Glial Fibrillary Acidic Protein Immunoreactivity in the Prefrontal Cortex Distinguishes Younger from Older Adults in Major Depressive Disorder

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Background: Recent postmortem studies in major depressive disorder (MDD) provide evidence for a reduction in the packing density and number of glial cells in different regions of the prefrontal cortex; however, the specific types of glia involved in those morphologic changes are unknown.

Methods: The territory occupied by the astroglial marker glial fibrillary acidic protein (GFAP) was measured as an areal fraction in cortical layers III, IV, and V in sections from the dorsolateral prefrontal cortex (dIPFC) of MDD and control subjects. In addition, the packing density of GFAP-immunoreactive somata was measured by a direct three-dimensional cell counting method.

Results: The mean areal fraction and packing density of GFAP-immunoreactive astrocytes in the dIPFC of MDD subjects were not significantly different from those in control subjects; however, in MDD there was a significant strong positive correlation between age and GFAP immunoreactivity. When the MDD group was divided into younger (30–45 years old) and older (46–86) adults, in the five younger MDD adults, areal fraction and packing density were smaller than the smallest values of the control subjects. In contrast, among older MDD subjects these parameters tended to be greater than in the older control subjects.

Conclusions: The present results suggest that the GFAP-immunoreactive astrocytes are differentially involved in the pathology of MDD in younger compared with older adults.


Key Words: Astroglia, dorsolateral prefrontal cortex, human postmortem, neuropathology, aging, quantitative immunohistochemistry.

Introduction

Alterations in the physiology and morphology of neurons or glial cells in the cerebral cortex are hallmarks of neurodegenerative disorders such as Alzheimer’s disease and Huntington disease. These alterations are manifested by loss of neurons and glial proliferation and hypertrophy. In psychiatric disorders, however, changes at the cellular level are not as prominent; consequently, strong evidence that those changes may be a part of the neuropathology of these disorders is fairly recent (Benes 1993; Bogerts 1993; Falkai et al 1988; Heckers 1997; Öngür et al 1998; Rajkowska et al 1998, 1999; Selemon et al 1995, 1998). Postmortem histopathologic studies in mood disorders reveal significant morphologic alterations in glial cells in several regions of the prefrontal cortex (Öngür et al 1998; Rajkowska et al 1999). In major depressive disorder (MDD), a significant reduction in the packing density of glial cells is observed in layers III and V of the dorsolateral prefrontal cortex among other prefrontal regions (Rajkowska et al 1999). This reduction is accompanied by an increase in the average size of glial cell nuclei in layer III. In addition, cell packing density and size of neurons are decreased in layer III of the dIPFC in MDD. Thus, structural alterations of glial cells may be related to the abnormal morphology of cortical neurons and to the gross functional (metabolic) and structural alterations observed with neuroimaging techniques in the dIPFC in MDD (for reviews, see Ketter et al 1996; Winsberg et al 2000). Nonetheless, in MDD, it is not yet known whether a specific type of neuroglia is mainly affected by the observed morphologic and functional changes.

The three major types of neuroglia found in the central nervous system, microglia, oligodendroglia, and astroglia, are known to respond to variations in the neural environment (Coyle and Schwarcz 2000; de Vellis 1993; Müller 1995). Microglia are mainly involved in immune responses within the nervous system and in the elimination of cellular debris. Oligodendroglia are crucial for the
conduction of action potentials along axons. Among glial cells, astrocytes deserve special attention because they are involved in numerous and complex functions, such as response to injury, participation in immune defense (together with microglia), promoting the transfer of nutrients to neurons, maintenance of synaptic transmission, ionic homeostasis of the neuropil, and the support of cerebral glucose metabolism (Coyle and Schwarz 2000; Magistretti and Pellerin 1999; Magistretti et al 1999). Moreover, astrocytes are known to express transporters and receptors for the neurotransmitters glutamate, γ-aminobutyric acid, serotonin, and norepinephrine (Blankenfeld and Kettenmann 1992; Cohen et al 1999; Flott and Seifert 1991; Hirst et al 1998; Kimelberg and Katz 1985; Russ et al 1996; Schousboe and Westergaard 1995; Sutin and Shao 1992). Finally, astroglia generate specific changes in plasma membrane ionic conductance and second messenger responses when exposed to these neurotransmitters (McCarthy et al 1995; Walz 1995). Thus, astrocytes are able to respond with functional and morphologic changes to alterations in the neurotransmitter systems implicated in both the pathophysiology of mood disorders and the therapeutic response to psychotropic medications.

