Involvement of medullary regions controlling sympathetic output in Lewy body disease

Eduardo E. Benarroch, 1 Ann M. Schmeichel, 1 Phillip A. Low, 1 Bradley F. Boeve, 1,2 Paola Sandroni 1 and Joseph E. Parisi 1,3

1Department of Neurology, 2Robert H. and Clarice Smith and Abigail Van Buren Alzheimer’s Disease Research Program of the Mayo Foundation and 3Department of Anatomic Pathology, Mayo Clinic, Rochester, MN, USA

Correspondence to: Eduardo E. Benarroch, MD, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
E-mail: benarroch.eduardo@mayo.edu

Summary
We sought to determine the involvement of medullary regions controlling sympathetic output in pathologically confirmed diffuse Lewy body disease (LBD). We studied eight limbic or neocortical stage LBD and eight multiple system atrophy (MSA) cases, confirmed neuropathologically, and eight age-matched controls. Five of the LBD cases and all MSA cases had orthostatic hypotension. Serial 50-μm sections obtained from the medulla rostral to the obex were immunostained for tyrosine hydroxylase, tryptophan hydroxylase and α-synuclein. Analysis was focused on the ventrolateral medulla and medullary raphe nuclei. In LBD cases, there were Lewy bodies and neurites, as well as dystrophic neurons in the ventrolateral medulla, but the number of catecholaminergic and serotonergic neurons was not significantly reduced. All these groups were depleted in MSA. There were Lewy body pathology and dystrophic neurons in the raphe in all LBD cases. Cell numbers were reduced in both the raphe obscurus and raphe pallidus. Our findings suggest that, although LBD affects medullary autonomic areas, it does so less severely than MSA, particularly in the case of the VLM, which controls sympathetic outputs maintaining arterial pressure. In LBD, orthostatic hypotension may be due primarily to involvement of sympathetic ganglion neurons rather than ventrolateral medulla neurons.

Keywords: autonomic failure; synucleinopathy; raphe; ventrolateral medulla

Abbreviations: LBD = Lewy body disease; MSA = multiple system atrophy; OH = orthostatic hypotension; ROb = raphe obscurus; RPa = raphe pallidus; TH = tyrosine hydroxylase; TrOH = tryptophan hydroxylase; VLM = ventrolateral medulla


Introduction
Lewy body disorders, including dementia with Lewy bodies and Parkinson’s disease, like multiple system atrophy (MSA), are synucleinopathies that may manifest with prominent autonomic failure (Hishikawa et al., 2000; Larner et al., 2000; Horimoto et al., 2003). In most cases, Lewy body pathology appears to start in the medulla (Del Tredici et al., 2002), and therefore Lewy body disease (LBD) has been subdivided into brainstem-predominant, limbic (transitional) and neocortical stages, the first corresponding to the clinical syndrome of Parkinson’s disease and the other two to dementia with Lewy bodies (Cummings, 2004; McKeith et al., 1996). A recent retrospective study (Thaisetthawatkul et al., 2004) indicates that autonomic failure, documented with quantitative autonomic laboratory testing is frequent in patients with clinical diagnosis of dementia with Lewy bodies and its severity, as a group, is intermediate between that observed in MSA and Parkinson’s disease patients. However, in some patients, LBD may manifest with prominent autonomic failure, mimicking pure autonomic failure or MSA, even before development of parkinsonism and dementia (Hishikawa et al., 2000; Larner et al., 2000). Although Lewy bodies are found in brainstem autonomic areas in patients with dementia with Lewy bodies associated with autonomic failure (Hishikawa et al., 2000), the involvement of specific medullary groups controlling sympathetic cardiovascular function in late stage LBD is still incompletely defined.

In MSA, there is severe depletion of catecholaminergic neurons in the rostral ventrolateral medulla (VLM)
(Benarroch et al., 1998) as well as loss of serotonergic neurons in the nucleus raphe obscurus (ROb), raphe pallidus (RPa) and VLM (Benarroch et al., 2004). Since these neurons project to and differentially control the activity of target-specific subgroups of preganglionic sympathetic neurons (Morrison, 1999), neuronal loss in these medullar regions may contribute to orthostatic hypotension (OH) and other manifestations of sympathetic impairment in MSA. These catecholaminergic and serotonergic medullary groups were found to be much less severely affected in cases with Parkinson’s disease, despite the abundance of Lewy body pathology in these regions (Benarroch et al., 2000, 2004). However, these groups may be more severely affected and potentially contribute to sympathetic failure in patients with limbic and neocortical predominant LBD, associated or not with dementia, parkinsonism, or both. In this study, we sought to determine whether there is involvement of medullary catecholaminergic and serotonergic neurons controlling sympathetic function in cases with neuropathologically confirmed limbic and neocortical stages of LBD and compare its severity with that found in MSA.

