Diffuse Lewy body disease: correlative neuropathology using anti-ubiquitin immunocytochemistry

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SUMMARY Diffuse Lewy body disease is an important pathological substrate of the common syndrome of parkinsonian dementia. The new technique of anti-ubiquitin immunocytochemistry has been used in a correlative quantitative neuropathological study of fifteen cases of diffuse Lewy body disease, showing that the severity of dementia is related to cortical Lewy body density, whilst subcortical abnormalities make a much less significant contribution. Cortical senile plaques also appear to be part of the pathology of diffuse Lewy body disease and should not therefore be used as an isolated diagnostic criterion for Alzheimer's disease. Diagnostic criteria for diffuse Lewy body disease are discussed.

Diffuse Lewy body disease is a common neuropathological condition which is associated with clinical features of parkinsonian dementia.^{1,2} At least 20% of all patients with Parkinson's disease develop dementia.³ Conversely at least 30% of patients with dementia develop signs of parkinsonism.⁴⁻¹¹ The pathological basis of these parkinsonian dementia syndromes is poorly understood. The dementia has in the past generally been attributed to the pathological changes of Alzheimer's disease but recent clinico-pathological studies have provided little or no support for this explanation, finding either no evidence of Alzheimer's disease^{12,13} or only sparse changes^{14,15} which fail to meet diagnostic criteria for Alzheimer's disease^{16,17} and do not correlate with the severity of the dementia.¹⁸ Diffuse Lewy body disease provides an alternative explanation.

Diffuse Lewy body disease is characterised by the presence of eosinophilic hyaline inclusion bodies (that is, Lewy bodies) in both subcortical and cortical areas.¹⁹⁻²³ The brainstem Lewy bodies closely resemble those seen in Parkinson's disease both in morphology

and distribution (figure 1) and it seems reasonable to suppose that the brainstem pathology may be the basis of the parkinsonian features of diffuse Lewy body disease. The cortical Lewy bodies (which are unusual in uncomplicated Parkinson's disease²⁴) may be the pathological basis of the dementia in diffuse Lewy body disease. However, quantitative studies to test this hypothesis have been hampered by the fact that cortical Lewy bodies are poorly circumscribed and difficult to identify using conventional haematoxylin and eosin (H and E) stains. In the present study of a series of fifteen cases of diffuse Lewy body disease we have therefore used the new technique of anti-ubiquitin immunocytochemistry, which identifies cortical Lewy bodies with greatly increased sensitivity and reliability²⁵⁻²⁷ and which also identifies neurofibrillary tangles and senile plaques.²⁸⁻³² The technique allows quantitative study of large areas of cerebral cortex for the first time. We show a clear relationship between the cortical pathological features and the severity of dementia in diffuse Lewy body disease.

Preliminary data from this study have been reported elsewhere.^{2,33,34}

Materials and methods

Pathological material

All 216 brains referred to this department from within the

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Nottingham Health District (population 616,000) during April 1985 to March 1986 were screened for the presence of cortical Lewy bodies using conventional H and E stains. Fifteen cases of diffuse Lewy body disease were identified on the basis of classical brainstem Lewy bodies and widespread cortical Lewy bodies. All 15 came from within the subgroup of 57 brains known to be from patients with dementia, a parkinsonian syndrome, or both. Diffuse Lewy body disease was identified in 27% of the cases with dementia in this survey, and was thus the commonest form of dementia after Alzheimer's disease. The clinical features of these cases have been described in detail elsewhere¹ and are summarised in table 1. The remaining 201 cases included a broad range of neuropathological conditions and ages.

The brains (or randomly selected half-brains in the eleven cases where the brain had been bisected sagittally in the fresh state and one half frozen for neurochemistry) were fixed in 10% formol saline for at least six weeks and then dissected in a standardised fashion. The cerebral cortex was divided into one centimetre coronal slices and blocks were taken from the major neocortical regions and from entorhinal cortex, periamygdaloid cortex and hippocampus; insular cortex and basal nucleus of Meynert at the level of the midpoint of the anterior commissure; and anterior cingulate gyrus from the same slice. The brainstem was sliced transversely at the level of the oculomotor nerve to obtain a substantia nigra block; longitudinally through pons for locus ceruleus; and transversely through medulla for dorsal vagal nucleus. These blocks were processed in paraffin wax and sectioned at 10 micrometres.

