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# Glial fibrillary acidic protein-immunoreactive astrocytosis in elderly patients with schizophrenia and dementia

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**Abstract** Clinical and neuropsychological studies of chronically institutionalized patients with schizophrenia indicate that severe cognitive impairment and functional disability in late life are very prevalent. The biological substrates for this dementia remain unknown. While subtle cytoarchitectural and morphometric abnormalities have been described in patients with schizophrenia and interpreted as reflecting aberrant neurodevelopment, postmaturational injury or neurodegeneration associated with gliosis remain as plausible explanations of at least some of the clinical manifestations of schizophrenia. We monitored astrocytosis and neurofibrillary tangle (NFT) formation in 21 elderly patients with schizophrenia (14 with concurrent dementia, 7 without), and in 12 normal and 5 Alzheimer's disease (AD) control cases. Astrocytes in ventromedial temporal, frontal, and calcarine cortices were immunohistochemically identified with monoclonal antibodies directed at glial fibrillary acidic protein (GFAP) and vimentin, and NFTs were labeled with an anti-tau antibody specific for paired helical filaments. There were no increases in GFAP- or vimentin-immunoreactive astrocyte counts, GFAP optical density, or NFT counts for the schizophrenic group as a whole compared to the non-neuropsychiatric group, while both groups differed from AD. When patients with schizophrenia were divided into demented and non-demented subtypes, those with dementia demonstrated significantly greater numbers of GFAP-positive astrocytes than those without dementia. These data may reflect an up-regulation of GFAP in normal astrocytes or the presence of reactive astrocytosis in a subgroup of schizophrenics. In the absence of any diagnostic neuropathological findings in this subgroup, the implications of these observations for the pathogenesis of schizophrenia remain to be determined.

**Key words** Vimentin · Neurofibrillary tangle · Subiculum · Orbitofrontal cortex

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## Introduction

Recent clinical and neuropsychological studies of elderly, chronically institutionalized patients with schizophrenia have identified a high prevalence of severe cognitive impairment and functional disability. Davidson and colleagues [24, 36, 37] reported that negative symptomatology progressively increases with age to a severe degree of impairment in association with a slow decline in cognitive function on the Mini-Mental State Examination (MMSE) [30]. In a demographically similar population of elderly patients with schizophrenia who are participants in a prospective clinicopathological studies program, we reported severe illness on the Brief Psychiatric Rating Scale (BPRS [50]), severe cognitive impairment on the MMSE, severe negative symptomatology on the Scale for the Assessment of Negative Symptoms (SANS [4]), and severe functional impairment on the Physical Self-Maintenance Scale ("Functional Assessment Scale", FAS [44]), all of which correlated with age [12]. Mild to questionable positive symptomatology on the Scale for the Assessment of Positive Symptoms (SAPS [5]) was observed which did not correlate with age. Psychometric study of this group [compared to a sample of Alzheimer's disease (AD) patients matched for age, education, and global dementia severity] with the CERAD neuropsychological battery

[47] revealed a neuropsychological profile of dementia that is similar to that of primary degenerative dementia [32, 46].

The cause of the cognitive and functional impairments seen in this population of schizophrenia patients remains unknown. Post-mortem studies have attempted to identify a neuropathological basis for dementia in elderly schizophrenia patients. For example, Arnold et al. [10] and Purohit et al. [53] conducted autopsy examinations on the brains of such patients but no diagnostic neuropathological abnormalities were identified that might explain the severe dementia. Indeed, there was a remarkable paucity of neurofibrillary tangles (NFTs) and senile plaques (SPs) that are characteristic of AD, even though abundant NFTs and SPs are seen in 50–60% of a general population-based dementia sample [43]. Further studies failed to identify excess AD-related protein [51] or decreased cholinergic activity [35]. These findings are in agreement with two other studies which used carefully diagnosed patients [18, 27], but they differ from several other studies which found an increased prevalence of AD, albeit in less well characterized patients with schizophrenia [52, 67]. Davidson et al. [24, 37] proposed that the cognitive decline in schizophrenia in late life was due to developmental events rather than a degenerative process because of the slow rate of decline in MMSE scores in their studies and the lack of neurodegenerative disease pathology at autopsy.

