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Stage-Specific Changes in Neurogenic and Glial Markers in Alzheimer's Disease

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ABSTRACT

BACKGROUND: Reports of altered endogenous neurogenesis in people with Alzheimer's disease (AD) and transgenic AD models have suggested that endogenous neurogenesis may be an important treatment target, but there is considerable discrepancy among studies. We examined endogenous neurogenesis and glia changes across the range of pathologic severity of AD in people with and without dementia to address this key question.

METHODS: Endogenous neurogenesis and glia in the subventricular zone and dentate gyrus neurogenic niches were evaluated using single and double immunohistochemistry and a validated antibody selection for stage-specific and type-specific markers in autopsy tissue from a representative cohort of 28 participants in the Medical Research Council Cognitive Function and Ageing Study. Immunopositive cells were measured blinded to diagnosis using bright-field and fluorescent microscopy.

RESULTS: The number of newly generated neurons significantly declined only in the dentate gyrus of patients with severe tau pathology. No other changes in other neurogenic markers were observed in either of the neurogenic niches. Alterations in astrocytes and microglia were also observed in the dentate gyrus across the different stages of tau pathology. No change in any of the markers was observed in individuals who died with dementia compared with individuals who did not die with dementia.

CONCLUSIONS: Alterations in endogenous neurogenesis appeared to be confined to a reduction in the generation of new neurons in the dentate gyrus of patients with AD and severe neurofibrillary tangle pathology and were accompanied by changes in the glia load. These data suggest that intervention enhancing endogenous neurogenesis may be a potential therapeutic target in AD.

Keywords: Alzheimer's disease, Glia, Human brain, Neural progenitors, Neurogenesis, Tangles

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Dementia currently affects >34 million people worldwide, with estimations that >110 million people will be affected by dementia in 2050 (1). Alzheimer's disease (AD), the most common form of dementia, causes enormous personal, social, and financial burdens on the patients, their caregivers, and society. Current pharmacologic treatments offer symptomatic benefits only, and effective disease-modifying therapies are urgently needed. Because AD is a neurodegenerative disease, cell replacement strategies are a potential target for therapeutic intervention, such as promoting endogenous neurogenesis.

Endogenous neurogenesis is evident in two areas of the brain: the hippocampal dentate gyrus (DG) and the wall of the lateral ventricles (subventricular zone [SVZ]) (2–5). In mammals, neural progenitors at the base of the DG granular layer (subgranular layer) give rise to neurons that can be functionally integrated in the granular cell layer, whereas the SVZ neural progenitors follow a distinct pathway, the rostral migratory stream, to the olfactory bulb where they create interneurons. In the healthy adult brain, SVZ neurogenesis maintains cellular turnover in the olfactory bulb, contributing to olfactory adaptation and learning (6–8), whereas in DG, endogenous neurogenesis is crucial for hippocampal-dependent spatial learning and memory throughout life (8–11). Groundbreaking work over the last 2 decades has demonstrated the presence of the same neurogenic niches in the adult human brain, including the temporal horn of the lateral ventricles, located adjacent to the hippocampal formation (12–15). Consequently, there has been evolving interest in the therapeutic potential of strategies that aim to enhance endogenous neurogenesis. Many available compounds, of which some are already in clinical use, such as retinoid agonists, cannabinoids, selective serotonin reuptake inhibitors, cholinesterase inhibitors, and certain hormones, have a substantial positive impact on neurogenesis in animals either by stimulating proliferation of endogenous neural stem cells or by increasing their differentiation into neurons (16,17).

The potential clinical relevance for patients with AD is less clear, with contradictory results from the small number of

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human autopsy studies that have been undertaken. Ziabreva *et al.* (18) and Perry *et al.* (19) identified an increase at the proliferation stages of neurogenesis in the anterior SVZ and the temporal horn SVZ and DG, respectively, but a reduction in the early-stage neural progenitors in the SVZ of patients with AD compared with age-matched controls (18). In a previous study from our group focusing on a different cohort of patients with AD, including patients with concurrent cerebrovascular disease, no statistically significant difference was observed in early neuronal marker immunoreactivity between patients with AD and controls (19). In contrast, in another study, increased numbers of neural progenitors were detected in the DG of patients with AD, which resulted in an unsuccessful maturation to newly generated neurons (20). In a report focusing on younger patients with AD, increased glial proliferation was reported in the subgranular layer, but no alteration in neurogenesis was identified (21). Other studies suggested that both concurrent cerebrovascular pathology (22–26) and the severity of cortical cholinergic system deficits (18,19,27) are likely to represent key mediating factors in increasing and decreasing endogenous neurogenesis, respectively. The influence of age-associated neuropathologic changes on neurogenesis is not fully elucidated, in particular with respect to the early stages of the AD process.

