Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer’s disease

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Abstract

Neurofibrillary degeneration in the nucleus basalis and a loss of its cortical cholinergic projections are prominent components of the neuropathology in Alzheimer’s disease (AD). The AD brain is also associated with a degeneration of the noradrenergic projections arising from the nucleus locus coeruleus (LC), but the time course of this lesion is poorly understood. To determine whether the LC displays neurofibrillary abnormalities early in the course of events leading to AD, we examined tissue specimens from seven cognitively normal controls and five subjects at the stages of mild cognitively impairment (MCI) or early AD. Tyrosine hydroxylase immunochemistry was used as a marker of LC neurons while AT8 immunolabeling visualized abnormal tau associated with neurofibrillary tangles and their precursors. Thioflavine-S was used as a marker for fully developed tangles. We found that AT8-positive labeling and thioflavine-S positive tangles were present in both groups of specimens. However, the percentage of neurons containing each of these markers was significantly higher in the cognitively impaired group. The MMSE scores displayed a negative correlation with both markers of cytopathology. These results indicate that cytopathology in the LC is an early event in the age-MCI-AD continuum and that it may be listed among the numerous factors that mediate the emergence of the cognitive changes leading to dementia.

Keywords: Alzheimer’s disease; Mild cognitive impairment; Locus coeruleus; Norepinephrine; Neurofibrillary tangles

1. Introduction

Alzheimer’s disease (AD) leads to severe cognitive and behavioral impairments that compromise independent daily activities. Neurofibrillary tangles (NFTs) and β-amyloid plaques in cortical and subcortical areas are its prominent histopathological markers. Other significant features include neuronal death, synaptic loss and depletion of transmitters such as acetylcholine, serotonin and noradrenaline (NA) [15]. The loss of neurotransmitters has attracted a great deal of attention because it is the one aspect of the disease most amenable to pharmacological intervention. Determining the time course of transmitter-specific pathologies helps to differentiate the degenerative changes that are part of the initial pathogenetic cascade from those that may reflect end-stage events. This is an important distinction since changes that can be traced to the early and prodromal stages of the disease become potential targets for therapies aimed at disease prevention. Addressing this question requires postmortem examination of subjects who have had longitudinal neuropsychological characterization until shortly before death.

We recently conducted such an investigation on cholinergic pathways in a sample of 12 rigorously characterized subjects. We found that cytoskeletal abnormalities in the nucleus basalis (NB), the origin of cholinergic innervation of the cerebral cortex, begin during the course of normal aging and that...
they become significantly more prominent at a prodromal stage of the disease known as mild cognitive impairment or MCI. We also determined that the extent of cytopathology in the NB was significantly correlated with performance on a delayed memory test, suggesting that pathological alterations in cortical cholinergic pathways may contribute to the declining memory performance seen in the course of the age-MCI-AD continuum [26].

The present study aimed to determine whether a similar conclusion can be reached in the case of the noradrenergic projections originating in the nucleus locus coeruleus (LC). This nucleus displays severe cytopathology in advanced stages of AD, leading to a prominent loss of noradrenergic markers in the cerebral cortex [3,18,22,46]. However, the status of the LC at the earlier stages of the aging-MCI-AD continuum has yet to be investigated in a neuropsychologically well characterized sample of individuals.

The LC is the primary source of cortical, thalamic, cerebellar, brain stem and spinal cord NA. Its ascending projections branch extensively and innervate all parts of the cerebral cortex [14,27,35]. Investigations in several animal models have demonstrated that the activity of LC neurons and the resultant release of NA influence attention, arousal and memory [39]. This noradrenergic innervation is thought to optimize the signal-to-noise ratio at the stage of information processing and to influence response selection at the stage of decision making [8,11]. Increasing LC neuronal activity mediates changes from EEG synchronization to low voltage fast activity, from slow wave sleep to REM, and from behavioral drowsiness to vigilance. In humans, clinical experience suggests that a deficiency of cortical monoamines, including NA, promotes symptoms of depression [7,45,46]. A dysfunction of LC neurons and its resultant effects on cortical NA could therefore contribute to the deterioration of mood as well as cognition in AD.