### Methods

#### Subjects

Brains were obtained at autopsy from 24 subjects (Table 1). All patients had signed informed consent for autopsy according to the Institutional Review Board guidelines. Eight subjects (four men and four women, age 66 ± 6 years) had no history of neurological disease; eight cases (six men and two women, age 64 ± 2 years) had the clinical diagnosis of MSA, confirmed neuropathologically; and eight cases (six men and two women, age 80 ± 4 years) with

---

**Table 1 Patient population**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex</th>
<th>PMD</th>
<th>Neurological features</th>
<th>Autonomic features</th>
<th>Disease duration</th>
<th>Clinical diagnosis</th>
<th>Pathological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>72/F</td>
<td>25</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>NHL</td>
<td>NHL</td>
</tr>
<tr>
<td>Control 2</td>
<td>73/M</td>
<td>18</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>CHF</td>
<td>Ischaemic cardiomyopathy</td>
</tr>
<tr>
<td>Control 3</td>
<td>78/F</td>
<td>9</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>CAD</td>
<td>CAD</td>
</tr>
<tr>
<td>Control 4</td>
<td>74/M</td>
<td>12</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>CAD</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Control 5</td>
<td>32/F</td>
<td>6</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>CHD</td>
<td>CHD</td>
</tr>
<tr>
<td>Control 6</td>
<td>51/M</td>
<td>22</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>CHF</td>
<td>Hypertrophic cardiomyopathy</td>
</tr>
<tr>
<td>Control 7</td>
<td>66/F</td>
<td>18</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>Pulmonary fibrosis, CHD</td>
<td>Pulmonary fibrosis</td>
</tr>
<tr>
<td>Control 8</td>
<td>84/M</td>
<td>6</td>
<td>None</td>
<td>None</td>
<td>N/A</td>
<td>Atrial fibrillation</td>
<td>CAD</td>
</tr>
<tr>
<td>LBD 1</td>
<td>84/M</td>
<td>13</td>
<td>Dementia</td>
<td>OH</td>
<td>10</td>
<td>Dementia</td>
<td>LBD, neocortical</td>
</tr>
<tr>
<td>LBD 2</td>
<td>77/F</td>
<td>8</td>
<td>Parkinsonism, dementia, dysphagia</td>
<td>None</td>
<td>18</td>
<td>PD, dementia</td>
<td>LBD, limbic</td>
</tr>
<tr>
<td>LBD 3</td>
<td>72/M</td>
<td>8</td>
<td>Parkinsonism, dementia</td>
<td>OH, urinary retention</td>
<td>20</td>
<td>MSA</td>
<td>LBD, neocortical</td>
</tr>
<tr>
<td>LBD 4</td>
<td>90/F</td>
<td>24</td>
<td>Parkinsonism, dementia</td>
<td>None</td>
<td>16</td>
<td>PD, dementia</td>
<td>LBD, limbic</td>
</tr>
<tr>
<td>LBD 5</td>
<td>79/M</td>
<td>11</td>
<td>Parkinsonism, dementia</td>
<td>None</td>
<td>6</td>
<td>DLB</td>
<td>LBD, neocortical</td>
</tr>
<tr>
<td>LBD 6</td>
<td>81/M</td>
<td>18</td>
<td>Parkinsonism, dementia</td>
<td>OH, urinary retention</td>
<td>8</td>
<td>MSA</td>
<td>LBD, neocortical</td>
</tr>
<tr>
<td>LBD 7</td>
<td>80/M</td>
<td>2</td>
<td>Parkinsonism</td>
<td>OH</td>
<td>10</td>
<td>MSA</td>
<td>LBD, neocortical</td>
</tr>
<tr>
<td>LBD 8</td>
<td>79/M</td>
<td>7</td>
<td>Parkinsonism</td>
<td>OH, cardiac vagal anhidrosis</td>
<td>4</td>
<td>MSA</td>
<td>LBD, limbic</td>
</tr>
<tr>
<td>MSA 1</td>
<td>55/F</td>
<td>26</td>
<td>Ataxia, stridor</td>
<td>OH, anhidrosis</td>
<td>3</td>
<td>MSA-C</td>
<td>MSA</td>
</tr>
<tr>
<td>MSA 2</td>
<td>69/F</td>
<td>13</td>
<td>Parkinsonism</td>
<td>OH, incontinence</td>
<td>10</td>
<td>MSA-P</td>
<td>MSA</td>
</tr>
<tr>
<td>MSA 3</td>
<td>59/M</td>
<td>5</td>
<td>Ataxia, stridor</td>
<td>OH, incontinence, impotence</td>
<td>7</td>
<td>MSA-C</td>
<td>MSA</td>
</tr>
<tr>
<td>MSA 4</td>
<td>67/M</td>
<td>8</td>
<td>Parkinsonism</td>
<td>OH, incontinence, impotence</td>
<td>7</td>
<td>MSA-P</td>
<td>MSA</td>
</tr>
<tr>
<td>MSA 5</td>
<td>70/M</td>
<td>24</td>
<td>Parkinsonism</td>
<td>OH, incontinence, GI dysmotility</td>
<td>4</td>
<td>MSA-P</td>
<td>MSA</td>
</tr>
<tr>
<td>MSA 6</td>
<td>54/M</td>
<td>13</td>
<td>Ataxia, stridor</td>
<td>OH, incontinence, impotence, anhidrosis</td>
<td>7</td>
<td>MSA-C</td>
<td>MSA</td>
</tr>
<tr>
<td>MSA 7</td>
<td>68/M</td>
<td>23</td>
<td>Parkinsonism, ataxia</td>
<td>OH, incontinence</td>
<td>4</td>
<td>MSA-M</td>
<td>MSA</td>
</tr>
<tr>
<td>MSA 8</td>
<td>67/M</td>
<td>1</td>
<td>Parkinsonism</td>
<td>OH, incontinence</td>
<td>3</td>
<td>MSA-P</td>
<td>MSA</td>
</tr>
</tbody>
</table>

