Nucleus Raphe Dorsalis in Alzheimer’s Disease: Neurofibrillary Tangles and Loss of Large Neurons

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Diffuse serotonergic fibers are presumed to project to the telencephalon from the nucleus raphe dorsalis (NRD) of the midbrain, in a manner similar to the cholinergic projections from the nucleus basalis of Meynert (nbM) to the cerebral cortex. Neuropathological changes in both of these nuclei have been reported in Alzheimer’s disease (AD). Although many morphometric studies of the nbM in AD have been documented, only one such study of the NRD has been conducted so far; it demonstrated a sixfold increase in neurofibrillary tangles in AD but no statistically significant difference in the number of neurons in patients with AD and age-matched controls.

A study of the NRD utilizing different stains and wider anatomical boundaries is detailed in this report of 5 patients with AD and 7 age-matched controls. In AD the NRD showed 39 times more neurofibrillary tangles and the number and cell density of large neurons were reduced to 23% and 28%, respectively, of those in the controls. A small number of senile plaques were found in the NRD in all patients with AD but none were found in the controls.


The nucleus basalis of Meynert (nbM) is known to provide the major source of cholinergic innervation to the cerebral cortex. Recent studies have suggested that the severe neuronal loss in the nbM and the marked decrease in choline acetyltransferase in the cerebral cortex may play an important role in Alzheimer’s disease (AD) and in other dementing conditions [2, 20, 21, 26, 27].

The cholinergic nbM, the noradrenergic locus ceruleus, and the presumably serotonergic nucleus raphe dorsalis (NRD) constitute the three major sources of diffuse projection fibers to the forebrain [6, 8, 11, 16, 24]. In experimental animals the NRD was shown to provide fibers to the hypothalamus, thalamus, amygdaloid nucleus, hippocampal formation, septum, caudate, putamen, and cerebral cortex, especially the frontal cortex [6]. Although biochemical studies of the serotonergic system in AD have created controversy [1, 3, 4, 9, 10, 17, 18, 22, 23], a morphometric study of the NRD [7] has called attention to this nucleus as another possible important site of abnormality in AD.

We describe here a morphometric study of the NRD in patients with AD and in age-matched controls.

Materials and Methods

Transverse sections at the level of the trochlear nucleus and without vascular abnormalities were chosen from the autopsy files of Montefiore Medical Center. Among them, five typical cases of AD with clinical evidence of severe dementia of several years’ duration were available for examination. All five patients had had numerous senile plaques (SPs) and neurofibrillary tangles (NFTs) in the neocortex and hippocampus (Table).

Seven patients without dementia served as controls. The controls did not have SPs or NFTs in the neocortex, and there were no (Patients 6–10) or just a few (Patients 11 and 12) NFTs in the hippocampus. Patient 6 had cytomegalovirus encephalitis, Patient 9 had tabes dorsalis, and Patients 10 and 11 had small cerebral and cerebellar infarcts; the remaining three patients had no neuropathological abnormalities.

Formalin-fixed, paraffin-embedded tissue was cut in 8 and 10 μm sections and stained using modified Bielschowsky [12] and Nissl methods, respectively. One slide of each preparation was selected for counting. Because there is no clear-cut border between the NRD and the surrounding periaqueductal gray matter, we defined the NRD arbitrarily (Fig 1) as the area bordered ventromedially by the trochlear nuclei, the medial longitudinal fascicles, and their thin median connection; laterally by the mesencephalic tracts and nuclei of the trigeminal nerves; and dorsally by a horizontal...
Counts of Neurofibrillary Tangles and Large Neurons in the Nucleus Raphe Dorsalis of Patients with Alzheimer's Disease and Age-matched Controls

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>Brain Weight (gm)</th>
<th>NFTs in NRD</th>
<th>Large Neurons in NRD</th>
<th>Area of NRD (mm²)</th>
<th>Large Neuron Density (per mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58/F</td>
<td>950</td>
<td>70</td>
<td>28</td>
<td>7.9</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>73/F</td>
<td>1,110</td>
<td>42</td>
<td>14</td>
<td>8.0</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>73/F</td>
<td>1,000</td>
<td>258</td>
<td>26</td>
<td>11.7</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>81/F</td>
<td>1,050</td>
<td>67</td>
<td>42</td>
<td>12.1</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>86/F</td>
<td>970</td>
<td>208</td>
<td>54</td>
<td>13.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>74.2 ± 10.6</td>
<td>1,020 ± 60</td>
<td>129 ± 97</td>
<td>32.8 ± 15.5</td>
<td>10.7 ± 2.6</td>
<td>2.98 ± 0.92</td>
</tr>
</tbody>
</table>

For control patients, the number of NFTs per section was 3.3, with a range of 0-10, and the mean number of large neurons was 142, with values ranging from 73 to 248. The mean cell density of large neurons in the controls was 10.6 per mm².

Results

The age of the patients and the area of the NRD did not significantly differ between AD and control groups (see the Table). In the controls the mean number of NFTs was 3.3 per section, with a range of 0 to 10, and the mean number of large neurons was 142, with values ranging from 73 to 248. The mean cell density of large neurons in the controls was 10.6 per mm².