Data from post-mortem studies of gliosis in the central nervous system (CNS) have figured prominently in discussions of developmental versus degenerative or post-maturational processes in schizophrenia. In the post-natal brain, reactive astrocytosis occurs as an acute, dynamic, non-specific response to injury due to a variety of causes [26, 49]. It is characterized by increased expression of glial fibrillary acidic protein (GFAP) and increased GFAP mRNA levels, proliferation and somatic enlargement of astrocytes, and extension and thickening of astrocytic processes. In addition, vimentin, a developmentally regulated intermediate filament protein normally seen in fetal neurons and glial cells [7, 21], is often re-expressed in reactive astrocytes, albeit at lower levels than GFAP. Studies of schizophrenia in younger patients using traditional histological stains (e.g., Holzer's) have reported focal gliosis in several subcortical areas, most notably in periventricular and periaqueductal regions [48, 59]. In contrast, reports using immunohistochemistry with antibodies directed against GFAP have so far failed to find any evidence of gliosis [19, 54, 55, 58]. Special attention has been paid to the presence or absence of gliosis in the ventromedial temporal lobe where a number of cytoarchitectural and morphometric abnormalities have been reported [8, 11, 16, 23, 29, 39, 42]. The failure to find evidence of gliosis in this region has been interpreted to support the hypothesis that abnormal development rather than post-maturational injury or neurodegeneration is the basis for schizophrenia, since the latter processes would be associated with gliosis.

Whether there is gliosis in schizophrenia or not remains controversial. It has been argued that the GFAP-immunohistochemical methods that failed to find gliosis in schizophrenia are less sensitive than the Holzer's method for demonstrating chronic, fibrillary gliosis [61], especially in the archival, aldehyde-fixed, paraffin-embedded tissue used in most studies to date. On the other hand, the traditional stains lack molecular specificity and are capricious, and thus could be staining non-specifically [61].

To overcome these problems and to determine if gliosis is present in the CNS in schizophrenia, we probed GFAP, vimentin, and paired helical filament (PHF) immunoreactivity in ventromedial temporal, frontal, and visual cortices in a prospectively accrued, chronically institutionalized sample of elderly patients with schizophrenia. Many of the patients exhibited a deterioration in cognition and functional abilities in the years prior to death sufficient to warrant an additional diagnosis of primary degenerative dementia. This population may be particularly instructive to examine neuropathologically since these patients required chronic hospitalization for many years and are likely to represent the severe end of the schizophrenia spectrum or a subgroup with particularly malignant symptoms. Thus, neuropathological findings would be expected to be more evident than in a less-afflicted sample. Further, if accumulated neural injury or neurodegeneration is part of the pathophysiology of schizophrenia, then this would be most prominent in an elderly group.

## Materials and methods

### Case material

Brain tissue was obtained at autopsy from 21 patients with chronic schizophrenia, 5 patients with AD, and 12 non-neuropsychiatric controls. Demographic data on these subjects are found in Table 1. Patients with schizophrenia were derived from our prospective accrual registry of elderly patients who had been chronically institutionalized with a primary psychiatric diagnosis of schizophrenia. Diagnoses of schizophrenia and primary degenerative dementia

**Table 1** Demographic data. Neuroleptic exposure represents chlorpromazine equivalent daily dosage 1 month prior to death (*N/A* not applicable, *PMI* post-mortem interval)

| | Non-neuropsychiatric controls | Schizophrenia/ dementia | Schizophrenia/ no dementia | Alzheimer's disease |
|---|---|---|---|---|
| *n* | 12 | 14 | 7 | 5 |
| Age (years) | 74.7 (3.8) | 82.2 (2.4) | 73.7 (2.4) | 77.6 (2.2) |
| Sex (M/F) | 5/7 | 6/8 | 3/4 | 3/2 |
| PMI (h) | 11.5 (1.6) | 10.2 (0.8) | 18.4 (2.8) | 10.3 (1.4) |
| Duration of illness (years) | N/A | 58.4 (2.6) | 47.4 (2.6) | N/A |
| Neuroleptic exposure (mg) | N/A | 171 (111) | 240 (103) | N/A |

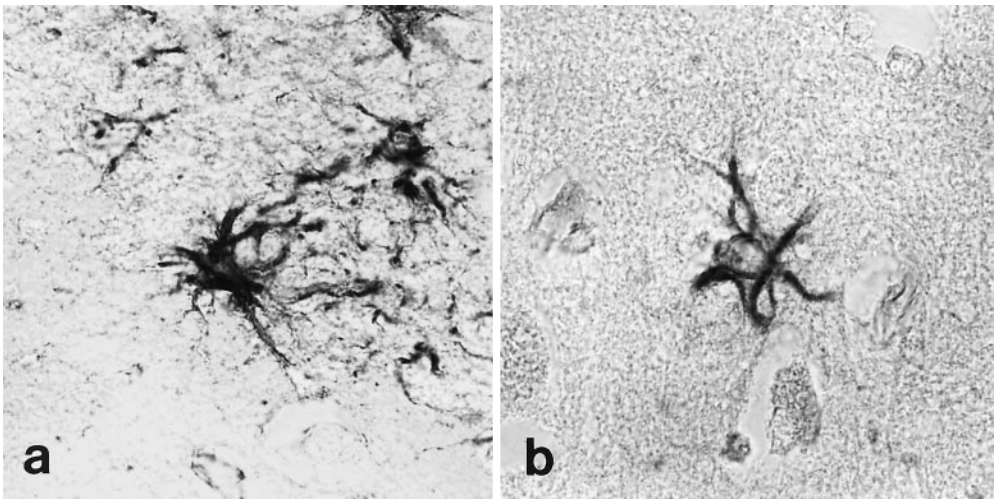


**Fig. 1** Astrocytes immunohistochemically labeled for **a** glial fibrillary acidic protein (GFAP); and **b** vimentin. **a**, **b** × 1000