**CAD** = coronary artery disease; **CHD** = congenital heart disease; **CHF** = congestive heart failure; **LBD** = Lewy body disease; **MSA** = multiple system atrophy (MSA-C = cerebellar type; MSA-P = parkinsonian type; MSA-M = mixed type); **NHL** = non-Hodgkin lymphoma; **PD** = Parkinson’s disease; **OH** = orthostatic hypotension; **PMD** = post-mortem delay (h).
neuropathological diagnosis of LBD had received the clinical diagnosis of MSA (six cases), Alzheimer disease (one case) and Parkinson’s disease with dementia (one case). The LBD group was older than the control and MSA groups (P < 0.05). The clinical and neuropathological diagnoses of LBD and MSA were made according to current consensus criteria (McKeith et al., 1996; Gilman et al., 1999). The main cause of misdiagnosis of MSA in LBD cases was the presence of prominent OH and other manifestations of autonomic failure, as represented in this illustrative case.

**Illustrative case**

A 75-year-old man was evaluated in May 2000 for a 10-year history of OH, decreased sweating, sexual dysfunction, urinary urgency, and early satiety and constipation. His routine blood studies were unremarkable, but plasma norepinephrine was low at rest in the supine position (21 pg/ml, normal 70–750 pg/ml) and failed to increase upon standing (25 pg/ml). Thermoregulatory sweat testing showed global anhidrosis. Autonomic reflex screen revealed diffusely impaired sudomotor axon reflex responses, indicative of severe postganglionic sudomotor failure; impaired heart rate responses to deep breathing and the Valsalva manoeuvre, indicative of severe cardiac failure; and absence of late phase II and phase IV during the Valsalva manoeuvre which, together with OH during tilt-table testing, indicated severe adrenergic failure (Low, 1997). His initial neurological evaluation was otherwise normal, leading to the clinical diagnosis of pure autonomic failure. The patient was treated symptomatically with dietary and postural manoeuvres, midodrine and fludrocortisone and remained essentially stable for 2 years. Subsequently, his condition deteriorated due to increasing frequency of syncopal episodes and development of progressive parkinsonism, leading to the presumptive clinical diagnosis of MSA. Although he apparently did not develop any cognitive or behavioural changes, he required placement in a nursing home in 2003. The patient died of disease progression in May 2004. Neuropathological diagnosis revealed limbic-predominant LBD.