In MDD, the packing density and size of different types of glial cells as determined by Nissl-staining is altered in several regions of the prefrontal cortex, including the dorsolateral prefrontal cortex (dlPFC; Rajkowska et al 1999). Interestingly, in cortical layer III of the dlPFC, there is a reduction in the packing density of medium-sized glial cells associated with an increase in the density of glial cells with extra large nuclei; in layer V, there is a reduction in the density of glial cells with large nuclei. Glial cells with medium to large nuclei are known to include mainly astrocytes, whereas cells with medium to small nuclear sizes are most likely to be oligodendrocytes or microglia ( Blinkov and Glezer 1968, 237–253). Enlargements in the size of glial cell nuclei occur in some neurodegenerative brain pathologies, and, in the case of astrocytes, those changes parallel the changes in GFAP expression (Berciano et al 1995; Levinson et al 1998; Weis et al 1993). Thus, the changes in the density of glial cell nuclei with the largest sizes observed in Nissl material in MDD suggest that there may be a reduction in the packing density of astrocytes in this disease, or that astrocyte morphology and the expression of the astrocytic cytoskeletal marker glial fibrillary acidic protein (GFAP) are different in MDD compared with normal control subjects. Astrocytes react to neuronal injury or degeneration by increasing the synthesis of the cytoskeletal component GFAP and by expanding and elongating their processes (Bignami and Dahl 1995; Norenberg 1996). In astrocytes, GFAP immunoreactivity also is regulated through activation of serotonergic and adrenergic receptors (Griffith and Sutin 1996; Le Prince et al 1990), which are considered to play a crucial role in the pathophysiology of mood disorders (Potter 1996). Accordingly, the aim of our study was to examine in postmortem tissue the relative extent of GFAP immunoreactivity and the packing density of GFAP-immunoreactive astrocytes in the dlPFC from subjects with MDD compared with psychiatrically normal control subjects.

**Methods and Materials**

**Human Subjects**

All human postmortem brain tissue was collected from autopsies that took place at the Cuyahoga County Coroner’s Office in Cleveland, Ohio. In all cases, retrospective psychiatric assessments were conducted in accordance with the Institutional Review Board policies, and written consent was obtained from the next of kin. Consensus diagnoses of MDD were made in accordance with the Diagnostic and Statistical Manual of Mental Disorders-Revised (DSM-III-R; American Psychiatric Association 1987). Additional information about previous hospital medical records, prior medical or substance abuse problems, medication history, and postmortem toxicology also was gathered. Evidence of head trauma, neurologic disease, or psychoactive substance use disorder within the last year of life was sufficient basis to exclude a case from the study. Only brains with a postmortem delay of less than 30 hours and a fixation time no longer than 4 years were included (Table 1). Further details about the diagnostic procedures and methods for collecting information on the subjects are provided elsewhere (Rajkowska et al 1999).

**Tissue Sampling**

The tissue samples were obtained from the left prefrontal cortex of 14 subjects retrospectively diagnosed with MDD (nonpsychotic) and 15 psychiatrically normal control subjects. Morphometric parameters were measured in the left dlPFC (Brodmann’s area 9). Previously established cytoarchitectonic criteria for area 9 (Rajkowska and Goldman-Rakic 1995a, 1995b) were used to select the cortical regions of interest for the present study. Blocks of tissue (2 × 2 cm) sampled from the dlPFC were embedded in celloidin and cut into 40 μm thick sections. Three sections evenly spaced 400 μm apart were chosen from each subject to be immunostained for GFAP. These sections were adjacent to or within 100 μm of the Nissl-stained sections that were used in our previous morphometric study demonstrating reduced glial cell packing density in MDD (Rajkowska et al 1999). The Nissl-stained sections were then used to draw the boundaries between individual cortical layers. These laminar boundaries were imposed as guides on the GFAP immunostained sections to determine the laminar distribution of GFAP-immunoreactive cells.

