Archival Report

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

Antigoni Ekonomou, George M. Savva, Carol Brayne, Gillian Forster, Paul T. Francis, Mary Johnson, Elaine K. Perry, Johannes Attems, Alyma Somani, Stephen L. Minger, and Clive G. Ballard, on behalf of The Medical Research Council Cognitive Function and Ageing Neuropathology Study

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 ≥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.