Loss of thalamic intralaminar nuclei in progressive supranuclear palsy and Parkinson's disease: clinical and therapeutic implications

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Summary

Whilst many reports mention neurofibrillary tangle pathology in the thalamus in progressive supranuclear palsy, there has been little detailed regional analysis of the distribution and density of thalamic pathology in this disease or in other parkinsonian syndromes. The caudal intralaminar thalamic nuclei are the major thalamic regulators of the caudate nucleus and putamen, areas known to be dysfunctional in progressive supranuclear palsy and Parkinson's disease. We investigated whether these thalamic nuclei degenerate in patients with these disorders compared with age-matched, neurologically normal controls. Neurofibrillary tangle and Lewy body pathology was assessed and unbiased optical disector methods were used to quantify total neuronal number. Despite different thalamic pathology, there was a dramatic reduction in the total neuronal number in the caudal intralaminar nuclei in both progressive supranuclear palsy and Parkinson's disease (40–55% loss). In contrast, there was no loss of volume or total neuronal number in the limbic thalamic nuclei in either disease group, indicating selective degeneration of the caudal intralaminar nuclei. In Parkinson's disease, Lewy bodies were found in these regions, while in progressive supranuclear palsy abundant intracellular neurofibrillary tangles and glial tangles concentrated in the caudal intralaminar nuclei. However, tangle formation accounted for only a small proportion of cell loss (≤10%) in the thalamus in progressive supranuclear palsy. These findings have several implications. The caudal intralaminar thalamus appears to be one of three basal ganglia sites commonly affected in both progressive supranuclear palsy and Parkinson's disease. These sites are the dopaminergic substantia nigra, the cholinergic pedunculopontine tegmental nucleus and, from our results, the glutamatergic caudal intralaminar thalamus. In both diseases these sites contain characteristic but different pathologies, indicating disease-specific mechanisms of neurodegeneration. Interestingly, the proportion of remaining neurons affected by these pathologies is low. This may indicate additional (possibly common) cellular mechanisms responsible for the degeneration in these regions. Both the dopaminergic nigra and the glutamatergic caudal intralaminar thalamus are the major regulators of basal ganglia function via the caudate nucleus and putamen. The pedunculopontine tegmental nucleus has major projections to both of these regulators. These findings indicate that dysregulation of two neurotransmitter systems within the basal ganglia may underlie common parkinsonian symptoms in these disorders. For patients with Parkinson's disease, this loss of glutamate regulation may help explain some problems with dopamine replacement therapies, particularly over time. For patients with progressive supranuclear palsy, more widespread degeneration of basal ganglia structures would contribute to poor treatment outcomes.

Introduction

Clinical features common to Parkinsonian disorders include bradykinesia, rigidity and postural instability (Litvan *et al.*, 1997). Patients with Parkinson's disease also commonly exhibit resting tremor, while patients with progressive supranuclear palsy (PSP) exhibit additional early falls and vertical gaze palsy (Litvan *et al.*, 1997). Parkinson's disease is characterized by the degeneration of brainstem basal ganglia structures associated with α-synuclein Lewy body and Lewy neurite formation (Spillantini *et al.*, 1997). PSP is characterized by brainstem and basal ganglia degeneration associated with abundant formation of tau-positive neurofibrillary tangles (NFT) and neuropil threads (Litvan, 1998). The tau pathology of PSP can be differentiated from that of Alzheimer's disease and Pick's disease by its subcortical distribution and the exclusive presence of exon 10 in the hyperphosphorylated tau isoforms found in the NFT (Pasquier and Delacourte, 1998; Sergeant *et al.*, 1999).

The predilection of pathology for basal ganglia regions probably underlies many of the motor and cognitive deficits present in Parkinsonian disorders (Litvan, 1998; Lozano *et al.*, 1998). However, the normal expression of basal ganglia-related behaviours also requires an intact thalamus. In the original paper describing the clinical and pathological features of PSP, tangle formation was documented in the thalamus, with the greatest concentration in the caudal intralaminar nuclei (Steele *et al.*, 1964). The caudal intra-laminar thalamic nuclei (parafascicular and centromedian nuclei) have important connections with the basal ganglia, regulating the output of the caudate nucleus and putamen in concert with the dopaminergic substantia nigra (Percheron *et al.*, 1994). Because of their functional similarity in basal ganglia regulation, degeneration in both these regions may be important for clinical disease expression. There have been no further studies of the caudal intralaminar thalamus in PSP, and before our recent analysis (Henderson *et al.*, 2000) there has been only limited information on these regions in Parkinson's disease (Xuereb *et al.*, 1990, 1991). To determine whether these regions are selectively involved in parkinsonism, comparison with other thalamic nuclei is also required. The nearby mediodorsal and the anterior principal nuclei project largely to the frontal lobe and are considered major limbic nuclei (Engelborghs *et al.*, 1998), and are therefore selected as internal control nuclei for the specificity of degeneration. The present study describes the neurodegenerative changes within these thalamic nuclei in six patients with PSP compared with nine patients with Parkinson's disease and 10 age-matched, neurologically normal controls.

Methods

Patient selection

Patients were selected from participants in our brain donor programme at the Prince of Wales Medical Research Institute who died between 1990 and 1996. This programme was approved by the institutional ethics committee in accordance with the National Health and Medical Research Council of Australia and complied with the Declaration of Helsinki on human experimentation. Consent of both patient and next of kin were required for entry into the study.

The brain was removed at post-mortem, weighed, then fixed by suspension in 15% buffered formalin. After 2 weeks the brains were reweighed, the brainstem and cerebellum removed, and the cerebrum was cut into 3 mm coronal slices using a rotary slicer. Blocks were then taken from the frontal, motor, anterior cingulate and temporal cortices, hippocampus, basal ganglia, midbrain, pons, medulla and cerebellum for paraffin-embedding and sectioning. Sections were stained with haematoxylin–eosin, Bielschowsky silver stain and immunohistochemistry for ubiquitin, as described previously (Harding and Halliday, 1998; Henderson *et al.*, 2000).