were determined by application of DSM-III-R criteria [3] to clinical symptoms documented in hospital records, and by interviews with caretakers and family, as previously described [12]. Diagnosis of AD was based on National Institute of Aging Consensus Criteria [41]. As can be seen in Table 1, the groups did not differ with regard to age [$F(2, 35) = 0.83$, not significant (NS)], post-mortem interval (PMI) [$F(2, 35) = 0.52$, NS], or gender ($\chi^2 = 0.55$, $df = 2$, NS).

## Tissue processing and immunohistochemistry

After whole hemisphere coronal sectioning, blocks from the ventromedial temporal lobe, midfrontal gyrus, gyrus rectus, and calcarine fissure were dissected, fixed in ethanol (70% EtOH, 150 mM NaCl) for 24 h and paraffin-embedded according to a previously described protocol [57]. Sections (6 μm thick) were processed for immunohistochemical identification of astrocytes with 2.2B10, a monoclonal antibody with demonstrated specificity for GFAP that has been used extensively in biochemical and immunocytochemical studies [63]. In addition, we identified AD-related neurofibrillary pathology with PHF-1, a monoclonal antibody that labels abnormally phosphorylated tau, the principal component of PHF [33] and monitored vimentin-immunoreactive astrocytosis in hippocampal sections with anti-vimentin (DAKO). Immunocytochemistry was performed using a previously described peroxidase-antiperoxidase/diaminobenzidine (PAP/DAB) procedure [6, 9], in single, precisely timed runs for each antibody and region.

## Selection of regions and image analysis

Guided by examination of adjacent Nissl-stained sections, eight regions of interest were delineated by cytoarchitectural criteria [1] and chosen for quantitative analyses. These regions were: the inner and middle thirds of the molecular layer of the dentate gyrus (DG), CA3, CA1, subiculum, layers II and III of the entorhinal cortex (EC), layer V of midfrontal cortex, layer V of orbital frontal cortex, and layer IVc of primary visual cortex. For the hippocampal region, sections from the rostral-most hippocampal block in which all ammonic subfields, the DG, and the EC together were selected for processing and analysis. This occurs approximately at the level of the posterior mamillary bodies and is composed of mid-hippocampus and the posterior portion of the EC. In most cases at this level, a transitional cytoarchitecture from the EC to posterior parahippocampal cortex was evident, so sections from the next most rostral block were used for the EC regions. The EC was defined cytoarchitecturally on the basis of clusters of stellate neurons in layer II [45] and the presence of a distinct lamina dissecans and corresponded to cytoarchitectural subdivision area 28a [66] or $E_C/E_{CL}$ [2].

After coding slides for blind measurements, each region was visually scanned with a low-power objective (× 2.5). The boundaries of CA3, CA1, and subiculum were determined by cytoarchitectural criteria [1], and a central field selected along the horizontal and vertical axes. The EC was divided into lateral and medial domains of equal breadth, and fields at the horizontal center of each domain chosen for analysis. For EC layer II, the full vertical depth and horizontal width of each cluster was delineated as the field. For EC layer III, the superficial-most portion of the layer (that portion closest to layer II) along the vertical axis was chosen. Neurons at this level, together with EC layer II neurons, serve as the origin of the perforant pathway [64]. For midfrontal cortex layer V, orbital frontal cortex layer V, and visual cortex layer IVc, the field was captured at the vertical center of the cortical layer. Without moving the slide from the chosen position, the objective was then raised to × 20 to obtain a field in the region's central portion. A Sony CCD 72 video camera module attached to a Leitz DM RB Research Microscope captured and transferred the field to a Macintosh Quadra 700 using Image 1.55 software (W. Rasband, NIH). The two immediately adjacent microscopic fields were also captured for each region of interest. Because the clusters of stellate cells that constitute EC layer II are discontinuous, the two immediately adjacent clusters, rather than fields, were selected. Measurement values from all the EC regions and both DG regions were averaged into composite EC and DG values, respectively, for analysis.

