Neuronal Loss Is Greater in the Locus Coeruleus Than Nucleus Basalis and Substantia Nigra in Alzheimer and Parkinson Diseases

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Context: Alzheimer disease (AD) and Parkinson disease (PD) are associated with neuronal degeneration in major subcortical nuclei, but few studies have examined the neuronal degeneration in these nuclei concurrently.

Objective: To identify clinical and pathological correlates of neuronal loss in the nucleus basalis (NB), locus coeruleus (LC), and substantia nigra pars compacta (SN) in AD and PD.

Design: The study sample comprised 86 cases with pathologically confirmed AD, 19 cases with PD, and 13 healthy elderly control subjects. The number of nucleated neurons was counted in representative sections of the NB, LC, and SN. Effect sizes (ES) were computed to determine the standardized difference in cell counts relative to healthy controls.

Results: Cases of AD showed the greatest neuronal loss in the LC (ES=3.16) followed by the NB (ES=1.10), but variable loss in the SN (ES=0.16). Cases of PD also showed the greatest neuronal loss in the LC (ES=6.47), followed by the SN (ES=2.58) and the NB (ES=0.85). Significant correlations were found between the number of neurons in the NB and LC in PD ($r=0.54$, $P<.05$), as well as AD ($r=0.24$, $P<.05$). The duration of illness correlated with greater neuronal loss in the LC and NB in AD, and greater neuronal loss in the SN in PD.

Conclusions: For both AD and PD the greatest neuronal loss was found in the LC. In AD, neuronal loss was most severe and best correlated with the duration of illness in the LC, rather than in NB as traditionally expected. Correlations between neuronal loss in the LC and NB (but not SN) in both PD and AD suggest that the former 2 nuclei may share common pathogenetic susceptibilities. Given the prominent loss of neurons in the LC, detection and treatment of noradrenergic deficiencies warrant attention in both AD and PD.

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Both Alzheimer disease (AD) and Parkinson disease (PD) are associated with neuronal degeneration in subcortical nuclei. Degeneration of the cholinergic nucleus basalis (NB) is characteristic of AD, but also occurs in PD.1 Neuronal loss in the dopaminergic substantia nigra pars compacta (SN) is the pathologic hallmark of PD,2 but occurs to a variable degree in AD.3 Depletion of neurons in the noradrenergic locus coeruleus (LC) is also well recognized in both disorders.4,5

In the present study, we compared the severity of neuronal loss in AD and PD relative to healthy elderly control subjects across 3 nuclei (NB, LC, and SN). Determining the relative severity of neuronal loss as well as clinical and pathologic correlates of neuronal loss across nuclei could shed light on underlying pathogenetic mechanisms and inform pharmacological treatment.
cresyl violet to visualize neurons. After reviewing these subserial sections at low magnification using a stereoscope (Bausch & Lomb, Tampa, Fla), 1 section from each nucleus was chosen for counting. Magnocellular NB neurons were counted in the Ch4 region containing the site of the maximum neuronal density.8 Pigmented LC neurons were counted in the section containing the maximum number of neurons. Pigmented SN neurons were counted in a section containing the intra-axial portion of the third cranial nerve.9 Nucleated neurons were identified under ×200 magnification and recorded using a camera lucida.

Neurons in the SN and LC were counted bilaterally and were doubled in the few cases with only 1 side available. Counts obtained from right and left sides of the LC and SN were similar (LC: r=0.93, n=99, P<.001; SN: r=0.85, n=97, P<.001). The NB was only available from 1 hemisphere, but is bilaterally symmetric in healthy controls and in cases with AD,10 so NB counts were doubled. Counts are expressed as the number of neurons per section.

STATISTICAL ANALYSES

Analysis of variance (ANOVA) was used to compare neuron counts in each subcortical region in AD cases vs PD cases vs controls. The Tukey follow-up multiple comparison test was used if overall group differences were found. Demographic features with significant differences across groups (age at death, postmortem delay, duration of illness, and brain weight Table 1) were used as covariates in separate analyses of covariance (ANCOVA). Age at onset was omitted from ANCOVA because it is highly correlated with 2 other covariates (duration of illness and age at death).