Patients were selected using definitive neuropathological diagnosis according to published criteria (Hauw *et al.*, 1994; Gelb *et al.*, 1999). Patients with neurodegenerative diseases other than Parkinson's disease or PSP (e.g. Alzheimer's disease, corticobasal degeneration, multisystem atrophy) were excluded. Six cases of PSP, nine cases of Parkinson's disease and ten controls were selected. Controls had no neurological or neuropathological abnormality. All patients selected were followed longitudinally and were clinically reviewed at least 12 months before death with standardized scores for motor severity of disease (Hoehn and Yahr, 1967). Patients with dementia according to the Clinical Dementia Rating Scale (Hughes *et al.*, 1982) were excluded. Many of the cases reported have been analysed previously in studies of basal ganglia regions (Halliday *et al.*, 1996; Hardman *et al.*, 1996, 1997*b*; Hardman and Halliday, 1999*a*, *b*; Henderson *et al.*, 2000).

All PSP patients studied met the NINDS–SPSP (National Institute of Neurological Disorders and Stroke–Society for Progressive Supranuclear Palsy, Inc.) criteria (Hauw *et al.*, 1994) for the clinical and pathological diagnosis of PSP. All had bradykinesia, rigidity, falls and/or supranuclear gaze palsy, and little or no sustained response to levodopa. At the time of death, two PSP patients had early bilateral limb signs (Hoehn and Yahr stage 2–3) and four were severely disabled or were wheelchair- or bed-bound (Hoehn and Yahr stage 4–5). All nine Parkinson's disease patients who were selected fulfilled the diagnostic criteria for Parkinson's disease (Gelb *et al.*, 1999), were levodopa-responsive with bradykinesia and rigidity, and six had tremor at rest. At the time of death, two Parkinson's disease patients exhibited only early bilateral limb signs (Hoehn and Yahr stage 2), whilst the remainder had severe disability and were wheelchair- or bed-bound (Hoehn and Yahr stage 4–5). Ten age-matched controls without neurological disease (from detailed clinical histories) and who did not have evidence of any neuropathological disease were selected for comparison. Five cases of

Table 1 *Demographic features of cases and quantitation of thalamic nuclei*

	Control (*n* = 10)	PD (*n* = 9)	PSP (*n* = 6)
Age at onset (years)	–	70.2 ± 1.4	62.7 ± 3.7*
Age at death (years)	71.0 ± 3.6	79.0 ± 0.9	69.8 ± 4.8
Disease duration (years)	–	7.2 ± 2.0	9.3 ± 1.5
Disease severity (Hoehn and Yahr)		Stage 4–5 (*n* = 7)	Stage 4–5 (*n* = 4)
		Stage 2 (*n* = 2)	Stage 2–3 (*n* = 2)
Volume (mm^3)			
Thalamus (whole)	13 680 ± 710	13 670 ± 760	11 720 ± 490
Pf	180 ± 10	140 ± 10*	100 ± 10**
CM	310 ± 20	290 ± 10	220 ± 20*
AP	150 ± 10	130 ± 10	140 ± 10
MD	820 ± 20	780 ± 30	790 ± 20
Total number of neurons ($\times 10^3$)			
Pf	657 ± 37	460 ± 50*	326 ± 44**
CM	664 ± 35	397 ± 42**	305 ± 25**
AP	488 ± 16	498 ± 24	497 ± 26
MD	2611 ± 72	2881 ± 135	2616 ± 83

*$P < 0.05$ versus controls (or versus Parkinson's disease group for age); **$P < 0.01$ versus controls.

Parkinson's disease, four PSP patients and two controls died of pneumonia, four Parkinson's disease and six controls died of heart failure and one control and one PSP patient died of cancer. Agonal state was similar in all groups, and the postmortem delay was ≤62 h in all patients (group mean ± standard error of the mean: control, 20 ± 4, Parkinson's disease, 31 ± 4; PSP, 18 ± 6). Further patient details are given in Table 1.

Tissue preparation and analysis

After fixation and 3 mm coronal sectioning of the brain, the thalamus was blocked from the mammillothalamic tract to the beginning of the pulvinar, cryoprotected in 30% sucrose in 0.1 M Tris–HCl buffer (pH 7.4) for 2 days, frozen to –20°C and serially sectioned on a Leica freezing microtome at 50 μm intervals. Nine adjacent series of sections spaced 750 μm apart were taken and stained using (i) cresyl violet, (ii) haematoxylin–eosin, (iii) luxol fast blue, (iv) nickel peroxidase (Cullen, 1994), and immunohistochemistry performed for (v) tau II [T5530, Sigma (St Louis, Mo., USA), diluted 1 : 10 000], (vi) ubiquitin (U5379; Sigma, diluted 1 : 100), (vii) α-synuclein [18–0215, Zymed (San Francisco, Calif., USA), diluted 1 : 3000] and the calcium-binding proteins (viii) calbindin (C8666; Sigma, diluted 1 : 2000) and (ix) parvalbumin (P3171; Sigma, diluted 1 : 10 000). Routine immunohistochemistry was used with peroxidase visualization as described previously (Henderson *et al.*, 2000). A selection of sections were counterstained with cresyl violet. The specificity of the immunohistochemical reaction was tested by omitting the primary antibody on test sections. No peroxidase reaction was observed.

The criteria of Hirai and Jones (Hirai and Jones, 1989) were used for the boundaries of thalamic nuclei. Nuclear boundaries were delineated in serial sections stained for different substances, including the calcium-binding proteins,

as described recently (Münkle *et al.*, 1999; Henderson *et al.*, 2000), and the boundaries were plotted using an integrated computerized microscope system (Neurolucida, MicroBrightField, Colchester, Vt., USA). The cross-sectional area of the nucleus within each section was calculated by the computer and the volume for each nucleus determined by multiplying the sum of the areas by the distance between the sections using Cavalieri's principle. There was <5% variation in 10 repeat measures of the cross-sectional area for the smallest nucleus by the same investigator. There was <10% inter-rater variation in cross-sectional area and volume measurements of the same nuclei in four control cases. Therefore, boundaries of the nuclei delineated in this study are reliable and accurate.

