

Reduced Glial Cell Density and Neuronal Size in the Anterior Cingulate Cortex in Major Depressive Disorder

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Background: Glial cells are more numerous than neurons in the cortex and are crucial to neuronal function. There is evidence for reduced neuronal size in schizophrenia, with suggestive evidence for reduced glial cell density in mood disorders. In this investigation, we have simultaneously assessed glial cell density and neuronal density and size in the anterior cingulate cortex in schizophrenia, major depressive disorder, and bipolar disorder.

Methods: We examined tissue from area 24b of the supracallosal anterior cingulate cortex in 60 postmortem brain specimens from 4 groups of 15 subjects, as follows: major depressive disorder, schizophrenia, bipolar disorder, and normal controls. Glial cell density and neuronal size and density were examined in all subjects using the nucleator and the optical disector.

Results: Glial cell density (22%) ($P = .004$) and neuronal size (23%) ($P = .01$) were reduced in layer 6 in major depressive disorder compared with controls. There was some evidence for reduced glial density in layer 6 (20%) ($P = .02$) in schizophrenia compared with controls, before adjusting for multiple layerwise comparisons, but there were no significant changes in neuronal size. There was no evidence for differences in glial density or neuronal size in bipolar disorder compared with controls. Neuronal density was similar in all groups to that found in controls.

Conclusion: These findings suggest that there is reduced frontal cortical glial cell density and neuronal size in major depressive disorder.

Arch Gen Psychiatry. 2001;58:545-553

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HERE ARE 3 main types of glial cell populations in the central nervous system and together they constitute well over half of all cells in the brain. Until recently, they have been largely viewed as “passive handmaidens” to neurons, and their central role in cortical and neuronal function has not been fully appreciated.¹ They have important roles in synaptic function,²⁻⁴ clearance of extracellular ions⁵ and transmitters,⁶ neuronal metabolism,⁷⁻⁹ and neuronal migration.¹⁰ There is also some evidence that cortical glial cell numbers may be increased by neuroleptic medication in primates,¹¹ that glial cell density is reduced in the prefrontal cortex,¹² and that the subgenual anterior cingulate cortex (ACC)¹³ is reduced in major depressive disorder (MDD) and the orbitofrontal¹⁴ cortex in schizophrenia. These findings suggest that glial cell dysfunction may be involved in the pathophysiology of major psychiatric disorders.

Macroscopic investigations of schizophrenia, bipolar disorder (BPD), and MDD

show many similarities in brain pathology, with the differences being quantitative rather than qualitative. For example, ventricular dilatation and reduced hippocampal and cortical volumes are seen in schizophrenia,¹⁵ but also to a less marked degree in MDD and BPD.^{16,17} These investigations also indicate which cortical regions are predominantly affected in MDD and schizophrenia.¹⁶⁻²⁰ In MDD, there is reduced metabolism in the ACC²¹ on the left side.²²⁻²⁴ Abnormalities of the prefrontal cortex are also described in schizophrenia,²⁵ and while the ACC is again implicated,²⁶⁻²⁸ the changes are neither so marked nor so lateralized as in MDD. The presence of these ACC abnormalities in MDD and schizophrenia are consistent with the known functions of this cortical region in information processing, attention, and in the expression and modulation of emotion.^{29,30} As these functions are altered in schizophrenia³¹ and MDD,¹⁹ this area is a candidate region in which to search for the presence of distinct microscopic neuroanatomical substrates for these disorders.

SUBJECTS AND METHODS

SUBJECTS

Human brain specimens from Brodmann area 24 were obtained from the Stanley Foundation Brain Consortium.³⁷ The sample consisted of 60 subjects (15 normal controls, 15 subjects with schizophrenia, 15 with BPD, and 15 with MDD) and is the same as that used previously to investigate subgenual ACC.¹³ Diagnoses were made according to DSM-IV³⁸ criteria. Detailed case summaries were provided on demographic, clinical, and histological information (see **Table 1** for group summary details). All brains underwent clinical neuropathological examination and none demonstrated evidence of neurodegenerative changes or other pathological lesions. Messenger RNA levels of the housekeeping gene *glyceraldehyde phosphate dehydrogenase* were measured in the Stanley Foundation Brain Consortium laboratory by the reverse transcription polymerase chain reaction and they were excellent to good in all groups, demonstrating good tissue preservation.

Tissue was available from only 1 hemisphere of each brain, with roughly equal numbers sampled in a random manner from each side of the brain (Table 1). Hemispheres were fixed in 10% phosphate-buffered formalin and then cut in coronal sections of roughly 1-cm thickness. From these slices, a block was taken from the supracallosal ACC approximately 2-cm caudal to the tip of the genu of the corpus callosum and processed to paraffin wax. From these blocks, a series of 20 sections of 30- μ m thickness were taken and 5 sections were systematically randomly sampled for analysis. All sections were then stained with cresyl violet according to standard methods.