The sections were stained using H and E, cresyl-fast violet, solochrome-cyanin, thioflavine S, the Cross modification of Palmgren's silver stain³⁵ and anti-ubiquitin immunocytochemistry.³⁶ Anti-ubiquitin immunocytochemistry was performed using a rabbit polyclonal antibody which showed high specificity for ubiquitin-protein conjugates in vitro and in tissue sections as previously described,^{25,36} with a streptavidin-biotin-peroxidase detection system.³⁷ The antibody clearly identified both brainstem and cortical Lewy bodies (figures 1 to 3), as well as neurofibrillary tangles and neuritic elements of senile plaques (figures 2 and 3).²⁸

Immunocytochemical staining was limited in two cases by the accidental discarding of some of the tissue after preliminary histology had been completed.

Additional pathological material

In addition to the large number of miscellaneous neuropathological cases examined in this series, seven brains were examined from patients who satisfied clinical and pathological criteria for the diagnosis of Parkinson's disease, matched for age and duration of illness, who showed no evidence of dementia (based on retrospective case note examination using the criteria described below). They were examined in the same way as the cases of diffuse Lewy body disease and serve as a non-demented Parkinson's disease comparison group. Two of these cases came from the 1985-86 series; the remainder from more recent files. Ten brains from age-matched patients who died with no evidence of neurological or psychiatric disease (by the same criteria) were used as normal controls for cell counts in substantia nigra and nucleus basalis of Meynert.

Quantitative neuropathology

Numbers of pigmented cells and eosinophilic inclusion bodies in the substantia nigra were counted in between one and four unilateral H and E sections. The area of maximum cell density in the nucleus basalis of Meynert was identified and marked on H and E or cresyl-fast violet sections; the number of large (>25 micrometre diameter), Nissl-containing, nucleated cells was counted in between 10 and 20 graticule fields (area 0.23 mm²) within this area to obtain an estimate of cell density, expressed as the number of cells/mm².

Cortical Lewy body density was assessed using anti-ubiquitin immunocytochemistry in the superior frontal gyrus, superior parietal lobule, anterior cingulate gyrus, inferior and middle temporal gyri, insular cortex, entorhinal cortex and in the cornu Ammonis and subiculum of the hippocampus. In the hippocampus the numbers of Lewy bodies, tangles and plaques were counted using an eyepiece graticule (area 0.23 mm²) in a minimum of 20 graticule fields selected at random throughout all four divisions of the cornu Ammonis, and at least a further 20 graticules from all four

Table 1 Clinical features in cases of diffuse Lewy body disease^a

Case	Age at onset	Sex	Dur ^a (yrs)	Presentation	Motor features and levodopa response ^b		Dementia severity ^c
1	74	F	2	Parkinsonism	RBTGP	Definite	Severe
2	82	M	3	Parkinsonism	RBTG	Definite	Mild
3	58	M	5	Parkinsonism	RBTGP	Definite	Severe
4	74	F	5	Parkinsonism	RBTG	Definite	Moderate
5	68	F	19	Parkinsonism	RBTGP	Definite	Severe
6	72	F	8	Parkinsonism	RBTGP	Definite	Very severe
7	73	M	3	Both	RBTGP	No trial	Severe
8	62	M	4	Both	RBTGP	Definite	Very severe
9	75	M	5	Both	RBG	Probable	Very severe
10	83	F	3	Dementia	P	No trial	Severe
11	65	F	2	Dementia	RGP	No trial	Very severe
12	73	M	8	Dementia	RBGP	Definite	Severe
13	71	F	6	Dementia	RTG	Definite	Very severe
14	70	F	7	Dementia	RTGP	Definite	Very severe
15	83	M	3	Dementia	G	No trial	Very severe

a: further clinical details are given in reference 1.

b: R = extrapyramidal rigidity, B = bradykinesia, T = resting tremor,

G = parkinsonian gait, P = flexed postural deformity.