The total number of neurons and NFT within the parafascicular nucleus (Pf) and the centromedian (CM), anterior principal (AP) and mediodorsal (MD) nuclei was quantified using the unbiased optical disector technique (Harding *et al.*, 1994; Henderson *et al.*, 2000). Briefly, the disector frame used for the neuronal counts was 100 × 100 μm at a magnification of ×400. These disectors were sampled in a grid array separated by 1 mm and the full section thickness was evaluated (50 μm). We have demonstrated previously that the use of the full section thickness does not alter the disector calculations significantly (Harding *et al.*, 1994). The number of sections upon which disector frames were placed varied from 10 to 26 between cases. The number of disector frames sampled varied from 81 to 162 for Pf, 135 to 243 for CM, 43 to 103 for AP and 102–189 for MD. Nissl-stained neurons whose nucleolus fell entirely within the sampling frame or on one of each of three adjacent inclusion borders (in the *x*, *y* and *z* planes) were counted. The number of neurons counted varied between 127 and 427 for the Pf nucleus, 79 and 255 for the CM nucleus, 74 and 174 for the AP nucleus and 162 and 306 for the MD nucleus. Repeated measures of the number of neurons within

the sampling frames in multiple sections from multiple subjects consistently gave similar results, even between different investigators. Because of variations in the distribution and density of NFT between subjects, all tau-immunopositive NFT within the target region whose tip came into focus through the section were counted. Repeated measures of the number of tau-positive NFT within the same sections from various subjects gave similar results between investigators. Neuronal density was estimated by dividing the total number of neurons counted by the total sample volume. The total number of neurons was estimated by multiplying the density of the neurons (coefficient of error range 0.03–0.11) by the volume of the region in which they were contained. The total number of NFT was estimated by summing the number counted per section in each region (coefficient of error range 0.03–0.16) and multiplying by the sampling frequency (15).

Statistical analysis was conducted using the Statview 5.0 program (Abacus Concepts, Berkeley, Calif., USA). Data are expressed as mean $\pm$ standard error of the mean. For statistical analysis, a value of $P < 0.05$ was accepted as significant. Analysis of variance (ANOVA) was used to test for differences between the PSP, Parkinson's and control groups. *Post hoc* analysis using Fisher's protected least significant difference (PLSD) test was applied if differences were found. Regression analysis was used to investigate any correlations between pathologies and disease duration.

Results

There was no significant difference in age at death between the patient and control groups (ANOVA, $P = 0.09$; Table 1) or in disease duration between the patient groups (ANOVA, $P = 0.40$; Table 1). However, most of the PSP patients had an earlier age of disease onset than the Parkinson's patients, as described in the literature (Litvan, 1998).

Pathological lesions in the medial thalamus

As expected, tau immunohistochemistry revealed large numbers of intracellular NFT in the medial thalamus of PSP patients, in contrast to controls and Parkinson's disease patients (Fig. 1A, B and D–H). The majority of tau-positive NFT were globose and significantly larger than glial tangles (Fig. 1D). Tau-positive glial tangles were mainly coiled bodies (Fig. 1D–H) or tufted-, star- or thorn-shaped astrocytes containing many processes. NFT density was increased in the intralaminar nuclei compared with the limbic thalamus in PSP (Fig. 1A). NFT were absent in the control and Parkinson's disease groups (Fig. 1B). The number of NFT present in the PSP group was 30 500 $\pm$ 6000 in the Pf, 33 000 $\pm$ 4500 in the CM, 26 000 $\pm$ 10 000 in the MD and 1500 $\pm$ 500 in the AP nucleus. The caudal intralaminar nuclei showed a higher percentage of NFT relative to the

total number of remaining cells ($\leqslant$10%) when compared with the limbic thalamic nuclei ($\leqslant$1%). Despite these relative differences in concentration, the number of NFT in one thalamic region correlated with the number of NFT in the other regions ($r^2 = 0.55$–0.97l) (Fig. 1K).

In contrast to the tau immunohistochemical staining, ubiquitin immunohistochemistry stained fewer cellular elements in either disease group. In PSP patients, ubiquitin was found in a small proportion of extracellular, globose NFT. In Parkinson's disease patients, ubiquitin- and α-synuclein-positive Lewy bodies and Lewy neurites were observed in the intralaminar nuclei (Fig. 1C, I and J). These pathological structures were more commonly observed in sections stained immunohistochemically for α-synuclein than for ubiquitin. Many immunohistochemically stained pathological structures were axonal Lewy bodies (Fig. 1), although neuronal Lewy bodies as well as the entire neuronal cytoplasm contained α-synuclein immunoreactivity in a number of instances (Fig 1I and J). Similar pathology was also observed in the limbic and motor thalamic nuclei, but to a lesser degree. Although neuronal Lewy body structures were relatively sparse, they were consistent with the pathological diagnosis of Parkinson's disease in these patients.

Quantitation of volume and neuronal loss

The total volume of the thalamus in the PSP and Parkinson's disease groups was not significantly different from that in the controls (ANOVA, $P = 0.15$) (Table 1). The MD and AP nuclei were readily delineated from surrounding thalamic nuclei (Fig. 2A, D and G) and were composed of darkly stained, large neurons in all patients analysed (Fig. 2B, C, E, F, H and I), with a neuronal density of ~3200/mm^3. There was no significant difference in the volume of either the MD or the AP nucleus between the three groups (all ANOVA, $P > 0.10$) (Table 1). Consistent with the lack of atrophy found in these regions, there was no reduction in the total neuronal number compared with control values (ANOVA, $P = 0.12$) (Fig. 4A and B).

