Neuron Volumes in Hippocampal Subfields in Delayed Poststroke and Aging-Related Dementias

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Abstract
Hippocampal atrophy is widely recognized in Alzheimer disease (AD). Whether neurons within hippocampal subfields are similarly affected in other aging-related dementias, particularly after stroke, remains an open question. We investigated hippocampal CA3 and CA4 pyramidal neuron volumes and densities using 3-dimensional stereologic techniques in postmortem samples from a total of 67 subjects: poststroke demented (PSD; n = 11), nondemented stroke survivors (PSND) and PSD patients from the CogFAST (Cognitive Function After Stroke) cohort (n = 13), elderly controls (n = 12), and subjects diagnosed as having vascular dementia (n = 11), AD (n = 10), and mixed AD and vascular dementia (n = 10). We found that CA3 and CA4 neuron volumes were reduced in PSD samples compared with those in PSND samples. The CA3 and CA4 neuron volumes were positively correlated with poststroke global cognitive function but were not associated with the burden of AD pathology. There were no differences in total neuron densities in either subfield in any of the groups studied. Our results indicate that selective reductions in CA4 and to a lesser extent CA3 neuron volumes may be related to poststroke cognitive impairment and aging-related dementias. These data suggest that CA4 neurons are vulnerable to disease processes and support our previous finding that a reduction in hippocampal neuron volume predominantly reflects vascular mechanisms as contributing to dementia after stroke.

INTRODUCTION
Stroke is a major risk factor for dementia (1), and up to 50% of initially nondemented stroke survivors (PSND) will ultimately go on to develop delayed poststroke dementia (PSD) (2). However, the underlying mechanisms that increase the vulnerability of stroke survivors to delayed PSD from months to years after a stroke are unclear. Medial temporal lobe atrophy and hippocampal neurodegeneration are key pathologic correlates of dementia, particularly in Alzheimer disease (AD), but little is known of how these degenerating structures that are associated with learning and memory change in dementias caused by vascular disease.

We previously found that the volumes of hippocampal CA1 and CA2 neurons were related to poststroke cognitive function, and that delayed PSD subjects had 10% to 20% smaller neuron soma volumes versus PSND and age-matched controls. Neuron volumes in CA1 and CA2 were similarly reduced in patients with vascular dementia (VaD), AD, and mixed AD with VaD (MD) (3). We reasoned that this reduction in neuron volume reflected disease processes that disrupted hippocampal circuitry leading to cognitive impairment. Our finding that neuron volumes were equally reduced in CA2 was surprising because neurons in the CA1 subfield are selectively vulnerable to damage after hypoxia and in AD, whereas the CA2 is considered to be more resistant to damage (4–6). This suggests that neurons in the other hippocampal subfields may also be similarly affected.

Pyramidal neurons in the CA3 and CA4 form extensive contacts with CA1 and CA2 as part of the hippocampal circuit. CA3 neurons in particular are closely physiologically linked to CA1 through the Schaffer collaterals synapsing on CA1 dendrites as part of the classical trisynaptic hippocampal circuit (7). CA3 and CA4 neurons are also exposed to similar pathologic insults as those of CA1 and CA2 because of their close proximity within the hippocampal formation. Therefore, we investigated neuron volume and density in CA3 and CA4 to determine whether neuron changes within these subfields were also implicated in the pathogenesis of poststroke and aging-related dementias.
MATERIALS AND METHODS