2. Methods

2.1. Participants

Postmortem brainstem tissue was obtained from participants who enlisted in a longitudinal study at the University of Miami and who were cognitively normal at the time of entry. All subjects agreed to annual neuropsychological testing and consented to brain donation. From the study’s data bank, we identified all subjects who had converted from normalcy to impairment and who had at least two full neuropsychological tests within the enrolment period. The criteria also required that one of these examinations be within 18 months of death. We excluded specimens with significant neuropathological changes other than plaques and tangles. Subjects who had medical diseases or medication intake that could interfere with cognitive function were also excluded. Five individuals (three women, two men; mean age, 92.4) fulfilled the criteria and were included in the present study. Four individuals were at the stage of MCI and one at the early stages of AD (8-CI–12-CI). Seven subjects (four women, three men; mean age 89.7) for whom we had definite evidence of cognitive normalcy throughout the follow-up period comprised the control group (1-CN–7-CN). Serial neuropsychological examinations on six of these subjects and Telephone Interviews of Cognitive Status (TICS) for one subject (7-CN) served as evidence for cognitive normalcy [29]. Other salient individual characteristics can be found in Table 1. All of these subjects, except 7-CN, were also reported in a study investigating the NB [26].

Table 1
Subject characteristics

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>MMSE (mpd) a</th>
<th>Age at death, sex</th>
<th>Autopsy interval (h) b</th>
<th>Braak stage</th>
<th>CERAD (Ent/STS)</th>
<th>Brain weight (g)</th>
<th>LC (A6) AT8 (%) c</th>
<th>LC (A6) tangles (%) c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-CN</td>
<td>25 (12)</td>
<td>88, F</td>
<td>9</td>
<td>2</td>
<td>S/S</td>
<td>1104</td>
<td>12/218 (5.5)</td>
<td>3/218 (1.4)</td>
</tr>
<tr>
<td>2-CN</td>
<td>29 (10)</td>
<td>89, F</td>
<td>9</td>
<td>1–2</td>
<td>S/S</td>
<td>1180</td>
<td>16/554 (2.9)</td>
<td>3/554 (0.54)</td>
</tr>
<tr>
<td>3-CN</td>
<td>30 (13)</td>
<td>90, M</td>
<td>2.5</td>
<td>2</td>
<td>S/S</td>
<td>1155</td>
<td>14/252 (5.6)</td>
<td>3/252 (1.2)</td>
</tr>
<tr>
<td>4-CN</td>
<td>28 (18)</td>
<td>91, M</td>
<td>12</td>
<td>2</td>
<td>S/S</td>
<td>1309</td>
<td>4/196 (2.0)</td>
<td>3/196 (1.5)</td>
</tr>
<tr>
<td>5-CN</td>
<td>28 (15)</td>
<td>92, M</td>
<td>7</td>
<td>3</td>
<td>S/S</td>
<td>1290</td>
<td>14/581 (2.4)</td>
<td>11/581 (1.9)</td>
</tr>
<tr>
<td>6-CN</td>
<td>28 (4)</td>
<td>95, F</td>
<td>3.2</td>
<td>3</td>
<td>M/F</td>
<td>1096</td>
<td>15/267 (5.6)</td>
<td>7/267 (2.7)</td>
</tr>
<tr>
<td>7-CN</td>
<td>NA c (11)</td>
<td>83, F</td>
<td>13</td>
<td>2</td>
<td>S/S</td>
<td>1175</td>
<td>14/415 (3.4)</td>
<td>8/415 (1.9)</td>
</tr>
<tr>
<td>8-CI</td>
<td>24 (16)</td>
<td>89, F</td>
<td>4.5</td>
<td>2</td>
<td>M/S</td>
<td>1280</td>
<td>15/217 (6.9)</td>
<td>9/217 (4.1)</td>
</tr>
<tr>
<td>9-CI</td>
<td>24 (1)</td>
<td>90, M</td>
<td>3</td>
<td>3</td>
<td>M/S</td>
<td>1380</td>
<td>15/253 (5.9)</td>
<td>10/253 (4.0)</td>
</tr>
<tr>
<td>10-CI</td>
<td>27 (9)</td>
<td>92, F</td>
<td>3.5</td>
<td>3–4</td>
<td>S/S</td>
<td>1084</td>
<td>24/548 (4.4)</td>
<td>22/548 (4.0)</td>
</tr>
<tr>
<td>11-CI</td>
<td>23 (11)</td>
<td>92, M</td>
<td>4.5</td>
<td>5</td>
<td>F/F</td>
<td>1100</td>
<td>58/323 (18)</td>
<td>65/323 (20)</td>
</tr>
<tr>
<td>12-CI</td>
<td>27 (1)</td>
<td>99, F</td>
<td>5</td>
<td>3–4</td>
<td>S/M</td>
<td>1060</td>
<td>27/353 (7.6)</td>
<td>13/353 (3.7)</td>
</tr>
</tbody>
</table>