In controls, CM neurons were small and faintly stained for Nissl substance compared with the larger, more closely packed and deeply stained Pf neurons (Fig. 3B and C). The neuronal density was ~3600/mm^3 in the Pf and 2200/mm^3 in the CM nucleus. The Pf nucleus exhibited atrophy in both patient groups, the degree of atrophy being greatest in PSP (45% relative to controls; Fisher's PLSD, $P = 0.011$) (Table 1). Whilst atrophy of the Pf nucleus in PSP was, on average, approximately twice that observed in Parkinson's disease, there was considerable individual variation such that this difference just failed to reach significance (Fisher's PLSD, $P = 0.053$). The Pf nucleus sustained obvious neuronal loss in both disease groups (Fig. 3B, E and H and Fig. 4C) (Table 1). The volume and total neuronal number were strongly correlated for the densely cellular Pf region ($r^2 = 0.84$). On average, 30–50% of Pf nucleus neurons degenerated in Parkinson's disease (control versus Parkinson's disease,
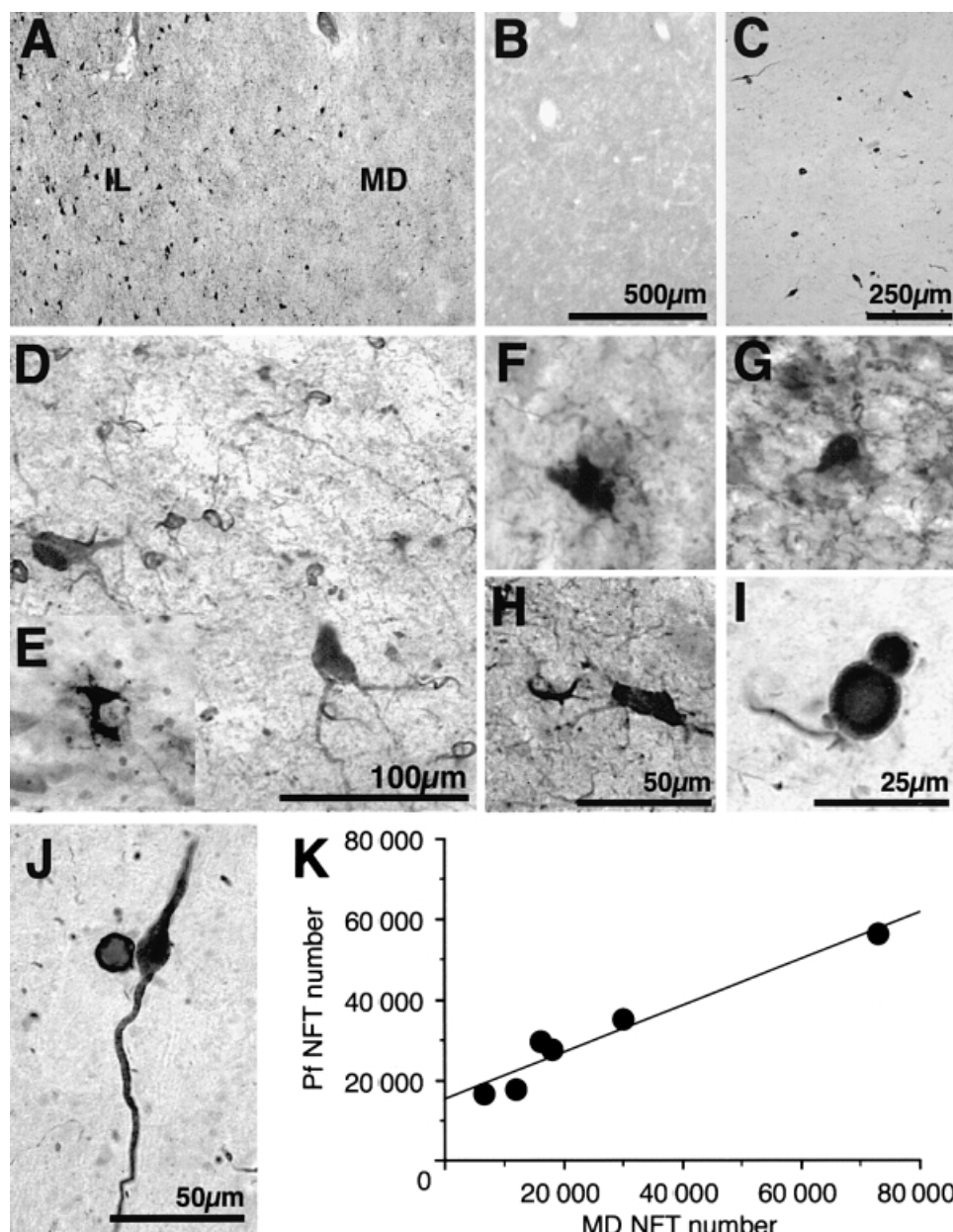
Fig. 1 Representative photomicrographs of sections through the caudal intralaminar nuclei in patients with Parkinson's disease (**B**, **C**, **I**, **J**) or PSP (**A**, **D–H**), and graph showing the correlation between NFT number in different thalamic nuclei in PSP (**K**). (**A**) Differential density of tau NFT pathology in the caudal intralaminar (IL) and MD nuclei in a PSP patient. (**B**) Absence of tau pathology in the IL nuclei of a patient with Parkinson's disease. (**C**) Serial section to **B** stained immunohisto-chemically for α-synuclein. Many Lewy bodies and some neurons were immunoreactive for α-synuclein in the IL in Parkinson's disease. Scale in **C** is equivalent to that in **A** and **B**. (**D**) High magnification of the IL nuclei stained immunohistochemically for tau, showing NFT (in larger cells) and coiled glial bodies (small cells) in PSP. (**E**) High magnification of intracellular NFT in the IL nuclei in PSP stained with nickel peroxidase and counterstained with cresyl violet. The majority of NFT had clearly labelled nuclei when the cresyl violet counterstain was used. (**F**, **G**) High magnification of IL nuclei stained immunohistochemically for tau and counterstained with cresyl violet, showing intracellular