The effect size (ES) was calculated as the difference between the 2 means (mean of the controls minus the mean of the AD cases or PD cases) divided by the pooled SD. An ES of 1.0 represents 1 SD difference between 2 groups. An ES of 0.2 is considered small; 0.5, medium; and 0.8 or greater, large.11 Pearson product-moment correlations were used to relate demographic variables to the numbers of neurons among the 3 subcortical nuclei within each diagnostic group. Analyses were 2-tailed with significance set at P<.05 and were carried out with the interactive software SAS Version 8.0 (SAS Institute, Cary, NC). Data are given as mean (SD). DSTAT12 was used to compute ESs.

RESULTS

Significant differences were found in sex, age at onset, duration of illness, age at death, postmortem delay, and brain weight among the 3 groups (Table 1). Age at death for all cases ranged from 55 to 92 years (75.0 [8.9] years). Cases of PD were significantly younger than cases of AD at the time of death, but age at death for cases of PD or cases of AD did not differ from controls (Tukey). The mean brain weight was significantly lower for cases of AD, but higher for cases of PD vs controls (Tukey). Although the PD group was preponderantly male, a significant difference remained when brain weights of males with PD were compared with male controls (t=4.24,17; P<.001). Although brain weight is correlated with body length, body length was unavailable for analysis. In both AD and PD, age at onset was inversely correlated with the duration of illness (AD, r=−0.39, P<.001; PD, r=−0.68, P<.001) but positively correlated with age at death (AD, r=0.54, P<.001; PD, r=0.50, P<.001).

NEURON LOSS IN THE NB, LC, AND SN

The severity of neuronal loss in NB, LC, and SN varied considerably between and within groups (Table 2 and Figure). In the NB, the mean loss of nucleated neurons in the AD group was 41.1% vs control (ES=1.10) and 37.3% in the PD group vs control (ES=0.85). Although both groups differed significantly from the controls, they did not differ from each other. Significant group differences remained when age at death, duration of illness, postmortem delay, and brain weight were covaried in ANCOVA analyses.

In both AD and PD, the most severe loss was seen in the LC (Table 2 and Figure). Mean cell loss was 67.9% in AD (ES=3.16) and 83.2% in PD (ES=6.47). The loss was uniformly severe across PD cases. By contrast, neuronal loss was more variable in AD, where 22 AD cases (25.5%) fell within the reference range for controls.

Neuronal loss in the SN was uniformly severe in the PD group: 77.8% mean loss compared with control (ES=2.58). In the AD group, neuronal loss in the SN was mild (6.7% overall, ES=0.16) but highly variable. In 6 cases (7%) neuronal loss was as severe as in the PD group, while in 37 cases (43%), the numbers of neurons were in the 75th percentile or higher of the controls (Figure).

WITHIN-GROUP CORRELATIONS IN NB, LC, AND SN NEURON COUNTS

A small but statistically significant correlation was found between the number of neurons in the NB and LC in AD (r=0.24, P<.05). A somewhat stronger correlation was found in PD (r=0.54, P<.05). Fewer neurons in the NB correlated with female gender (r=0.27, P<.02).

Table 1. Demographic Features*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases of AD (n = 86)</th>
<th>Cases of PD (n = 19)</th>
<th>Control Subjects (n = 13)</th>
<th>Statistical Test</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, F/M</td>
<td>39/47</td>
<td>1/18</td>
<td>6/7</td>
<td></td>
<td>.004</td>
</tr>
<tr>
<td>Age of onset, y</td>
<td>67.9 (9.2) [84]</td>
<td>57.7 (10.5) [15]</td>
<td>...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness, y</td>
<td>8.8 (3.9) [84]</td>
<td>12.4 (6.0) [15]</td>
<td>...</td>
<td></td>
<td>.003</td>
</tr>
<tr>
<td>Age at death, y</td>
<td>76.8 (8.6) [86]</td>
<td>70.0 (7.1) [19]</td>
<td>71.1 (10.3) [13]</td>
<td></td>
<td>.003</td>
</tr>
<tr>
<td>Postmortem delay, h</td>
<td>9.8 (14.5) [82]</td>
<td>15.2 (8.3) [14]</td>
<td>28.1 (19.2) [12]</td>
<td></td>
<td>.001</td>
</tr>
<tr>
<td>Brain weight, g</td>
<td>1103 (165.0) [84]</td>
<td>1423 (99.0) [13]</td>
<td>1229 (91.0) [12]</td>
<td></td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; ellipses, not applicable; PD, Parkinson disease.