Similar to the often contradictory data from studies on human tissue, studies investigating neurogenesis in transgenic animal models carrying the human mutations for amyloid precursor protein or presenilin 1 or presenilin 2 or tau proteins reported increased, decreased, or unchanged progenitor activity (28–32). To elucidate the role of neurogenesis in AD further, we examined postmortem brain tissue from a subset of participants of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS), including individuals who died with and without dementia and who showed all neuropathologic stages of AD-associated tau pathology (i.e., Braak stages 0–VI), without any other neuropathology such as cerebrovascular disease. For the first time, the levels of astrocytic and microglia cell numbers were also identified in the different Braak stages. Our study primarily aimed to identify alterations in the various phases of endogenous neurogenesis in relation to dementia and pathology associated with AD.

METHODS AND MATERIALS

Tissue was obtained from brains donated to the United Kingdom MRC CFAS. Details of the study have been described elsewhere (33,34) and can be found at the study website (www.cfas.ac.uk). Briefly, MRC CFAS included an initial cohort of 13,004 individuals, representative of the population $\geq$65 years old recruited from general practice lists in five areas of England and Wales. The cohort for the current study consists of 28 brain samples selected from participants of the MRC CFAS who agreed to donate their brain on death and among whom a successful autopsy was performed. At the time of sampling, 456 brain donations had been made to the study, 114 of which were available and had sufficient tissue for the current analysis (brain donations from the Cambridgeshire and Newcastle centers). Brains that received a neuropathologic diagnosis of “normal brain,” “possible AD,” “probable AD,” or “definite AD” were considered for selection into the study. Brains with any diagnosis of Lewy body disease, cerebrovascular disease, or other neuropathology were excluded. Neurofibrillary tangle pathology was assessed using Braak staging (35–37) after histochemistry by experienced neuropathologists working in the MRC CFAS study who were blinded to clinical findings (Figure S1 in Supplement 1). Neurofibrillary Braak stages are based on the topographical distribution of neurofibrillary tangles and neuropil threads, which are neuropathologic hallmark lesions of AD; at Braak stages I–II, neurofibrillary tangles are confined mainly to the transentorhinal region of the brain; at stages III–IV, they are also found in limbic regions such as the hippocampus; and at the severe stages V–VI, they are extensively located in other brain areas, including the neocortex (35,36). The neuropathologic diagnosis of AD was done according to internationally accepted criteria that include the assessment of amyloid-β pathology, which progresses spatially and temporally differently than tau pathology. Similar to many other studies, we used Braak stages to indicate the overall severity of AD pathology but not to compare the severity of tau pathology directly with neurogenesis; in this study, neither tau nor amyloid-β pathology was directly compared with neurogenesis in the same topographical locations.

Diagnosis of Dementia

Dementia status at death was determined based on interviews during the last years of life, including the full Geriatric Mental State–Automated Geriatric Examination for Computer Assisted Taxonomy diagnostic algorithm that was equivalent to that in DSM-III-R, interviews with informants after the respondent’s death when this was possible, and death certification (37). Of the 28 individuals included in the present study, 13 received a study diagnosis of dementia at death. Demographic data are shown in Table 1.

Immunohistochemistry

Paraffin-embedded 8-μm-thick sections were obtained at the level of basal ganglia, including the anterior SVZ, and at the level of the hippocampus, including the temporal horn of the SVZ. Slides were processed for immunohistochemistry and double immunofluorescence (described in Methods and Materials in Supplement 1), according to previously published procedures (22,23,26,27).

Cell Counts

Cell counting was performed twice, blind to the clinical and neuropathologic diagnosis, using a Nikon Eclipse E800 micro-

Table 1. Demographic Data

	Braak Stage		
	0–II	III–IV	V–VI
	n = 12	*n* = 11	*n* = 5
Age (Years), ± SD	80.3 ± 8.4	88.9 ± 8.2	86.8 ± 5.3
Gender	F, 5; M, 7	F, 8; M, 3	F, 1; M, 4
Dementia	*n* = 3	*n* = 5	*n* = 5
Gender	F, 2; M, 1	F, 4; M, 1	F, 1; M, 4
PM Delay (Hours), Median (IQR)	17.5 (12–28)	25 (7–27)	17.5 (9.5–33)

Data represent the mean or median in each group.
F, female; IQR, interquartile range; M, male; PM, postmortem.