Tangle densities of the entorhinal, transentorhinal, fusiform, and inferotemporal corticies were used to determine the Braak & Braak stage. CERAD classification indicates sparse (S), moderate (M) and frequent (F) distribution of plaque densities in the entorhinal cortex (Ent) and the superior temporal sulcus (STS). TICS: Telephone Interview of Cognitive Status; MMSE: Mini-Mental State Examination; CERAD: Consortium to Establish a Registry for Alzheimer’s Disease.

a Scores from the final testing session in the months prior to death (mpd).

b Time elapsed between death and autopsy.

c The numerator denotes the number of tangles (or AT8-positive cells) while the denominator reflects the number of locus coeruleus (LC[A6]) neurons from an adjacent section.

TICS was used instead of MMSE testing. Score = 38/50.
2.2. Neuropsychology

The psychological testing assessed memory, language, visuospatial skills and executive functions of the subjects [1,2,13,30,43]. We also had dementia severity scores based on the Mini-Mental State Examination. A complete list of tests and the subjects’ respective scores during the first and last sessions is presented in Table 2. Determining what is considered normal performance for those above 89 years of age is a complex process as normative ranges above this age are rarely available and can vary by age, gender and education level. In this study, the normative values were determined using the eldest available group data, the 70–89-year-old age range. Additionally, individual scores were compared against normative scores of 50–59-year-old (the exception being the CERAD 15-item BNT where we used the 50–69-year-old age range for normative scores) [9,10,19,20,40]. Based on available age- and education-related ranges, test scores were converted into a non-linear six-point scale (Fig. 1). A grade of 1 and 2 indicated impairment, 3 and 4 signified average performance and a grade of 5 and 6 denoted superior performance (i.e. performance that is considered normal for the 50–59-year-old range). Although only the results of the first and last testing sessions are presented (Table 2), all sessions were considered for determining the individual’s cognitive status. Behavioral questionnaires and family interviews yielded data on daily living activities. Cognitive impairment that involved memory but was not severe enough to significantly interfere with independent daily living activities led to a MCI diagnosis while the presence of more severe impairments and corresponding disruptions of daily living activities led to a diagnosis of early AD [31,36,37].

2.3. Histology

Fixation of tissue occurred following a postmortem interval of 2.5–13 h as formerly described [24]. Available brainstem tissue was sectioned coronally into 1–2 cm blocks, frozen on dry ice, cut into 40 μm sections using a sliding microtome and collected in phosphate buffer. The identification of the LC was based on adjacent sections stained with (1) cresyl violet, (2) tyrosine hydroxylase (TH) using standard methods and a modified (3) acetylcholinesterase (AChE) procedure [24]. Cresyl violet staining was used to identify the characteristically magnocellular and neuromelanin-rich LC neurons while TH immunoreactivity was used as a marker for the noradrenergic nature of these neurons [35]. The AChE histochemistry yielded the characteristic AChE-rich staining pattern of LC neurons and also permitted the macroscopic delineation of the LC from other major structures at that level of the brainstem [21]. In keeping with numerous reports in the literature, these three stains provided unequivocal identification of the LC, also known as the A6 cell group [46]. Thioflavine-S histofluorescence identified fully formed NFTs whereas AT8 (Pierce Endogen),...
immunohistochemistry recognized NFTs as well as abnormally phosphorylated tau that precedes tangle formation [5]. Most of the AT8 immunolabeling was performed using the brown DAB reaction product. However, in order for the AT8 immunoreactivity to be differentiated from neuromelanin with even greater clarity, some labeling was based on an alkaline phosphatase blue reaction product. Immunohistochemical and histofluorescence procedures used in this study are described elsewhere [16]. The AT8 and TH primary antibodies were substituted with an irrelevant IgG in negative controls. Brainstem sections from two cognitively intact and neurologically healthy 35-year-old were utilized as positive controls. The anteroposterior extent of the LC is approximately 1 cm. We confined our observations to the anterior 0.5 cm of the LC for which we had tissue available in all subjects.