*Data are given as mean (SD) [number studied] unless otherwise indicated.
It is hypothesized that more malignant disease is associated with earlier age at symptom onset and fewer surviving neurons. This relationship was found in the AD group between age at onset and the numbers of neurons in the LC ($r = 0.25$, $P < .05$). Similar trends were found between age at onset and numbers of NB neurons in the AD group ($r = 0.19$, $P = .09$) and between age at onset and numbers of SN neurons in PD ($r = 0.44$, $P = .10$). In the AD group, the duration of illness was inversely correlated with the number of neurons in the NB ($r = -0.28$, $P < .01$) and LC ($r = -0.30$, $P < .01$), but not SN. In the PD group, duration of illness was inversely correlated with the number of neurons in SN ($r = -0.53$, $P < .05$), but not NB or LC.

**COMMENT**

This study identifies the LC as the major site of subcortical neuronal loss in both AD and PD. It confirms previous reports of loss of neurons in the NB, LC, and SN in AD and PD, but extends these findings by clarifying the relative severity of neuronal loss across all 3 nuclei. The greatest loss of neurons in AD occurs in the LC (ES = 3.16), followed by the NB (ES = 1.10), and to a variable extent the SN (ES = 0.16). Cases of PD showed the greatest loss of neurons in the LC (ES = 6.47), followed by the SN (ES = 2.58) and NB (ES = 0.85). Neuronal loss in the NB was comparable in AD and PD, even when differences in age at death, postmortem delay, or duration of illness were considered. As has been the case for AD, these findings suggest that greater attention should be given to ameliorating cholinergic deficits in PD.

To our knowledge, this is the largest single report to date of neuron counts in the NB, LC, and SN in AD and PD. Other large series with sample sizes greater than 25 cases of AD are summarized in Table 3. In the single report of a large series of PD cases, neuronal loss in both the LC and NB was more severe in demented vs nondemented PD. Consistent with the present findings, Perry et al observed greater neuronal loss in the LC (83%) vs SN (69%) in PD.

A number of authors have compared pathologic features in individual nuclei between AD and PD (Table 4). Sample sizes have been small, with few consistent differences in relative vulnerability. While loss in the SN is consistently greater in PD, LC neuronal loss is reportedly similar in both diseases, greater in AD than PD or greater in PD than AD. Overall, our findings are consistent with the literature. However, examination of neuronal loss across
all 3 nuclei highlights the greater vulnerability of the LC and modifies the common impression that the NB is the primary site of subcortical neurodegeneration in AD, and that the SN is the primary site in PD.

A potential limitation of this study is our use of representative sections rather than unbiased stereological methods to count neurons in each subcortical nucleus. Design-based stereological methods are necessary to estimate total numbers of neurons in a nucleus. However, for the purpose of estimating ESs, a recent meta-analysis demonstrates that representative sections yield estimates similar to those obtained using nonbiased stereology. Comparability between the 2 methods has also been demonstrated by applying both methods to the same study material.  

Our finding that the LC is more severely affected in both PD and AD was unexpected. It is generally assumed that the SN is the most vulnerable target nucleus in PD as the NB is in AD. In the present study, duration of illness did correlate with the severity of neuronal loss in the SN in PD and in the NB in AD. The latter supports the traditional notions that involvement of these nuclei are core features of these diseases, without necessarily implying that they are also the most vulnerable.

In both disease groups, severities of neuronal loss in LC and NB were intercorrelated, whereas the numbers of neurons in SN were not. These findings suggest that the NB and LC, but not the SN, share some underlying susceptibility. The LC and NB neurons normally have fewer dendritic ramifications than the SN neurons, a condition which may confer greater susceptibility to degenerative processes. Alternatively, the shared vulnerability of the LC and NB may lie in their anatomical connectivity with large areas of cerebral cortex.