IDENTIFICATION OF CORTICAL LAMINA

For each case, Brodmann area 24b of the ACC was identified and selected for analysis according to macroscopic and microscopic criteria.³⁹ This region has clear laminar boundaries, making it a particularly suitable region for the delineation of laminar specific variations in cortical cytoarchitecture.³⁹ Layer 5 is relatively easily divided into distinct sublayers (layers 5a and 5b), which we assessed separately, in addition to the remaining 4 layers. The width of each cortical layer was assessed using an image analysis

system (Image-Pro Plus; Media Cybernetics, Baltimore, Md),⁴⁰ with which we obtained a series of contiguous images (20 \times 25 images) at 20-times objective magnification, from which a single composite image was formed. The laminar boundaries were identified on this image and the mean laminar width was calculated. The percentage of total cortical width contributed by each layer in each group was then calculated (**Table 2**).

3-DIMENSIONAL CELL COUNTING AND NEURONAL SIZE ESTIMATES

In this investigation, neurons were identified by the presence of a cresyl violet-stained cytoplasm, a single nucleolus, and their generally larger shape and nonspherical outline. Glia were identified by the absence of stained cytoplasm, the presence of a thicker nuclear membrane, and more heterogeneous chromatin within the nucleus.

Sections were viewed using a BH2 Olympus microscope (Olympus Optical Co [UK] Ltd, London, England) with a 100-times (numerical aperture, 1.4) oil-immersion objective lens, to which was attached a color video camera (TK1280-E; Microinstruments Ltd, Oxon, England), a z-axis depth gauge (Heidenhain [GB] Ltd, London, England) (accurate to <1 μ m), and an Olympus x- and y-axis movement gauge. After the mounting and the staining of tissue sections, the thickness of the tissue sections was assessed. This had reduced from 30 μ m to a mean (SD) of 23.4 (2.8) μ m. Consequently, using an optical disector with a depth of 15 μ m, our guard volumes above and below the disector averaged 4.8 μ m. Cell-density estimations were made with the aid of image analysis software (Stereology 2.5; Kinetic Imaging, Liverpool, England) according to the stereological optical disector method.⁴¹ The dimensions of the disector used were 50.5 \times 37.5 μ m in the x- and the y-axis, respectively. There was 1 disector per field.

A systematic random sampling strategy was optimized before the investigation, so that an equal proportion of sampled neurons was obtained from each of the 5 sections used in each case. This involved estimating the number of fields required to give more than 100 sampled neurons per layer (and sublayers, in the case of 5a and 5b) per case, and then calculating the required size of the steps (taken in a sine wave fashion with random start) between fields, so that the entire region of each cortical layer of

Microscopically, investigations of schizophrenia point to abnormalities of neuronal cytoarchitecture and neuropil,¹⁵ with evidence for reduced neuronal size.^{15,32} Whether such changes are also present in MDD and BPD is not yet clear, for there have been few investigations. In MDD, reduced glial density has been described,^{12,13} but this has not been a consistent finding in schizophrenia.^{13,33-36} It has been proposed that reduced glial cell density may be specific to MDD and BPD,¹² and alternatively, that if reduced glial cell density is a feature of schizophrenia, then it exhibits a region-specific distribution.³⁴ In this study, we set out to characterize neuronal and glial cell density and neuronal size in the ACC in normal human brain, schizophrenia, BPD, and MDD.

RESULTS

ANALYSIS OF NEURONAL DENSITIES

Two predictors of neuronal density were identified at the model selection stage: age of the patient at death (Wald test: $\chi^2_1=6$, $P=.01$; estimated increase in neuronal density per 10 years' survival, 3.4%; 95% confidence interval [CI], 0.67%-6.3%) and the brain hemisphere from which the sections were taken (Wald test: $\chi^2_1=5.5$, $P=.02$; estimated increase in neuronal density for left hemisphere relative to right hemisphere, 7.5%; 95% CI, 1.2%-14.2%).

Layerwise comparisons between the 3 patient groups and the control group were adjusted for the effect of age

Brodman area 24b in the tissue section was sampled. An average of 48 fields were counted per layer per case for neuronal estimations, and 33 fields for glial estimations. A mean (SD) of 103 (3.2) neurons and 85 (15) glia were counted in each layer of each case. Values for the coefficient of error of the neuronal and glial density estimates in the different cortical layers were less than 5% and 6%, respectively. Neuronal and glial cell densities are expressed as cell per $\text{mm}^3/10^3$.

The neuronal size of all disector sampled neurons were estimated using the stereological estimator of number-weighted volume: the nucleator.⁴² Thus, we calculated the size (expressed in cubic micrometers) of more than 100 neurons from each individual layer of each subject. As glial cytoplasm was unstained, glial cell size was not assessed.

STATISTICAL ANALYSIS

Analysis of Neuronal and Glial Cell Densities

The objective of the statistical analysis was to compare within each cortical layer the neuronal and glial cell densities of the 3 patient groups (schizophrenia, BPD, and MDD) with the control group. Layerwise density data (cell counts and sizes of search area) were obtained by combining the 5 sections and sampled fields per layer. Because of their possibly skewed distributions, the density data are summarized by their medians (**Table 3**). A number of subject-specific clinical and demographic variables with potential to affect cell densities were recorded (these are listed in Table 1 in italics). To compare groups using an adequate model, a forward-selection procedure was employed to identify variables that could be shown empirically to predict densities. Within each layer, groups were then compared using models that adjusted for these variables.

