Kruskal-Wallis analysis of variance test (Statistica 5.0) There were no significant differences in astrocytic density between the schizophrenic, bipolar disorder, major depressive and control groups ($H = 1.80$; $p = 0.62$; Kruskal-Wallis test) (see Table 4).

There were no significant differences between any of the subject groups with regard to age at death, gender, PMI, side of brain (alternate sides were examined), brain weight, nuclear diameter of GFAP- positive astrocytes, or age of onset. However, the time in formalin fixative for the schizophrenic, bipolar disorder, and major unipolar depressive groups was about twice as long as for the nonpsychiatric controls ($H = 9.12$; $p = 0.03$; Kruskal-Wallis test). Gender, PMI, brain weight, nuclear diameter, and age of onset had no effect on cell density, but the correlation between cell density and age was significant (Spearman, $r = 0.38$; $p = 0.04$). The correlation between density and duration of the disease approached but did not reach significance ($r = 0.28$; $p = 0.07$). This was most likely related to the association of increased numbers of GFAP-positive cortical astrocytes with more advanced age [11,40].

Differences in Results Between the CBDB and SFNC Cases

We investigated the reasons for the marked differences in astrocytic density between the controls in the two cohorts and between the schizophrenic subjects in these cohorts. A Spearman rank order correlation, covarying astrocytic density with age (in all