tau-positive NFT. (**H**) High magnification of the IL nuclei stained immunohistochemically for tau and counterstained with cresyl violet, showing an extracellular NFT (smaller structure) and a neuron filled with tau immunoreactivity. Scale in **H** is equivalent to that in **D–G**. (**I**) High magnification of the IL nuclei stained immunohistochemically for α-synuclein, showing two Lewy bodies. (**J**) High magnification of the IL nuclei stained immunohistochemically for α-synuclein, showing a Lewy body and a Lewy neurite. (**K**) Regression plot demonstrating positive relationship between NFT number in the thalamic Pf and MD nuclei.
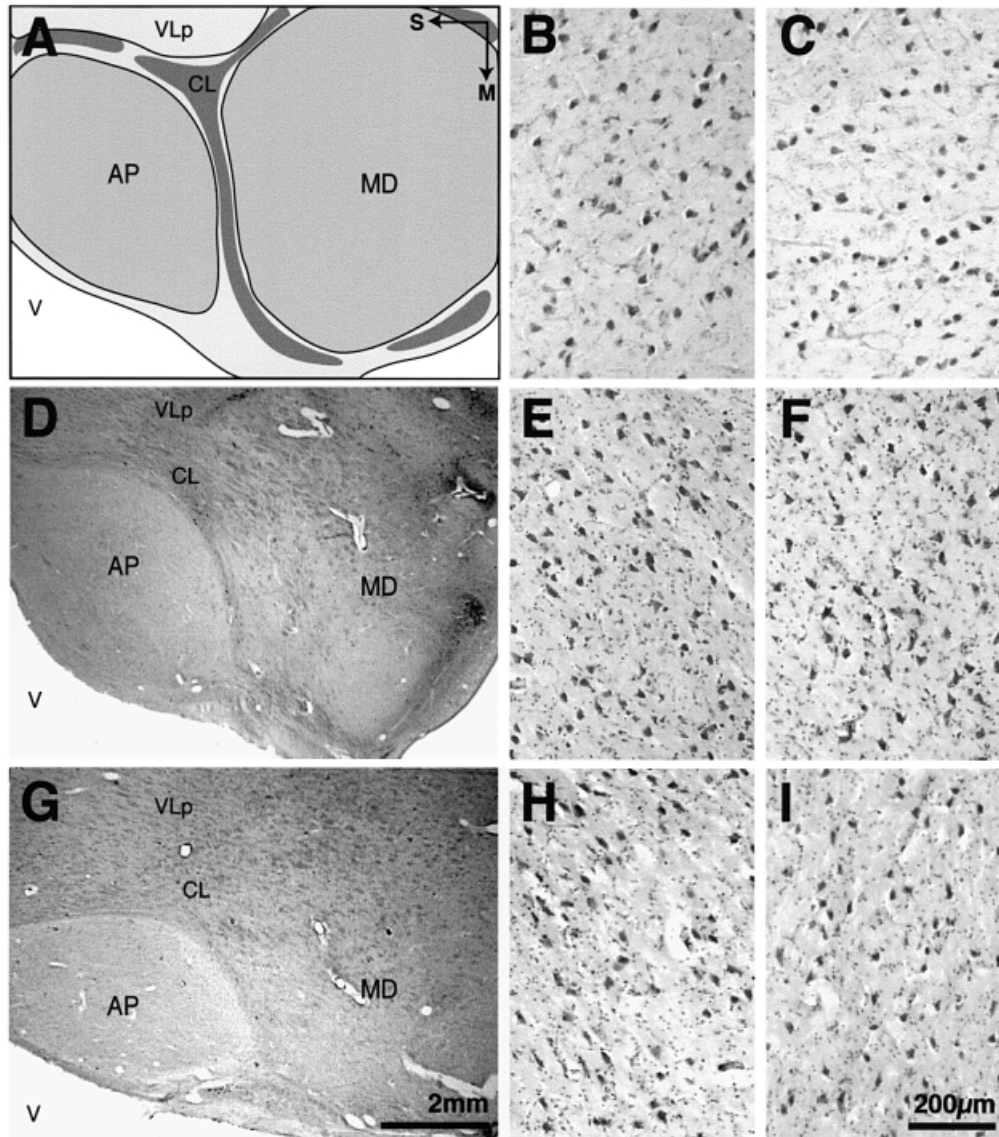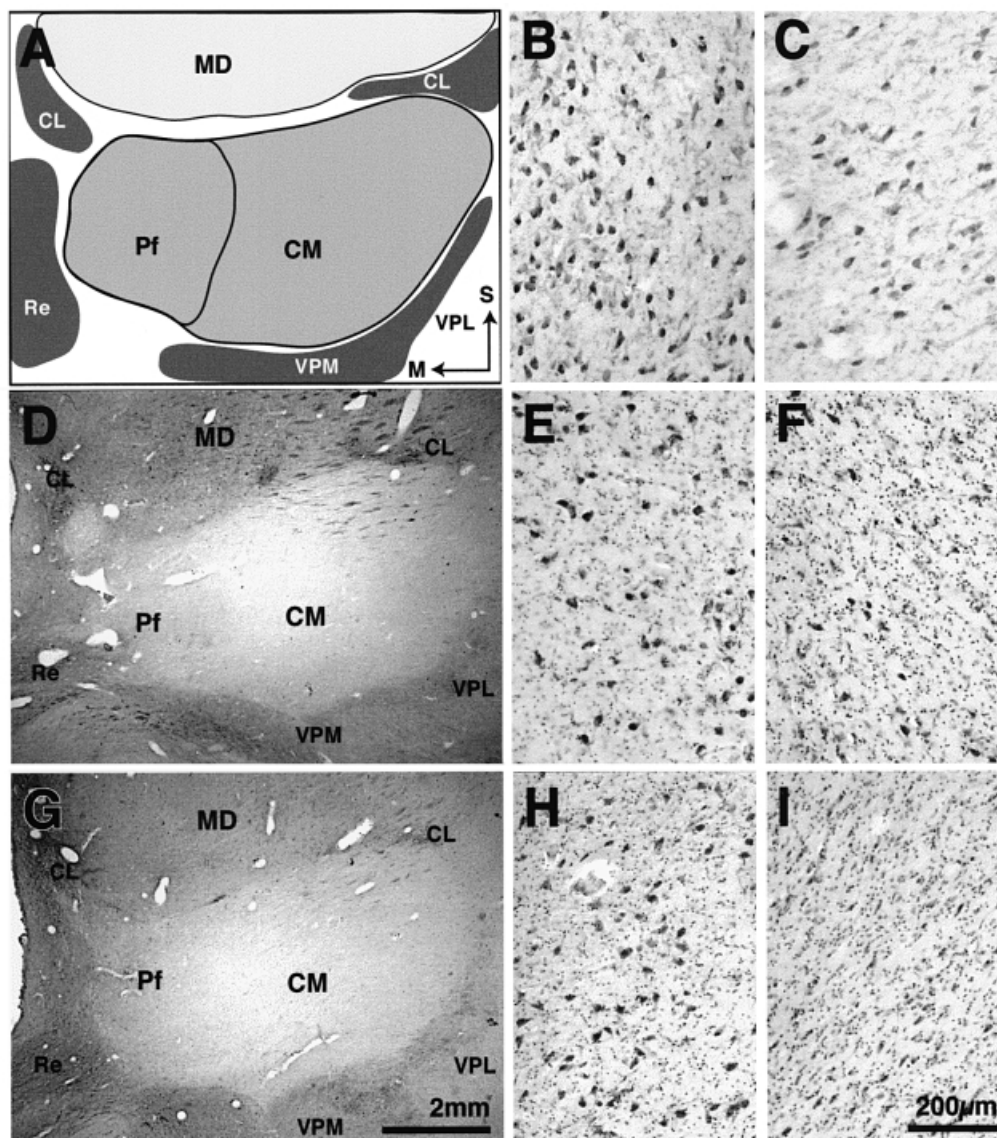
Fig. 2 Anatomy and cell composition of the limbic thalamus in controls (**A–C**), Parkinson's disease patients (**D–F**) and PSP patients (**G–I**). Scale in **G** is the same as that in **A** and **D**; scale in **I** is the same as that in **B**, **C**, **E**, **F** and **H**. (**A**) Diagrammatic representation delineating the normal boundaries of the limbic thalamic nuclei, AP and MD. These nuclei are separated by the internal medullary lamina. Neurons of the centrolateral thalamic (CL) nucleus are found between the MD and ventral lateral posterior thalamic (VLp) nuclei. Anatomical positioning is indicated by arrows showing the direction of the superior surface of the brain (S) versus the midline (M). (**D** and **G**) Low-power sections from representative Parkinson's disease (**D**) and PSP (**G**) patients, stained immunohistochemically for the calcium binding-protein calbindin, showing clear delineation of the AP and MD nuclei with respect to the surrounding thalamic regions. (**B**, **E** and **H**) High-power cresyl violet-stained sections of Nissl-stained neurons in the AP in representative control subjects (**B**), Parkinson's disease patients (**E**) and PSP patients (**H**). Note the similar morphologies and densities of neurons in the three groups. (**C**, **F** and **I**) High-power cresyl violet-stained sections of Nissl-stained neurons in the MD in representative control subjects (**C**), Parkinson's disease patients (**F**) and PSP patients (**I**). Note the similar morphologies and densities of neurons in the three groups. V = third ventricle.