c: classified according to the stage model of Strub and Black.³⁸

Fig 1 *Substantia nigra (Case 15) showing cell loss, gliosis, pigment incontinence and classical Lewy bodies (arrows) in a high proportion of remaining neurons (H and E, magnification $\times 210$). Inset: brainstem Lewy body stained with anti-ubiquitin immunocytochemistry, showing intense peripheral immunoreactivity (magnification $\times 800$).*

divisions of the subiculum. In the other cortical regions, large areas of the cortical ribbon were marked in ink on the coverslips before microscopic examination. Lewy bodies, tangles and plaques were counted by systematic parallel sweeps across the marked area at a magnification of $\times 250$ using the eyepiece graticule. The marked area was then projected and measured using a Kontron MOP Videoplan computerised planimetry system. The mean area counted in each case was 4.2 mm^2 (range $2.3\text{--}9.2 \text{ mm}^2$) in the hippocampus and 56.2 mm^2 (range $11.7\text{--}122.0 \text{ mm}^2$) in each region of the cortex. Cortical Lewy bodies were defined as rounded, non-fibrillar, intensely immunoreactive inclusion bodies. Preliminary studies determined that this definition excluded neurofibrillary tangles and other forms of inclusion body;²⁵ it also excluded extracellular cortical Lewy bodies. Fibrillar perikaryal inclusion bodies were counted as neurofibrillary tangles (figure 3): this definition included small "wisps" of fibrillar material as well as classical "flame-shaped" tangles. Senile plaques with a clear neuritic component or compact core were counted; less clearly defined forms of senile plaque (so-called primitive or early plaques) were excluded.

In all cases Lewy body density was also assessed using conventional H and E stains of adjacent sections. The densities obtained by this method were proportional to those obtained using anti-ubiquitin immunocytochemistry but invariably lower, reflecting the difficulty in identifying

cortical Lewy bodies using H and E stains. Densities obtained with the anti-ubiquitin technique are therefore presented here. Neurofibrillary tangle and senile plaque densities were also assessed using conventional thioflavine S and Cross/Palmgren stains in similar areas of adjacent sections. The ubiquitin and thioflavine S techniques generally showed close agreement in the larger cortical regions and reasonable agreement in the hippocampus where the smaller areas studied produce a greater tendency to sampling error. Cross/Palmgren stains gave much lower tangle and plaque counts. Densities obtained from thioflavine S stains are presented here to facilitate comparison with other studies.

In the anterior cingulate gyrus the proportion of neurons containing Lewy bodies and the distribution of Lewy bodies within the cortical layers were determined in a minimum of 1500 neurones counted and examined using parallel sweeps, perpendicular to the surface of the gyrus, with an eyepiece graticule.

All neuropathological assessments were carried out on laboratory-coded slides without knowledge of the patient's identity or clinical details.

Assessment of dementia severity

All of these cases had spent time under the care of the Department of Health Care of the Elderly and had undergone detailed assessment of cognitive function. However,

Fig 2 Entorhinal cortex (Case 8) stained with anti-ubiquitin immunocytochemistry showing cortical Lewy bodies in small neurons within the deeper cortical layers (arrows) and the neuritic elements of a senile plaque (arrow heads) (magnification $\times 210$). Inset: cortical Lewy body showing that anti-ubiquitin immunoreactivity is again most intense at its periphery (magnification $\times 1100$).

several different tests were employed with different patients and many patients became too profoundly demented to attempt any test in the later stages of their illness. Thus while a wealth of cognitive data had been assembled for these patients, no single standardised prospective measure of preterminal dementia severity could be applied to the entire group. Since the clinical progression of dementia severity conformed to the stage models proposed by Strub and Black³⁸ and by Reisberg *et al*³⁹ (in cases of primary degenerative dementia), cases were retrospectively classified according to these scales, using the standardised cognitive tests where relevant, supplemented by hospital medical and nursing records and general practitioner case notes. Many cases showed fluctuation in cognitive impairment in the early stages of their illnesses, but these disappeared with time. Classification was performed independently by two of us (GL and EJB) and verified by a third (RBGA): a very high degree of interobserver agreement was obtained. For simplicity the five point scale of Strub and Black (normal, mild, moderate, severe and very severe dementia) was used in correlations with the neuropathological data, but similar results were obtained using the Reisberg scale. In this series the distinction between severely and very severely demented patients is important; severely demented patients were dis-

orientated in time and place, showed clear evidence of global cognitive impairment, required substantial assistance with activities of daily living and scored five or less on a 10 point mental status questionnaire, whilst very severely demented patients were in a vegetative state, being unable to communicate (either mute or uttering only incoherent noises), incontinent and bedbound, were totally dependent upon nursing support and scored 0 on a 10 point mental status questionnaire.