scope and NIS-Elements software for bright-field microscopy (version 2.3; both from Nikon Europe, Amsterdam, The Netherlands) and a Carl Zeiss Axioplan 2 microscope and ApoTome and AxioVision software for immunofluorescence (version 4.7.2; all from Zeiss, Cambridge, United Kingdom). The length of the hippocampus and length of three areas of the SVZ and the neighboring ependymal cell layer in either the anterior ventricle horn or the temporal ventricle horn were measured on each slide section under a very low magnification and using the relevant microscope software tools. Results were expressed as the number of immunopositive cells for each antibody/mm length for all the markers to adjust for variances owing to different lengths of the areas measured.

Statistical Analysis

For all markers except double cortin, negative binomial regression analysis was used to model the difference in the number of immunopositive cells/mm length across groups defined by Braak stage. Regression models were adjusted for age at death and for gender. Differences in cell counts/mm between subjects with and without dementia at autopsy were assessed by negative binomial regression adjusting for age at autopsy, gender, and Braak stage. Negative binomial regression is a count-based regression model and allows a number of events (immunopositive cells) to be modeled in terms of covariates of interest (Braak stage and dementia diagnosis) as well as potentially confounding covariates (age and sex), while also taking into account differences in an "exposure" variable (length of tissue sample being examined). Negative binomial regression is a generalization of Poisson regression in that it allows for heterogeneity in the number of cells/mm across individuals within a group. Poisson regression assumes an even distribution of immunopositive cells across samples within groups and would lead to type I errors because this assumption is highly unlikely to be met. Negative binomial regression results in an estimate of "rate" (cells/mm) in each group standardized to remove the effect of any differences in age and sex across groups and an estimate of difference in terms of an "incident rate ratio" corresponding to the ratio of the rates across groups, assuming all other covariates held constant. The rate estimate is very similar to the raw count of cells/mm in each case, suggesting that the effect of any difference in age and sex across groups in this analysis is minimal.

As a secondary, albeit more conventional analysis, we conducted an analysis of variance across groups treating total cells/mm as a continuous outcome. This analysis led to substantively similar results; however, it is likely that the outcome measure of cells/mm violates the assumptions of an analysis of variance (normally distribution with equal variances across groups), and so we prefer the negative binomial regression as the primary analysis. The numbers of cells positive for double cortin were too small for meaningful multivariate analysis or analysis using a count-based regression model, and so for each brain area the proportion of cases with any double cortin-positive cells was compared across groups using Fisher's exact tests.

An initial p value $< .05$ was set for statistical significance; however, owing to the large number of hypotheses considered, we subsequently corrected for multiple testing using the method of Benjamini and Hochberg (38) by setting a false discovery rate of $q = .05$, leading to a revised critical value of $p < .0016$ for each individual hypothesis. Finally, Spearman correlation coefficients were estimated to identify any association between the detected changes. For the data analysis, IBM SPSS (version 19; Portsmouth, United Kingdom) and STATA (version 12.1; College Station, Texas) statistical software were used.

RESULTS

Demographic data for the cohort are presented in Table 1. Across the entire study cohort, the mean age at death was 84.8 years ($\pm$8.6; range, 71–103 years), and 50% of the participants were female (Table 1). Statistical analysis showed that the postmortem delay was not related either to the Braak stage and dementia diagnosis ($p > .05$ for both) or to the cell counts of any of the markers examined (data not shown).

The pattern of immunoreactivity of the neural stem/progenitor cells and their progeny was consistent with our previous descriptions (22,23). Nestin and double cortin immunoreactivity was observed in cellular somata and processes, although in the case of double cortin, immunoreactivity in the processes was rarely observed, possibly secondary to the postmortem delay, as described previously (21). Because double cortin is also expressed in astrocytes (39), any double cortin-positive cells with astrocytic appearance (multiple processes) were excluded from counting. Proliferating cell nuclear antigen (PCNA) and human neuronal proteins C and D (HuC/D) immunoreactivity were detected in the cell nucleus. Figure 1A and B show the different cell types, as detected by 3,3'-diaminobenzidine immunohistochemistry and double immunofluorescence in the hippocampal DG and the SVZ.

Dentate Gyrus

The estimated number of immunopositive cells/mm length in the DG and the estimated frequency of cells across Braak stages standardized to the average age at the time of autopsy and for gender is shown in Figure 2A and B for both neurogenic markers and glia. There was a lower number of HuC/D-positive postmitotic early neurons in the DG in individuals with Braak stages V–VI (severe neuropathology) compared with individuals with Braak stages 0–II ($p = .032$) (Figures 1B and 2A).