In the model selection stage, all layerwise counts were modeled simultaneously, employing a log-link Poisson model that used the size of the search area as an offset. A dispersion parameter was introduced to account for spatial clustering within fields or sections. In addition, a random effect for subject was included to account for layerwise densities of the same subject being more similar than densities from different subjects. The factor layer was always included in the model, since by definition densities vary between cortical layers. In the stepwise-forward procedure, the *P* value threshold for inclusion

of a new variable was chosen to be 10%. The random-effects Poisson models were fitted using the procedure generalized linear mixed model in the statistical package Genstat 5,⁴³ which employs the Schall method⁴⁴ to fit a generalized linear mixed model.

Having identified empirical predictors, a log-link Poisson model, using size of search area as an offset and allowing for overdispersion because of spatial clustering, was fitted to the cell count data in each layer. Density ratios between each of the 3 psychiatric groups and the control group adjusted for the empirical predictors were estimated. Since hemisphere was identified as a predictor of neuronal as well as glial density (see the "Results" section), we allowed for the density ratios to differ between hemispheres. Accumulated analysis of deviance,⁴⁵ using the experimental method, was employed to test for differences between the patient groups and the control group. To account for multiple layerwise testing, the *P* values of the group comparisons can be compared with $.05/6=0.008$ to achieve an experiment-wise type I error probability of 5% according to the Bonferroni correction. This Poisson modelling was again carried out in Genstat 5.

Analysis of Neuronal Sizes

The objective of the statistical analysis was to compare within each cortical layer neuronal sizes of the 3 patient groups with the control group. Size was recorded for each neuron identified, yielding approximately 100 data points per case and layer (from 5 tissue sections with approximately 10 fields per section and layer). Because of their positively skewed distributions, the neuronal size data are summarized by their medians (Table 2) and analyzed on the log-scale, in which empirical distributions were well approximated by normal distributions. We employed robust SEs when fitting our regression models. Such inferences are robust against correlations between repeated observations on the primary sampling units. Here the cases constituted the (independent) primary sampling units. The analysis of the neuronal size data was carried out in analogy to the analyses of the density data. Out of the variables marked with italics in Table 1, empirical predictors were identified using a forward-selection procedure and group comparisons adjusted for these variables. The robust model fitting was carried out in Stata 6.⁴⁵

and hemisphere (see Table 3 for observed median neuronal densities within categories defined by brain hemisphere, cortical layer, and patient group). There was some evidence that the ratio of neuronal density between the BPD group and the control group depended on the hemisphere of the brain in layer 1 at the single test significance level of 5% ($F_{1,51}=4.31$, $P=.04$). However, this evidence of an interaction disappeared after adjusting for the 6 layerwise comparisons. None of the other comparisons were significant, even at the unadjusted level of 5%.

ANALYSIS OF GLIAL CELL DENSITIES

Two predictors of glial density were identified at the model selection stage; the pH of the tissue (Wald test: $\chi^2_1=7.6$

$P=.006$; estimated increase in glial density of 4% per 0.1 increase in pH; 95% CI, 1.6%-6.6%) and the brain hemisphere (Wald test: $\chi^2_1=5.1$, $P=.02$; estimated increase in glial density for left hemisphere relative to right hemisphere, 14%; 95% CI, 1.8%-27.6%).

Layerwise comparisons between the 3 patient groups and the control group were adjusted for the effect of pH and hemisphere (see Table 3 for observed median glial densities within categories defined by brain hemisphere, cortical layer, and patient group). Tests for overall differences between patient groups and control group as well as their interactions with hemisphere were carried out (**Table 4**). Not adjusting for multiple layerwise comparisons, there seemed to be a significant difference between the schizophrenic and the control group

Table 1. Group Summaries of Demographic, Clinical, and Histological Information on the Brains Donated by the Stanley Foundation*

Variable‡	Group†			
	Controls	Scz	BPD	MDD
Demographics				
Sample size	15	15	15	15
<i>Age at death in years, mean ± SD</i>	48.1 ± 10.7	44.5 ± 13.1	42.3 ± 11.7	46.5 ± 9.3
Histological				
<i>Postmortem interval in hours, mean ± SD</i>	23.7 ± 9.94	33.7 ± 14.6	32.5 ± 16.1	27.5 ± 10.7
<i>Brain hemisphere</i>				
Right	7	6	8	6
Left	8	9	7	9
<i>pH, mean ± SD</i>	6.27 ± 0.24	6.16 ± 0.26	6.18 ± 0.24	6.20 ± 0.23
<i>Cause of death</i>				
CPD	13	7	3	7
Traffic accident	2	0	0	1
Suicide	0	7	9	4
Alcohol intoxication	0	1	0	1
Pneumonia	0	0	1	1
Subdural hematoma	0	0	1	0
Malnutrition	0	0	1	0
Drowning	0	0	0	1
Clinical				
<i>Duration of disorder in years, mean ± SD</i>	0 ± 0	21.3 ± 11.4	20.1 ± 9.7	12.7 ± 11.1
<i>Fluphenazine milligram equivalents§</i>				
Minimum	0	0	0	0
Median	0	35 000	7500	0
Maximum	0	200 000	60 000	0
<i>Past alcohol/drug abuse or dependence</i>				
No	13	12	12	14
Yes	2	3	3	1
<i>Current alcohol/drug abuse or dependence</i>				
No	15	12	11	12
Yes	0	3	4	3
<i>Treated with antidepressants at death</i>				
No	15	10	9	6
Yes	0	5	6	9
<i>Lithium treatment at death</i>				
No	15	12	5	13
Yes	0	2	4	2
Other mood stabilizer	0	1	6	0
<i>Family history of disorder </i>				
None	15	10	8	3
Scz	0	3	1	0
BPD	0	0	3	1
MDD	0	2	3	8
Unknown	0	0	1	3
<i>Death by suicide</i>				
No	15	11	6	8
Yes	0	4	9	7