Images of the captured fields from the 38 cases underwent analyses in random order by a single operator. GFAP-positive astrocytes and PHF-1-positive tangles were counted in the three × 20 fields representative of each region, and vimentin-positive astrocytes counted in each hippocampal region. Astrocytes were identified according to criteria which included the presence of positive immunolabeling, a visible nucleus and characteristic processes (Fig. 1). In addition, the optical density (OD) of GFAP labeling was assessed in each selected region. The illumination of the blank, coverslipped portion of each slide was adjusted to a standard level before capture of the selected fields, a flat-field correction for unevenness in background illumination was applied and then a mean gray value of each field was determined and converted to an OD value, which served as the basis of analysis.

## Statistical analyses

To assess for normal aging and PMI effects, correlations between age, PMI, and all neuropathological measures were examined within the non-neuropsychiatric control group. Within the schizophrenia group, patients were further subdivided into those with dementia and those without dementia, and these subgroups were

compared with two-tailed *t*-tests for age, PMI, duration of illness, and neuroleptic exposure. Any significant between-group differences or correlations for these potentially confounding variables prompted their inclusion as regressors in subsequent analyses. Group differences for each neuropathological measure were analyzed in separate ANOVAs (or ANCOVAs) with diagnostic group as the independent variable and neuropathological measure in different regions as the repeated-measures dependent variable. These were followed by post hoc Scheffe tests to assess individual between-group differences, and individual ANOVAs with each region's measure as the dependent variable to assess regional differences. The association of a neuropathological measure with dementia or non-dementia subtypes within the schizophrenia group was analyzed in a repeated-measures ANCOVA with subtype as the independent variable and neuropathological measure as the independent variable followed by post hoc one-tailed *t*-tests of individual regions, with the hypothesis being that the dementia subgroup would have greater astrocytosis or NFT values. Analyses were performed using Statview 4.1 and SuperANOVA statistical software (Abacus, Berkeley).

## Results

Assorted minor abnormalities had been noted on diagnostic neuropathological examination in two of the non-neuropsychiatric control cases (lacunar infarcts; small contusions of temporal poles) and four of the schizophrenic cases (left lateral occipital lobe infarct; right cerebellar infarct, posterior fossa meningioma, left acoustic neuroma; lacunar infarct; lacunar infarct). None of these findings can account for the patient's mental status nor do they influence the neuropathological measures in the regions evaluated in this study, all of which were remote from these abnormalities. Analyses were performed both including and excluding these cases and they yielded equivalent results.

Within the normal control group, a correlation of age with GFAP astrocyte count occurred only in visual cortex ($r = 0.57$, $P = 0.05$) and GFAP OD only in subiculum ($r = 0.68$, $P = 0.01$). There were no significant age correlations for GFAP astrocyte count and OD for any other region, nor for PHF-1 NFT or vimentin-immunoreactive astrocyte counts in any region, and there were no significant correlations for PMI. Individual *t*-tests also revealed no sex differences within the non-neuropsychiatric group for any measure in any region.

Within the schizophrenia group, patients with dementia were older than those without dementia [$t$ (19) = –2.251, $P = 0.04$] and the former patients had a shorter PMI [$t(19) = 3.634$, $P = 0.02$]. Because of these differences, age and PMI were used as regressors in subsequent

