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Neuronal Loss and α-Synuclein Pathology in the Superior Colliculus and Its Relationship to Visual Hallucinations in Dementia with Lewy Bodies


Objective: Patients with dementia with Lewy bodies (DLB) often experience visual hallucinations, which are related to decreased quality of life for patients and increased caregiver distress. The pathologic changes that contribute to visual hallucinations are not known, but several hypotheses implicate deficient attentional processing. The superior colliculus has a role in visual attention and planning eye movements and has been directly implicated in several models of visual hallucinations. Therefore, the present study sought to identify neurodegenerative changes that may contribute to hallucinations in DLB.

Methods: Postmortem superior colliculus tissue from 13 comparison, 10 DLB, and 10 Alzheimer disease (AD) cases was evaluated using quantitative neuropathologic methods.

Results: α-Synuclein and tau deposition were more severe in deeper layers of the superior colliculus. DLB cases had neuronal density reductions in the stratum griseum intermedium, an important structure in directing attention toward visual targets. In contrast, neuronal density was reduced in all laminae of the superior colliculus in AD.

Conclusion: These findings suggest that regions involved in directing attention toward visual targets are subject to neurodegenerative changes in DLB. Considering several hypotheses of visual hallucinations implicating dysfunctional attention toward external stimuli, these findings may provide evidence of pathologic changes that contribute to the manifestation of visual hallucinations in DLB.

Key Words: Dementia, Lewy, visual, superior colliculus, neuropathology, neuropsychiatry
Dementiap– with Lewy bodies (DLB) is the second most common progressive neurodegenerative dementia after Alzheimer disease (AD), accounting for approximately 4.2% of all dementia cases. DLB is clinically characterized by three core symptoms of fluctuating cognition, parkinsonism, and complex visual hallucinations. The defining histologic feature of DLB, along with Parkinson disease and Parkinson disease dementia, is the presence of intracytoplasmic α-synuclein–containing inclusions termed Lewy bodies. The topographic distribution of Lewy body–related pathology in the respective disorders is believed to underlie the patterns of clinical manifestations. Lewy body pathology affects brainstem, limbic, and neocortical regions in DLB, often in combination with the two hallmark histologic features of AD, β-amyloid and tau pathology.

Visual attention is more abnormal in DLB than in AD or Parkinson disease, and DLB patients show greater impairment in filtering distracting visual stimuli compared with AD or comparison patients. Deficits in visual attention have been suggested to contribute to the manifestation of complex visual hallucinations in DLB. Complex visual hallucinations are common in DLB, occurring in 60%–80% of cases, and typically consist of objects, such as animals, people, and faces. Visual hallucinations are related to impaired quality of life for DLB patients, contribute to caregiver distress, and thus represent an important therapeutic target. However, their pathologic etiology is poorly understood.

The superior colliculus has a distinctive laminar structure consisting of seven layers in three major gray matter strata, the stratum griseum superficiale (SGS), the stratum griseum intermedium (SGI) and the stratum griseum profundum (SGP), interspersed with predominantly fiber-rich layers. The superior colliculus may be subject to neurodegenerative changes in DLB because increased saccadic latency, which has been reported in DLB, has also been observed in nonhuman primates after chemical inactivation of the superior colliculus. The superior colliculus is also involved in attentional functions that are impaired in DLB, such as target selection and the filtering of distracting stimuli. One model has directly implicated dysfunction of the dorsal attentional network, with which the superior colliculus is believed to interact, in the manifestation of visual hallucinations in DLB. The pathway from the retina to the inferior pulvinar, routed through the SGS, has also been directly implicated in the manifestation of hallucinations in Lewy body disease.