*Scz indicates schizophrenia; BPD, bipolar disorder; MDD, major depressive disorder; and CPD, cardiopulmonary disease.

†There were 9 males and 6 females in each group.

‡Italics indicate that the variable is a potential predictor of cell density and neuronal size in the analysis.

§Fluphenazine milligram equivalents is an estimate of the lifetime neuroleptic dose in fluphenazine equivalent dose in milligrams.

||Family history was defined as a positive for Scz, BPD, or MDD if 1 or more first-degree relatives had a diagnosis of that same disorder, negative if there was no history, and unknown if insufficient information was available. One subject with BPD had a family history of BPD and MDD.

and between the depressed patients and the control group in layer 6 (**Figure**). In addition, the ratio of glial density between the depressed group and the control group seemed to depend on the hemisphere of the brain in layers 1 and 3. However, after adjusting for the 6 layerwise comparisons, at the experiment-wise 5% level, the only significant difference was between the depressed group and the control group in layer 6 ($F_{1,51}=8.9, P=.004$). In layer 6, the glial density was estimated to be reduced by

22% in depressed patients compared with controls (uncorrected 95% CI, 7%-35%) (Table 4).

ANALYSIS OF NEURONAL SIZE

During the model selection stage, the age of the patient was identified as a predictor of neuronal size (t test using robust SEs: $t_{59}=-1.95, P=.05$; estimated decrease in neuronal density per 10 years' survival, 4.7%; 95% CI,

Table 2. Observed Median Neuronal Sizes in Square Micrometers and Cortical Height (Expressed as Percentage of Total Cortical Thickness) Within Categories Defined by Cortical Layer and Patient Group (n = 15 per Group)*

	Group	Layer†					
		1	2	3	5a	5b	6
Neuronal size	Control	106 (64, 166)	263 (129, 529)	493 (203, 952)	737 (310, 1635)	804 (337, 1778)	741 (358, 1386)
	Scz	103 (66, 170)	276 (148, 525)	547 (243, 1024)	684 (274, 1443)	756 (302, 1679)	649 (311, 1223)
	BPD	109 (65, 177)	286 (141, 547)	505 (216, 993)	687 (293, 1480)	686 (309, 1510)	602 (285, 1202)
	MDD	92 (58, 152)	289 (158, 538)	494 (199, 900)	710 (256, 1556)	676 (286, 1471)	596 (279, 1092)
Cortical height	Control	10.8 (9.2, 11.9)	10.3 (9.1, 12.0)	25.2 (23.6, 26.2)	11.8 (10.8, 12.9)	14.7 (12.7, 16.7)	26.7 (23.3, 28.5)
	Scz	10.0 (7.3, 12.3)	9.6 (8.2, 11.1)	26.2 (21.7, 27.3)	12.1 (11.2, 13.2)	13.4 (13.1, 14.8)	27.7 (23.2, 35.2)
	BPD	10.9 (7.9, 12.0)	10.4 (8.3, 11.3)	25.5 (21.8, 27.0)	11.3 (10.9, 12.0)	14.0 (13.3, 15.4)	27.3 (23.6, 35.0)
	MDD	11.3 (9.7, 11.8)	10.0 (9.0, 12.0)	24.9 (23.7, 29.2)	11.8 (11.2, 12.8)	13.6 (12.8, 14.2)	26.2 (24.4, 28.8)

*Scz indicates schizophrenia; BPD, bipolar disorder; and MDD, major depressive disorder.

†The lower and upper quartiles are included in parentheses.

-0.1% to 9.2%). This finding is in keeping with previous literature.⁴⁶

Layerwise comparisons of neuronal size between the 3 patient groups and the control group were adjusted for the effect of age (see Table 2 for observed median neuronal sizes within categories defined by cortical layer and patient group). Tests for overall differences between patient groups and control group were undertaken (Table 5). These show that at the single-test 5% level neuronal size differed between the depressed patients and controls in layers 5b and 6. These comparisons do not remain significant after adjusting for multiple layerwise comparisons. However, the value for comparing depressed patients with controls in layer 6 of $P = .01$ is only slightly exceeding the adjusted significance level of $P = .008$ and is interpreted as mild evidence for a difference between these groups, taking into account the conservative nature of the Bonferroni procedure. In layer 6, the neuronal size was estimated to be reduced by 23% in depressed patients compared with controls (uncorrected 95% CI, 6%-37%) (Table 5).