A significantly higher number of microglia cells, as identified by ionized calcium binding adapter molecule 1 immunohistochemistry, were detected in individuals with Braak stage III–IV compared with the other two groups ($p = .033$) (Figures 2B and 3). However, the differences in early neurons and microglia cells detected were not statistically significant after adjusting for multiple testing.

There was a significantly lower number of glial fibrillary acidic protein–positive cells (identifying both neural stem cells and astrocytes) among individuals with Braak stage III–IV (incidence rate ratio = .6; 95% confidence interval = .4–.9) compared with individuals with Braak stage 0–II but a higher number among individuals with Braak stage V–VI (incidence rate ratio = 1.9; 95% confidence interval = 1.3-2.8). This

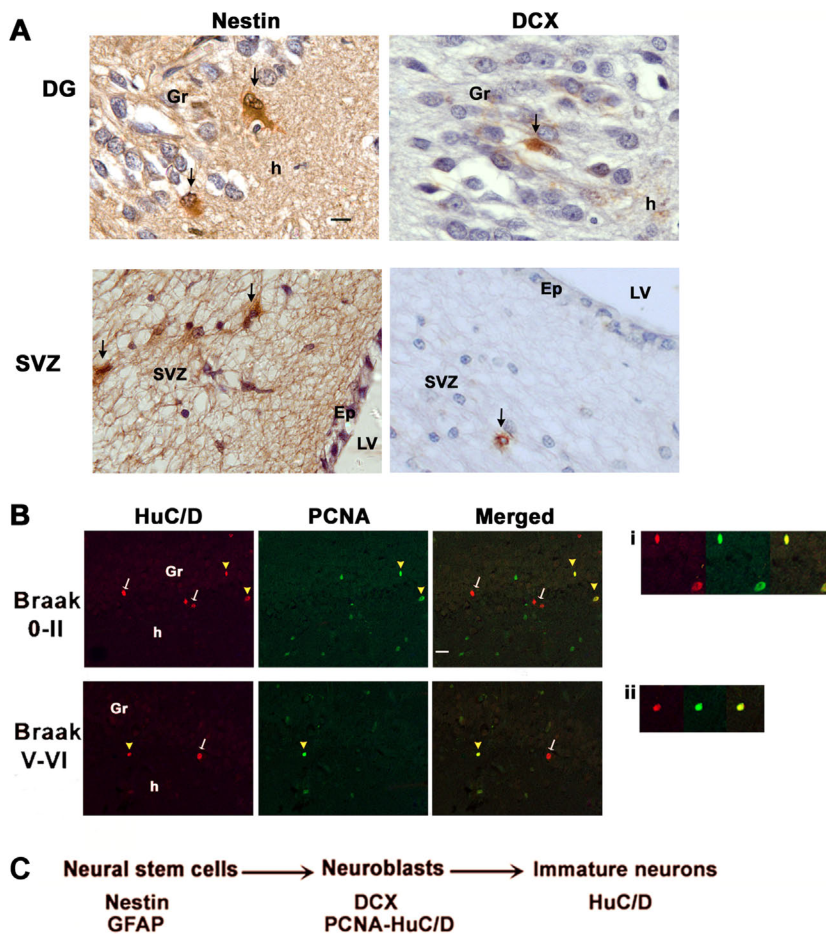

Figure 1. Immunohistochemistry showing neurogenesis in the dentate gurus and the subventricular zone of the adult human brain. **(A)** 3,3'-Diaminobenzidine immunohistochemistry for nestin (top left, arrows) and DCX (top right, arrow) depicting neural stem cell/progenitors and late neural progenitors, respectively, in both neurogenic niches. Scale bar = 5 μm. **(B)** Double immunofluorescence for HuC/D (red) and PCNA (green) in patients with severe AD (Braak stage V–VI) and age-matched controls (Braak stage 0–II). There are significantly fewer postmitotic immature neurons (arrows, HuC/D-immunopositive cells) in the DG of patients with severe AD compared with age-matched controls. Yellow arrowheads indicate proliferating neuronal progenitors/neuroblasts immunopositive for both PCNA (a proliferating marker) and the HuC/D antigen. Images i and ii show higher magnification of double-labeled cells at Braak stage 0–II (image i) and V–VI (image ii). Scale bar = 20 μm. **(C)** Schematic representation of the markers used for characterization of the different stages of neurogenesis in the present study. DCX, doublecortin; DG, dentate gyrus; Ep, ependymal cell layer; GFAP, glial fibrillary acidic protein; Gr, dentate gyrus granular layer; h, hilus; HuC/D, human neuronal proteins C and D; LV, lateral ventricle; PCNA, proliferating cell nuclear antigen; SVZ, subventricular zone.