**Neuropathological assessment**

Post-mortem delay was similar among controls (15 ± 3 h), cases with the final neuropathological diagnosis of LBD (12 ± 3 h), and those with MSA (14 ± 3 h).

The brains were immediately immersion-fixed in 2% buffered paraformaldehyde or 5% formalin for 24 h at 4°C and cryoprotected in a buffered 30% sucrose solution for 5–7 days prior to processing. For the purpose of this study, a block of the brainstem including the caudal pons and medulla was separated from the rest of the brain, which was processed for routine neuropathological studies.

Of the eight cases with the final neuropathological diagnosis of LBD, five had neocortical and three limbic stages of disease. Lewy body pathology and neuronal loss in the substantia nigra pars compacta and locus coeruleus was severe in four cases and moderate in the other four LBD cases. The spinal cord was available in three cases with final diagnosis of LBD. In one case, it showed moderate accumulation of Lewy bodies in the intermediolateral cell column, but no apparent cell loss; in the other two cases the spinal cord was normal. Autonomic ganglia were not available for examination. All MSA cases showed neuronal loss in the substantia nigra pars compacta, putamen, basis pontis and cerebellum in various combinations and the presence of α-synuclein-immunoreactive oligodendroglial cytoplasmic inclusions.

**Immunohistochemistry**

For the purposes of this study, serial 50-µm cryostat sections of the medulla were obtained between 0 and 13 mm rostral to the obex. Every eighth section was processed for immunoreactivity for tyrosine hydroxylase (TH; mouse monoclonal antibody 1 : 1500; Immunostar, Hudson, WI, USA), tryptophan hydroxylase (TrOH; sheep polyclonal antibody 1 : 1000, Chemicon, Temecula, CA, USA), and α-synuclein (goat polyclonal antibody 1 : 400; Santa Cruz Biotechnologies, Santa Cruz, CA, USA). Diaminobenzidine/glucose oxidase solution with nickel enhancement (Sigma, St Louis, MO, USA) was used for the substrate reaction. Omission of the primary antibody or incubation with normal sera resulted in a lack of immunostaining. After immunoreactivity had been detected and analysed, sections were contained with thionin.

**Quantitation and data analysis**

The sections were examined under bright field microscopy. The numbers of sections analysed were 23, 29, 28, 29, 30, 31, 34, and 28, respectively, for the eight controls; 29, 21, 27, 22, 26, 31, 21 and 33, respectively, for the eight LBD cases; and 29, 31, 23, 30, 34, 29 and 31, respectively, for the eight MSA cases. The TH- and TrOH-immunoreactive cells were counted in the regions corresponding to the intermediate reticular zone as well as in the nucleus RPa and ROb. These areas were identified on the basis of the atlas of Paxinos and Huang (Paxinos and Huang, 1995).

Image analysis was performed using a KS400 image analysis system (Carl Zeiss, Thornwood, NY, USA). There was no significant difference in size between the surviving TH and TrOH neurons between the LBD, MSA and control cases (for TH cells in the VLM, 28 ± 1 µm in LBD, 29 ± 1 µm in MSA and 27 ± 1 µm in controls, n = 5 each; for TrOH neurons in the ROb, 30 ± 1 µm in LBD, 30 ± 3 µm in MSA and 31 ± 2 µm in controls, n = 5 each; and for TrOH neurons in RPa, 27 ± 1 µm in LBD, 27 ± 6 µm in MSA and 26 ± 3 µm in controls, n = 5 each). Since our aim was to compare the numbers of cells per section between controls, LBD and MSA cases, we did not use stereology, which would be best if the intent was to count total numbers of cells. We instead performed ‘profile’ counting using an automated method under direct microscopic guidance. Since we are aware of potential drawbacks of the classical counting method used in our study, we took several steps to minimize potential pitfalls of profile counting, including, double counting, shrinkage effects and sampling error. We counted only neurons that could be identified by their intense immunoreactivity and the presence of processes. Since the maximum size of the cells counted using this criterion (30 ± 3 µm) was much smaller than the thickness of each section (50 µm), the possibility of counting parts of the same neuron twice is probably minimal. Although unevenness of section thickness or tissue shrinkage could have affected our results, they are not expected to have done so differently in each of these three groups, and therefore to have altered the comparison between groups and therefore our conclusions. Furthermore, the maximum cell size was not different between controls, LBD and MSA cases. To avoid sampling errors, we analysed, for a given immunocytochemical marker, all sections obtained serially at 400 µm intervals between the plane of the obex and 12 mm rostral to it so as to obtain a comparable representation of the medulla in all cases. All counts were performed by the same investigator, blinded to the definitive neuropathological diagnosis.