COMMENT

In this investigation, we have found evidence for reductions in glial cell density and neuronal size in layer 6 of the ACC in subjects with MDD. The estimated sizes of these reductions are 22% and 23%, respectively (Tables 4 and 5). As glia have important metabolic influences on neurons⁷⁻⁹ and contribute to synaptic function²⁻⁴ and neurotransmission,⁶ the findings imply that abnormalities of glial function may undermine neuronal function and predispose to MDD.

There have been 2 previous stereological investigations in MDD, and both support our finding of reduced glial cell density in MDD.^{12,13} The first study, that of Ongur and colleagues,¹³ found reduced glial cell density in the subgenual ACC of the same set of brains as examined in our investigation of supracallosal ACC. The second, by Rajkowska et al,¹² found reduced glial cell density in the dorsolateral prefrontal cortex and the caudal orbitofrontal cortex. As in our investigation, these latter changes were most prominent in the deeper cortical layers and were accompanied by reduced neuronal size. However, there are some differences between the 3 investi-

gations. First, Rajkowska et al¹² found decreased neuronal density in the prefrontal cortex in MDD, while our study of the ACC and that of Ongur et al¹³ did not, suggesting a possible region-specific effect. Second, Ongur et al¹³ found no reduction in neuronal size in MDD. This difference may relate to the lack of laminar specific data in their study and to the fact that subgenual rather supracallosal ACC was assessed. Ongur et al¹³ also found reduced glial cell density to be most prominent in familial MDD and BPD groups, while we found no evidence for any changes in microscopic neuroanatomy in BPD, and we refrained from smaller subdivisions of the patient groups according to family history. Third, the absolute values for neuronal density differ between the 3 studies. The reasons for these differences are likely to relate to processing differences between our study, which used paraffin-embedded material, and the other studies, which used cryosections from fixed tissue¹³ and celloidin sections,¹² respectively.

Our investigation found a trend for an estimated reduced glial cell density of 20% in layer 6 in schizophrenia (Table 4). While contrasting with some,¹³ it is in keeping with previous work showing glial cell reductions in the orbitofrontal,⁴⁷ anterior cingulate,^{35,36} and primary motor cortices.³⁶ These reductions have been moderate,³⁶ with evidence that schizophrenic subjects with affective symptoms are more likely to show reduced glial cell density.³⁵ Reductions in the levels of glial fibrillary acidic protein,⁴⁸ which labels astrocytes, and myelin basic protein,⁴⁹ which labels oligodendroglia in the anterior frontal cortex in schizophrenia and MDD, have also been demonstrated; these findings point to the potential cellular basis of the glial cell deficit described in our study.

We found no change in neuronal density in the ACC in schizophrenia (Table 3). This contrasts with the studies of Benes and colleagues,^{35,36} which found reduced density of small neurons in layers 2 through 6,³⁵ and reduced density of all neurons in layer 5 of the ACC³⁶ in schizophrenia. These contrasting results may be caused by differences in the region within the ACC that was assessed; for example, Benes and colleagues^{35,36} may have examined perigenual rather the supracallosal ACC as assessed in our investigation. Additionally, the smaller size of the sampled fields in our investigation may have affected our sensitivity to detect changes in the density of

Table 3. Observed Median Neuronal and Glial Densities (Cells per Cubic Millimeter/10³) Within Categories Defined by Brain Hemisphere

Cell Type	Group	Hemisphere					
		1		2		3	
		Right	Left	Right	Left	Right	Left
Neurons	Control	58.3 (47.5, 67.0)	53.0 (47, 59.9)	184.8 (169.9, 202.6)	199.7 (184.8, 231)	92.4 (81.2, 99.4)	101.1 (92.1, 109.3)
	Scz	53.6 (47.2, 57.6)	51.8 (45.8, 60.6)	158.7 (142.2, 193.7)	203.3 (188.9, 247.1)	93.1 (81.8, 105.6)	92.4 (88.1, 97.3)
	BPD	49.5 (40.2, 56.2)	71.1 (57, 73.5)	161.5 (145.9, 199)	217.2 (179.8, 228.3)	75.5 (73.8, 96.8)	106.6 (99.8, 106.6)
	MDD	50.4 (47.2, 54.7)	49.7 (45.3, 66.3)	167.5 (144.7, 191.9)	179.4 (168, 201.6)	87 (83.5, 91)	88.9 (75.8, 91.6)
Glial	Control	113.9 (86.3, 132.5)	144.9 (113.7, 182.3)	65.2 (36.9, 79.6)	72.4 (59.7, 85.5)	73.8 (57.5, 88.9)	94.9 (85.1, 113.9)
	Scz	123.4 (106.6, 154)	126.8 (86.4, 135.8)	62.7 (39.1, 77.5)	61.5 (46.7, 73.6)	79.2 (56.9, 89.7)	87.9 (75.7, 97.1)
	BPD	122.7 (91.5, 151.5)	118.1 (105.7, 120.4)	61.9 (52.4, 84.4)	71.1 (58.4, 76.5)	72.5 (67.4, 88.7)	85.7 (81.1, 90.9)
	MDD	111.3 (92.4, 147.9)	106.6 (71.3, 117.6)	72.7 (63.3, 79)	61.1 (50.2, 64.7)	85.7 (68.6, 92.4)	59.8 (53.7, 90.4)

*Scz indicates schizophrenia; BPD, bipolar disorder; and MDD, major depressive disorder.
 †Lower and upper quartiles are included in parentheses.