The aim of the present study was to identify whether the superior colliculus is subject to neurodegenerative changes in DLB using stereologic measures of cell density and densitometric analysis of neuropathologic lesions. Cell density and neuropathologic data were then compared with neuropsychological data obtained during life, where available, and the known function of the individual laminae of the superior colliculus to determine the relationship between neurodegenerative changes and clinical features. DLB cases were compared with aged comparison cases and “disease comparison” AD cases to elucidate whether degenerative changes occur to the superior colliculus in DLB and their relationship to its distinct clinical manifestation.

**METHODS**

**Tissue Preparation**

Human postmortem tissue was obtained by a convenience sample from the Newcastle Brain Tissue Resource, and ethical approval was granted by the Newcastle University ethics board and the Joint Ethics Committee of Newcastle and North Tyneside Health Authority (reference no. 08/H0906/136). DLB and AD subjects had been part of several prospective clinical studies and had received detailed clinical assessments according to international consensus guidelines and case note review after death. Cases with psychiatric or neurodegenerative comorbidities were not included in the present study. Neuropathologic assessment was performed according to standardized neuropathologic diagnostic procedures. Clinical and pathologic data were collated to establish a clinico-pathologic consensus diagnosis. Three groups of cases were included in the present study: 10 DLB cases, 10 AD cases, and 13 clinically confirmed aged comparison cases that showed no, or only low, age-associated neurodegenerative pathology at postmortem examination. Case details are presented in Table 1.

At autopsy, the upper midbrain was dissected from the cerebrum at the level of the third cranial nerve, along a line from the junction of the mammillary body running posteriorly to the upper part of the superior colliculus (Figure 1). For stereologic analysis, 3 × 30 μm adjacent
TABLE 1. Demographic Information

<table>
<thead>
<tr>
<th>Case Identification</th>
<th>Gender</th>
<th>Age at Death (yr)</th>
<th>PM Interval (hr)</th>
<th>Braak NFT</th>
<th>McKeith LB</th>
<th>Clinicopathologic Diagnosis</th>
<th>NPI (hall)</th>
<th>NPI Interval to Death (mo)</th>
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<td>DLB</td>
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<tr>
<td>Mean ± standard deviation</td>
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<td></td>
<td></td>
<td>0.4 ± 1</td>
<td>11.1 ± 3</td>
</tr>
</tbody>
</table>

Note: N/A stands for not applicable. PM interval represents the interval from death to fixation; Braak NFT represents Braak neurofibrillary pathology stage as outlined in Braak et al.; McKeith LB represents Lewy body pathology stage as outlined in McKeith et al.; clinicopathologic diagnosis represents the consensus diagnosis made by senior clinicians and a neuropathologist, following case note review and autopsy findings; NPI (hall) represents the score on the hallucinations subscale of the Neuropsychiatric Inventory, as detailed by Cummings et al.
sections were cut and stained with cresyl violet. Then, 6-μm sections were stained with antibodies (KM51 anti-α-synuclein [Leica Biosystems, Milton Keynes, UK], 1:250; AT8 anti-phosphorylated tau [Autogen, Holliston, MA], 1:4,000; 4G8 anti-β-amyloid [Covance, Princeton, NJ], 1:15,000) using Menapath polymer detection kits (Menarini Pharma, Berkshire, UK).

**Stereology**

Stereologic estimates of neuronal density were made in each of the three prominent gray matter laminae of the superior colliculus (SGS, SGI, and SGP) based on their cytoarchitecture and laminar organization (Figure 2). In cresyl violet sections, neurons were differentiated from glia by the presence of Nissl substance within cytoplasm, a pale nucleus, and a single identifiable nucleolus. Traced an outline around the region of interest (i.e., SGS, SGI, or SGP) using a 2.5 × objective. Dissector frames were placed in a uniform, random arrangement to calculate the density of cells within a defined region (as described previously). Neuronal counts were conducted at 63 × oil-immersion objective using the optical dissector probe. Glial cell counts were calculated in all laminae in dissector frames of 3,500 μm², with neuron counts calculated in dissector frames of 1,900 μm². Section thickness did not vary across disease groups in any layer. The mean coefficients of error for neuronal and glial cell estimates was calculated using the Gundersen-Jensen method. The mean coefficient of error values for all stereologically obtained data showed acceptable levels of accuracy (<0.15).