All assessments of dementia severity were made blind with regard to the neuropathological findings.

Statistics

Comparisons between cell counts and densities in cases and controls have been made using the Mann-Whitney U test. Correlations between dementia severity and neuropathological measures have been made using the Spearman rank correlation coefficient (chosen because the dementia severity scale is non-linear and the cases are not evenly distributed within it). The correlation coefficients cited refer to the cases of non-demented Parkinson's disease and diffuse Lewy body disease taken as a whole, but similar, highly significant correlation coefficients are obtained if the cases of non-demented Parkinson's disease are excluded from the analysis.

Fig 3 Adjacent neurons in the inferior temporal cortex (Case 15) containing a rounded cortical Lewy body (left) and linear fibrillar material constituting a neurofibrillary tangle (right; arrows) (magnification $\times 800$).

Results

Brainstem pathology

All fifteen cases of diffuse Lewy body disease showed loss of pigmented cells in the substantia nigra together with gliosis and classical Lewy body formation (figure 1). These changes were similar to those found in idiopathic Parkinson's disease. Lewy bodies, cell loss and gliosis were also found in other brainstem nuclei, in a distribution which again resembled that of Parkinson's disease: affected structures included the

Lennox, Lowe, Landon, Byrne, Mayer, Godwin-Austen nucleus paranigralis, locus ceruleus and dorsal vagal nucleus. In each case the severity of these changes was similar to that found in the substantia nigra.

Quantitatively the degree of substantia nigra pigmented cell loss was moderate to severe when compared with normal controls; there was no significant difference between substantia nigra cell counts from the cases of diffuse Lewy body disease and Parkinson's disease (table 2), although a greater proportion of the remaining neurones contained Lewy bodies in the cases of diffuse Lewy body disease (mean 13.8 per section versus 4.6 in the cases of Parkinson's disease), perhaps indicating an increased tendency to Lewy body formation. There was no significant correlation between substantia nigra cell or Lewy body counts and dementia severity.

No case showed neurofibrillary tangles within brainstem structures.

Nucleus basalis of Meynert pathology

The nucleus basalis of Meynert also showed moderate to severe cell loss, gliosis and Lewy body formation in both diffuse Lewy body disease and non-demented Parkinson's disease. Cell density was significantly reduced in both diseases compared with controls. The reduction was slightly greater in cases of diffuse Lewy body disease than in cases of Parkinson's disease, although this difference was not statistically significant (table 2). There was no significant correlation between dementia severity and cell density or Lewy body counts. Occasional neurofibrillary tangles were seen in all three groups, but in no case numbered more than two per section.

Cortical Lewy body pathology

Cortical Lewy bodies were not detected in the normal controls. Cortical Lewy bodies were very rare in the non-demented Parkinson's disease cases: none was identified using H and E stains, but with the more

Table 2 Subcortical and cortical pathology in diffuse Lewy body disease and Parkinson's disease

Cases (number)	Age at death (mean years)	Substantia nigra cell count ^a Mean, SE (range)	Nucleus basalis of Meynert cell density ^b Cells/mm ² , SE (range)	Mean cortical Lewy body density ^c Lewy bodies/mm ² , SE (range)
Normal controls (n = 10)	76.3	814.8, 38.9 (489 - 926)	89.1, 9.9 (61.7 - 116.5)	0, 0
Non-demented PD ^d (n = 7)	75.2	190.9, 42.0 (87 - 384)	38.6, 4.5 (23.7 - 57.1)	0.006, 0.003 (0 - 0.02)
DLBD ^e (n = 15)	77.7	170.4, 30.1 (47 - 394)	31.3, 2.3 (18.7 - 50.4)	1.27, 0.24 (0.07 - 2.94)

Notes:

a: number of pigmented cells per unilateral section at the level of the third nerve.

b: density of large, Nissl-containing cells in the area of maximum cell density below the mid-point of the anterior commissure.

c: mean density of cortical Lewy bodies detected using anti-ubiquitin immunocytochemistry in the superior frontal, superior parietal, inferior temporal, entorhinal, insular and anterior cingulate regions.

d: Parkinson's disease.

e: Diffuse Lewy body disease.