2.4. Neuropathology

Thioflavine-S staining of entorhinal and superior temporal sulcus (STS) areas determined the Braak and Braak NFT [6] and CERAD plaque staging [28]. A Nikon Eclipse E800 scope with a computer driven stage and Stereoinvestigator-linked software (Microbrightfield Inc., Colchester, VT) was used to view, trace and digitize stained sections that were then viewed on a computer screen. For examining NFT distribution, thioflavine-S sections were scanned under a violet filter (380–420 nm) with a 20× objective and 10× ocular. The stage was guided automatically in a frame-by-frame manner; digital markers were placed on the identified NFTs, preventing them from being counted twice. Using the same approach, brightfield illumination was used in viewing cresyl violet, AChE, and TH sections (10× objective and 10× ocular) for the purpose of determining the boundaries of the LC (by cresyl violet and AChE staining) and counting TH-positive LC neurons within these boundaries. These values were used to calculate the percentage of TH-positive (that is, noradrenergic) neurons that contained fully formed NFT or AT8-positive tauopathy. Digitized files of adjacent thioflavine-S and AT8 sections were merged and aligned with matching fields in the TH-labeled sections for the purpose of illustrating the anatomical relationships among these markers. Table 1 illustrates the quantitative results.

3. Results

3.1. Neuropsychological subject characterizations

Tables 1 and 2, respectively, provide a summary of the pathological and neuropsychological characteristics of the cognitively normal (CN) and cognitively impaired (CI) subjects. In all five cases with cognitive impairment, there was indication of a decline in cognitive performance so that the approximate time of conversion from normal to MCI or from MCI to early AD could be determined. Examples of CN and CI neuropsychological profiles are depicted in Fig. 1.

3.2. Neuropathology

When examined with the stains noted above, the specimens displayed no major neuropathological lesions (such as infarction, neoplasm or infection) other than amyloid plaques and NFTs. The cognitively normal (CN) control group displayed tangles largely confined to medial temporal structures, corresponding to Braak stages 1–3. Six of the seven subjects in the CN group had a very light density of mature plaques in the entorhinal area and the cortex of the superior temporal sulcus (STS). In the MCI cases, the Braak NFT stage ranged...
from 2 to 4. Subject 11-CI, who had the clinical findings of early AD, displayed a Braak stage of 5, indicating a definite spread of NFT to neocortical areas. Neuritic plaque densities also tended to be higher in the CI group. However, there was also considerable overlap between the two groups: 10-CI had distinctly fewer plaques than 6-CN and 8-CI had fewer NFTs than 5-CN or 6-CN.

3.3. Cytoarchitecture

The LC was identified by its characteristic hyperchromic magnocellular and melanin-containing neurons in cresyl violet sections and TH-reactive perikarya in the matching immunoreacted sections. Sections treated with a non-specific IgG instead of the TH antibody did not yield immune active perikarya in that region. As neurons of the LC are also AChE-rich, the enzymatic histochemical procedure was particularly useful for the macroscopic identification of the LC as well as the delineation of other nuclear groups and their adjacent fiber fascicles. The LC was cytoarchitectonically differentiated from the nucleus subcoeruleus, which also contains TH-positive perikarya, on architectonic grounds. As all LC neurons are homogeneously TH-positive [42], the number of TH-positive neurons was used as the denominator in calculating the percentage of LC neurons containing NFTs or AT8 immunoreactivity.