To evaluate whether neuronal density changes occur as a result of increasing age, postmortem delay, or length of time fixed in formalin, correlational analyses were conducted between these variables and neuronal density in each layer of the superior colliculus across all cases. To assess whether neuronal density is altered based on the duration of disease, correlation analyses were conducted between neuronal density in each layer and duration of disease in DLB and AD cases.

**Neuropathology**

To quantify neuropathologic lesions, images of each gray matter stratum of the superior colliculus (Figure 2) were taken on a Zeiss AxioVision Z.1 microscope using a DsFi1 camera (Nikon, Tokyo, Japan). Stereologer software was used to delineate a region of interest with a 2.5 × objective, before placement of dissector frames in a uniform, random arrangement. This method prevented the introduction of bias by giving every area of the region of interest an equal probability of being sampled for analysis. In all cases β-amyloid, tau, and α-synuclein were measured using a 20 × objective. Approximately eight images were taken per lamina per case within the dissector frames and analyzed using ImagePro Plus v.4.1 image analysis system (Media Cybernetics, Bethesda, MA). Using previously published techniques, the mean percentage area of immunopositivity was determined by standardizing red-green-blue thresholds per antibody and applying to all sections per case. Each case thus had a mean value generated per antibody for each stratum. The percentage area immunoreactive for α-synuclein, tau, and β-amyloid was compared across disease groups.
to evaluate the vulnerability of the superior colliculus with neurodegenerative pathology in DLB, AD, and comparison cases. Correlational analyses were conducted between neuropathologic and stereologic data to evaluate the relationship between neuropathology and neuronal density in each disease group.

**Clinicopathologic Correlation**

DLB patients had been recruited for clinical research studies, during which some had received serial assessments of visual hallucination severity and frequency at frequent intervals until death. Because of the hypothesized role of the superior colliculus in the manifestation of visual hallucinations in Lewy body disease, neuronal density and pathologic burden were correlated with final Neuropsychiatric Inventory Hallucinations Subscale score (NPI [hall]) in all layers of the superior colliculus in DLB cases, where available. Final NPI (hall) scores were available for 8 of 10 DLB cases. The mean interval from final NPI assessment to death was 7.81 ± 3.45 months. The relationship between visual hallucinations and the superior colliculus was not explored in AD because visual hallucinations are not a core feature of AD and were less frequently assessed. Additionally, as a separate disease entity, it is possible that visual hallucinations in AD, when present, have a different underlying pathogenesis to that in DLB.

**Statistics**

Inspection of Q-Q plots and Shapiro-Wilk tests suggested that demographic, stereologic, and neuropathologic data were either not normally distributed or did not have homogeneity of variance. Therefore, Kruskal-Wallis (KW) tests with post-hoc Mann-Whitney U tests were used for these data. Because of the relatively small sample size, corrections for multiple comparisons were not applied. However, effect sizes were reported using Cohen’s d. For comparisons...
between different laminae, Friedman tests with post-hoc Wilcoxon signed-ranks tests were performed.

RESULTS

Demographics

No significant differences were found between groups in age at death (KW $\chi^2 = 2.461$, df = 2, $p = 0.292$), post-mortem delay (KW $\chi^2 = 2.708$, df = 2, $p = 0.258$), or length of time fixed in formalin (KW $\chi^2 = 0.472$, df = 2, $p = 0.790$; Table 1).

Stereology

In the SGS there was a significant main effect of diagnosis on neuronal density (KW $\chi^2 = 6.579$, df = 2, $p = 0.037$). No significant difference was found between comparison cases and DLB. AD cases (mean $\times 100 = 0.0054$ [95% confidence interval [CI]: 0.0047–0.0061]) compared with DLB (mean $\times 100 = 0.0062$ [95% CI: 0.0053–0.0071]) was observed (U = 26.000, df = 1, $p = 0.07$, d = 0.60). There was no significant main effect of diagnosis on glial density in the SGS (Figure 3).