Subject Selection, Clinical Diagnosis, and Tissue Acquisition

Neuron volumes and densities were measured in the CA3 and CA4 subfields of the same hippocampal sections that we previously studied (3). Subject demographics and pathologic findings are summarized in Table 1. Analysis was performed on postmortem hippocampal tissue from 24 subjects from the prospective Cognitive Function After Stroke (CogFAST) study (8). Nondemented stroke survivors older than 75 years were recruited 3 months poststroke and received annual clinical assessments and neuropsychologic testing from baseline 3 months poststroke, including the Cambridge Assessment of Mental Disorders in the Elderly (CAMCOG) test, which generated subscores for cognitive domains including memory and executive function (9, 10). To investigate the effects of different disease processes, analysis was also carried out in 12 cognitively normal elderly controls and 11 VaD, 10 MD, and 10 AD subjects. Final diagnoses of dementia were assigned based on Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM III-R) criteria for dementia and established neuropathologic diagnostic criteria. Hematoxylin and eosin staining was used for assessment of structural integrity and infarcts, cresyl violet and Luxol fast blue for cellular and myelin loss, respectively, Bielschowsky silver impregnation for Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) rating of neuritic plaques (11), and tau immunohistochemistry for Braak staging of neurofibrillary tangles (12). A diagnosis of VaD was made based on the presence of multiple or cystic infarcts, lacunae, microinfarcts, and small-vessel disease with Braak stage less than III (13). A diagnosis of AD was made when there was significant AD-type pathology (Braak stage V–VI and moderate to severe CERAD score) in the absence of severe vascular pathology. A diagnosis of MD was made when there was evidence of VaD with AD. In patients from the CogFAST study, the burden of global vascular pathology was also calculated from the sum of ratings of vascular lesions (including arteriosclerosis, amyloid angiopathy, perivascular space dilation, myelin loss, and infarcts) in the hippocampus, frontal lobe, temporal lobe, and basal ganglia to generate a score/20 (Vincent Deramecourt, Raj Kalaria), as described (14). Control subjects were selected if they had demonstrated no evidence of cognitive impairment or any neurologic or psychiatric disease. Neuropathologic examinations of the control samples demonstrated no significant pathologic alterations.

Tissue Acquisition

Brain tissues were acquired from the Newcastle Brain Tissue Resource (Newcastle, UK), except 4 control cases that were obtained from the Medical Research Council London Brain Bank for Neurodegenerative Diseases (Institute of Psychiatry, London, UK). Ethical approval and permission for postmortem research using brain tissue were granted for this project. Three 30-μm-thick sections were cut from predefined paraffin-embedded blocks of the hippocampus according to the Newcastle Brain Map (15) at the level of the pre-geniculate nucleus and the pulvinar at which the emergence of the ventricle is visible. Sections were stained using cresyl violet to visualize neuron cell bodies and nucleoli and checked for quality and staining consistency. All cases were collected, treated, and analyzed in a standardized manner to minimize differential effects from processing and staining.

Stereologic Analysis

Stereologic analysis of neuron soma volumes and densities was carried out using identical equipment and techniques as previously described (3). Slides were coded so that analyses were carried out blind to disease group. Sections were viewed using a 2.5× objective, and the reference areas were delineated using stereologic analysis software (Visiopharm Integration System, Horsholm, Denmark). CA3 and CA4 subfields were defined according to The Human Hippocampus (16), where the CA4 was completely enclosed by the dentate gyrus and CA3 began at the opening of the dentate gyrus where neurons became densely packed in a curve leading to the thinner band of CA2 neurons (Fig. 1). Three-dimensional stereologic analysis of neuron volume and density was carried out at 100× magnification. Pyramidal neuron density was estimated using the optical dissector method (17). Each dissector frame had an x-y area of 2,548.66 μm² and a depth of 18 μm, excluding a guard volume of 4 μm or higher from the top and bottom of each section, measured using a Heidenhain z axis micrometer accurate to 0.5 μm (Heidenhain GB, Ltd, London, UK). Pyramidal neurons were identified using established criteria, that is, characteristic triangular soma, with darkly stained single nucleolus (18). Neuron soma volumes were measured using an independent, uniform, random-oriented nuclear probe when the nucleolus came into focus as the probe was traversed.