Table 4. Approximate F Tests and Comparison of Glial Cell Density Between Patient Groups and the Control Group (n = 15 per Group) From Accumulated Analysis of Deviance (Experimental Method), and Single 95% Confidence Intervals (CI) for Glial Cell Density Ratios Between Patient Groups and the Control Group (Adjusted for pH and Hemisphere)*

Patient Group	Cortical Layer	Test for Overall Difference Between Patient Group and Control Group		Estimate of Effect, Estimated Ratio (95% CI)	Test for Interaction With Hemisphere	
		F _{1,51}	P		F _{1,51}	P
		Scz (n = 15)	1	0.72	.40	0.91 (0.73-1.13)
	2	0.24	.63	0.95 (0.76-1.19)	0.71	.40
	3	0.15	.70	0.96 (0.80-1.15)	0.12	.73
	5a	3.05	.09	0.83 (0.71-0.99)	2.25	.14
	5b	2.89	.10	0.87 (0.74-1.02)	0.04	.84
	6	5.92	.02†	0.80 (0.67-0.96)	0.27	.61
BPD (n = 15)	1	0.16	.69	0.95 (0.77-1.18)	2.56	.12
	2	0.56	.46	1.09 (0.88-1.35)	0.39	.54
	3	<0.001	>.99	1.01 (0.85-1.21)	1.18	.28
	5a	0.18	.67	0.97 (0.83-1.13)	0.76	.39
	5b	0.22	.64	0.96 (0.82-1.13)	0.01	.92
	6	1.70	.20	0.89 (0.75-1.06)	0.13	.72
MDD (n = 15)	1	2.91	.09	0.85 (0.69-1.06)	5.33	.02†
	2	<0.001	>.99	1.02 (0.82-1.26)	2.06	.16
	3	2.64	.11	0.90 (0.75-1.07)	5.78	.02†
	5a	2.66	.11	0.89 (0.76-1.05)	0.35	.56
	5b	3.90	.05	0.86 (0.73-1.01)	0.81	.37
	6	8.90	.004‡	0.78 (0.65-0.93)	1.58	.21

*Scz indicates schizophrenia; BPD, bipolar disorder; and MDD, major depressive disorder.
 †Significant at the single-test significance level of 5%; not adjusted for multiple layerwise comparisons.
 ‡Significant at the experiment-wise significance level of 5% (ie, adjusted for multiple layerwise comparisons).

the larger neurons. However, this would not explain the difference in the results regarding smaller neurons; we used a 100-times oil-immersion lens that maximized our ability to distinguish glia from small neurons. Similar discrepancies have also been reported regarding neuronal density in the prefrontal cortex.^{33,50} Neuronal size has previously been reported to be reduced in the prefrontal cortex in schizophrenia,¹⁸ but we found no evidence for this reduction in our study (Table 5).

In our investigation, tissue was available from 1 hemisphere of each subject; consequently, we were unable to assess true laterality effects within our subjects. We did find, however, that neuronal and glial cell densities were increased in the left hemispheres compared with the right hemispheres (Table 3). This finding may reflect geneti-

cally determined structural asymmetries in the normal brain,⁵¹ such as may be responsible for the recently reported left-lateralized increase in the ACC fissurization in normal subjects.⁵² We also found a trend for a dependence on hemisphere of the changes in glial cell density in layers 1 and 3 between controls and MDD. This finding is consistent with left-lateralized changes reported in the ACC in MDD in functional neuroimaging investigations.²²⁻²⁴

There are several methodological advantages of this study. These include the pragmatic application of stereologically derived methods, the assessment of all cortical layers, and the presence of 3 psychiatric groups with good sample size, clinical details, and careful pathological characterization. There are a number of potential con-

Hemisphere, Cortical Layer, and Subject Group (15 per group)*

Group†

5a		5b		6	
Right	Left	Right	Left	Right	Left
118 (100, 126.2)	117.4 (110.5, 127.4)	89.2 (71.9, 90.4)	85.1 (80.7, 93.6)	58.7 (52.3, 63)	62.4 (55.1, 64.8)
118.7 (109.9, 127.5)	123 (113.3, 128.4)	85.8 (82.9, 87.8)	84.3 (77.2, 90.9)	62.1 (53, 80.1)	61.2 (51.5, 64.4)
97.5 (89.5, 112.6)	119.2 (113.9, 139.6)	79.6 (70.9, 81)	102.4 (86.6, 102.4)	51.1 (49.3, 53.1)	63.6 (61.4, 70.4)
116.5 (108.7, 121.9)	117.7 (109.1, 119.2)	82.5 (77, 86.8)	86.3 (83.5, 93.2)	56.9 (56.3, 61.1)	61.1 (57, 70.5)
87.4 (67.7, 102.1)	104.8 (95.4, 113.2)	89.7 (74.5, 112.1)	108.4 (99.5, 130)	126.5 (98.8, 140.3)	169.6 (138, 177.9)
64.3 (46.5, 76)	96.3 (77.7, 114.6)	88.9 (68.5, 96.9)	93.6 (75.2, 115.6)	102 (81.1, 129.7)	121.2 (103.3, 130.9)
83.6 (67, 104.7)	99.9 (81.6, 105.3)	84.9 (68.7, 109.2)	107.3 (99.8, 116.6)	132.2 (105.1, 142.2)	135.8 (120, 150.7)
77.7 (70, 87)	82.5 (67.8, 111.4)	81.8 (71.1, 105.6)	83.2 (72.1, 111.5)	105.4 (99.9, 109.6)	100.9 (89.9, 121.1)