TABLE 2

DEMOGRAPHICS OF STANLEY FOUNDATION NEUROPATHOLOGY CONSORTIUM SUBJECT GROUPS

Groups	Age	Sex	Race	Cause of Death	PMI	Brain Weight (g)	Lat	Onset/Duration (age/yrs.)	Formalin (mo.)
Schizophrenics									
Br#1	52	M	W	Cardiac	61	1530	L	20 / 32	31
Br#2	30	M	W	Pneumonia	32	1620	L	13 / 17	8
Br#3	62	F	A	Motor vehicle accident	26	1270	L	38 / 24	7
Br#4	60	F	W	Cardiac	40	1395	L	15 / 45	13
Br#5	60	M	W	Accidental drowning	31	1340	R	27 / 33	9
Br#6	32	M	A	Acute alcohol intoxication	19	1590	L	27 / 5	18
Br#7	31	M	W	Suicide: jumped	14	1555	L	18 / 13	9
Br#8	58	F	W	Cardiac	26	1410	R	42 / 16	9
Br#9	25	M	W	Suicide: hanged	32	1555	L	20 / 5	8
Br#10	44	M	W	Cardiac	50	1640	R	17 / 27	7
Br#11	44	M	W	Pulmonary disease	29	1500	L	21 / 23	5
Br#12	56	F	A	Suicide: overdose	12	1420	R	24 / 32	9
Br#13	35	M	W	Cardiac	35	1380	R	19 / 16	3
Br#14	49	F	W	Cardiac	38	1440	L	25 / 24	3
Mean ± SD	46 ± 13	9M / 5F			32 ± 13	1475 ± 112	6R / 9L	23 / 22	9.93 ± 7.16
Bipolar									
Br#1	25	F	W	Suicide: hanged	24	1540	R	19 / 6	8
Br#2	48	F	W	Pneumonia	22	1260	L	16 / 32	8
Br#3	37	F	W	Suicide: overdose	29	1130	R	14 / 23	14
Br#4	54	M	W	Subdural hematoma	39	1690	R	39 / 14	13
Br#5	30	M	W	Pneumonia and myocarditis	31	1350	R	22 / 8	10
Br#6	30	M	W	Suicide: carbon monoxide	56	1580	R	7 / 23	16
Br#7	57	M	W	Cardiac	19	1440	L	30 / 27	13
Br#8	48	M	W	Suicide: immolation	13	1540	R	27 / 21	9
Br#9	30	M	W	Suicide: overdose	45	1590	L	14 / 16	10
Br#10	50	F	B	Malnutrition and dehydration	18	1180	L	34 / 16	8
Br#11	61	F	W	Suicide: overdose	60	1415	L	18 / 43	9
Br#12	50	M	W	Suicide: jumped	19	1380	L	17 / 23	4
Br#13	50	F	W	Pulmonary emboli	62	1320	L	25 / 25	2
Mean ± SD	44 ± 12	7M / 6F			34 ± 17	1417 ± 168	6R / 7L	22 / 21	9.54 ± 3.89
Depressive									
Br#1	32	F	W	Suicide: overdose	47	1500	L	32 / 1	11
Br#2	53	F	W	Acute alcohol intoxication	40	1320	R	11 / 42	19
Br#3	44	F	W	Suicide: overdose	32	1410	L	27 / 17	18
Br#4	65	M	W	Cardiac	19	1360	R	45 / 20	14
Br#5	46	M	W	Suicide: carbon monoxide	26	1720	R	28 / 18	9
Br#6	42	F	W	Cardiac	25	1340	R	39 / 3	9
Br#7	51	M	W	Suicide: gunshot	26	1550	R	50 / 1	15
Br#8	39	M	W	Suicide: carbon monoxide	23	1530	L	17 / 22	2
Br#9	42	M	W	Suicide: hanged	7	1350	L	32 / 10	5
Br#10	56	M	W	Cardiac	23	1240	L	52 / 4	1
Br#11	56	F	W	Pulmonary emboli	28	1520	L	54 / 2	1
Br#12	30	F	W	Suicide: overdose	33	1370	L	19 / 11	4
Br#13	43	M	W	Cardiac	43	1460	L	30 / 13	2
Br#14	47	M	W	Cardiac	28	1740	L	27 / 20	1
Mean ± SD	46 ± 10	8M / 6F			29 ± 10	1458 ± 147	5R / 9L	33 / 13	7.93 ± 6.57
Controls									
Br#1	52	M	W	Cardiac	28	1700	L		13
Br#2	44	F	W	Cardiac	25	1490	R		10
Br#3	59	M	W	Cardiac	26	1560	R		10
Br#4	52	M	W	Cardiac	8	1840	L		8
Br#5	52	M	W	Cardiac	22	1330	R		2
Br#6	53	M	W	Cardiac	28	1400	L		2
Br#7	44	M	W	Cardiac	10	1510	L		2
Br#8	35	F	W	Cardiac	23	1340	R		2

(Continued)

TABLE 2
CONTINUED

Groups	Age	Sex	Race	Cause of Death	PMI	Brain Weight (g)	Lat	Onset/Duration (age/yrs.)	Formalin (mo.)
Br#9	41	M	B	Pulmonary embolus	11	1305	R		2
Br#10	42	M	W	Cardiac	27	1500	R		2
Br#11	35	F	W	Pulmonary embolus	40	1560	L		2
Br#12	68	F	W	Pulmonary embolus	13	1360	L		5
Br#13	58	M	W	Cardiac	27	1780	L		1
Br#14	29	F	W	Motor vehicle accident	42	1440	L		3
Br#15	57	F	W	Motor vehicle accident	26	1400	R		2
Mean ± SD	48 ± 11	9M/6F			24 ± 10	1501 ± 164	7R / 8L		4.40 ± 3.87

M, Male; F, Female; W, White; B, Black; A, Asian; PMI, postmortem interval in hours; Lat, laterality. Brain weights were obtained before fixation. Formalin fixative as in Table 1.

subjects in both cohorts), found no significant correlation in the CBDB cohort ($r = 0.38$, $p = 0.15$), but a significant correlation in the SFNC cohort ($r = 0.38$; $p = 0.004$). Using an analysis of covariance, the difference in mean density between the two groups of subjects, when covaried for age in the normals in both cohorts, was significant ($F = 6.82$; $p < 0.017$). When control and schizophrenic subjects over age 60 in the SFNC cohort were dropped, there were also no significant differences in the corrected means between the two cohorts (analysis of variance/multivariate analysis of variance $p < 0.45$). (We did not apply this test in the CBDB cohort because three of the seven schizophrenic subjects were 60 years and older.) The Mann-Whitney *U*-test did not show significant differences in astrocytic density in the control subjects between CBDB and SFNC cohorts ($p = 0.052$), but a significant difference was found between schizophrenic subjects of the two cohorts ($p = 0.04$).