Fig 4 Correlation between dementia severity and cortical Lewy body (LB) density in cases of non-demented Parkinson's disease (non-dem PD) and diffuse Lewy body disease, in (a) inferior temporal cortex, (b) insular cortex, (c) anterior cingulate cortex, (d) entorhinal cortex, (e) superior frontal cortex and (f) superior parietal cortex. All Lewy body densities expressed as numbers of cortical Lewy bodies per square millimetre. n = number of cases examined in each category (see text), r and p = Spearman's correlation coefficient and probability of the null hypothesis respectively.

Fig 5 Correlation between dementia severity and mean cortical Lewy body density for all six cortical regions (Lewy bodies per square millimetre).

sensitive anti-ubiquitin immunocytochemistry small numbers were detected in three cases (that is, a total of 1–4 cortical Lewy bodies per case in the six regions quantified; maximum density 0.06 per mm²) (table 2, figures 4 and 5).

In the cases of diffuse Lewy body disease cortical Lewy bodies were found most abundantly in entorhinal, inferior temporal, insular and anterior cingulate cortex, mainly in small and medium sized pyramidal neurons in the deeper cortical layers. Lewy bodies affected up to 4% of all neurones in the anterior cingulate gyrus. There was a highly significant correlation between dementia severity and cortical Lewy body density in each of these cortical regions ($p < 0.001$) (figure 4 a–d). They were less abundant in

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superior frontal and superior parietal lobes, but here too there was a significant correlation between cortical Lewy body density and dementia severity ($p < 0.005$) (figure 4 e–f). A highly significant correlation was also found between the mean cortical Lewy body density in all six cortical regions and dementia severity ($p < 0.001$) (figure 5). Lewy bodies were rare in the hippocampus (and occurred almost exclusively in the subiculum: no Lewy bodies were identified in the dentate gyrus), but again there was a significant correlation between subiculum Lewy body density and dementia severity ($p < 0.001$) (not shown).

Cortical senile plaques

Senile plaques were found in all fifteen cases of diffuse Lewy body disease (table 3). Senile plaque density generally reflected cortical Lewy body density ($p = 0.02$) (figure 6); their density did not correlate with neurofibrillary tangle density as might be expected if the senile plaques were due to coexistent Alzheimer's disease. Indeed, senile plaques were often found in large numbers in cases of diffuse Lewy body disease which showed no other features to support a diagnosis of Alzheimer's disease, such as neurofibrillary tangles, granulovacuolar degeneration or Hirano bodies.

Senile plaques were found in the non-demented Parkinson's disease and control cases in small numbers and therefore compatible with normal ageing.⁴⁰

Cortical neurofibrillary tangles

Eleven of the fifteen cases of diffuse Lewy body disease had either no hippocampal and neocortical tangles or only small numbers consistent with age (table 3). The remaining four cases had neurofibrillary tangles in numbers which satisfy quantitative criteria for the

Table 3 Senile plaque and neurofibrillary tangle density in cases of diffuse Lewy body disease

Case number	Hippocampus ^a		Middle temporal gyrus		Superior parietal lobule	
	Plaques	Tangles	Plaques	Tangles	Plaques	Tangles
1	0.6	0	1.09	0	0	0
2	1.6	0.4	1.14	0	not measured	
3	0.9	0.3	0.28	0.07	0	0
4*	2.4	4.4	3.69	0.12	1.08	0.10
5	0	0	0.30	0	0	0
6	4.8	4.2	1.79	0.08	1.51	0
7	2.4	0.4	1.80	0	0	0
8	4.8	0.6	1.66	0	2.71	0
9	0	1.6	1.23	0.07	0.91	0.06
10	1.6	0.8	1.74	0.09	0.60	0
11	2.1	1.8	2.44	0.07	4.09	0
12	0	0	0.47	0.13	not measured	
13*	7.6	13.3	4.31	2.56	0.84	0.59
14*	5.0	14.5	3.83	0.04	1.76	0.41
15*	4.2	2.7	3.47	0.71	2.40	0.50

All plaque and tangle densities assessed using thioflavine S (see text) and expressed as numbers per square millimetre of cerebral cortex.

a: mean subiculum

*: cases where a second diagnosis of Alzheimer's disease was considered (see text).