**Table 2** Measurements of astrocytosis. All values shown are mean ± SEM

| | | Normal ($n$ = 12) | Schizophrenia/ no dementia ($n$ = 7) | Schizophrenia/ dementia ($n$ = 14) | AD ($n$ = 5) |
|---|---|---|---|---|---|
| GFAP-positive astrocyte counts (per mm$^2$) | Entorhinal cortex | 15.3 ± 4.6 | 8.9 ± 2.8 | 26.8 ± 12.0 | 151.7 ± 33.6 |
| | Subiculum | 18.0 ± 4.7 | 11.8 ± 3.4 | 39.3 ± 6.8 | 95.6 ± 26.4 |
| | CA1 | 8.9 ± 6.8 | 3.1 ± 1.8 | 9.2 ± 5.9 | 74.3 ± 26.5 |
| | CA3 | 124.6 ± 15.8 | 75.6 ± 21.2 | 114.1 ± 21.2 | 154.4 ± 27.0 |
| | Dentate gyrus | 17.9 ± 6.4 | 35.8 ± 14.1 | 24.3 ± 6.3 | 93.9 ± 30.1 |
| | Midfrontal cortex | 63.2 ± 13.2 | 69.8 ± 11.1 | 86.5 ± 11.1 | 122.3 ± 31.7 |
| | Orbital frontal cortex | 36.5 ± 8.2 | 17.6 ± 5.4 | 53.8 ± 11.2 | 95.6 ± 22.0 |
| | Visual cortex | 60.9 ± 9.2 | 44.6 ± 16.8 | 70.2 ± 12.9 | 140.0 ± 34.1 |
| GFAP-positive optical density | Entorhinal cortex | 0.021 ± 0.002 | 0.024 ± 0.003 | 0.027 ± 0.002 | 0.060 ± 0.014 |
| | Subiculum | 0.018 ± 0.002 | 0.019 ± 0.003 | 0.029 ± 0.004 | 0.046 ± 0.003 |
| | CA1 | 0.015 ± 0.001 | 0.013 ± 0.002 | 0.012 ± 0.001 | 0.033 ± 0.012 |
| | CA3 | 0.033 ± 0.003 | 0.039 ± 0.005 | 0.042 ± 0.004 | 0.048 ± 0.007 |
| | Dentate gyrus | 0.026 ± 0.002 | 0.033 ± 0.003 | 0.033 ± 0.005 | 0.045 ± 0.006 |
| | Midfrontal cortex | 0.023 ± 0.003 | 0.030 ± 0.005 | 0.027 ± 0.004 | 0.035 ± 0.007 |
| | Orbital frontal cortex | 0.025 ± 0.001 | 0.027 ± 0.004 | 0.029 ± 0.003 | 0.035 ± 0.004 |
| | Visual cortex | 0.022 ± 0.002 | 0.027 ± 0.005 | 0.026 ± 0.004 | 0.053 ± 0.013 |
| Vimentin-positive astrocyte counts (per mm$^2$) | Entorhinal cortex | 0.7 ± 0.5 | 0.0 ± 0.0 | 1.1 ± 0.8 | 25.8 ± 12.7 |
| | Subiculum | 2.0 ± 1.2 | 0.0 ± 0.0 | 0.2 ± 0.2 | 5.9 ± 2.7 |
| | CA1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.2 ± 0.2 | 6.6 ± 3.4 |
| | CA3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Dentate gyrus | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| PHF-1-positive tangle counts (per mm$^2$) | Entorhinal cortex | 13.3 ± 6.3 | 35.0 ± 17.7 | 14.7 ± 5.4 | 97.3 ± 22.9 |
| | Subiculum | 2.1 ± 1.4 | 7.4 ± 2.8 | 4.0 ± 1.1 | 23.9 ± 11.1 |
| | CA1 | 4.2 ± 2.0 | 6.2 ± 2.4 | 5.9 ± 2.1 | 59.4 ± 14.3 |
| | CA3 | 1.3 ± 1.1 | 0.6 ± 0.4 | 0.4 ± 0.2 | 10.6 ± 5.7 |
| | Midfrontal cortex | 0.1 ± 0.1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 38.7 ± 15.0 |
| | Orbital frontal cortex | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 25.9 ± 10.0 |
| | Visual cortex | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.6 ± 2.6 |