Fisher's PLSD, $P = 0.0031$) and PSP (control versus PSP, Fisher's PLSD, $P < 0.001$) (Table 1). Again, there was considerable individual variation (Parkinson's disease versus PSP, Fisher's PLSD, $P = 0.058$) (Fig. 3E and H).

The PSP group also exhibited a 30% reduction in the volume of the CM (PSP versus control, Fisher's PLSD, $P =$ 0.0050) (Table 1). This was significantly different from the Parkinson's disease group (Parkinson's disease versus PSP, Fisher's PLSD, $P = 0.037$), which did not differ from control values (Parkinson's disease versus control, Fisher's PLSD, $P = 0.44$) (Table 1). While atrophy of the CM was consistently found only in the PSP group, neuronal loss within the CM

Fig. 3 Anatomy and cell composition of the caudal intralaminar thalamus in controls (**A–C**), Parkinson's disease patients (**D–F**) and PSP patients (**G–I**). Scale in **G** is the same as that in **A** and **D**. Scale in **I** is the same as that in **B**, **C**, **E**, **F** and **H**. (**A**) Diagrammatic representation delineating the boundaries of the caudal intralaminar thalamic nuclei. Anatomical positioning is indicated by arrows showing the direction of the superior surface of the brain (S) versus the midline (M). These nuclei are separated from the MD by the internal medullary lamina. Neurons of the centrolateral thalamic nucleus (CL) nucleus are found between the MD and these nuclei. (**D** and **G**) Low-power sections from representative Parkinson's disease (**D**) and PSP (**G**) patients stained immunohistochemically for the calcium-binding protein calbindin, showing clear delineation of the CM and Pf nuclei with respect to the surrounding thalamic regions. (**B**, **E** and **H**) High-power cresyl violet-stained sections of Nissl-stained neurons in the Pf nucleus in representative control subjects (**B**), Parkinson's disease patients (**E**) and PSP patients (**H**). Note the decreased density of neurons and increased gliosis in the two patient groups compared with the controls. (**C**, **F** and **I**) High-power magnification of cresyl violet-stained sections from representative control subjects (**C**), Parkinson's disease patients (**F**) and PSP patients (**I**), showing neurons and glial cells in the CM. Note the different morphologies and densities of Pf and CM neurons in **B** and **C**. The CM neurons (**C**) are smaller and further apart than those of the Pf (**B**). In both Parkinson's disease (**F**) and PSP (**I**), there was marked neuronal loss and gliosis of the CM compared with controls (**C**). Re = reuniens thalamic nucleus; VPM = ventral posterior medial thalamic nucleus; VPL = ventral posterior lateral thalamic nucleus.
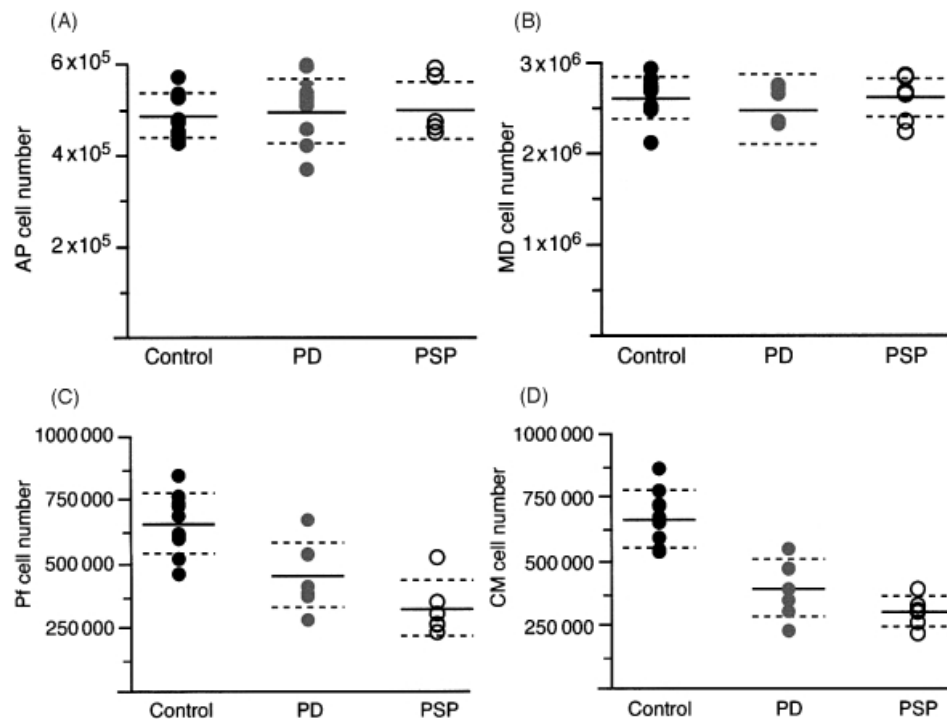
Fig. 4 Graphs of total neuronal number in the limbic (**A** and **B**) and caudal intralaminar (**C** and **D**) thalamic nuclei in the control subjects, Parkinson's disease patients and PSP patients. (**A** and **B**) Total neuronal number in the limbic thalamus in the three groups. There was no difference in total neuronal number in either the AP (**A**) or the MD (**B**) nuclei in the three groups of patients. (**C** and **D**) Total neuronal number in the caudal intralaminar thalamus. There was a significant reduction in total neuronal number in both the Pf (**C**) and the CM (**D**) nuclei in the Parkinson's disease and PSP groups relative to controls.