Fig. 1. The nucleus raphe dorsalis (Patient 5) is outlined by the dashed line and bordered by the medial longitudinal fascicles (MLF), trochlear nuclei (IV), nuclei and tracts of the trigeminal nerves (V), and a horizontal line drawn across the center of the aqueduct (A). (Inset) Same section at lower power. (IC = inferior colliculus; SCP = decussation of the superior cerebellar peduncles.) (Modified Bielschowsky; x7.8; inset, x1.4 before 25% reduction.)
Fig. 2. Severe loss of large neurons in the nucleus raphe dorsalis of a patient with Alzheimer's disease (Patient 5) (A), compared with a control (Patient 7) (B). Note preservation of the trochlear nuclei in A. (C, D) Higher magnification of sections indicated by arrowheads in A and B, respectively. A white line on the right in A is an artifact. (A = aqueduct; IV = trochlear nucleus.) (Nissl; A, B, x 16; C, D, x 78, all before 25% reduction.)

There were few to several SPs in the NRD (see Fig 3B, C) in all cases of AD. SPs, with or without amyloid cores, were frequent in the lateral portions of the NRD. They were even more common in the dorsal part of the periaqueductal gray matter and inferior colliculi (Fig 4A, B). The diameter of the largest SP observed in the NRD was 30 μm. They were generally smaller than the SPs in the adjacent dorsal area. It was sometimes difficult to distinguish SPs from NFTs because of their similarity in shape and argentophilia. In the inferior colliculi small plaques without surrounding neuritic processes were frequently observed (Fig 4C). Neither typical SPs nor those without surrounding neuritic processes were detected in the controls.

Obvious astrocytic gliosis was not recognized in the NRDs of patients with AD.

Discussion
Numerous NFTs have been observed in the midbrain tegmentum of patients with AD [13–15, 19, 28]. A quantitative analysis of NFTs in this area was recently documented by Curcio and Kemper [7]. In a morphometric study of the NRD, utilizing Bodian and Luxol fast blue/cresyl violet stains, they observed a six-fold increase in NFTs in patients with dementia of the Alzheimer type compared with age-matched controls. However, they did not observe a significant difference in the number of neurons, either large or medium-

Yamamoto and Hirano: Nucleus Raphe Dorsalis in AD 575
Fig 3. (A) Abundant neurofibrillary tangles (NFTs) (dark dots indicated by arrows) in the nucleus raphe dorsalis of a patient with Alzheimer's disease (Patient 3), with a preponderance in the medial portion. (B) Numerous NFTs and two small senile plaques (SPs) (arrow and arrowhead, respectively) in the nucleus raphe dorsalis of a patient with Alzheimer's disease (Patient 5). (C) Higher magnification of SP indicated by arrow in B. A typical SP and two NFTs are shown. (A = aqueduct; IV = trochlear nuclei; V = a blood vessel.) (Modified Bielschowsky; A, ×16 before 20% reduction; B, ×78 before 25% reduction; C, ×630 before 25% reduction.)

Fig 4. (Patient 3). (A) Many senile plaques (arrows) in the dorsal half of the periaqueductal gray matter and inferior colliculus (IC) of a patient with Alzheimer’s disease. (B) A cluster of typical senile plaques with amyloid cores and (C) a plaque without a surrounding neuritic corona in the inferior colliculus. (A = aqueduct.) (Modified Bielschowsky; A, ×16; B, ×310; C, ×630, all before 25% reduction.)

small, or in the neuronal cell packing density between the two groups. They did find a decrease in the relative percentage of large neurons in their patients with dementia of the Alzheimer type. Our morphometric study of the NRD was done on thicker sections and with a modification of the Bielschowsky silver impregnation method [12] instead of Bodian's. We observed a 39-fold increase in NFTs and a marked reduction in the large neuron population, as well as large neuron density, in AD.

Four factors in combination provide a plausible explanation for the quantitative difference between our data and those of Curcio and Kemper. The choice of stain is one factor; the modified Bielschowsky staining method may be a more sensitive method for the demonstration of NFTs and SPs. Severity of dementia at the time of death is another factor; our patients may have had a more severe form of the disease, accounting for the marked loss of neurons. The arbitrary area of the NRD is the third factor. Our NRD occupied a wider zone that included the most lateral portions of the ventral periaqueductal gray matter. Takahashi and colleagues [25] have shown that the serotonin-positive
neurons in this area in human fetuses are distributed more laterally than in other mammals. Finally, the definition of large neurons may be different in the two studies. We defined large neurons as those with a largest diameter of 25 μm or more, whereas Curcio and Kemper do not mention the actual size of the neurons they counted as large.

Of related interest is the recent report by Mann and Yates [19], which stated that the serotonergic nerve cells in "the nucleus supratrochlearis and dorsal tegmental nucleus" were affected in AD. They reported a significant decrease in the nucleolar volume and cytoplasmic RNA content of these neurons compared with those of controls. On the basis of Mann and Yates’s description of "the medial and dorsal surfaces of the medial longitudinal fasciculus—at the level of nucleus of IV cranial nerve," the two nuclei they examined seem to correspond to our definition of the NRD. Bowen and co-workers [4] found considerable loss of markers of serotonergic terminals from the neocortex in association with NFT formation in the NRD, and on this basis predicted cell loss in this nucleus in AD.

SPs were not described in the NRD in the previous reports [13–15, 19, 28]. We found a small number of SPs, with or without a core, in the NRD in all cases of AD. We also noted SPs in the adjacent dorsal areas. Small plaques without neuritic processes as well as typical SPs were also observed frequently in the inferior colliculi. These SPs and small plaques were not found in the controls.

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References

Yamamoto and Hirano: Nucleus Raphe Dorsalis in AD 577