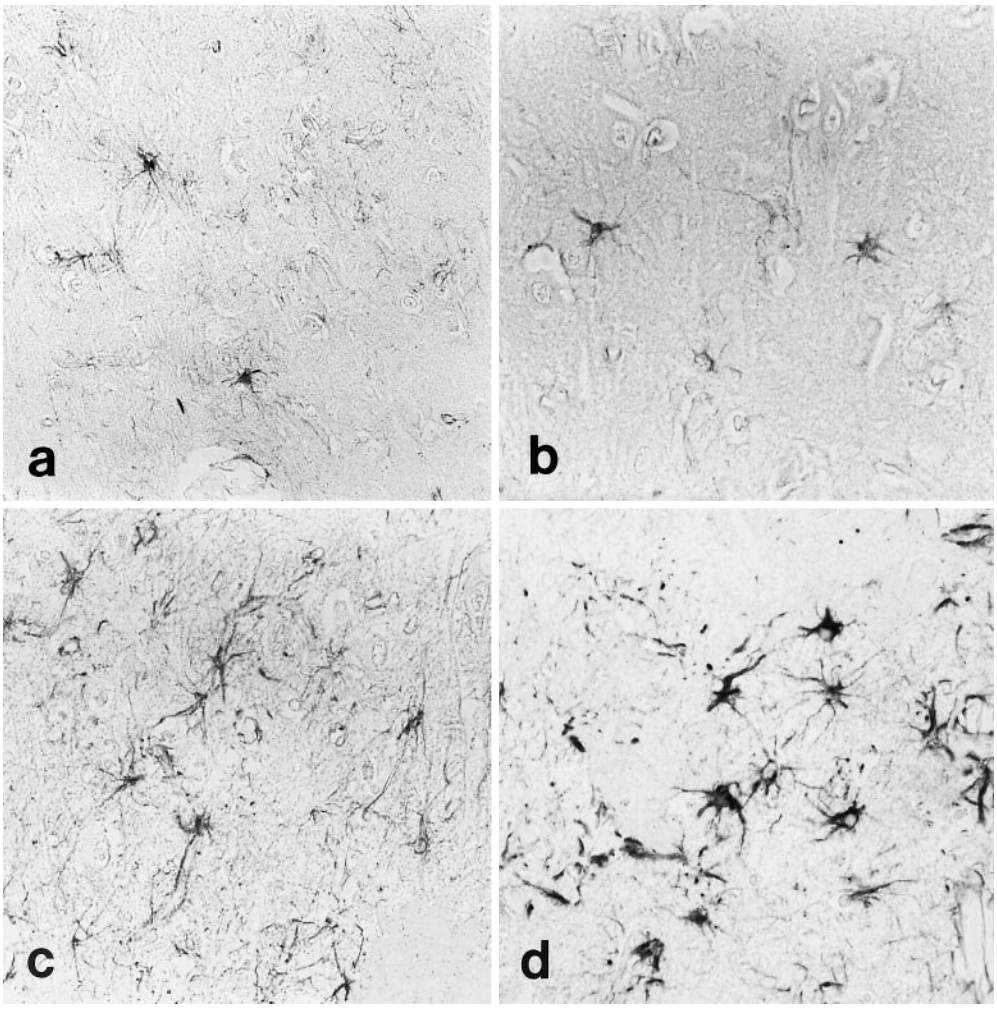


**Fig. 2 a–d** GFAP-immunoreactive astrocytosis in sections from the subiculum. **a** A 91-year-old normal control subject; **b** an 85-year-old schizophrenic subject without dementia; **c** an 86-year-old subject with schizophrenia and dementia; **d** a 79-year-old subject with Alzheimer's disease (AD). Note the larger and more intensely immunoreactive-appearing astrocytes in AD compared to the schizophrenic case in **c**. This was seen in most AD cases, and likely indicates a stronger astrocytic reaction. **a–d** × 400

analyses. There was no difference in neuroleptic exposure (chlorpromazine equivalent dosage 1 month prior to death) between the two subgroups [$t(19) = 0.397$, NS] and they were balanced for sex ($X^2 = 0.00$, $df = 1$, NS).

## GFAP-immunoreactive astrocyte counts

A summary of results for all measures is presented in Table 2. Regional GFAP astrocyte counts are graphically represented in Fig. 3. Analysis of GFAP-immunoreactive astrocyte counts revealed a significant main effect of diagnosis [$F(2, 35) = 13.96$, $P < 0.0001$]. Post hoc comparisons between groups showed significant differences between AD and each of the other diagnostic groups but not between schizophrenics and normals. Individual comparisons for each region revealed significant increases in astrocyte counts for AD compared to the other two groups in all regions except CA3 and mid-frontal cortex. Astrocyte counts were higher in the schizophrenia group than in the normals in the EC, subiculum, DG, mid- and orbitofrontal cortices and visual cortex, although no differences were significant with ANOVAs.

Analyses of the schizophrenia group were performed using dementia status as the grouping variable, age and PMI as regressors, and GFAP astrocyte counts as the repeated-measures dependent variable. As can be seen in Fig. 3, a significant increase in the GFAP-positive astrocyte counts for the dementia subgroup was observed [$F(1, 17) = 5.068$, $P = 0.038$]. Post hoc tests for each cortical region found significantly higher astrocyte counts in subiculum [$t(19) = 2.748$, $P = 0.006$] and orbitofrontal cortex [$t(19) = 2.207$, $P = 0.020$].

## GFAP optical density

Global mean values for GFAP-immunoreactive OD significantly correlated with astrocyte counts ($r = 0.595$, $P < 0.0001$). Repeated-measures ANOVA found a highly significant effect of diagnosis [$F(2, 35) = 14.924$, $P < 0.0001$] with post hoc comparisons revealing a similar pattern to that of astrocyte counts. Individual regional ANOVAs found significantly greater GFAP OD in AD than in schizophrenia and non-neuropsychiatric controls for EC, subiculum, CA1, DG, and visual cortex, but not