These differences between astrocytic density in the two cohorts may be due to the fact that a greater percentage of the CBDB cohort (3 of 7 subjects) were 60 years or older, while in the SFNC only 3 out of 14 subjects were 60 years or older. Other factors that may have contributed to the difference in astrocytic density are that the immunohistochemical reactions were carried out on two different occasions separated by a period of about 2 years (the CBDB cohort being processed first), and/or that there were possible differences in fixation factors. In regard to the latter, although the same formalin fixative was used initially, differences in potency or in pH of the fixative occurring over time may have resulted from differences in ambient temperature during storage, changing the formalin at different intervals, or from other subtle factors.

DISCUSSION

The ERC has been considered one of several components of the hippocampal formation [2,39] and an important relay station from cortical sensory information to the hippocampus, with extensive projections to the medial thalamic region and prefrontal cortex as reviewed in Krimer et al. [37]. There has been increasing clinical, experimental, neuroimaging, and neuropathological data implicating the cortical area of the medial temporal lobe in the pathology of schizophrenia [8,13,31,53]. Of all the data implicating the ERC in schizophrenia, the results of a few qualitative studies are noteworthy [4,33–35]. The latter studies have reported disorganization and displacement in several subareas of the ERC of neuronal

TABLE 3
NUMBER AND DENSITY OF GLIAL FIBRILLARY ACIDIC PROTEIN-POSITIVE ASTROCYTES IN ENTORHINAL CORTEX FOR CLINICAL BRAIN DISORDERS BRANCH SUBJECT GROUPS (INTERMEDIATE SUBAREA)

Schizophrenic				Control			
Case	No.	N.D.	C.D.	Case	No.	N.D.	C.D.
Br#1	526	6.03	8.14	Br#8	335	6.25	5.11
Br#2	1041	6.00	16.14	Br#9	415	5.95	6.45
Br#3	452	6.38	6.85	Br#10	280	6.08	4.32
Br#4	252	5.80	3.96	Br#11	336	5.93	5.23
Br#5	282	6.20	4.32	Br#12	329	6.05	5.08
Br#6	234	6.28	3.57	Br#13	256	5.93	3.99
Br#7	1447	6.03	22.39	Br#14	268	5.95	4.17
				Br#15	232	5.73	3.66
Mean		6.10	9.34			5.98	4.75
SD			7.21				0.90

Cortical layers 2 through 6 were examined; No., number of glial fibrillary acidic protein (GFAP)-positive astrocytes observed; N.D., nuclear diameter of GFAP-positive cells; C.D., corrected density of immunopositive astrocytes/mm^2 (see text). No significant differences were noted between the two subject groups ($U = 20$, $n_1 = 7$, $n_2 = 8$, $p = 0.35$ Mann-Whitney *U*-test).

TABLE 4

NUMBER AND DENSITY OF GLIAL FIBRILLARY ACIDIC PROTEIN-POSITIVE ASTROCYTES IN ENTORHINAL CORTEX FOR STANLEY FOUNDATION NEUROPATHOLOGY CONSORTIUM SUBJECT GROUPS (INTERMEDIATE SUBAREA)