founding factors. For example, reduced glial cell density or neuronal size could be secondary to pharmacological treatments or group differences in tissue pH. In our analysis, we found that increasing tissue pH was predictive of increasing glial density. Consequently, we corrected for this potential confounding factor in our analysis. We found no evidence that pharmacological treatments (neuroleptics, antidepressants, or mood stabilizers) had predictive effects on cell density or neuronal sizes. Indeed, the literature that is available indicates that pharmacological treatments increase rather than decrease their densities. For example, a recent investigation suggests that chronic exposure to neuroleptics increases glial density in the prefrontal cortex.¹¹ There is also preliminary in vitro evidence that antidepressants activate microglia and cause proliferation of oligodendroglial cells,⁵³ and that lithium treatment is associated with gliosis.^{54,55} Together, these findings suggest that our finding of reduced glial cell density is unlikely to be a consequence of pharmacological treatments or group differences in tissue pH.

There are at present no clear biological mechanisms to explain our findings. However, there may be a clue in the shared neuroanatomy in schizophrenia and MDD. Reduced hippocampal and cortical volumes,¹⁵⁻¹⁷ neuronal size,^{12,32} and dendritic spine density⁵⁶ are features of both schizophrenia and MDD, and we have now shown reduced glial cell density in both disorders. These similarities suggest that there may be a shared pathophysiological mechanism. One intriguing possibility is stress-related, glucocorticoid-mediated toxic effects.⁵⁷ The consequences of elevated levels of glucocorticoids⁵⁸⁻⁶¹ are consistent with both the macroscopic and microscopic neuroanatomy described in MDD¹²⁻¹⁷ and schizophrenia.^{15,17} Elevated levels of glucocorticoids are known to reduce astrocyte activity and function,⁶² and reduced levels of the messenger RNA for the glucocorticoid receptor are reported in the frontal cortex and hippocampus of subjects with MDD and schizophrenia.⁶³ Consequently, glucocorticoids may act directly on neurons, or indirectly through glia to undermine neuronal and cortical function in both disorders.

However, although the etiology of the glial cell loss is not clear, the consequences of such loss are poten-

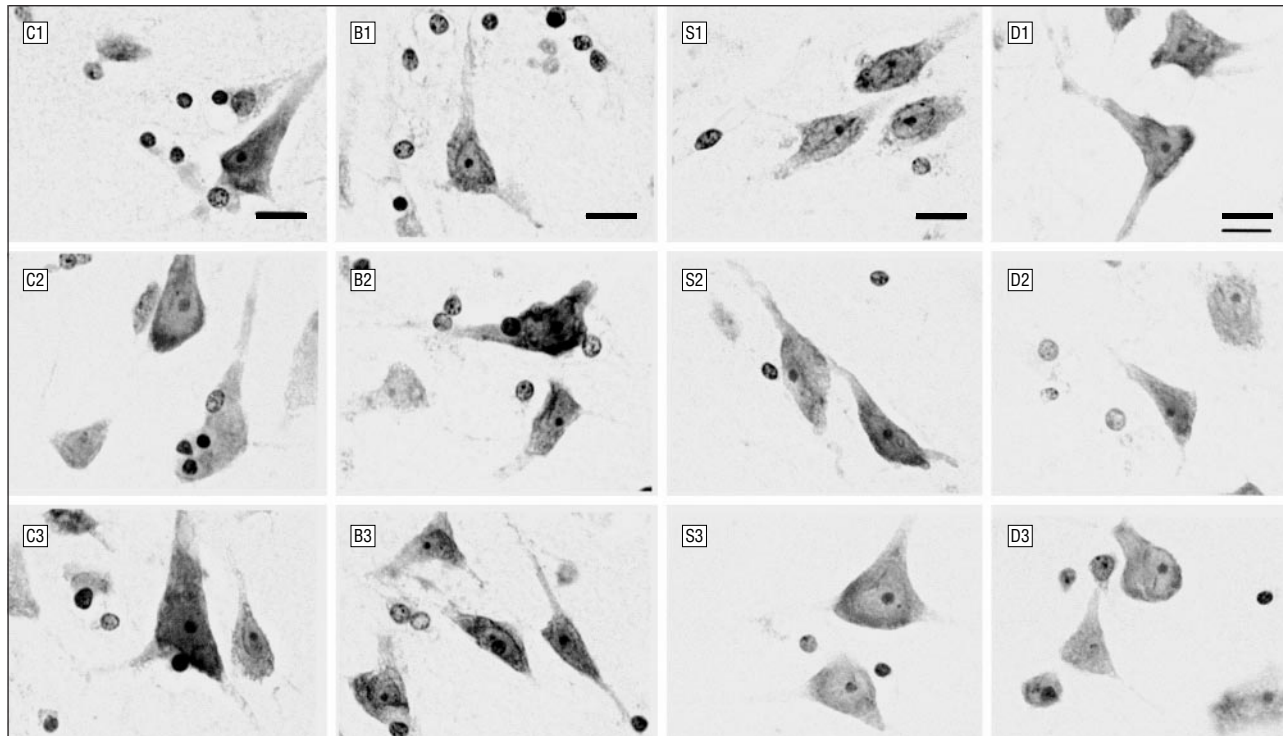
Table 5. Single 95% Confidence Intervals (CI) and *t* Tests Based on Robust SEs for Comparing Neuronal Size Between Patient Groups and the Control Group (n = 15 per Group) (Comparisons Are Adjusted for Age at Death)*