Although a split cell error would not affect the results, given the similar cell size in controls, LBD and MSA cases, a correction factor was calculated and was 0.96. Cell numbers (mean ± SEM) were
compared among control, LBD and MSA groups using analysis of variance. A *P* value <0.05 was considered significant.

**Results**

**Distribution of Lewy bodies in medullary autonomic regions**

In all LBD cases, there were abundant α-synuclein-immunoreactive Lewy bodies and Lewy neurites in all medullary regions controlling sympathetic outflow, including VLM, ROb and RPa. The distribution of Lewy bodies overlapped that of catecholaminergic and serotonergic neurons in these regions (Fig. 1). In addition, there were Lewy bodies and Lewy neurites in the dorsal vagal nucleus and nucleus reticularis gigantocellularis.

**Involvement of the VLM**

In addition to Lewy bodies, there were occasional dystrophic changes in TH- and TrOH-immunoreactive neurons in the VLM in LBD cases (Fig. 2). However, the cell numbers were not significantly reduced compared with controls (Fig. 3). There were no significant differences in VLM cell numbers between the five LBD cases presenting with OH and the three cases without OH (for TH, 36 ± 6 and 41 ± 7 cells/section, respectively; for TrOH, 41 ± 6 and 55 ± 4 cells/section, respectively). In contrast, in all MSA cases there was marked depletion of catecholaminergic and serotonergic VLM neurons (Fig. 3) as well as glial cytoplasmic inclusions in this region (Fig. 2).

**Involvement of the medullary raphe nuclei**

There were abundant Lewy bodies and Lewy neurites in the medullary raphe, including the ROb and RPa in all LBD cases (Fig. 2). In these regions, there was a significant reduction in the numbers of TrOH-immunoreactive neurons compared with controls (Fig. 4). Since, as a group, LBD cases were a decade older than controls, we also compared the eight LBD with six of the eight controls to match for age (74 ± 2 years in controls versus 80 ± 2 years in LBD, *P* = 0.07). Even after correcting for age differences, the numbers of medullary serotonergic raphe neurons was significantly reduced in LBD compared with controls (for ROb, 16 ± 2 and 36 ± 5 cells/section, respectively, *P* = 0.002; for RPa, 8 ± 2 and 16 ± 1 cells/section, respectively, *P* = 0.004). There were no differences in the number of medullary serotonergic neurons between the five LBD cases with OH and the three cases without OH (for ROb, 16 ± 2 and 15 ± 3 cells/section, respectively; for RPa, 7 ± 3 and 8 ± 1 cells/section, respectively).

Although the degree of depletion of serotonergic neurons in the ROb was slightly less severe in LBD than in MSA cases (16 ± 2 and 12 ± 1 cells/section, respectively, *P* = 0.04), there was no significant difference in the number of serotonergic cells in the RPa between LBD and MSA cases.

**Discussion**

The main findings of this study are that (i) although Lewy body pathology is prominent in the VLM in LBD, the degree of involvement of catecholaminergic and serotonergic neurons in this region, even at late stages of disease, is much less severe than in MSA; (ii) in limbic and neocortical stage LBD, there is a significant loss of serotonergic neurons in the medullary raphe; and (iii) the degree of involvement of catecholaminergic VLM or serotonergic raphe neurons in LBD is independent of the presence or absence of OH during life.