difference across Braak groups remained statistically significant after correction for multiple testing ($p < .0001$) (Figures 2B and 3). Table 2 shows the difference in cell numbers/mm between individuals with and without dementia and the rate ratio standardized for age, gender, and Braak stage. There is some evidence of an increase in HuC/D-positive cell numbers in individuals with dementia (adjusted incidence rate ratio = 2.1; 95% confidence interval = 1.0–4.3; $p = .05$).

Because the number of double cortin-positive cells was very low in the DG, a different statistical analysis was performed, as described in the Methods and Materials section. Table 3 shows the proportion of cases with double cortin-immunopositive cells in each brain area examined, with some evidence that these are more commonly found in the DG of individuals with higher Braak stages (Fisher's exact test $p = .05$). All of the samples with double cortin-immunopositive cells in the DG were from participants who died with dementia (Fisher's exact test $p = .04$) (Table 3); however, owing to small numbers of samples and the large number of hypotheses considered, these findings should be considered with caution.

To examine potential correlations between the neurogenic and glial markers, we performed Spearman correlation (ρ) analysis. There was a significant positive association between newly generated neurons and the activated microglia ($R = .52$, $p = .005$) but a negative association with the changes of astrocytic cell numbers ($R = -.396$, $p = .045$) in the DG (Table 4).

SVZ and Ependymal Cell Layer (Anterior and Temporal Horn)

The neurogenic markers remained unchanged at the anterior (at the level of basal ganglia) and temporal (at the level of hippocampus) horns of the SVZ and the ependymal cell layer adjacent to those when analyzed by Braak stage and by the presence of dementia (Table S1 in Supplement 1; Table 3). The PCNA-HuC/D double and HuC/D single immunolabeled cells were not detected in these areas. The astrocytic and microglia markers also remained unchanged in the SVZ neurogenic niche and neighboring ependymal cell layer in Braak stages 0–VI (Table S1 in Supplement 1).

DISCUSSION

A detailed analysis of endogenous neurogenesis in both neurogenic niches at various stages of tau pathology in 28 individuals with and without AD was undertaken from a representative community cohort of patients followed to autopsy (MRC CFAS). This is a population-based representative pilot study of endogenous neurogenesis in AD using