The clinical correlates of neuronal degeneration in the LC are often overshadowed by extrapyramidal symptoms in PD and cognitive disturbances in AD. However, affective disturbances and changes in sleep-wake cycles have been recognized in both disorders and merit closer

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**Table 3. Neuron Counts in Large Series of Alzheimer Disease– and Parkinson Disease–Affected Brains**

<table>
<thead>
<tr>
<th>Source</th>
<th>Total Sample Size</th>
<th>% Loss in NB</th>
<th>% Loss in LC</th>
<th>% Loss in SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arendt et al., 1997</td>
<td>64</td>
<td>70.5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bondareff et al., 1987</td>
<td>46</td>
<td>NA</td>
<td>37.7</td>
<td>NA</td>
</tr>
<tr>
<td>Forstl et al., 1994</td>
<td>42</td>
<td>38.5</td>
<td>47.9</td>
<td>43.1</td>
</tr>
<tr>
<td>Gibb, 1988</td>
<td>37</td>
<td>NA</td>
<td>NA</td>
<td>25.6</td>
</tr>
<tr>
<td>Liu et al., 1997</td>
<td>28</td>
<td>NA</td>
<td>NA</td>
<td>12.0</td>
</tr>
<tr>
<td>Mann et al., 1985</td>
<td>32</td>
<td>48.6</td>
<td>65.1</td>
<td>7.6</td>
</tr>
<tr>
<td>Younkin et al., 1986</td>
<td>26</td>
<td>61.1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Present study, 2002</td>
<td>86</td>
<td>41.1</td>
<td>67.9</td>
<td>6.7</td>
</tr>
</tbody>
</table>

**Table 4. Studies Comparing Neuronal Loss Between Alzheimer Disease– and Parkinson Disease–Affected Brains**

<table>
<thead>
<tr>
<th>Source</th>
<th>Subsample Size</th>
<th>% Loss in NB</th>
<th>% Loss in LC</th>
<th>% Loss in SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study, 2002</td>
<td>86</td>
<td>41.1</td>
<td>37.3</td>
<td>67.9</td>
</tr>
<tr>
<td>Chui et al., 1986†</td>
<td>5</td>
<td>61.9</td>
<td>44.8</td>
<td>60.2</td>
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<tr>
<td>Perry et al., 1990</td>
<td>14</td>
<td>39.3</td>
<td>67.9</td>
<td>68.3</td>
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<tr>
<td>Chan-Palay et al., 1993</td>
<td>6</td>
<td>54.8</td>
<td>57.9</td>
<td>69.3</td>
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<tr>
<td>Perry et al., 1993</td>
<td>4</td>
<td>8.0</td>
<td>57.1</td>
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<tr>
<td>Rogers et al., 1985</td>
<td>3</td>
<td>70.0</td>
<td>54.0</td>
<td>68.3</td>
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<td>Chan-Palay and Asan, 1989</td>
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<td>52.0</td>
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<td>Chan-Palay, 1990</td>
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<td>25.6</td>
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<td>Goto et al., 1990</td>
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<td>Liu et al., 1997</td>
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<td>57.1</td>
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<tr>
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<td>1.0</td>
<td>68.4</td>
<td>68.3</td>
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<tr>
<td>Tompkins et al., 1997</td>
<td>5</td>
<td>35.4</td>
<td>68.0</td>
<td>68.3</td>
</tr>
<tr>
<td>Victoroff et al., 1996†</td>
<td>69</td>
<td>21.6</td>
<td>83.3</td>
<td>68.3</td>
</tr>
</tbody>
</table>

**Abbreviations:** LC, locus coeruleus; NA, not applicable; NB, nucleus basalis; and SN, substantia nigra pars compacta.

*These are all series that have more than 25 cases of Alzheimer disease.

†Some cases in these reports are included in the present study by Zarow et al.
attention.\textsuperscript{21,22} Depression occurs in 40\% of the patients with PD and in similar percentages of patients with mild dementia due to AD.\textsuperscript{23,24} Greater loss of LC neurons has been reported among patients with AD who are depressed\textsuperscript{15,25} and patients with PD\textsuperscript{15} by some, but not all, investigators.\textsuperscript{26} Forstl et al\textsuperscript{25} noted that the subset of patients with AD and depression had the combination of relatively lower number of LC, but relatively higher number of NB neurons. Given the vulnerability of the LC and NB in AD and PD, the role of combined noradrenergic and cholinergic therapy warrants additional study.

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**Author contributions:** Study concept and design (Drs Zarow and Chui); acquisition of data (Drs Zarow and Mortimer); analysis and interpretation of data (Drs Zarow and Chui and Mr Lyness); drafting of the manuscript (Drs Zarow and Chui); critical revision of the manuscript for important intellectual content (Drs Zarow, Mortimer, and Chui and Mr Lyness); statistical expertise (Mr Lyness); obtained funding (Dr Chui); administrative, technical, and material support (Drs Mortimer and Chui).

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## REFERENCES