Fig 6 Correlation between mean cortical senile plaque density and mean cortical Lewy body density (both expressed as numbers per square millimetre) in the fifteen cases of diffuse Lewy body disease.

diagnosis of Alzheimer's disease in the hippocampus (Cases 13 and 14) and neocortex (Cases 4 and 15).^{16 17 41} However, one should be cautious in interpreting the significance of tangle counts in isolation: other evidence suggests that a second diagnosis of Alzheimer's disease may be inappropriate in these cases. Firstly, in the cortex Lewy bodies were present in much greater densities than tangles in all four cases. Secondly, dementia severity did not correlate with neurofibrillary tangle density in any region. Thirdly, the morphology and distribution of tangles differed qualitatively from that seen in cases of Alzheimer's disease; individual tangles frequently consisted of very fine perikaryal "wisps" (figure 3) with few classical flame-shaped structures and were confined largely to the mesial temporal lobe.

Other pathology

No other neuropathological diagnosis was made in the cases of diffuse Lewy body disease. Case 2 had a small lacunar infarction (8 mm × 2 mm × 4 mm in maximum extent) in the left medial thalamus, but there was no other significant cerebrovascular lesion and no evidence of Binswanger's disease. Two cases (cases 11 and 13) had moderate spongiform change with vacuolation of the neuropil in the mesial temporal lobe; there was no status spongiosus and no spongiform change in the cerebellum to support a diagnosis of Creutzfeldt-Jakob disease. Five cases (Cases 1, 5, 8, 12 and 13) had small or moderate numbers of swollen achromatic neurons in the temporal cortex. No case, however, showed the pathological features of Pick's disease; there were no strongly argyrophilic inclusion bodies (that is, Pick

bodies) in the neocortex and no inclusion body of any kind in the dentate gyrus of the hippocampus. Care was taken to exclude other potentially confusing differential diagnosis; there was no evidence of post-encephalitic parkinsonism, progressive supranuclear palsy, Hallervorden-Spatz disease, corticobasal or striatonigral degeneration.

Discussion

The common syndrome of parkinsonian dementia has in the past been attributed to the cortical and sub-cortical pathology of Alzheimer's disease, either alone or in combination with brainstem Parkinson's disease pathology.^{11 13 14 15 42} This study of diffuse Lewy body disease shows that a less complex explanation may suffice: parkinsonism may be related to brainstem Lewy bodies and dementia to their cortical counterparts. Before turning to the evidence supporting this explanation, it is worth emphasising it has only become a plausible possibility with the demonstration that diffuse Lewy body disease is common.¹² In the year surveyed for this study, 15 cases of diffuse Lewy body disease were identified amongst 55 cases of dementia (in a series of 216 unselected referrals), making diffuse Lewy body disease the commonest cause of dementia after Alzheimer's disease. Further studies of subsequent years have confirmed that diffuse Lewy body disease is not rare, and that in cases coming to post mortem it has an incidence comparable to that of vascular dementia (unpublished observations). These observations have been confirmed elsewhere: Forno and Langston identified 13 cases in an unselected series of 260 brains⁴³ whilst Perry *et al* have re-examined a series of 91 brains from elderly demented patients in Newcastle, identifying 14 cases similar to those described here (which they term senile dementia of Lewy body type).⁴⁴

Pathological basis of dementia in diffuse Lewy body disease

The most striking finding in this series is that dementia severity is closely related to cortical Lewy body density (figures 4 and 5). This suggests that cortical Lewy bodies are a marker for the pathological process responsible for the dementia of diffuse Lewy body disease and that the process is primarily cortical. This accords well with the clinical observation that patients with diffuse Lewy body disease (like severely demented patients with Parkinson's disease^{45 46}) generally show features of cortical dementia such as aphasia, agnosia and apraxia.¹ The Lewy bodies may not strictly speaking be the direct cause of the dementia, but since the presence of a Lewy body is likely to disrupt the function of a neuron and since they are found in up to 4% of all cortical neurons (and

a much higher percentage of the deeper cortical neurons in which they are concentrated) the Lewy bodies themselves may well contribute significantly to the devastating loss of intellectual function suffered by these patients. However, associated pathological features such as cortical neuron loss and senile plaques (discussed below) may also contribute; cortical neuron density has not been measured in this study but the finding of areas of cortical gliosis and spongiosis suggests neuronal loss. In this respect (as with the brainstem Lewy body where massive cell loss in the substantia nigra is associated with relatively small numbers of Lewy bodies in remaining neurons) the cortical Lewy body may be simply a marker of a process which is causing more widespread neuronal damage.