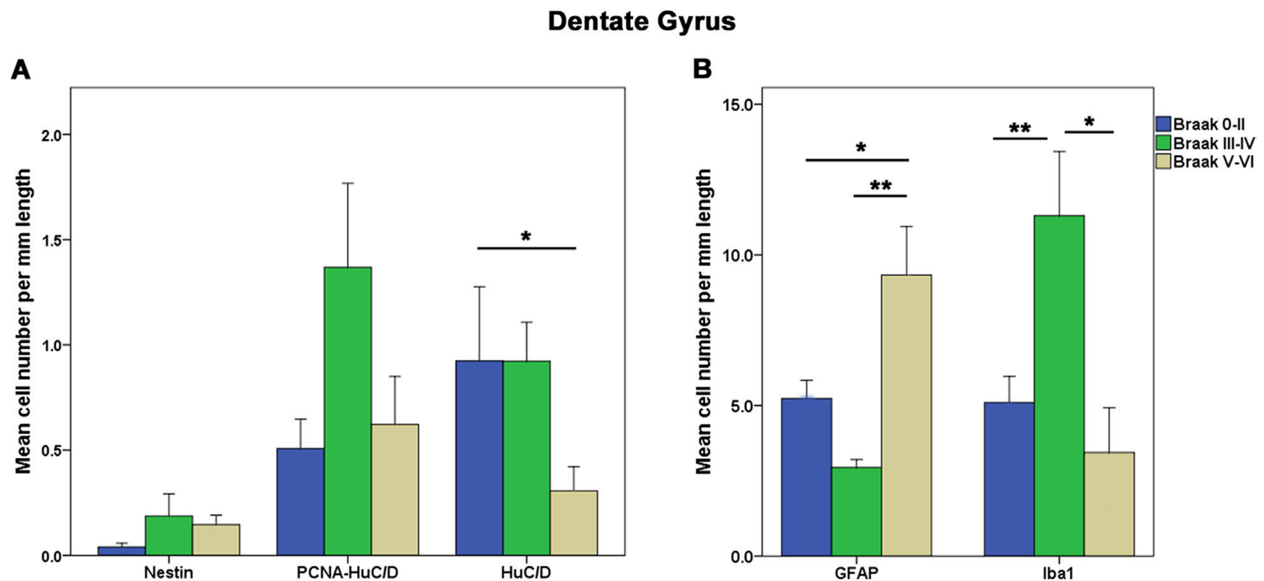

Figure 2. Graph bars represent the adjusted mean number (± SE) of immunopositive cells per mm of length of dentate gyrus for neurogenic **(A)** and glial **(B)** markers in patients with AD at different Braak stages, adjusted for age and dementia status. *$p < .05$. **$p < .001$. GFAP, glial fibrillary acidic protein; HuC/D, human neuronal proteins C and D; Iba1, ionized calcium binding protein adapter molecule 1; PCNA, proliferating nuclear antigen.

human autopsy tissue and compared with our previous studies (18,19) focuses on the full range of pathologic disease severity, the cognitive status, and both neurogenic niches from each individual. The present study has the methodologic advantage of excluding patients with concurrent cerebrovascular pathology, Lewy body disease, and any other pathology that has been found to affect adult endogenous neurogenesis (22–27,40), as opposed to previous studies of endogenous neurogenesis in AD (20,21), including our previous studies focusing on different patient cohorts (18,19), in which the percentage area of immunoreactivity was measured rather than the number of immunopositive cells.

There were limited but statistically significant changes in one of the markers used in this study in individuals with dementia or at the different Braak stages. Specifically, we observed a significant reduction in newly generated neurons, as determined by single HuC/D immunohistochemistry, but not neural progenitors, as determined by nestin, double cortin, and double PCNA-HuC/D immunolabeling, in individuals with severe tau pathology (Braak stage V–VI) compared with individuals with no significant tau changes (Braak stage 0–II). Consistent with other, more recent studies about the role of microglia in modulating endogenous neurogenesis, there was a significant relationship with activated microglia.

There were no changes in neural stem cells or progenitors in the anterior and temporal horn of the SVZ, which is in contrast with the increased numbers of neural stem cells and progenitors observed in the SVZ of patients with stroke, vascular dementia, dementia with Lewy bodies, and small vessel disease, as our previous studies have shown (22,23,26,27,40). Markers of neurogenesis did not vary between individuals with and without dementia after adjusting for Braak stage; the reduced numbers of new neurons were specifically associated with severe AD pathology.

Earlier studies examining endogenous neurogenesis in the course of AD produced conflicting results, possibly because of the predominant focus on more severe AD, variable concurrent vascular changes, and the limited focus on the later stages of neurogenesis (i.e., the newly generated neurons). The only other study to examine early neurons as well as progenitors reported a significant reduction in their maturation as marked by the decreased levels of microtubule-associated protein 2a and 2b isoforms in the DG of AD patients (20), consistent with our current findings.

The present study has some limitations, mainly secondary to methodology. Because there are no reliable or applicable markers to "visualize" and follow the fate of neural stem cells in the adult brain, it is impossible to draw any conclusions about adult endogenous neurogenesis during the life span of the participants in the study, so all our results represent adult neural stem cells and their progeny at a single time point—that of autopsy. Optimistically, the development of new technologies would facilitate drawing such conclusions and clarify more the role of adult neurogenesis and its involvement in cognitive decline in aging and AD. Because exclusion criteria were applied to the cases in reference to the presence of other neurodegenerative diseases, our sample was modest ($n = 28$), but it was a population-representative pilot study of endogenous neurogenesis focusing on "pure" AD in human autopsy tissue, including individuals with different stages of AD pathology.

The use of antibodies as markers of endogenous neurogenesis on human autopsy tissue represents a challenge, but we employed a validated battery of antibodies for the identification of progenitors and newly developed neurons at the various stages of neurogenesis in human autopsy tissue. Long postmortem delay, a common factor in obtaining human tissue, has been shown to alter but not eliminate the