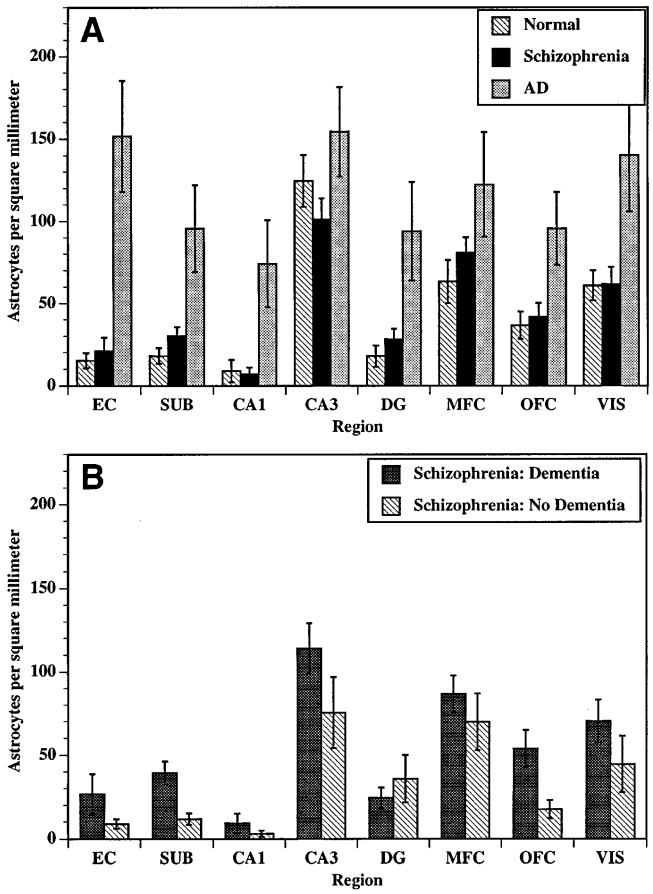


**Fig. 3A, B** GFAP-immunoreactive astrocyte counts per square millimeter (± SE). **A** Data comparing normal control, schizophrenia, and AD diagnostic groups in different regions. **B** Data comparing demented and non-demented schizophrenia subgroups (*EC* entorhinal cortex, *SUB* subiculum, *DG* dentate gyrus, *MFC* mid-frontal cortex, *OFC* orbitofrontal cortex, *VIS* calcarine visual cortex)

CA3, orbitofrontal and mid-frontal cortices. The schizophrenia group exhibited higher GFAP OD values than normal controls in all regions measured except CA1, but again, these differences were not significant with ANOVAs.

In contrast to astrocyte counts, repeated-measures ANCOVA of OD with age and PMI as regressors revealed no overall difference between dementia and non-dementia schizophrenia subgroups for GFAP OD [$F(1, 17) = 0.120$, NS]. Individual *t*-tests demonstrated a significantly higher GFAP OD only for subiculum [$t(19) = 1.779$, $P = 0.046$] in the schizophrenia with dementia subgroup.

### PHF-1 NFT counts

As expected, the AD cases exhibited much higher numbers of PHF-1 NFTs than the schizophrenia and normal comparison groups in repeated-measures ANOVA [$F(2, 35) = 43.641$, $P < 0.001$]. Non-neuropsychiatric and schizophrenic samples did not differ in post hoc analyses. Significantly higher numbers of NFTs were present in the AD group in ANOVAs of all individual regions assessed (DG molecular layer was not assessed as it contains no neurons), while the schizophrenia and normal groups did not differ.

The non-demented schizophrenic subgroup exhibited slightly more neurofibrillary pathology in each of the hippocampal subfields than the patients with dementia, although there were no significant differences overall in repeated-measures ANCOVA [$F(1, 17) = 1.719$, NS].

### Vimentin-immunoreactive astrocyte counts

Immunohistochemical studies with the anti-vimentin antibody conducted on the hippocampal region identified reactive astrocytes (Fig. 1), but the number of vimentin-positive astrocytes was less than 10% of GFAP-labeled astrocytes in the AD group. Further, the normal and schizophrenia groups exhibited far fewer vimentin-immunoreactive astrocytes than the AD group [$F(2, 34) = 17.593$, $P < 0.0001$]. No difference between demented and non-demented schizophrenics was observed [$F(1, 17) = 1.534$, NS].

## Discussion

These data demonstrate that, while elderly, chronic patients with schizophrenia as a whole show only slightly increased astrocytosis compared to non-neuropsychiatric elderly controls, the subgroup with severe cognitive impairment shows significantly greater astrocyte numbers compared to the less-impaired subgroup. GFAP OD and vimentin-immunoreactive astrocyte counts proved to be less robust discriminators of these groups of subjects. The increased GFAP-immunoreactive astrocyte numbers were not associated with an increase of AD-related or any other diagnostic neuropathological findings, and they appear to be unrelated to any other potentially confounding variables.

Although the observations reported here are based on well-established methods and procedures, it is important to point out the limitations of this study and our interpretation of the findings. First, we used a highly select population of patients with schizophrenia and, while there are some advantages to using this sample, one must exercise caution in generalizing results to broader populations of schizophrenics. Second, the dementia and non-dementia schizophrenia subsamples differed in age. This was expected given our previous finding of a negative correlation between age and MMSE in the population from which this sample was derived [12]. The age difference is potentially relevant to our findings since increased astrocytosis has been described as an aging phenomenon [34] (although we did not find this in our non-neuropsychiatric control sample in any region except for a weak correlation in visual cortex). We controlled for age by including it (and PMI) as regressors in analyses of covariance. A third limitation is that most of the patients in this study died

prior to a direct, standardized antemortem clinical assessment. Thus, diagnoses were based on clinical data obtained from extensive chart review as well as interviews with caretakers. However, 5 of the 21 schizophrenics had had antemortem clinical assessment. One of these patients was non-demented with a MMSE score of 28 and the other 4 were severely demented with MMSE scores ranging from 4 to 8. While no meaningful statistical correlation is possible with 5 cases, it was noted that the non-demented case had minimal astrocytosis, while all of the demented cases had moderate to high astrocyte counts. As further brains are accrued from patients who have had antemortem assessments, correlation analysis will be able to clarify more directly how cognitive impairment in schizophrenia is related to astrocytosis.