**Immunohistochemistry**

The specific protocol for the immunostaining of celloidin-embedded sections from human postmortem brain tissue was...
developed in our laboratory as a modification of existing protocols (Miguel-Hidalgo and Rajkowska 1999). Briefly, celloidin was removed from the sections with a saturated 50% solution of NaOH in methanol, and sections were rinsed three times in 100% methanol. The sections were then washed in phosphate buffer saline (pH 7.4) followed by Tris-HCl buffer saline (pH 7.6).

Subsequently, the floating sections were incubated with a mouse monoclonal GFAP antibody at a 1:500 dilution, and the binding of this antibody was detected with a biotinylated secondary antibody according to the ABC method (ABC kit, Vector Laboratories, Burlingame, CA). The monoclonal antibody against GFAP used as first antibody in our study was obtained

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from Sigma (catalogue no. G 3893, clone G-A-5). This antibody was originally developed in mouse using GFAP from pig spinal cord as immunogen. It specifically stains GFAP in immunoblots and in brain sections (Debus et al 1983; Franke et al 1991). In our experiments, omission of the first antibody or the biotinylated secondary antibody resulted in the absence of any immunostaining. It is important to stress that the Nissl-stained sections used in our previous study for estimates of neuronal and glial densities were closely adjacent to the sections processed for immunohistochemistry. To reduce to a minimum the variability in the intensity of staining due to uncontrollable changes from experiment to experiment, each immunostaining experiment involved sections from both the control group and the MDD group. In each of three of the experiments, 23 sections were selected per experiment, all of them processed simultaneously. Out of these sections, 11 were from different subjects of the control group, and 12 were from the corresponding subjects of the MDD group. In each of three additional experiments, six sections were selected per experiment. Out of these sections, four were from the control group, and two were from the MDD group. In total, six experiments were conducted resulting in three sections per subject used for the quantitative immunohistochemistry studies.

Extent of GFAP Immunoreactivity

The area occupied by GFAP-immunoreactivity in astrocytes on histologic sections was examined according to a modification of the method for the measurement of GFAP-immunoreactivity described by Zilles et al (1991). The area occupied by GFAP-immunoreactivity in layers III+IV and in layer V was examined. These layers were chosen for analysis because significant changes in glial cell density and glial nuclear size in these same layers have been previously reported (Rajkowska et al 1999). Layers III and IV were analyzed together since the pattern of distribution of immunoreactivity is very similar in the lower layer III and in layer IV. Moreover, in area 9 the boundary between layer III and layer IV is considerably tortuous, which prevents establishing a reliable separation between these two layers in the GFAP-immunoreactive sections. In contrast, there is a much denser appearance to GFAP immunoreactivity in layer V, which permits a reliable distinction of layer V from the neighboring layers.

An image analysis system working with images from a video camera attached to a microscope was used. A box of fixed width (1530 μm) was positioned spanning either layers III+IV or layer V. In each of those boxes, an image was obtained with the video camera and digitized into gray level images. Immunopositive structures were segmented by defining a background level in an area of the section with no specific immunoreactivity and thresholding with a fixed level of 20 gray values over the background; gray levels were from 0 (brightest) to 255 (darkest). By this procedure, a binary image of the area occupied by immunoreactivity was obtained. The areal fraction occupied by glial immunoreactivity was calculated by dividing the immunoreactive area by the total area occupied by the cortical layers in the outlined box. The immunoreactivity was expressed as a percentage of the total area. The average of three measurements obtained in the samples per subject was considered as the value of areal fraction and used for statistical analysis.