was similar in the two patient groups (average neuronal loss compared with controls, 40–55%) (Table 1; Fig. 3C, F and I; Fig. 4D). Thus, the neuronal degeneration within the intralaminar thalamic nuclei was similar in the two disease groups (Fisher's PLSD: control versus Parkinson's disease, $P < 0.0001$; control versus PSP, $P < 0.001$; Parkinson's disease versus PSP, $P = 0.12$) (Fig. 4C and D), even though considerably more atrophy occurred in the CM in PSP. This suggests additional degeneration of CM neuropil in the PSP cases. There were no significant correlations between the number of neurons in one thalamic region and the number found in another.

Correlations between pathologies and clinical variables

In both disease groups, characteristic pathology was found within regions of neurodegeneration (Fig. 1A–J). However, in PSP patients, characteristic pathology was also found in regions without considerable neurodegeneration, suggesting a more widespread disease mechanism. In the PSP patients analysed, ≤1% of neurons in the MD and AP contained NFT, whereas ~10% of the remaining CM and Pf neurons contained NFT.

Comparisons between clinical and cellular variables revealed no significant relationship between disease severity

using the Hoehn and Yahr score and the magnitude of the degeneration in either the Pf or the CM nucleus in the disease groups (Fig. 5). Similar atrophy and cell loss were found irrespective of whether Parkinson's disease or PSP patients had mild or more severe disease (Fig. 5). Similarly, no relationship was found between disease duration and the degree of cell loss in the patient groups (Pf, $r^2 = 0.12$; CM, $r^2 = 0.39$). The degree of neuronal loss in these nuclei was similar regardless of these clinical parameters and whether tremor was present or absent. In contrast, there was a negative correlation between NFT number and disease duration ($r^2 = -0.63$ to -0.97), in that the longer the disease was present the fewer NFT were found within all thalamic nuclei (Fig. 5).

Discussion

The present study describes previously under-recognized degeneration of the caudal intralaminar thalamus in both PSP and Parkinson's disease. For both diseases, the characteristic disease pathology (NFT or Lewy bodies) was observed throughout the thalamus. Although the pathological substrate differed between these Parkinsonian disorders, the thalamic regions with neuronal loss were the same. In both Parkinson's disease and PSP, prominent cell loss was restricted to the caudal intralaminar nuclei (averaging 40–55% cell loss). This cell loss was very selective as pathology was found in the
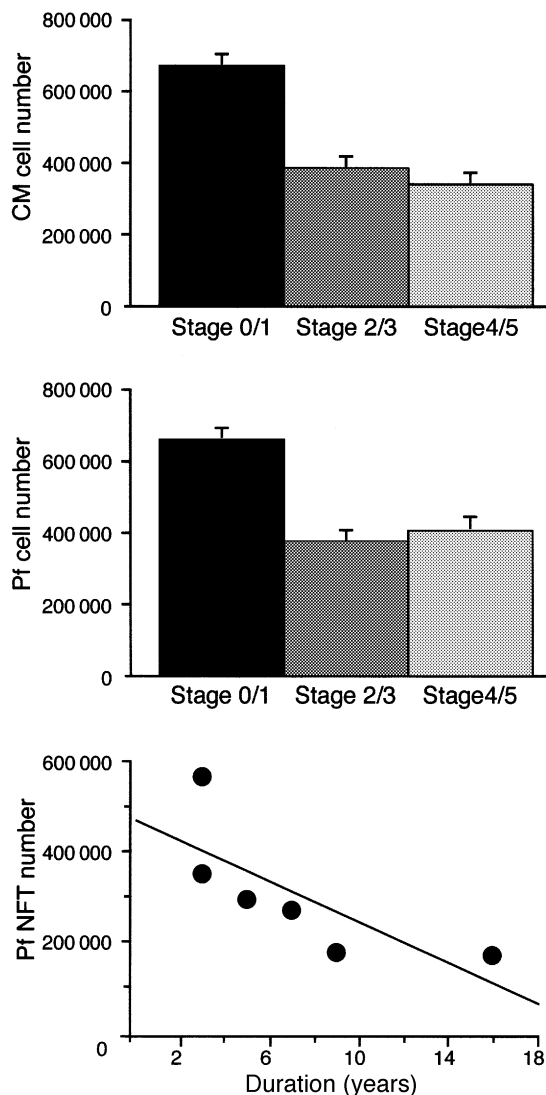
Fig. 5 Graphs of the relationship between cell (top and middle) or NFT (bottom) number and clinical disease indices. (*Upper two panels*) The number of neurons in the three groups plotted against Hoehn and Yahr stage at death. In parkinsonian patients (stages 2–5) there was a significant reduction in total neuronal number in the CM (top) and Pf (middle) nuclei relative to controls. This cell loss occurred irrespective of whether patients had milder (stage 2–3) or more severe (stage 4–5) disease. (*Bottom*) The number of NFT declined significantly with increasing duration of PSP.