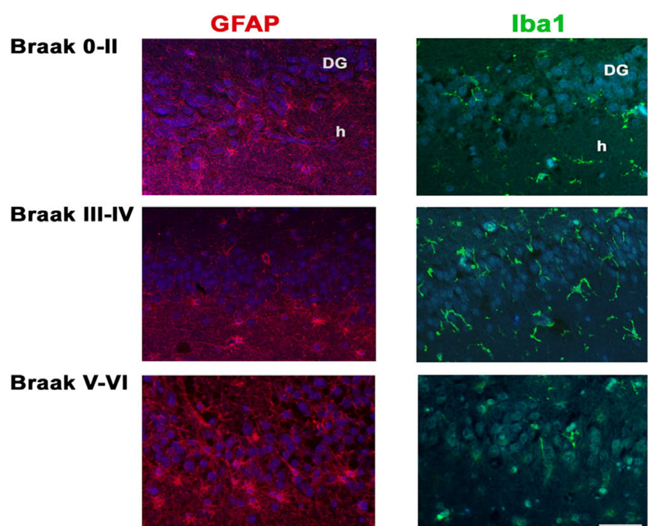

Figure 3. Immunofluorescence for astrocytes (GFAP) and microglia (Iba1) in age-matched individuals at Braak stage 0–II and patients with moderate (Braak stage III–IV) and severe (Braak stage V–VI) tangle pathology. Scale bar = 50 μm. DG, dentate gyrus; GFAP, glial fibrillary acidic protein; h, hilus.

immunostaining pattern for double cortin, with similar overall levels of staining but reduced staining within the soma. For example, Boekhoorn *et al.* (21) showed that postmortem delay reduced immunoreactivity within the dendrites of double cortin-positive neuroblasts. A similar pattern was seen for double cortin in our study, but importantly the changes in the overall pattern of staining by dementia stage were similar for other markers of neuroblasts/immature neurons (HuC/D with PCNA). Because it has been suggested that doublecortin can also be expressed in astrocytes (39) and in dormant cells in non-neurogenic areas (41,42), the use of HuC/D as an additional marker for neuronal progenitors/early neurons is important and limits the possibility of overinterpreting results obtained from doublecortin immunohistochemistry. In addition, postmortem delay was not significantly correlated with the overall level of staining. Many hypotheses were explored, and when a correction was applied to ensure a false discovery rate of $<.05$, only the changes detected in the numbers of glial fibrillary acidic protein–immunopositive cells in the DG remained statistically significant across groups.

Because there is an age-related decline in neurogenesis observed in humans (43), our estimates have also been adjusted for age, which further confirms the validity of our results. Physical activity and certain pharmacologic treatments such as selective serotonin reuptake inhibitors (16,17) can have an impact on neurogenesis in rodent models (16,17,44–51). These were not specifically examined in the current study, but it is unlikely that the magnitude of these effects would be sufficient to confound the analysis.

The PCNA immunohistochemistry (Figure 1B) also revealed numerous cells that are not coexpressing the neuronal fate marker HuC/D (green only cells in Figure 1B). Although these cells were not counted for the study, we can speculate that they could represent astrocytic, microglial, or endothelial cell proliferating progenitors or cells reentering the cell cycle according to Yang *et al.* (52), although that was not observed in the DG.

Our results still showed some evidence that although the early stages of endogenous neurogenesis remained unchanged throughout the different Braak stages, severe AD pathology had a detrimental effect on the numbers of newly generated neurons in the DG of the affected individuals. In contrast, endogenous neurogenesis at all stages and areas remained unchanged in individuals with dementia compared with individuals without dementia, suggesting that severe AD pathology impaired only the production of new neurons.

We used Braak staging, a neurofibrillary tangle–based staging system, to describe the overall severity of AD. Amyloid and tau pathology both increase with increasing disease severity, and many other concurrent pathways related to a broad range of changes, including inflammation and mitochondrial function, are activated. The specific mechanisms associated with the reduced production of new neurons in people with severe AD are difficult to unravel from the results of the current study. However, previous work has suggested that tau transgenic mice (29,53) do have reduced neurogenesis, supporting the potential role of tau pathology as a contributor to this effect.

There was a significant positive correlation between the cell numbers of activated microglia and those of the newly

Table 2. Cell Numbers and Area Lengths (mm) for Neurogenic Markers in Human Dentate Gyrus by Study Diagnosis of Dementia at Death

	No Dementia (n = 15)			Dementia (n = 13)		
Antibody	Cells/mm (Raw Data)	Adjusted Cells/mm	IRR	Cells/mm (Raw Data)	Adjusted Cells/mm	IRR[a]
Nestin	.094	.13 (.08)	Ref	.10	.09 (.05)	.7 (.1–4.0)
HuC/D-PCNA	.88	1.0 (.3)	Ref	.61	.6 (.2)	.6 (.2–1.6)
HuC/D	.74	.6 (.1)	Ref	.73	1.3 (.4)[b]	2.1 (1.0–4.3)
GFAP	4.79	5.2 (.7)	Ref	2.46	4.9 (.5)	.9 (.7–1.4)
Iba1	7.82	7.3 (1.9)	Ref	5.57	7.0 (2.2)	1.0 (.4–2.3)

Rates and incident rate ratios for the difference in cell density in the dementia group compared with the no dementia group are estimated by negative binomial regression, adjusted for Braak stage. Cells per mm are standardized to the sample age and gender, and numbers in parentheses represent standard error.