### TABLE 1. Demographic Features of the Subject Groups

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>PSND</th>
<th>PSD</th>
<th>VaD</th>
<th>Mixed</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean age (range), years</td>
<td>81.9 (72–92)</td>
<td>84.5 (80–94)</td>
<td>88.7 (80–98)</td>
<td>86.4 (71–97)</td>
<td>84.6 (76–93)</td>
<td>82.4 (70–91)</td>
</tr>
<tr>
<td>Mean PMD (range), hours</td>
<td>22.9 (8–48)</td>
<td>44.8 (24–96)</td>
<td>40.4 (10–96)</td>
<td>51.2 (24–84)</td>
<td>34.6 (11–63)</td>
<td>40.9 (6–72)</td>
</tr>
<tr>
<td>Mean section thickness (±2 SE), μm</td>
<td>25.1 (1.4)</td>
<td>26.3 (0.4)</td>
<td>27.1 (0.2)</td>
<td>27.3 (1.6)</td>
<td>25.9 (2.4)</td>
<td>25.8 (1.6)</td>
</tr>
<tr>
<td>Mean Braak stage* (range)</td>
<td>0–1</td>
<td>2.8 (1–5)</td>
<td>2.3 (0–4)</td>
<td>2.1 (1–4)</td>
<td>4.4 (1–6)</td>
<td>5 (4–6)</td>
</tr>
<tr>
<td>Mean CERAD score* (range)</td>
<td>0–1</td>
<td>1.6 (0–2)</td>
<td>1.0 (0–3)</td>
<td>1.2 (0–2)</td>
<td>2.4 (1–3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Mean vascular pathology score (range)</td>
<td>N/A</td>
<td>12.5 (10–16)</td>
<td>11.5 (8–16)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Significant (p < 0.05) differences between group means versus controls.

AD, Alzheimer disease; CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; mixed, mixed VaD and Alzheimer disease; N/A, no data available or not applicable; PMD, postmortem delay; PSD, poststroke dementia; PSND, poststroke nondemented; VaD, vascular dementia.
through the z axis (19). An average of 116 neurons (±2 SE, 5) in CA3 and 106 neurons (±2 SE, 8) in CA4 were analyzed per subfield per case. Coefficient of error values were within the acceptable range, demonstrating a high level of precision (neuron volume in CA3, p = 0.052, and CA4, p = 0.073; neuron density in CA3, p = 0.051, and CA4, p = 0.07) (20). Further details of the equipment used are described in Gemmell et al. (3).

Statistical Analyses

Statistical analyses were conducted using SPSS version 19.0. Data were checked for normal distribution and homogeneity of variance using the Shapiro-Wilk and Levene tests. Group means were analyzed using one-way analysis of variance with post hoc Tukey honest significant difference. Correlations were performed using Pearson rank correlation. Results were considered significant when p < 0.05.

RESULTS

Subject Demographics

Subject demographics are presented in Table 1, and clinical features of poststroke subjects are presented in Table 2. Fixation length and postmortem delay were different across all groups (F₅,83 = 2.9, p = 0.019 and F₅,56 = 2.7, p = 0.028, respectively). Post hoc comparisons using the Tukey honestly significant difference test indicated that the control group mean postmortem delay was shorter than that of the PSD group (p = 0.054), and the mean fixation time of the MD group was significantly longer than that of the PSD group (p = 0.015). However, neither correlated with CA3 or CA4 neuron measurements. There were no significant differences in ages among the groups. There were no differences between PSD and PSND groups in CERAD, Braak, or vascular pathology scores. Most of the PSD cases met pathologic criteria for VaD, whereas 4 samples had some AD pathology and were classified as MD (21).

Neuron Densities and Volumes

Neuron densities were greater in CA3 than those in CA4 in all groups (p < 0.001). There were no differences in CA3 or CA4 neuron densities among the groups. Neuron volumes were greater in CA4 than those in CA3 in all groups, including all poststroke subjects (p < 0.01); except there was a trend in the PSND group (p = 0.059). Neuron volumes in both CA3 and CA4 were lower in all poststroke subjects versus controls, with a greater effect in the CA4 region, although this did not reach significance (p > 0.05) (Fig. 2).

CA3 neuron volumes were different among the groups (F₅,60 = 6.3, p < 0.001) (Fig. 2A). Compared with those in the controls, CA3 neuron volumes were less in the MD group (p < 0.001) and there was a trend in the PSD group (p = 0.065). Compared with those in the PSND group, CA3 neuron volumes were reduced in the PSD (p = 0.043) and MD (p < 0.001) groups. The CA3 neuron volumes in the MD group were lower than those in the VaD (p = 0.04) group (Fig. 2A).