In the SGI there was a significant main effect of diagnosis on neuronal density (KW $\chi^2 = 13.046$, df = 2, $p = 0.001$). DLB (mean $\times 100 = 0.0030$ [95% CI: 0.0025–0.0034]; U = 18.500, $p = 0.003$, d = 1.08) and AD (mean $\times 100 = 0.0029$ [95% CI: 0.0026–0.0032]; U = 13.5, $p = 0.001$, d = 1.24) had reduced neuronal density compared with comparison cases (mean $\times 100 = 0.0036$ [95% CI: 0.0033–0.0038]). There was no significant difference in neuronal density between DLB and AD in the SGI. There was no significant main effect of diagnosis on glial density in the SGI (Figure 3).

In the SGP there was a significant main effect of diagnosis on neuronal density (KW $\chi^2 = 6.600$, df = 2, $p = 0.037$). No significant difference was found between comparison cases and DLB. AD cases (mean $\times 100 = 0.0028$ [95% CI: 0.0025–0.0031]) had reduced neuronal density compared with comparison cases (mean $\times 100 = 0.0036$ [95% CI: 0.0031–0.0040]; U = 27.000, $p = 0.018$, d = 1.12). There was no significant difference in neuronal density between DLB and AD in the SGP. There was no significant main effect of diagnosis on glial density in the SGP (Figure 3).
In DLB cases duration of disease was significantly negatively correlated with neuronal density in the SGI ($r_c = -0.726$, $N = 10$, $p = 0.018$). However, no significant correlations were found between duration of disease and neuronal density in the SGS and SGP. In AD duration of disease was not significantly correlated with neuronal density in any layer.

**Neuropathology**

Comparisons of neuropathology between groups are detailed in Table 2. In DLB cases there was a significant difference in the degree of α-synuclein pathology across the three layers (Friedman $\chi^2 = 12.600$, df = 2, $p = 0.002$; Table 2). The SGI (mean = 0.0268 [95% CI: 0.0180–0.0356]; Wilcoxon $z = 2.803$, $p = 0.005$, $d = 0.85$) and the SGP (mean = 0.0317 [95% CI: 0.0108–0.0525]; Wilcoxon $z = 2.497$, $p = 0.013$, $d = 1.09$) had a significantly greater burden of α-synuclein pathology than the SGS (mean = 0.0090 [95% CI: 0.0025–0.0155]). There was no significant difference in the burden of α-synuclein pathology between the SGI and SGP. There were no significant correlations between α-synuclein pathology and neuronal density across DLB, AD, and comparison cases.

Across DLB, AD, and comparison cases, there was a significant difference in the degree of tau pathology between the three layers (Friedman $\chi^2 = 26.375$, $p < 0.001$; Table 2). The SGI (mean = 0.0929 [95% CI: 0.0074–0.1783]; Wilcoxon $z = 4.330$, $p < 0.001$, $d = 0.32$) and SGP (mean = 0.1241 [95% CI: 0.0130–0.2352]; Wilcoxon $z = 4.031$, $p < 0.001$, $d = 0.46$) had a significantly greater burden of tau pathology than the SGS (mean = 0.0207 [95% CI: 0.0038–0.0376]). There was no significant difference in the burden of tau pathology between the SGI and SGP. Tau pathology was significantly negatively correlated with neuronal density in the SGI in all cases ($r_c = -0.404$, $N = 33$, $p = 0.022$) and the SGP ($r_c = -0.451$, $N = 33$, $p = 0.009$). However, there was no significant relationship between tau pathology and neuronal density in the SGS.