Our results are in part consistent with previous reports of a lack of cortical gliosis in schizophrenia in general. While relatively few in number, no study of schizophrenia has found abnormal gliosis in cerebral cortex using either histochemical or immunohistochemical staining methods and quantitation of either astrocyte numbers or GFAP OD [14, 15, 19, 29, 54, 55, 59]. Some studies have found evidence of gliosis in subcortical regions [18, 48, 59], although others have not confirmed this [17, 40, 58]. It has been argued that "scatter", i.e., the variability in degree of gliosis from patient to patient or brain region to brain region, may wash out detection of neuropathological abnormalities due to the averaging of cases, regions, and measures for statistical analyses [60]. Given the likely heterogeneity of schizophrenia, defining subtypes based on psychiatric, neuropsychological, or any of a number of phenotypic profiles could temper this averaging effect and highlight distinctive biological processes. This report provides evidence for the presence of cortical gliosis in at least one select subgroup of patients with schizophrenia, namely those who evidenced a particularly malignant course culminating in dementia late in life.

Individual regional analyses revealed that astrocytosis was most prominent in the subiculum and orbitofrontal cortex. Within our case population, the subiculum has been a principal site of neuroanatomical abnormality with findings of decreased neuron size [11], diminished expression of microtubule-associated proteins MAP2 and MAP5 [9], and now increased astrocytosis in schizophrenics with dementia. Whether any of these abnormalities represents a primary pathology or whether they are secondary to as yet unknown processes in schizophrenia remains unclear. Nonetheless, the topographical location of the findings is of neuropsychological consequence. The majority of efferent connections of the hippocampal formation to association cortices in temporal, frontal and parietal lobes as well as numerous subcortical nuclei originate in the subiculum [56, 62]. Neural dysfunction in the subiculum would disturb communication between the hippocampal formation and these brain areas, resulting in memory and other cognitive impairments. This mechanism has been proposed as the basis for the amnesia in AD, where the selective distribution of neurofibrillary pathology in the subiculum, CA1, and EC "disconnect" the hippocampal formation from the rest of the brain [65]. While the neuropathology of AD and schizophrenia with dementia are clearly different, some of the clinical and neuropsychological similarities may be due to disturbances in common brain regions.

The orbitofrontal cortex is an area that has received relatively little attention in schizophrenia, despite its connectional neuroanatomical importance as a multimodal association area [20] and its role in the modulation of affective and motor responses [28, 31]. One study examined the orbitofrontal cortex in elderly schizophrenia patients with an antibody directed at ubiquitin, a protein that mediates proteolysis and is expressed in neurons in a range of neurodegenerative disorders [38]. Results were negative and interpreted as indicating no neuronal degeneration in elderly patients with schizophrenia. However, the extremely long average PMI (>100 h) of the cases used in this study might have resulted in significant epitope degradation. Another study found increased pre- and postsynaptic glutamatergic markers in orbitofrontal cortex [25], which could be due to developmental hyperinnervation or to neural injury or degeneration. Our data are consistent with neural injury in the orbitofrontal cortex, and this may have contributed to the deterioration in cognitive and personality function in these patients.

Reactive astrocytosis is a universal occurrence in a wide variety of CNS injuries including ischemia, CNS toxins, infectious diseases, autoimmune diseases, and perhaps most relevant to current etiological theories of schizophrenia, neurodegenerative and neurodevelopmental disorders. Neurodegenerative diseases as well as genetic CNS disorders that result in tissue damage such as lysosomal storage diseases produce intense and persistent GFAP and vimentin immunoreactivity [49], while non-progressive disorders such as fragile X syndrome do not evidence gliosis [68]. In human fetal development, anoxic-ischemic injury can cause reactive gliosis in the CNS only after 24 weeks' gestation [22]. This inability to mount a glial response to CNS injury in early gestation may be related to the immaturity of immune function since the manipulation of immunoregulatory cytokines in stab-wound experiments in neonatal mice has been found to induce a reactive gliosis where there otherwise would be none [13].

In our series, we found prominent GFAP- and vimentin-immunoreactive astrocytosis in the AD cases, and elevated GFAP astrocytosis in schizophrenics with dementia compared to those without. Vimentin-immunoreactive astrocytosis was minimal in both schizophrenic subgroups and in non-neuropsychiatric controls. In the absence of any obvious neurodegenerative or other static tissue damage, the etiology for the increased astrocytosis in this subsample of schizophrenics awaits elucidation.

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