THREE-DIMENSIONAL CELL COUNTING. To estimate the number of GFAP-immunoreactive cell bodies, a direct, three-dimensional cell counting setup was used (Williams 1989; Williams and Rakic 1988). This system is based on the optical dissector method, which allows the estimation of the number of cells without introducing biases due to cell size, cell shape, or section thickness. To avoid a selective bias when counting astrocytes in GFAP-immunostained sections, a probe was consistently located in the middle of the region used to measure the areal fraction of GFAP immunoreactivity. The probe consisted of a series of consecutive boxes spanning either layers III+IV or layer V. The dimensions of each box were 25 μm thick, 60 μm wide, and 90 μm high. In layers III+IV and in layer V, the packing densities of GFAP-immunoreactive cell bodies were obtained according to the procedure described elsewhere (Rajkowska et al 1999). The packing densities of glial cells are noted as the number of cells × 10^3 /mm^3. One probe per section was sampled in each of three sections per subject. The probe contained an average of 13 boxes for layers III+IV and three boxes for layer V. The averages of all morphometric parameters measured in the three sections per brain sampled from dlPFC (area 9) were subjected to a single-factor (disease) analysis of variance (ANOVA) between the depressed and control groups. Other pairwise comparisons were performed with the Student’s t test. Differences between the groups were defined at the level of p < .05. Influence of age, postmortem delay, storage time in formalin, duration of illness and onset of illness on the areal fraction of GFAP immunoreactivity, and the packing density of GFAP immunoreactive cells was examined by Pearson correlation analysis. The same correlation analyses were used to establish the relationship between GFAP parameters and the packing densities of glial cells in the Nissl-stained sections.

Results

Distribution of GFAP Immunoreactivity: Qualitative Observations

The extent of GFAP immunoreactivity and the packing density of GFAP immunoreactive cells was highly variable in different layers of the dlPFC (Figure 1) and between subjects in both studied groups (Figure 2). This variability was particularly marked in layers III, IV, and V. At the same time, there was a clearly stratified pattern of GFAP immunoreactivity across the cortical layers (Figure 1, A and B). The outer two thirds of layer I were fully covered by GFAP immunoreactivity, and it was not possible to distinguish individual astrocytes. Long, radial, GFAP-immunoreactive processes (interlaminar processes) were observed to arise in layer I and reach as far as layer III. The upper parts of layer III contained a relatively low level of GFAP immunoreactivity that increased toward the lower part of layer III and in layer IV, becoming more
dense in layer V. In these three layers, however, the processes and cell bodies of individual astrocytes were clearly distinguishable for the most part (Figure 1, C and D). Layer VI was covered by GFAP-immunoreactive processes, and although the overall immunoreactivity appeared less intense than in layer I, in most cases it was difficult to identify individual GFAP-immunoreactive cell bodies in this layer (Figure 1A).

The high variability in GFAP immunostaining was reflected in dramatic interindividual differences in the amount of tissue covered by GFAP-immunoreactive structures and in the packing density of GFAP-immunoreactive cell bodies (Figure 2). This difference was more prominent in the MDD group. Some subjects had a very small area occupied by GFAP immunoreactivity and almost no immunoreactive cell bodies; in others, the areal fraction covered was as high as 95%, and the cell packing density was maximal (Figure 2, A and B).

**Comparison of the Areal Fraction Covered by GFAP Immunoreactivity in Control and MDD Subjects**

**LAYERS III+IV.** The areal fraction covered by the processes and cell bodies of GFAP-immunoreactive astrocytes was measured in layers III+IV and in layer V both in subjects with MDD and in the control group. In layers III+IV, the average areal fraction (%) measured in control subjects (44.84 ± 18.46; mean ± SD) was lower although not significantly different (F = 0.018, p = .895) from that in MDD subjects (46.13 ± 32.30); however, the higher SD in MDD and the distribution of values (Figure 3) suggested that the subjects with MDD could be separated into two subgroups, one of them with very low GFAP immunoreactivity, the other with high immunoreactivity.
Demographic variables of the subjects were examined for possible correlations with the widely different values of the areal fraction covered by GFAP immunoreactivity. In the MDD subjects, there was a significant positive correlation \( r = .890, \ p < .0005 \) between age and the areal fraction occupied by immunoreactivity in layers III+IV (Figure 4A). In the control group, there was a tendency toward higher GFAP immunoreactivity in older ages in layers III+IV (Figure 4B) that reached statistical significance \( r = .521, \ p < .05 \). The slope of the regression line was smaller than that in the MDD group, however. It was also remarkable that the areal fraction values (range of 2.46–19.73%) in the five youngest individuals in the MDD group were lower than the lowest value (21.8%) in the control group. Based on these results, the control and MDD groups were both sorted into an older subgroup (46–86 years old) and a younger subgroup (23–45 years old). Within the control group, comparison of the younger subgroup with the older subgroup showed that there was no significant difference in the areal fraction \( t = -0.629, \ p = .540 \); within the MDD group, however, the analogous comparison revealed a significant difference between the younger and the older subgroup \( t = 5.786, \ p < .0005 \).