CA4 neuron volumes also differed among the groups (F₅,61 = 9.4, p < 0.001) (Fig. 2B). Compared with those in the controls, CA4 neuron volumes were reduced in PSD (p < 0.001), MD (p < 0.001), and AD (p = 0.001), and there was a trend to significance with the VaD group (p = 0.089). Compared with those in the PSND group, CA4 neuron volumes were reduced in PSD (p = 0.001) and MD (p < 0.001), and there was a trend to significance in AD (p = 0.052) versus controls (Fig. 2B). Neuron volumes in CA4 in MD were lower than those in VaD (p = 0.025).

Neuron volume group means are presented as a percentage of control means in Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A564. There were no differences in neuron volumes or densities between male and female subjects.

Clinicopathologic Correlations in Stroke Survivors

The CA3 and CA4 neuron volumes were positively correlated with CAMCOG scores (r = 0.526, p = 0.012 and r = 0.572, p = 0.004, respectively) (Fig. 3). There were no

### TABLE 2. Clinical Findings in Poststroke Subjects

<table>
<thead>
<tr>
<th></th>
<th>PSND</th>
<th>PSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean time from baseline to death (±2 SE), months</td>
<td>68.5 (32.6)</td>
<td>54.2 (14.4)</td>
</tr>
<tr>
<td>Mean total CAMCOG score (range), /100</td>
<td>88.5 (76–98)</td>
<td>63 (24–80)</td>
</tr>
<tr>
<td>Mean memory subscore (±2 SE), /27</td>
<td>22 (1.18)</td>
<td>17.3 (3.6)</td>
</tr>
<tr>
<td>Mean executive function subscore (±2 SE), /28</td>
<td>16.9</td>
<td>9.6 (3)</td>
</tr>
<tr>
<td>Hemisphere with visible lesion on CT scan (right, left, both, none)</td>
<td>(3, 1, 3, 4)</td>
<td>(2, 4, 2, 2)</td>
</tr>
</tbody>
</table>

CAMCOG, Cambridge Assessment of Mental Disorders in the Elderly test; CT, computerized tomography; PSD, poststroke dementia; PSND, Poststroke nondemented.
correlations between neuron volume and memory or executive function subscores. Neuron density was not correlated with CAMCOG scores. Neither CA3 nor CA4 neuron volumes were correlated with AD pathology (Braak staging or CERAD scores), global vascular pathology, or age. Correlations between neuron volumes and CAMCOG scores remained significant when corrected for age.

Correlations Between Hippocampal Subfields

Neuron volumes in CA3 and CA4 were positively correlated across all subjects ($r = 0.718$, $p < 0.001$) and also correlated with previous neuron volume measurements in CA1, CA2, and entorhinal cortex Layer V (ECV) (Table 3). In the poststroke subjects only, neuron volumes were positively correlated between CA3 and CA4 ($r = 0.718$, $p < 0.001$), CA3 and CA1 ($r = 0.612$, $p = 0.002$), CA3 and CA2 ($r = 0.418$, $p = 0.024$), CA4 and CA1 ($r = 0.750$, $p = 0.005$), and CA4 and CA2 ($r = 0.619$, $p = 0.002$).

Neuron densities in CA3 were not correlated with densities in CA4. CA3 neuron densities were, however, positively correlated with ECV neuron densities ($r = 0.481$, $p < 0.001$), and CA4 neuron densities were also correlated with CA1 neuron densities ($r = 0.317$, $p = 0.003$). In the poststroke subjects only, CA3 neuron densities were correlated with ECV neuron density ($r = 0.503$, $p = 0.02$). There were trends toward negative correlations between neuron volume and density in CA3 ($r = -0.373$, $p = 0.08$) and CA4 ($r = -0.403$, $p = 0.051$).