This study does not support the concept of a subcortical basis for the dementia in the nucleus basalis of Meynert. This nucleus has received considerable attention as it is the principal source of extrinsic cholinergic innervation to the cerebral cortex^{47,48} and shows massive cell depletion in cases of Alzheimer's disease.⁴⁹⁻⁵¹ Since cell loss from the nucleus basalis of Meynert also occurs in Parkinson's disease^{52,53} and is most marked in demented patients, it has been suggested that this might be an important cause of parkinsonian dementia. In the present series, cell density was slightly lower in the demented cases of diffuse Lewy body disease than in the non-demented cases of Parkinson's disease but the difference between the groups was not statistically significant (table 2). Furthermore, in individual cases there was no significant correlation between dementia severity and nucleus basalis of Meynert neuronal density. These findings suggest that cell loss from the nucleus basalis of Meynert does not make a major contribution to the dementia of diffuse Lewy body disease. However, a lesser contribution cannot be excluded on the basis of these limited estimates of cell density. Although the sampling of the nucleus basalis of Meynert was carefully standardised, it remains liable to error and a study of total cell counts in serial sections, together with neurochemical measurement of cholinergic projections to the cerebral cortex, would be necessary to resolve this important issue. Reductions in the cholinergic marker choline acetyltransferase have been reported in two small groups of cases of diffuse Lewy body disease^{21,54} but it is not yet clear if this reduction relates to the dementia and in particular if it is greater than the reduction seen in non-demented cases of Parkinson's disease.

Relationship between diffuse Lewy body disease and Alzheimer's disease

Dementia in these cases could not, in general, be attributed to Alzheimer's disease. None of the cases

showed neurofibrillary tangles in the dorsal raphe nucleus, the nucleus basalis of Meynert or in other subcortical nuclei in the manner which is characteristic of Alzheimer's disease.⁵⁵ Eleven of the cases did not have significant numbers of neurofibrillary tangles in the hippocampus and neocortex (table 3); in the four cases with larger numbers of cortical tangles, these were invariably exceeded by the numbers of cortical Lewy bodies and did not occur with the morphology and distribution normally seen in Alzheimer's disease.^{56,57}

Senile plaques were common. This should not, however, be taken as evidence of Alzheimer's disease. Plaques occur in a range of neurodegenerative disorders and are not a specific hallmark of Alzheimer's disease.⁵⁸ In this series, plaque density paralleled the density of cortical Lewy bodies (figure 6), and in many cases large numbers of plaques occurred without any other evidence of Alzheimer's disease (such as neurofibrillary tangles, granulovacuolar degeneration or Hirano bodies). Similar cases, with many cortical Lewy bodies and senile plaques but few or no neurofibrillary tangles, have been reported by others,^{20-22,59,60} suggesting that senile plaques are part of the pathology of diffuse Lewy body disease.

This conclusion has important practical implications for the study of Alzheimer's disease. Recent neuropathological criteria for the diagnosis of Alzheimer's disease have laid great weight upon estimates of plaque density especially in older cases.⁶¹ Some authors have even suggested that there may be a "plaque-only" subgroup of Alzheimer's disease lacking the other pathological features of the disease.⁶² Such cases must be examined carefully for cortical Lewy bodies, preferably using the technique of anti-ubiquitin immunocytochemistry since cortical Lewy bodies are difficult to identify on routine H and E stains. It is very likely that some of these cases are in fact examples of diffuse Lewy body disease.