Pairwise statistical comparisons revealed a significant 75% reduction in the GFAP areal fraction in layers III+IV.
between the six subjects of the younger control subgroup (41.08 ± 13.09) and the five subjects of the younger MDD subgroup (10.29 ± 7.95; Student’s t test, \( t = 4.796, p = .001 \)); however, no such significant difference was apparent between the older control subgroup (47.34 ± 21.72) and the older MDD subgroup (66.03 ± 20.39). On the contrary, a statistically nonsignificant tendency (\( t = -1.882, p = .078 \)) for higher values of the GFAP areal fraction appeared in the older MDD subgroup.

**Layer V.** The results for layer V followed a pattern similar to that observed in layers III+IV (Figure 3B). Although the average GFAP areal fraction did not differ between the studied groups, there was a highly significant positive correlation (\( r = .745, p = .002 \)) between age and the areal fraction covered by GFAP immunoreactivity (Figure 4C) in the MDD group. In layer V, however, the segregation of the MDD group into two age subgroups was even more marked than in layers III+IV. In contrast, among the control group, there was no correlation between age and the areal fraction of GFAP (\( r = .246, p = .378 \); Figure 4D). The average value of the GFAP areal fraction (38.22 ± 23.32) in the younger MDD subgroup was also significantly lower (\( t = -3.423, p < .01 \)) than in the younger control subgroup (73.35 ± 9.05). In addition, there was a nonsignificant increase in GFAP areal fraction (\( t = 1.972, p = .066 \)) in the older MDD subgroup (92.05 ± 6.99) compared with the older control subgroup (78.60 ± 19.24).

**Packing Density of GFAP-Immunoreactive Cell Bodies**

To establish whether the reduced GFAP areal fraction in the younger MDD subjects was related to a reduced packing density of GFAP-immunoreactive astrocytes, the density of GFAP-immunoreactive cell bodies was estimated in MDD and control subjects.

The packing density of GFAP-immunoreactive cell bodies in the MDD group (layers III+IV, 7.35 ± 5.6 cells × 10^3/mm^2; layer V, 9.57 ± 5.17 cells × 10^3/mm^2) was not significantly different (layers III+IV, \( F = 0.05, p = .82 \); layer V, \( F = 0.66, p = .43 \)) from that in the control group (layers III+IV, 6.96 ± 3.38 cells × 10^3/mm^2; layer V, 11.03 ± 4.55 cells × 10^3/mm^2; Figure 5, A and B); however, as in the case of the GFAP areal fraction, the packing density showed a strong significant correlation with age in the MDD group both in layers III+IV (\( r = .820, p < .0005 \)) and in layer V (\( r = .811, p < .0005 \)). In contrast, among the control group, there was no significant correlation with age (layers III+IV, \( r = .256, p = .358 \); layer V, \( r = .079, p = .780 \); Figure 6, A and B; graphs for layer V not shown).

The packing density of GFAP-immunoreactive cell bodies in layers III+IV was also positively correlated with the areal fraction of GFAP immunoreactivity in both the control group (\( r = .578, p < .03 \)) and the MDD group (\( r = .824, p < .0005 \)).

The areal fraction and the packing density of GFAP-immunoreactive cells in layers III+IV and in layer V were not correlated either with the postmortem interval or the time in formalin in either the MDD group or the control group. Although the average time the brain tissue was stored in formalin was significantly higher in the control group than in the depressed group (Table 1), there was no significant correlation of areal fraction or packing density and the time in formalin (for all comparisons \( p > .2 \)).

**Correlation of GFAP Immunoreactivity with the Duration and Onset of Illness**

The duration of illness was highly variable among the subjects (0.25–50 years; see Table 1), and the longest periods occurred in some of the oldest MDD subjects. Nevertheless, duration of illness was not significantly correlated with age (\( r = .429, p = .126 \)). Duration of illness was, however, significantly correlated with the packing density of GFAP-immunoreactive cell bodies in
layers III+IV \( (r = .679, p < .01) \) and in layer V \( (r = .604, p < .03) \); Figure 6, C and D). By contrast, neither the areal fraction (layers III+IV \( r = .487, p = .077 \); layer V \( r = .343, p = .231 \)) nor the GFAP cell packing density (layers III+IV \( r = .264, p = .361 \); layer V \( r = .319, p = .267 \)) was significantly correlated with the age of onset of the disease.