Across DLB, AD, and comparison cases, there was no significant difference in β-amyloid expression across the three layers of the superior colliculus (Table 2). There were no significant correlations between β-amyloid pathology and neuronal density across DLB, AD, and comparison cases.

**Clinicopathologic Correlation**

There were no significant correlations between age at death, postmortem delay or fixation time in formalin, and neuronal density in any layer of the superior colliculus. In DLB cases NPI (hall) score was significantly positively correlated with neuronal density in the SGI and SGP. There were no significant correlations between β-amyloid pathology and neuronal density across DLB, AD, and comparison cases.

**DISCUSSION**

This study demonstrates significant reductions in neuronal density in the SGI in DLB compared with significant neuronal loss in all laminae in AD. α-Synuclein

---

**Table 2. Neuropathologic Data Demonstrating Comparisons Between Experimental Groups**

<table>
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<tr>
<th>Lamina</th>
<th>Protein Target</th>
<th>Control</th>
<th>DLB</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGS</td>
<td>α-Synuclein</td>
<td>0.0005 ± 0.0013</td>
<td>0.0090 ± 0.0091</td>
<td>0.0000 ± 0.0001</td>
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<tr>
<td></td>
<td>Tau</td>
<td>0.0071 ± 0.0219</td>
<td>0.0153 ± 0.0250</td>
<td>0.0419 ± 0.0724</td>
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<td>SGI</td>
<td>α-Synuclein</td>
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<td>0.0268 ± 0.0123</td>
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</tr>
<tr>
<td></td>
<td>Tau</td>
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<td>0.0421 ± 0.0684</td>
<td>0.2215 ± 0.3804</td>
</tr>
<tr>
<td>SGP</td>
<td>α-Synuclein</td>
<td>0.0039 ± 0.0112</td>
<td>0.0317 ± 0.0292</td>
<td>0.0000 ± 0.0001</td>
</tr>
<tr>
<td></td>
<td>Tau</td>
<td>0.0228 ± 0.0606</td>
<td>0.0491 ± 0.1014</td>
<td>0.3110 ± 0.4838</td>
</tr>
<tr>
<td></td>
<td>β-Amyloid</td>
<td>0.0681 ± 0.2398</td>
<td>0.1260 ± 0.1385</td>
<td>0.3891 ± 0.5491</td>
</tr>
</tbody>
</table>

Notes: Data are the mean ratio of area of staining to total region of interest ± standard deviation.

* $p < 0.05 compared with control and AD.
* $p < 0.05$ compared with control.
* $p < 0.05$ compared with control and D LB.
pathology was not related to neuronal density in any layer in DBL, but tau was inversely related to neuronal density in the SGI and SGP. There was no relationship between $\alpha$-synuclein pathology and hallucination severity and frequency, as assessed by NPI (hall). However, neuronal density in the SGS was positively correlated with the severity and frequency of visual hallucinations in DBL. Distinct topographic patterns of deposition were observed for $\alpha$-synuclein and tau pathology, with the SGI and SGP affected more severely than the SGS.

The SGS receives retinal input and projects to the medial temporal visual area (area V5/MT) through the inferior pulvinar, in a pathway believed to be involved in the nonconscious perception of motion. In contrast, the SGI and SGP receive inputs from wide-ranging cortical and subcortical regions, such as motor and association sensory cortices, the locus ceruleus, and dorsal raphe, and have important roles in the selection of visual targets and in directing the resulting motor output.

Within the superior colliculus, neuropathologic lesions occurred in a stereotypical manner, with higher levels of $\alpha$-synuclein and tau found in the SGI and SGP compared with the SGS. This may reflect the SGI and SGP receiving inputs from the dorsal raphe, locus ceruleus, and pedunculopontine nucleus, which are vulnerable to the accumulation of Lewy body and tau pathology. In contrast, the SGS receives predominant innervation from the retina and primary visual cortex, which do not appear to accumulate significant Lewy body pathology in DBL and develop tau pathology only at the final stage of AD pathology. Because tau and $\alpha$-synuclein may spread throughout the brain in a manner reminiscent of prion protein, these distinct patterns of deposition may reflect greater connectivity with areas that are vulnerable to early neuropathologic lesion formation. However, a neuropathologic study of the superior colliculus has reported greater tau pathology in the SGI and SGP in individuals with chronic traumatic encephalopathy, indicating that these laminae may have an intrinsic vulnerability to tau pathology.