**FIGURE 2.** Neuron volumes in hippocampal subfields CA3 (A) and CA4 (B). *, difference versus controls; +, differences versus PSND; o, outliers; black, $p < 0.05$; gray, $p < 0.1$ (trend). PSND, poststroke nondemented; PSD, delayed poststroke dementia; VaD, vascular dementia; MD, mixed vascular and Alzheimer dementia; AD, Alzheimer disease; All PS, all poststroke survivors.

**DISCUSSION**

We report novel evidence of reduced neuron volumes in hippocampal subfields CA3 and CA4 in poststroke and aging-related dementias. The CA3 and CA4 neuron volumes were reduced by approximately 20% in PSD patients versus those in PSND and elderly controls, and neuron volumes were related to poststroke cognitive function. These results are consistent with those of our previous study on CA1 and CA2, in which neuron volumes were also found to be reduced by 10% to 20% in the dementia groups. Taken together, these findings suggest that neurons within all hippocampal CA subfields are similarly affected in PSD and reflect pathologic mechanisms contributing to cognitive decline.

The other dementia groups also had reduced neuron volumes versus those in controls and PSND. CA3 and CA4 neuron volumes were reduced in MD, and CA4 neuron volumes were reduced in AD and there was a trend in VaD. We

**TABLE 3. Neuronal Volume Correlations Between All Regions**

<table>
<thead>
<tr>
<th></th>
<th>CA3</th>
<th>CA2</th>
<th>CA1</th>
<th>ECV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA3</td>
<td>$r = 0.718$, $p &lt; 0.001$</td>
<td>$r = 0.627$, $p &lt; 0.001$</td>
<td>$r = 0.462$, $p &lt; 0.001$</td>
<td>$r = 0.373$, $p = 0.001$</td>
</tr>
<tr>
<td>CA4</td>
<td>$r = 0.555$, $p &lt; 0.001$</td>
<td>$r = 0.386$, $p &lt; 0.001$</td>
<td>$r = 0.325$, $p = 0.005$</td>
<td></td>
</tr>
<tr>
<td>CA2</td>
<td></td>
<td>$r = 0.406$, $p &lt; 0.001$</td>
<td>$r = 0.311$, $p = 0.012$</td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td></td>
<td></td>
<td>$r = 0.231$, $p = 0.05$</td>
<td></td>
</tr>
</tbody>
</table>

$r$ represents Pearson correlation coefficient. CA1 to CA4, hippocampal regions CA1 to CA4; ECV, entorhinal cortex Layer V.
did not find any relationships between neuron volumes and AD pathology including amyloid or neurofibrillary tangle burden (Braak stage or CERAD score), which suggests a role for non-AD–specific processes in neuron volume loss. The MD group had the most markedly reduced neuron volumes in all CA subfields, indicating that both vascular and neurodegenerative disease processes may have exacerbated mechanisms causing neuron soma shrinkage.

CA3 and CA4 neuron volumes were related to the stroke survivors’ global cognitive function but not memory scores; this is unlike our previous study in which CA1 and CA2 neuron volumes were associated with memory function. This may reflect different roles of the CA3/CA4 neurons versus CA1/CA2 neurons in hippocampal information processing. Indeed, CA1 forms major outputs from the hippocampus and has been shown to be able to function independently of CA3 inputs (7, 22).

Our findings suggest that reduced neuron volumes contribute to hippocampal atrophy widely observed in poststroke, vascular, and neurodegenerative dementias (23–28), particularly in early stages of cognitive impairment before significant neuron loss. However, the finding that CA3 neuron volumes were not reduced in AD and VaD subjects may suggest that CA3 neurons are more resistant to specific injuries associated with either vascular or neurodegenerative disease. In the MD group, the coexistence of both AD and CVD processes resulted in the most severely reduced neuron volumes in all CA subfields including CA3. This may simply reflect damage to CA3 neurons caused by collective insults from both disease processes or, alternatively, it may reflect increased damage to remote susceptible neurons that communicate with CA3 neurons, resulting in increased loss of targets and deafferentation. This may have caused the retraction of processes and loss of axo-dendritic arbors in CA3 neurons, which has previously been implicated as the cause of neuron volume loss (29, 30).