GFAP, glial fibrillary acidic protein; HuC/D, human neuronal proteins C and D; Iba1, ionized calcium binding adapter molecule 1; IRR, incidence rate ratio adjusted for age and gender; PCNA, proliferating cell nuclear antigen; Ref, reference.

[a]Numbers in parentheses represent 95% confidence interval.

[b]p = .045.

Table 3. Occurrence of Double Cortin Immunoreactivity in Adult Human Brain Neurogenic Niches

Antibody	Braak 0–II (n = 12)	Braak III–IV (n = 11)	Braak V–VI (n = 5)	Fisher's Exact Test p Value	No Dementia (n = 15)	Dementia (n = 13)	Fisher's Exact Test p Value
DG	0 (0%)	2 (20%)	2 (40%)	.05	0 (0%)	4 (31%)	.04
SVZ	1 (8.3%)	2 (20%)	0 (0%)	.57	1 (7%)	2 (15%)	.58
EP	1 (8.3%)	1 (10%)	0 (0%)	1.00	1 (7%)	1 (8%)	1.00
SVZ BG	4 (33%)	1 (9%)	0 (0%)	.22	2 (13%)	3 (23%)	.64
EP BG	7 (58%)	6 (54%)	1 (20%)	.47	9 (60%)	5 (38%)	.45

Numbers represent number of samples with double cortin immunopositive cells in each brain area by Braak stage and dementia status.

DG, dentate gyrus; EP, ependymal cell layer; EP BG, ependymal cell layer at the level of basal ganglia, adjacent to SVZ BG; SVZ, subventricular zone; SVZ BG, subventricular zone at the level of basal ganglia (anterior horn).

generated neurons in the DG, suggesting that the reduction in activated microglia in people with Braak stage V–VI tangle pathology may be a key driver for the decline in the newly produced neurons in these individuals, along with the presence of tangles. Microglia have an important role in adult neurogenesis in the healthy brain because they have been shown to control the numbers of newly produced neurons in the hippocampus through apoptosis (54) and can have both proneurogenic and antineurogenic effects, finely "tuning" adult neurogenesis (55).

For the first time, changes in glial fibrillary acidic protein–positive astrocytes have been examined at the various Braak stages. Although we have no knowledge of the causal factors of these changes, one can hypothesize that because astrocytes have a significant role in the support and protection of neurons in the healthy brain, the decrease identified at Braak stage III–IV, below the levels seen in individuals with Braak stage 0–II, could be another contributing factor to the disease progression and pathology. The twofold increase above the levels observed in the healthy brain in astrocyte numbers in Braak stage V–VI could have a detrimental effect on the diseased brain, as it has been described for certain neurodegenerative diseases, including dementia (56). A separate study investigating this hypothesis further could clarify that observation and the underlying mechanism.

In conclusion, our study examined the fate of neuronal progenitors and their progeny at the DG and the anterior and temporal horn SVZ and adjacent ependymal layers in various stages of AD, without any effect from concurrent cerebrovascular or other neuropathology. It was shown that specific and significant reductions in newly generated neurons were detected only in the DG of patients with severe AD pathology and were associated with the microglial load of the area. Previous studies (57–59) identified that abnormal endogenous neurogenesis relates to age-related learning impairment and indicated that the manipulation of endogenous neurogenesis may be a potential treatment target in people with AD. As a cross-sectional autopsy study, our results need to be interpreted cautiously, but our findings do support the concept of an enhancement of aspects of endogenous neurogenesis as a possible treatment target in AD.

Table 4. Correlation Analysis Among Observed Changes in the Dentate Gyrus

			GFAP DG	HuC/D DG	Iba1 DG
Spearman ρ	GFAP DG	R	1.000	−.396[a]	−.476[a]
	HuC/D DG	R	−.396[a]	1.000	.524[b]
	Iba1 DG	R	−.476[a]	.524[b]	1.000

Statistical analysis was performed using a two-tailed Spearman ρ correlation analysis. R is correlation coefficient.

DG, dentate gyrus; GFAP, glial fibrillary acidic protein; HuC/D, human neuronal proteins C and D; Iba1, ionized calcium binding adapter molecule 1;

[a] $p < .05$.

[b] $p < .01$.

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