The present study has demonstrated that significant neuronal loss in the superior colliculus was only found in the SGI in DBL cases. This was in contrast to AD, where neuronal density reductions were found in all laminae of the superior colliculus. However, no significant difference in neuronal density was found between DBL and AD in the SGS and SGP. DBL had significantly increased tau in the SGI, but not in other layers of the superior colliculus. Because tau was negatively correlated with neuronal density in the SGI and SGP across all cases, this may indicate that tau is driving neuronal density reductions in DBL. The greater levels of tau pathology in AD cases may similarly cause the reductions in neuronal density seen in all laminae of the superior colliculus.

Previous studies in the lateral geniculate nucleus and primary visual cortex have not demonstrated neuronal loss in DBL. In contrast, neuronal loss has been identified in the lateral pulvinar and in the SGI of the superior colliculus in DBL. One model of visual hallucinations has suggested that DBL patients have difficulty engaging the dorsal attentional network to focus attention on ambiguous stimuli, instead relying on the self-referential default mode network. Because the lateral pulvinar and SGI are implicated in visual attention and target selection, respectively, it is tempting to speculate that neuronal losses in these regions could impair directed attention toward visual targets. However, visual attentional measures were not conducted on the present cases, so it is not possible to confirm whether these patients were impaired in these faculties or whether this related to more severe visual hallucinations. Additionally, visual hallucinations in DBL are unlikely to result purely from neuronal loss in the SGI and lateral pulvinar, and these changes likely act in concert with degenerative changes elsewhere in the visual system to elicit visual hallucinations.

Visual dysfunction, including hallucinations, in Lewy body disease have been linked to dysfunctional pathways involved in blindsight, the phenomenon whereby a blind individual may react to moving or emotional visual stimuli. These pathways implicate the SGS, a region found in the present study to be preserved in individuals with more severe and frequent visual hallucinations. This may indicate that dysfunction of this region, or the pathway through it, is crucial for the occurrence of visual hallucinations. The present study was conducted on a relatively small sample, so it is difficult to draw strong conclusions about the relationship between neuronal number in the SGS and visual hallucinations.

This study was limited by the small sample size as a result of limitations on the availability of upper midbrain tissue. Additionally, the superior colliculus was sampled on three adjacent slides rather than serially...
through the entire z-axis. As a result, volume and total neuronal number could not be estimated. This was because of the superior colliculus being routinely dissected on two different planes (coronal and axial) and the inherent value of upper midbrain tissue. Few cases were available that had NPI data on visual hallucinations obtained during life, and this impacted the number of cases included in the study. However, the use of a comparator group that do not typically manifest visual hallucinations suggests that the reported findings may be related to the vulnerability of DLB patients to visual hallucinations.

In summary, the present study has demonstrated that the superior colliculus is subject to neurodegenerative changes in DLB. Specifically, neuronal loss was only found in the SGI in DLB. In contrast, AD cases had neuronal loss in all layers. Because the SGI is important in directing attention and aiding visual target selection, the present results may indicate dysfunction in these functions in DLB. Because dysfunctional visual attention and target selection have been related to visual hallucinations in DLB, the present results may provide evidence of neuropathologic changes that contribute to the manifestation of visual hallucinations in DLB. However, these changes are unlikely to induce visual hallucinations on their own; thus, continued study of the visual system is necessary to further understand the pathologic changes that contribute to visual hallucinations in DLB.

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References

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