Schizophrenic				Bipolar				Depression				Control			
Case	No.	N.D.	C.D.	Case	No.	N.D.	C.D.	Case	No.	N.D.	C.D.	Case	No.	N.D.	C.D.
1	44	5.75	0.49	1	197	5.75	2.18	1	136	5.85	1.23	1	443	5.8	4.36
2	221	6.1	2.37	2	156	6.15	2.13	2	451	5.8	5.03	2	407	5.95	5.70
3	1120	5.95	12.38	3	410	5.85	4.51	3	158	6	1.94	3	543	5.9	6.02
4	409	5.85	4.50	4	148	5.95	1.60	4	911	5.85	9.81	4	412	6.1	4.51
5	86	5.8	0.95	5	366	5.7	4.07	5	82	5.95	0.89	5	285	5.9	3.16
6	58	5.9	0.64	6	12	6.15	0.13	6	147	6	1.59	6	349	5.95	3.47
7	172	6.2	1.83	7	794	5.85	8.74	7	319	5.85	3.55	7	141	5.95	1.75
8	39	6.15	0.47	8	264	5.9	2.93	8	66	5.9	0.67	8	120	5.8	1.88
9	232	5.65	2.48	9	162	6.05	1.67	9	91	5.9	1.00	9	129	5.8	1.42
10	386	5.85	4.30	10	191	5.85	2.13	10	328	5.9	3.56	10	161	5.9	1.62
11	246	6.05	2.76	11	715	5.9	7.93	11	85	6.15	0.93	11	21	5.95	0.23
12	413	5.95	4.47	12	457	5.6	5.81	12	260	6.05	2.86	12	723	5.75	7.83
13	318	5.85	4.30	13	98	5.55	1.08	13	49	5.7	0.55	13	180	5.75	1.97
14	50	6	0.61					14	324	5.65	3.53	14	241	5.9	2.56
												15	213	6	2.22
Mean		5.93	2.84			5.87	3.45			5.9	2.65			5.89	3.25
SD			3.11				2.64				2.48				2.07

Cortical layers 2 through 6 were examined. No., number of glial fibrillary acidic protein (GFAP)-positive astrocytes observed; N.D., nuclear diameter of GFAP-positive cells; C.D., corrected density of immunopositive astrocytes/mm^2. No significant differences were found in astrocytic-corrected density between the schizophrenic, bipolar disorder, major depressive, and control groups ($H = 1.80$; $p = 0.62$ Kruskal-Wallis test).

clusters which are characteristically found in laminae 2 and superficial 3. Additionally a recent spatial point pattern analysis of schizophrenic brain has reported subtle changes in neuronal dispersion and clustering in layers 2 and superficial 3 in the rostral ERC (a region adjacent to the amygdala). Although Krimer et al. [38] were unable to detect changes in neuronal arrangement in the ERC laminae, they did demonstrate a mild nonsignificant quantitative reduction in neuronal number and density in the prorhinal and 28 L subareas of ERC in schizophrenic subjects (subareal designation per Krimer et al. [37]). Structural changes in the ERC have also been associated with other neuropsychiatric disorders, including Alzheimer's disease [15,32,52], autism [9,10], and temporal lobe epilepsy [19].

In the present study we have compared numbers per unit area of GFAP-positive astrocytes in schizophrenic, bipolar, major depressive, and control subjects in cortical laminae 2 through 6 of the intermediate subarea of the ERC. We have focused our attention on GFAP-positive astrocytes because it is well-established that after injury and/or disease in the central nervous system, astrocytes become reactive, and respond with the rapid synthesis of GFAP, a structural protein of astrocytes (reviewed in Eng and Ghirnikar [21]). An increase in GFA protein is often present within a few hours to 24 h of injury, and levels remain high in the vicinity of the damage over long periods of time [22,46]. GFA protein autolyzes after death, yet remains relatively stable antigenically (reviewed in Eng and Ghirnikar) [21] and is highly immunogenic [22]. Increased GFAP immunostaining, content, and/or production have been reported in a variety of experimental models of injury and neurotoxicities, as well as in human neurodegenerative disorders, demyelinating diseases, viral diseases (including slow virus infections), and in aging and other conditions [21].