The distribution of α-synuclein positive Lewy bodies and dystrophic neurites in the LBD cases found in our study is consistent with that found in previous studies (Gai et al., 1995; Braak et al., 2001). Lewy body pathology was concentrated in the area of the VLM containing catecholaminergic and serotonergic neurons, the medullary raphe nuclei, the nucleus reticularis gigantocellularis.
reticularis gigantocellularis and the dorsal vagal nucleus. For the purpose of this study, we focused our attention on the catecholaminergic neurons of the rostral VLM and the serotonergic neurons of the NRPa, NROb and VLM, as all these groups provide descending inputs to the intermediolateral cell column (Strack et al., 1989). Catecholaminergic neurons of the VLM are involved in modulation of activity of preganglionic sympathetic neurons controlling arterial pressure.

Fig. 2 Representative sections of the medulla obtained from a control subject (78-year-old woman with no history of neurological disease; post-mortem delay 9 h), a 79-year-old man with limbic-predominant Lewy body disease (LBD; post-mortem delay 7 h) and a 67-year-old man with multiple system atrophy (MSA; post-mortem delay 8 h). Sections were processed for α-synuclein to identify Lewy bodies or glial cytoplasmic inclusions, tyrosine hydroxylase to identify catecholaminergic neurons in the ventrolateral medulla (VLM), and tryptophan hydroxylase to identify serotonergic neurons in the nucleus raphe obscurus (ROb) and raphe pallidus (RPa). In the LBD case, there were Lewy bodies and Lewy neurites, but preservation of catecholaminergic cells in the VLM as opposed to the severe depletion of these cells in MSA. There was loss of serotonergic neurons with Lewy body pathology in the RPa and ROb in LBD and neuronal loss and glial cytoplasmic inclusions in these regions in MSA. Bar = 25 μm.

Fig. 3 Numbers of tyrosine hydroxylase (TH)- and tryptophan hydroxylase (TrOH)-immunoreactive cells per section in the ventrolateral medulla (VLM) in eight control subjects (C), eight patients with the pathological diagnosis of limbic or neocortical stage Lewy body disease (LBD), and eight patients with the pathological diagnosis of multiple system atrophy (MSA). There was relative preservation of TH- and TrOH-immunoreactive cells in the VLM in the LBD cases, despite the presence of Lewy body pathology and independently of the presence or absence of orthostatic hypotension. In contrast, there was severe depletion of both catecholaminergic and serotonergic VLM neurons in MSA. ***P < 0.001 compared with controls; |***|P < 0.001 compared with LBD.
(Guyenet et al., 1996), whereas the medullary raphe controls preganglionic sympathetic neurons involved in thermoregulatory skin vasoconstriction (Morrison, 1999).

Our present study indicates that, even at later stages (neocortical and limbic) of LBD, there is relative preservation of the numbers of TH-immunoreactive neurons in the VLM, as opposed to the severe depletion found in MSA. These differences could not be attributed to the effects of ageing or post-mortem delay. Catecholaminergic VLM neurons were relatively preserved even in LBD cases where there was a marked loss of catecholaminergic neurons in the substantia nigra pars compacta and locus coeruleus.

These results have two implications. First, they support previous findings (Benarroch et al., 2000) indicating that the catecholaminergic rostral VLM neurons, corresponding to those projecting to the sympathetic preganglionic neurons, are much more vulnerable in MSA than in LBD, regardless of the stage of the disease. Secondly, they provide further evidence that, in LBD, not all catecholaminergic groups are susceptible to the same degree, despite the presence of Lewy body pathology. This is consistent with previous findings in Parkinson’s disease (Halliday et al., 1990). This suggests that the presence of Lewy bodies and Lewy neurites, although a marker of vulnerable neuronal populations in LBD, may not be the only factor leading to neuronal loss.

The preservation of catecholaminergic neurons in the rostral VLM in LBD as opposed to their severe depletion in MSA, despite the clinical history of severe OH in both groups of patients, provides further evidence that the mechanism of OH is different in these two synucleinopathies. Orthostatic hypotension in MSA has been classically attributed to loss of noradrenergic neurons in the intermediolateral cell column (Bannister and Oppenheimer, 1972), but there is a poor correlation between the degree of intermediolateral cell column cell loss and the severity of OH (Gray et al., 1988). Loss of catecholaminergic neurons in the rostral VLM may contribute to sympathetic vasomotor failure in MSA (Benarroch et al., 1998). Some of these neurons contain glutamate as well as epinephrine. Glutamate is responsible for tonic excitation of preganglionic sympathetic neurons whereas epinephrine modulates the excitability of these cells (Guyenet et al., 1996). Whereas loss of rostral VLM catecholaminergic neurons may have a role in the pathogenesis of OH in MSA, our results indicate that this may not be the case in LBD. However, involvement of non-catecholaminergic sympathoexcitatory VLM neurons containing glutamate cannot be excluded.