Fig. 2. Neurofibrillary tangles in the locus coeruleus. The green circles depict the melanin-rich noradrenergic locus coeruleus neurons. The red stars indicate the neurofibrillary tangles. For each subject, the illustrated section contained the greatest number of tangles.
3.4. Locus coeruleus tangles

To determine NFT percentages in the LC, thioflavine-S stained sections were matched to their adjacent TH sections. The percentages in each case were derived from an examination of two or three sections through the LC, depending on the availability of tissue. The maps in Fig. 2 were generated by identifying TH-positive perikarya (as green circles) and NFTs (as red stars) in separate but matching sections and then overlapping the landmarks visible in both sections. In the CN group, the percentage of NFT-containing LC neurons ranged from 0.54 to 2.7, whereas in the CI group the range extended from 3.7 to 20%. There was no overlap between the two groups. The one outlier, subject 11-CI with 20% of its LC neurons containing NFT, was also the only subject in the sample with a diagnosis of early AD (Table 1).

3.5. Locus coeruleus AT8-immunopositive neurons

The AT8 antibody recognizes abnormally phosphorylated tau not only in NFTs but also at stages of tauopathy preceding NFT formation. Using an approach similar to that described in the previous paragraph, we determined the percentage of AT8 containing perikarya in the LC (Table 1; Figs. 3 and 4). Negative control sections showed that processing with an irrelevant IgG (instead of the AT8 antibody) did not generate immunoreactivity in the LC. Positive controls were obtained in two 35-year-old specimens with no known neurological disease where immunolabeling with AT8 exhibited characteristic staining in the LC but did not reveal definite AT8 immunoreactivity.

Both CN and CI individuals displayed AT8 immunoreactivity in perikarya and the neuropil (Fig. 3). The percentage of AT8 immunopositive neurons ranged from 2 to 5.6 in the CN group and from 6.9 to 18% in the CI group. The only overlap occurred in subject 10-CI who had 4.4% AT8-positive LC neurons, falling within the range of the CN group. As in the case of NFTs, subject 11-CI was the outlier with the highest percentage (18%) of AT8 neurons (Table 1). In general, the distribution of AT8 phosphotau labeling was more extensive that the thioflavine-S labeled NFTs, supporting the conjecture that AT8 recognized abnormally phosphorylated tau not only in NFTs but also in their precursors.

At the pontine levels that were examined, the LC (and its ventral subcoeruleus extension) was the only nucleus that consistently displayed AT8-positivity in all specimens. The serotonergic raphe nuclei (NR) were not always present in the sections that we examined. However, in the few cases where it was available, it was the only other nucleus at this level of the brainstem to contain AT8-positive neurons, with the cognitively impaired subjects clearly exhibiting greater degrees of abnormality (Fig. 4). Underscoring the remarkable selectivity in the distribution of tauopathy early in the age-MCI-AD continuum, vast regions of the pons, containing the pontine, pedunculopontine, laterodorsal tegmental, parabrachial and reticular nuclei were without NFT or AT8 immunolabeling.

3.6. Quantitative analysis

The nonparametric Mann–Whitney rank-sum test demonstrated a significant difference between the CN and CI group for the percentage of LC neurons containing NFT (median = 4.0 for CI and 1.5 for CN, \( p = 0.004 \)) and AT8
immunoreactivity (median = 6.9 for CI and 3.4 for CN, \( p = 0.018 \)). The Wilcoxon matched-pairs signed rank test revealed that the percentage of AT8-immunopositive neurons was higher than that of NFT-containing neurons (median = 5.5 for AT8 and 2.3 for NFT, \( p = 0.010 \)). The Spearman rank order correlation showed that the MMSE scores \((n = 11)\) possessed a strong negative correlation with both NFT-containing \((rs = -0.827; p = 0.002)\) and AT8-positive \((rs = -0.628; p = 0.038)\) neurons.