Neuron volumes in all CA subfields were significantly correlated with one another. The strongest correlations were generally found between adjacent subfields (CA4-CA3, CA4-CA2, CA3-CA2, and CA2-CA1), which make up the major connections within the hippocampal circuit (7). These relationships may be caused by similar levels of exposure to disease processes or may reflect secondary morphologic changes to neurons caused by loss of connections from or to the neurons they contact. Loss of axo-dendritic arbors has been suggested to cause reductions in neuron soma volume in dementia (30), and studies have found synapse loss to be an important correlate of cognitive impairment in dementia (31). However, further work is needed to determine whether neuron soma volume changes reflect loss of axo-dendritic arbors and/or synapses in PSD.

We did not find any differences in CA3 and CA4 neuron density in PSD, VaD, and MD or VaD versus controls. Interpretation of this finding is limited by the use of neuron density rather than total neuron numbers as an indicator of

FIGURE 3. Relationship between neuron volumes and cognitive function in poststroke survivors. (A) Hippocampal subfield CA3 neuron volumes versus total Cambridge Assessment of Mental Disorders in the Elderly (CAMCOG) score ($r = 0.526$, $p = 0.012$). (B) Hippocampal subfield CA4 neuron volume versus CAMCOG score ($r = 0.572$, $p = 0.004$); o, poststroke nondemented; x, poststroke dementia.
neuron loss, as previously discussed in detail (3). Studies of other brain disorders, for example, depression and HIV-AIDS with cognitive dysfunction, have also reported reductions in neuron volumes without neuron loss (32, 33). Our results build on these findings and suggest that neuron volume reductions can occur in response to a variety of disease processes, resulting in changes to neuron morphology and cognitive dysfunction even without significant neurodegeneration.

Although this study was of a relatively substantial size for a study of human brain tissue, it would require greater numbers to investigate the relationship between the observed neuron changes and factors such as age, risk factors, and number and size of infarcts. There were no associations between neuron shrinkage and age, however, because this study only investigated neuron volumes in 75-year-old and older subjects; further work in younger controls without age-associated neuropathology would be required to determine whether neuron volume loss also occurs in normal aging. We did not find any associations between the number of vascular risk factors and neuron changes in PSND and PSD subjects, which may have been limited by the sample size; a previous study of the whole CogFAST cohort (n = ~400) found that the presence of 2 or more vascular risk factors was a predictor of dementia (21). We also found that it was not possible to establish accurately whether further strokes had occurred at follow-up; therefore, it was not possible to investigate relationships between lesion number and hippocampal neuron changes in this subgroup. A further limitation of this study was that tissue from controls, VaD, MD, and AD subjects was collected from parallel prospective studies rather than part of the CogFAST study. However, the results demonstrating differences between the PSND and PSD subjects within the same cohort and almost equal burden of vascular pathology at baseline were not attributable to differences in tissue processing or other unidentified factors. Furthermore, all tissues were collected, treated, and analyzed in a standardized manner to minimize differential tissue effects from processing and staining all cases, thereby allowing accurate and valid comparisons to be made.

These findings provide further evidence that hippocampal neuron soma volumes are decreased in delayed PSD and aging-related dementias and that reduced neuron volumes are associated with impaired cognitive function. CA4 neuron volumes were similarly decreased in AD and VaD, indicating that neuron volume loss occurred as a response to pathologic mechanisms with distinct disease etiologies. We did not find any significant differences in CA3 or CA4 neuron density between controls, PSND, and dementia groups. Taken together, our findings suggest that the selectively reduced neuron volumes may reflect mechanisms contributing to dementia and poststroke cognitive impairment even in the absence of significant neuron loss or AD pathology. Further work is needed to establish the underlying vascular mechanism driving neuron volume loss.

ACKNOWLEDGMENTS

We are very grateful to the patients, families, and clinical staff for their cooperation in this study. We are indebted to Dr. Tuomo M. Polvikoski for his assistance with the pathologic diagnosis of the poststroke cohort. We thank Michelle Widdrington, Careen Todd, Jean Scott, Deborah Lett, and Anne Nicholson for assistance in managing and screening the cohort. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

REFERENCES


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