Distinguishing diffuse Lewy body disease (that is, brainstem and cortical Lewy bodies and cortical plaques) from mixed Parkinson's disease and Alzheimer's disease (that is, brainstem Lewy bodies and tangles, and cortical tangles and plaques) is also of major theoretical importance. Failure to do so would provide spurious support for the suggested association between Parkinson's disease and Alzheimer's disease,^{14,15} which in turn promotes speculation that the two diseases may share a common vulnerability or a common cause. Previous reports describing such an association should perhaps be reviewed in the light of current knowledge of the pathology of diffuse Lewy body disease. It is possible that once cases of diffuse Lewy body disease have been identified, remaining genuine cases of mixed Parkinson's disease and Alzheimer's disease will have a statistical rather than a biological explanation.⁶³

Relationship between diffuse Lewy body disease and Parkinson's disease

The clinical features in diffuse Lewy body disease are frequently very similar to those of Parkinson's disease¹ (table 1). This study confirms previous reports that there is also considerable pathological overlap. In particular the brainstem pathology of the two diseases is for practical purposes indistinguishable, with both showing cell loss, gliosis and classical Lewy body formation in the substantia nigra, locus ceruleus and other brainstem nuclei. The only difference identified by this study is that a greater proportion of the remaining substantia nigra neurons contain Lewy bodies in diffuse Lewy body disease, raising the possibility that there is an increased tendency to Lewy body formation in this disease.

There is also pathological overlap in the nucleus basalis of Meynert, as discussed above. The striking difference between non-demented Parkinson's disease and diffuse Lewy body disease lies in the numbers of cortical Lewy bodies. No cortical Lewy body was detected in four of the seven cases of non-demented Parkinson's disease despite an extensive search using anti-ubiquitin immunocytochemistry. In the remaining three cases Lewy bodies were not detected using routine H and E stains but anti-ubiquitin immunocytochemistry revealed small numbers, for example, between 1 and 3 Lewy bodies in the insular cortex (that is, 0.01–0.04 Lewy bodies/mm²) and either 0 or 1 Lewy body in entorhinal cortex (that is, 0–0.06 Lewy bodies/mm²) (table 2). Far larger numbers (and greater densities) of Lewy bodies were detected in each of the cases of diffuse Lewy body disease. The small number of cortical Lewy bodies found in non-demented cases of Parkinson's disease is consistent with the hypothesis that dementia is related to cortical Lewy body density with symptoms of cognitive impairment developing only when a certain threshold has been exceeded. This "spectrum hypothesis" was first proposed by Yoshimura *et al*^{20,64} who classified cases of Lewy body disease into three groups, from Group C who had classical Parkinson's disease without dementia and brainstem but not cortical Lewy bodies, through to Group A (which they termed diffuse Lewy body disease) who were demented and had large numbers of cortical Lewy bodies as well as brainstem Lewy bodies. Between these groups lay Group B termed "transitional Lewy body disease", which had brainstem Lewy bodies but fewer cortical Lewy bodies and a lower incidence of dementia. If one accepts this hypothesis (without necessarily accepting its nomenclature), it follows that diffuse Lewy body disease can only be distinguished pathologically from Parkinson's disease by applying essentially arbitrary diagnostic criteria based on quantitative estimates of cortical Lewy body density. It would seem reasonable

to select the density at which symptoms of dementia first appear, but this point is difficult to define clinically and is in any case poorly represented in this series. On the basis of the present series it is possible to propose provisional criteria which have the advantage of practical simplicity. For example, in the anterior cingulate gyrus (which is small, easy to scan and invariably affected in cases of diffuse Lewy body disease in our experience) the presence of more than 12 cortical Lewy bodies in an anti-ubiquitin immunocytochemistry preparation or more than five on an H and E preparation would include all the moderate to very severely demented cases (that is, those demented by conventional criteria⁶⁵), but exclude the non-demented Parkinson's disease cases. It must, however, be emphasised that such definitions are tentative and arbitrary. Until larger studies of non-demented PD patients and mildly affected cases of diffuse Lewy body disease are available, it might be prudent to retain the spectrum hypothesis and quantify cortical Lewy body density thoroughly in individual cases.

From this study, however, we can conclude that diffuse Lewy body disease is a common substrate of parkinsonian dementia and that the dementia is related to a cortical process which involves Lewy body and senile plaque formation. Further understanding is likely to develop rapidly, as awareness of the condition increases: in particular clinicians should consider diffuse Lewy body disease in the differential diagnosis of cases of parkinsonian dementia and neuropathologists should seek cortical Lewy bodies with a sensitive technique such as anti-ubiquitin immunocytochemistry.

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