Although a potential limitation of our study is the relatively small number of LBD cases without history of OH, our findings provide indirect support to the increasing evidence that OH in MSA may, at least in part, be due to peripheral mechanisms. PET and single photon computer emission tomography studies show reduced uptake of norepinephrine precursors in cardiac sympathetic terminals in disorders associated with Lewy body accumulation, including Parkinson’s disease, dementia with Lewy bodies, and pure autonomic failure, but not in MSA (Goldstein et al., 1997; Yoshita et al., 2001). Our illustrative case confirms previous observations that LBD may manifest with isolated sympathetic and parasympathetic failure for many years or even until death, resembling pure autonomic failure (Hague et al., 1997; Horimoto et al., 2003). Thus, there may be a ‘peripheral’ stage of LBD, with predominant involvement of autonomic ganglia and peripheral effector organs, which in some cases may dominate the clinical picture. Unfortunately, the autonomic ganglia were not available from the cases analysed in this study. The fact that most of our LBD cases had an atypical presentation resembling MSA could indicate that they constitute a specific subgroup within the spectrum of the LBD, and emphasize the difficulties and potential pitfalls in the clinical diagnosis of this group of disorders.

Our results indicate that, as in the case of MSA, there is loss of serotonergic neurons in the RPa and ROb in limbic- or

**Fig. 4** Numbers of tryptophan hydroxylase (TrOH)-immunoreactive cells per section in the nucleus raphe obscurus (ROb) and raphe pallidus (RPa) in eight control subjects (C), eight patients with the pathological diagnosis of limbic or neocortical stage Lewy body disease (LBD), and eight patients with the pathological diagnosis of multiple system atrophy (MSA). There was a significant reduction of serotonergic medullary raphe neurons in both LBD and MSA. **P < 0.001 and ***P < 0.01 compared with controls; ^P < 0.05 compared with LBD.**

Downloaded from https://academic.oup.com/brain/article-abstract/128/2/338/402584 by guest on 14 April 2019
neocortical-predominant LBD. In a previous study, we found that in cases with clinical and neuropathological diagnosis of Parkinson’s disease, there was a relative preservation of the numbers of TrOH-immunoreactive cell bodies in the medullary raphe, despite the presence of Lewy bodies and dystrophic neurites in this region (Benarroch et al., 2004). Thus, our present findings indicate that, with progression of LBD from brainstem to limbic and neocortical stages, there is progressive loss of serotonergic neurons in the medullary raphe. This does not appear to be a merely a reflection of ageing. The preservation of serotonergic cells in the VLM, as opposed to the RPa and ROB, observed in our study does provide further evidence that in LBD there are different susceptibilities of distinct neuronal populations synthesizing the same primary neurotransmitter.

The significance of loss of serotonergic raphe–spinal neurons in LBD is uncertain. It did not relate to the presence or absence of OH, which is consistent with evidence that these cells do not participate directly in the baroreflex arc or the maintenance of sympathetic vasconstrictor tone (Morrison, 1999). In contrast, the medullary raphe has been implicated in thermoregulatory sympathetic vasconstriction (Morrison, 1999) as well as in the automatic control of respiration (Feldman et al., 2003). These functions appear to be more affected in MSA than in Parkinson’s disease (Pierangeli et al., 2001; Tsuda et al., 2002), which is consistent with the severe involvement of medullary serotonergic neurons in MSA compared with Parkinson’s disease (Benarroch et al., 2004). However, to our knowledge these functions have not been systematically explored in dementia with Lewy body patients, reflecting limbic- or neocortical-predominant LBD. Our findings thus provide a testable hypothesis that involvement of medullary serotonergic neurons may result in impairment of thermoregulatory or respiratory functions at these late stages of LBD.

Acknowledgements
This work was supported by grants PO1 NS32352-P2 from the National Institute of Neurologic Disorders and Stroke (E.B., A.M.S.) and by grants AG 06786, AG15866, and AG 16574 from the National Institute of Aging (B.F.B.).

References