**Comparison with Nissl Morphometric Studies of Glia**

Among MDD subjects, 12 out of 14 individuals included in our study were the same as those used previously in the morphometric analysis of the dlPFC in MDD (Rajkowska et al 1999). In that study, the packing density of the entire population of glial cells stained nonspecifically with cresyl violet for the Nissl method was markedly reduced in the MDD group compared with control subjects. Moreover, when the Nissl-stained glia were divided into four size classes (small, medium, large, or extra large based on their nuclear size; details on size criteria are described in Rajkowska et al 1998, 1999), the reductions in densities were confined to extra large, large, and medium classes of glia. Therefore, we tested the hypothesis that the densities of specific cell classes in the Nissl-stained material correlated with the measures of GFAP immunoreactivity reported in our present study. Because all the samples in both studies were taken from the same region of area 9 and they were adjacent to one another, the areal fraction and packing density of GFAP-immunoreactive astrocytes in layers III+IV and in layer V were directly compared with the packing densities of the different size classes of glial cell nuclei studied in neighboring Nissl-stained sections. The comparison revealed that the GFAP areal fraction and the GFAP packing density in layers III+IV was positively correlated with the density of Nissl-stained large glial nuclei in all cortical layers combined (GFAP area \( r = .590, p < .05 \); GFAP packing density \( r = .636, p < .03 \)). The two GFAP parameters were also significantly correlated with the density of Nissl-stained large glial nuclei in layer III (GFAP area \( r = .622, p < .04 \); GFAP packing density \( r = .604, p < .04 \)). In layer V, the packing density of GFAP-immunoreactive astrocytes was also significantly correlated with the density of large glial nuclei in all layers combined \( (r = .640, p < .03) \) and in layer Va \( (r = .591, p < .05) \). In contrast, GFAP areal fraction and GFAP packing density were not correlated with the density of any of the other glial size classes (small, medium, extra large) measured in Nissl-stained preparations.

Reexamination of the data from the previous study in Nissl-stained sections showed a tendency, although non-
significant, for an age-dependent higher density of large glial nuclei in all layers combined \( (r = .508, p = .092) \) and in sublayer IIIa \( (r = .540, p = .07) \). Moreover, this correlation was significant when layers III \( (r = .591, p < .05) \) and V \( (r = .586, p < .05) \) and sublayers IIIc \( (r = .636, p < .03) \) and Va \( (r = .589, p < .05) \) were analyzed separately. In contrast, the packing density of small, medium, or extra large nuclei did not show any tendency to be correlated with age \( (p > .3) \).

**Discussion**

Our present study tested the hypothesis that changes in GFAP-immunoreactive astroglia in the dorsolateral prefrontal cortex are involved in the histopathology of MDD. The results revealed that the area occupied by GFAP immunoreactivity and the packing density of GFAP-immunoreactive astrocytes were strongly correlated with age in layers III+IV and in layer V in the MDD group; in the control group, however, the correlation was weaker, only reaching statistical significance for the areal fraction but not for packing density. The data further indicate that GFAP-positive structures are greatly reduced in depressive subjects younger than 45 years old, compared with both older depressive subjects and with the nonpsychiatric control group. In the MDD group, there was a tendency, although nonsignificant, for higher values of GFAP immunoreactivity in older subjects compared with the older subjects of the control group. Thus, the main difference between the MDD and control groups appears to rest on a very low density of GFAP immunoreactive structures in the younger depressives (under 45 years old).