Patient Group	Cortical Layer	Test for Difference Between Patient Group and Control Group		Estimate of Effect, Estimated Ratio (95% CI)
		<i>t</i> ₅₉	<i>P</i>	
Scz (n = 15)	1	-0.47	.64	0.97 (0.83-1.12)
	2	0.32	.75	1.04 (0.83-1.3)
	3	0.77	.44	1.09 (0.88-1.34)
	5a	-1.3	.20	0.89 (0.74-1.07)
	5b	-0.84	.40	0.92 (0.75-1.13)
	6	-1.82	.07	0.85 (0.71-1.02)
BPD (n = 15)	1	-0.1	.92	0.99 (0.81-1.21)
	2	0.74	.46	1.06 (0.90-1.26)
	3	0.12	.90	1.01 (0.81-1.27)
	5a	-0.87	.39	0.91 (0.75-1.12)
	5b	-1.33	.19	0.87 (0.71-1.07)
	6	-1.91	.06	0.80 (0.64-1.01)
MDD (n = 15)	1	-1.37	.18	0.91 (0.78-1.05)
	2	1.15	.25	1.10 (0.94-1.28)
	3	-0.88	.38	0.92 (0.76-1.11)
	5a	-0.95	.35	0.90 (0.72-1.13)
	5b	-2	.05†	0.84 (0.71-1.00)
	6	-2.66	.01†	0.77 (0.63-0.94)

*Scz indicates schizophrenia; BPD, bipolar disorder; and MDD, major depressive disorder.

†Significant at the single-test significance level of 5%; not adjusted for multiple layerwise comparisons.

tially far-reaching because of the crucial roles of glial cells in neurotransmission and synaptic function,²⁻⁴ buffering neurochemical messengers,⁵ and providing metabolic support for neurons.⁷⁻⁹ Furthermore, glial cells express receptors⁶⁴ and transporters⁵ that are implicated in the monoaminergic neurotransmission abnormalities of MDD⁶⁵ and schizophrenia.⁶⁶ With regard to laminar specificity of cellular pathology, we found that glial cell density and neuronal size were significantly reduced only in layer 6. The deeper cortical layers receive noradrenergic afferents from the locus ceruleus,⁶⁷ which is implicated in the pathology of MDD,⁶⁸ and they also project via glutamatergic path-



Glia and neurons in layer 6 of the anterior cingulate cortex. Glia are small, dark, and generally round, with no stained cytoplasm. Neurons are larger, have a nucleolus visible within the nucleus and Nissl-stained cytoplasm. C1 to C3, Control subject: male, aged 44 years. B1 to B3, Case with bipolar disorder: female, aged 48 years. S1 to S3, Case with schizophrenia: male, aged 44 years. D1 to D3, Case with major depressive disorder: female, aged 52 years. Fewer glial cells are present in major depressive disorder and schizophrenia. Although not qualitatively obvious, neurons are smaller in major depressive disorder (Nissl stain; bar, 12 μ m).

ways to subcortical structures involved in the control of motor functions.⁶⁹ Therefore, our findings of altered layer 6 cytoarchitecture are consistent with the clinicopathological picture of MDD.

The glial cells sampled in this investigation do not represent a homogeneous population. They are composed of distinct populations of oligodendrocytes, microglia, and astrocytes, whose crucial role in cortical function is being actively reevaluated.¹⁻⁵ From our current data, we cannot identify which of these populations are particularly affected. Future work will be directed at identifying which of the main glial populations is deficient and whether this deficiency is primary or secondary to the disease process.

Accepted for publication December 21, 2000.

Funded by a Clinician Scientist Fellowship from the Medical Research Council, London, England (Dr Cotter), and a project award from the Theodore and Vada Stanley Foundation, Bethesda, Md. Postmortem brains were donated by the Stanley Foundation Brain Bank Consortium, Bethesda, courtesy of Llewellyn B. Bigelow, MD, Juraj Cervenak, MD, Mary M. Herman, MD, Thomas M. Hyde, MD, Joel Kleinman, MD, Jose D. Paltan, MD, Robert M. Post, MD, E. Fuller Torrey, MD, Michael Knable, MD, Maree J. Webster, MD, and Robert Yolken, MD.

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