Thus, an increased production of GFAP is almost always an accompaniment of the astrocytic response to central nervous system damage. In addition to its general association with injury and disease, reactive astrocytosis, as detected by traditional histologic stains, sometimes including Holzer's stain for astrocytic fibrils, has been reported to be present in the schizophrenic brain by some workers [41,47] (reviewed in Dwork and in Harrison [20,27]), but not by others [20,27]. With the use of immunohistochemical methods, no increase in GFA protein- positive astrocytes has been detected in twenty different sites in the temporal lobe (including the parahippocampal gyrus) of 18 schizophrenics, employing quantitative computer densitometry [44]. Also, no increase was found in the ERC of chronic schizophrenics [24] or in seven nondemented schizophrenic patients in the ventromedial temporal cortex using GFAP-positive astrocytic counts per unit area and optical density analysis [5]; in the latter, layers 2 and 3 of the ERC were examined just rostral to the posterior ERC. In the present study we also have been unable to detect significant differences in the density of GFAP-positive astrocytes between schizophrenic and control subjects as well as between bipolar, depressive and control subjects.

Other reports [48–51] have raised several issues critical in any analysis of possible gliosis in the schizophrenic brain. Time in fixative and PMIs are comparable for both cohorts of schizophrenic and control subjects in the present study (see Tables 1 and 2). We used the GFAP immunohistochemical method because it is widely recognized to be the most specific assay for the identification of astrocytes in tissue sections, and because the perikarya of reactive astrocytes in the cortical grey matter stain more intensely with GFAP than with the Holzer's stain [18]. We counted only positive astrocytic cell bodies and not their processes, in order to further increase the specificity of our counts. As to outliers in our study, none were found in the CBDB cohort. In the SFNC cohort there were four subjects over 2 SD from the mean density values. These were the oldest in 3 of the subject groups: namely age 62 in the schizophrenic group, age 65 in the depressive group and age 68 years in the controls. In the bipolar group, the second oldest

subject, age 57, was over 2 SD from the mean density. We could not identify any other demographic or clinical variables common to these cases.

As alluded to earlier, in the Results section, there was a highly significant correlation between cell density and increased age in the SFNC cohort ($r = 0.38$; $p = 0.004$). The corrected GFAP-positive average cell density was greater (but not significantly so) in the schizophrenic compared to the control subjects in the CBDB cohort, while in the SFNC cohort the corrected average density values were similar in these two subject groups. This is probably due to an increased percentage of aged patients, as about 50% of the schizophrenic cases in the CBDB cohort were age 60 years and over, as compared to 20% of the SFNC schizophrenic cases. In the nonpsychiatric disease controls of the CBDB cohort, almost 40% of the subjects were 60 years and over, while in the SFNC cohort, less than 7% were over 60 years of age.

Increased astrocytosis was not found in the present study, either in the intermediate subarea or in the lateral subarea (CBDB series, data not shown) of the ERC. The lack of increased astrocytosis in a brain region regarded as one of the key sites involved in schizophrenia does not favor a later-onset insult or disease process where residual reactive astrocytosis would generally be present. Rather, this finding lends credence to an earlier event, such as an early intrauterine injury, where, during the first 20 weeks of gestation in the human, virtually no residual astrocytosis is reported to occur, even after extensive tissue destruction [25,46]. Larger series of well-characterized and carefully matched cases may aid in a more complete understanding of this interesting question.

ACKNOWLEDGEMENTS

Presented in part at the 6th International Congress on Schizophrenia Research, Colorado Springs, Colorado, April 12–16, 1997. We thank the Stanley Foundation Research Program for summer fellowship support for the late Dr. David A. Goldenson. Drs. Paul D. MacLean, and Maree J. Webster provided most useful reviews. We also acknowledge the excellent technical assistance provided by Dr. Juraj Cervenak and Mrs. Yeva Snitskovsky.

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