Other studies in the normal aging human brain using a larger number of subjects also indicate an age-dependent increase in the amount of GFAP immunoreactivity and GFAP mRNA in the neocortex and hippocampus (David et al 1997; Nichols et al 1993; Takashima and Becker 1983). These studies also report a high interindividual variability not explainable solely by age, however. In addition, the age-dependent increases in GFAP immunostaining are more marked in some brain regions than in others. They are more prominent in the hippocampus than in the neocortex (David et al 1994, 1997). They tend also to be noticeable only after the sixth decade of age (David et al 1997; Hansen et al 1987; Nichols et al 1993). On the other hand, the great age-independent variability of GFAP content in the brain of normal subjects is understandable in the light of animal studies showing that the richness of the environment, and the metabolic and nutritional status of the aging animals greatly influence the age-related increase in the GFAP level (Morgan et al 1999; Soffie et al 1999). It is proposed that in the brain of subjects diagnosed with MDD, the environmental circumstances related to their behavior and the brain metabolic and functional changes may influence the expression of GFAP in the frontal cortex. Conversely, a primary defect in the expression of GFAP or astrocytic function could influence the normal functioning of neurons and result in metabolic and structural alterations; however, this line of argument would not apply to the older depressive subgroup in our study, which showed a nonsignificant tendency for higher GFAP immunoreactivity than did the older control subjects.

One way to make compatible the low GFAP content in younger depressives with a high content in older adults would be to consider the lack of GFAP-immunoreactive astrocytes in younger depressives as a reflection of lowered activity or responsiveness in these cells. This diminished activity would lead to a reduced support of neuronal activity during the life span of the depressive subject as MDD progresses. Eventually, the lack of support would produce degenerative features in neurons and their connections that would trigger an enhanced response in the GFAP-immunoreactive astrocytes. Additionally, the enhanced GFAP response might be compounded with a normal age-related increase in the expression of GFAP. Supporting the above explanation is the fact that both in layers III+IV and in layer V, there is a significant positive correlation between the packing density of GFAP-immunoreactive astrocytes and the estimated duration of illness. Thus, the absence of correlation with the onset of disease further implies that even depressed subjects with a relatively early onset of disease would increase dramatically the content of GFAP-immunoreactive elements with a long duration of depression.

An alternative explanation for the age-dependent differences in GFAP immunoreactivity, not incompatible with those outlined above, is that functional alterations in the prefrontal cortex of the youngest MDD subjects are brought about by cellular mechanisms different from those in older MDD subjects. For instance, it is well established that vascular diseases increase the chance of developing depression, and vascular alterations are more prominent in the elderly (Alexopoulos et al 1997; Krishnan et al 1995, 1997; Kumar 1993). Because the rate of suicide and the overall mortality is considerably higher in depressives than in the general population (Clayton 1985), MDD subjects with a late onset of depression probably are disproportionately represented among the older depressives. Nonetheless, the number of subjects in each group of our study was not sufficient to have a significant representation from each decade of the life span. Consequently, further studies comparing groups at different ages are critical to distinguish between idiopathic depression and old age–related depression and to ascertain their
relationship to age-dependent differences in the prefrontal glial pathology of MDD.

Another possibility that cannot be ruled out is that prolonged antidepressant treatment increases astrocyte activation and GFAP expression. Most MDD subjects in our study had received treatment with antidepressants for variable periods of time and different combinations of psychotropic medications. Nearly all effective antidepressant drugs predominantly target the adrenergic and serotonergic neurotransmitter systems (for review, see Potter 1996), and there is evidence that changes in those systems influence the astroglial reaction and the expression of GFAP. Formation of reactive astrocytes in vivo is influenced by noradrenergic axons (Griffith and Sutin 1996), and blockade of β-adrenergic receptor greatly reduces reactive astrocyte formation, as determined by GFAP labeling (Sutin and Griffith 1993). Conversely, in the absence of injury, β-adrenergic receptor activation increases GFAP immunoreactivity (Hodges-Savola et al 1996). By contrast, serotonin decreases GFAP immunostaining in cultured brainstem astrocytes (Le Prince et al 1990). The direct influence of antidepressants on glial morphology has not been studied to date. Interestingly, increases in glial cell packing density have been reported recently in the prefrontal cortex of rhesus monkeys treated with neuroleptics (Selemon et al 1999); however, the augmentation was detected only in layer I (with typical antipsychotics) or layer IV (with atypical antipsychotics).