4. Discussion

Pathology in the LC and loss of cortical noradrenergic markers in AD have been reported previously [15]. For example, Zweig and colleagues reported significant neuronal loss and NFTs in the LC of patients with AD [46]. Furthermore, Mann et al. found a correlation between LC atrophy and frequency of NFTs in the cortex [23] while Bondareff et al. reported a positive correlation of NFTs with the duration and severity of the dementia [4]. In a study of 47 autopsied specimens from subjects with AD, Zubenko and colleagues linked the noradrenergic pathology in the LC to the emergence of clinical symptoms of depression [45]. More recently, lumbar punctures revealed an apparently paradoxical increase of CSF NA levels in AD [12,33,41], suggesting that there may be a compensatory up-regulation of NA synthesis in the unaffected LC neurons [38] or possibly a reduced reuptake capacity in the pathologically compromised axons [12].

Nearly all previous investigations on this subject were based on patients with fully established clinical and pathological AD. Although one of these investigations attempted to separate the specimens into early and late stages [3,4], the groups were formed on the basis of neuropathological rather than neuropsychological evidence. Our sample is relatively unique because of the longitudinal neuropsychological characterization of the subjects and the focus on MCI, a diagnosis that reflects a prodromal stage of AD when made on the basis of memory impairments in subjects where the only significant neuropathology is confined to NFT and amyloid plaques [31,37].

Our results show that the LC develops fully formed neurofibrillary tangles and their precursors in the course of normal aging but that these abnormalities become significantly more prominent at the stages of MCI and early AD. The extent of this LC cytopathology was correlated with overall cognitive state as measured by the MMSE, suggesting that noradrenergic dysfunction may be listed among the numerous factors mediating the onset of cognitive impairments in the aging-MCI-AD continuum. Several lines of
evidence have shown that even early stages of neurofibrillary degeneration can interfere with normal cellular functioning [17]. Such changes can have particularly far reaching consequences when they involve the LC, a small nucleus, containing no more than 20,000 neurons in the normal human brain [32], each of which sends widely divergent axons covering extensive cortical and thalamic regions. Cytopathology in even a small percentage of LC neurons can therefore affect noradrenergic physiology within widespread cortical and thalamic territories. The presence of fully formed NFT and their precursors in the LC even at very low Braak & Braak stages also establishes that the LC is one of the few regions in the brain with a very high susceptibility to neurofibrillary degeneration. We did not measure the volume of the LC and therefore cannot make a statement about atrophy. The presence of atrophy in the MCI and early AD brains would not have influenced our conclusions since our results are based on the percentage of LC neurons containing abnormal inclusions rather than on their densities.

A comparison to our observations in the cholinergic nucleus basalis (NB) shows that the LC has an NFT susceptibility that is equivalent to that of the NB. The one common denominator of these two nuclei is that they send extensively branching projections to the cerebral cortex, an anatomical feature that seems to determine vulnerability to neurofibrillary degeneration among subcortical nuclei [25]. Zarow and colleagues demonstrated that LC neuronal loss in AD can be more extensive than the cholinergic cell loss in the NB and that it correlates more strongly with the estimated duration of illness. However, their study was based on neuronal loss, which is a late marker of neuronal pathology, and also on subjects who had fully established AD [44]. Based on our observations, it would be reasonable to conclude that cytopathology within the cell groups that give rise to the cortical cholinergic and noradrenergic innervations are equally early events in the course of the neuropathological cascade that leads to AD.

In the brainstem, the anatomical selectivity of LC pathology was quite striking. At the pontine levels that were examined, only neurons located within the boundaries of the LC (including its subcoeruleus extension) invariably contained NFT or AT8 immunoreactivity in all samples. The numerous surrounding nuclei were all uniformly free of such pathology (Fig. 4). The only other nucleus with similar pathology was the serotonergic raphe nucleus, which notably is the only other pontine nucleus with extensive axonal projections directed to the cerebral cortex. However, few of our specimens contained the raphe nuclei in their entirety. Our initial impression, based on these limited observations, was that the raphe cytopathology occurred in the cognitively impaired subjects but not in those that were cognitively normal for age. This preliminary observation, however, requires further documentation.

In summary, our study conclusively demonstrated that LC tauopathy is a component of MCI and early stages of AD, supporting the possibility that noradrenergic therapies may have beneficial value in modifying disease progression. Combinations of cholinergic, noradrenergic and serotonergic therapies may therefore warrant closer investigation.

Note added in proof


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References