An argument could be made that our data are biased because of some unknown effect of prolonged exposure to formalin. Increasing the time in formalin tends to reduce immunoreactivity for some antigens (Battifora et al 1986); however, in a previous study using the same antigen recovery technique as that in our present study, we found no evidence of reduced immunoreactivity for various antigens in brain tissues from normal control subjects with periods in formalin up to 2 years (Miguel-Hidalgo and Rajkowska 1999). Furthermore, in our present study, the lowest values of immunoreactivity parameters were found in the MDD group, in which also the lowest values for areal fraction and packing density of GFAP immunoreactive were observed. This fact, together with the lack of any correlation or tendency toward correlation between GFAP immunoreactivity and fixation time, indicates that the differences in GFAP immunostaining found in our material cannot be attributed to differences in the fixation times.

Size Classes of Glial Cells and GFAP Immunoreactivity

Comparison of the present findings with the data from our previous study on Nissl-stained sections sampled from the same cortical area in MDD subjects suggests that the glial cell nuclei classified as large in that study correspond to the GFAP-immunopositive elements in our present study. This would further implicate that a reduced GFAP immunoreactivity coincides with a reduced packing density of astrocytes with large nucleus and, consequently, that changes in GFAP expression are related to changes in the size of the astrocyte nucleus. Because GFAP immunoreactivity increases with age, an age-related increase in the packing density of astrocyte nuclei should also be expected in MDD. In fact, a close examination of the data from our previous Nissl study revealed a significant correlation between age and the density of large glial cell nuclei in layer III and layer V; however, no correlation between age and the density of any of the other glial size classes (small, medium, and extra large) was found.

The involvement of the cells with large nuclei (probably astrocytes) in MDD suggested by the combined analysis of data from our present study and our previous report does not rule out the participation of other glial cell types in the pathophysiology of MDD. In our previous Nissl study, the density of medium-sized and extra large glia in layer III, and medium and large glia in layer V, was significantly different in the MDD brains compared with control brains. By contrast, in our present study, there was no significant difference in the average areal fraction or packing density of GFAP positive astrocytes either in layers III+IV or in layer V. These contrasting findings suggest that other types of glia (microglia or oligodendroglia) also might be implicated in the histopathology of major depression.

Comparison with Other Psychiatric Disorders

In schizophrenia, possible changes in GFAP have been examined with different methods of assessing in situ GFAP immunoreactivity (Arnold et al 1996; Falkai et al 1999; Roberts et al 1987). These studies reveal an absence of GFAP-related gliosis in schizophrenia, which is consistent with the lack of significant changes in the density of Nissl-stained glial cells or sizes or glial nuclei in postmortem brains of schizophrenics (Rajkowska et al 1998; Selemon et al 1995). The absence of major changes in glial parameters in schizophrenia would predict a relationship between GFAP immunoreactivity and age similar to the one observed in normal control subjects. This correlation has not been tested, however. Future studies on the relationship between GFAP expression and age in schizophrenia are needed to ascertain that prediction.

Glial density and number were analyzed recently in another type of mood disorder: bipolar illness (BPD). The only two postmortem studies published to date on cell morphometry in BPD uniformly report reductions in glia in the BPD prefrontal cortex similar to those
observed in corresponding regions of MDD (Öngür et al 1998; Rajkowska et al 1997; G. Rajkowska et al, unpublished data). In the subgenual prefrontal cortex of subjects with familial bipolar disorder, Öngür et al (1998) have demonstrated a decrease in the number and density of glial cells, although in this study neither a distinction between different cortical layers nor an analysis of specific size classes in individual layers was made. In contrast, analysis of Nissl-stained cells in individual layers of dIPFC revealed a reduction in glial cells with medium-sized nuclei in all six cortical layers (G. Rajkowska et al, unpublished data). Interestingly, no reductions in the other size classes of glial cells were observed in this material. In contrast to BPD, reductions in packing density in MDD involve both medium-sized and large glial cells (Rajkowska et al 1999). Thus, it seems that different types of glial cells are involved in MDD and BPD or that the changes in these types are specific for each mood disorder. Further research with specific markers or methods to distinguish types of neuroglia and with larger samples of subjects are needed to ascertain the relative involvement of the various types of glial cells in the neuropathology of different mood disorders. A link between the possible involvement of different glial cell types in MDD and BPD and the different medications used in the treatment of each of these disorders also remains to be ascertained.

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