limbic thalamus without neurodegeneration. To our knowledge, no studies have examined these thalamic regions in detail in PSP. A previous study in Parkinson's disease observed some cell loss in the CM, but it was proposed that this degeneration may be related to ageing (Xuereb *et al.*, 1991). Our results suggest otherwise (see also Henderson *et al.*, 2000) and show that similar selective degeneration of the thalamus occurs in both Parkinson's disease and PSP, which are movement disorders with overlapping extra-pyramidal symptoms. In Parkinson's disease, we have shown recently that neuronal degeneration occurs principally in neurons differentially expressing calcium-binding proteins [affecting both the parvalbumin-containing neurons in the Pf

nucleus and the non parvalbumin-containing neurons in the CM nucleus (Henderson *et al.*, 2000)]. In PSP, atrophy was also observed in the CM nucleus, probably reflecting the additional degeneration of basal ganglia output nuclei, resulting in thalamic deafferentation (Hardman *et al.*, 1997*b*; Litvan, 1998; Hardman and Halliday, 1999*b*). Our findings suggest that, in addition to the dopaminergic substantia nigra, the caudal intralaminar thalamus may be an important effector in the genesis of common extrapyramidal symptoms in Parkinsonian disorders, even though PSP and Parkinson's disease have different underlying pathologies.

Whilst many studies have reported the presence of NFT pathology in the thalamus in PSP (Steele *et al.*, 1964; Lantos, 1994; Matsumoto *et al.*, 1996), there has been little detailed regional analysis of their distribution and density. In our patients with pathology in the typical basal ganglion and brainstem predilection sites (Halliday *et al.*, 1996; Hardman *et al.*, 1996, 1997*b*; Hardman and Halliday, 1999*a*, *b*), tau pathology also concentrated in the caudal intralaminar nuclei of the thalamus. It has been proposed that NFT formation is an index of cell loss (Braak and Braak, 1991, 1998; Lantos, 1994). Interestingly, we found that NFT accounts for only a small proportion of cell loss ($\leqslant$10%) in these thalamic regions in PSP. This occurred primarily because of the loss of NFT over the duration of the disease, suggesting clearance of these structures during the typical clinical course of the disease (which is shorter than for many other neurodegenerative conditions). This NFT data in PSP differs significantly from what is thought to occur in Alzheimer's disease, where NFT remain in the extracellular space for decades after cell death (Braak and Braak, 1998), enabling their use in pathological grading (Braak and Braak, 1991). Our data suggest that NFT in PSP cannot be utilized in the same way. Considerable variability in the distribution of NFT pathology in PSP cases has been noted previously (Lantos, 1994; Daniel *et al.*, 1995), and our study suggests that this may reflect differences in the degree of cell loss in different regions of susceptibility. Interestingly, the relative density of NFT was similar throughout the thalamus and was concentrated in the intralaminar regions simply because of the cell loss and reduced volume. This suggests the possibility that a proportion of the selective cell loss may occur independently of NFT formation, possibly via an alternative pathogenic mechanism. The lack of an association between the degree of cell loss and NFT formation further supports this concept (and cell loss was not correlated with disease duration).

In idiopathic Parkinson's disease there was also prominent cell loss in the intralaminar thalamus (see also Henderson *et al.*, 2000). Lewy bodies were found in the thalamus, consistent with their location in degenerating brainstem regions in this disease (Daniel, 1995; Gelb *et al.*, 1999). In the brainstem, Lewy bodies are typically rounded, eosinophilic, intracytoplasmic inclusion bodies, whereas those present in the cerebral cortex are better seen with immunohistochemistry for α-synuclein or ubiquitin (Daniel, 1995; Forno, 1996;

Spillantini *et al.*, 1997). The Lewy bodies observed in the thalamus in the present study were more typical of those found in cortical regions since they were pale-staining in haematoxylin–eosin but intensely immunoreactive for α-synuclein, even without formic acid pretreatment (Takeda *et al.*, 1998). Interestingly, Lewy body pathology was rarely observed when ubiquitin immunohistochemistry was performed on parallel series of thick sections. This may explain why few previous studies have reported such thalamic pathology (Fearnley and Lees, 1994; Braak *et al.*, 1996).

Our results indicate that the caudal intralaminar thalamus appears to be one of three basal ganglia sites commonly affected in both PSP and Parkinson's disease. The two sites identified previously are the dopaminergic substantia nigra (Fearnley and Lees, 1991; Halliday *et al.*, 1996; Hardman *et al.*, 1997*a*) and the cholinergic pedunculopontine tegmental nucleus (Hirsch *et al.*, 1987; Jellinger, 1988; Gai *et al.*, 1991). The dopaminergic substantia nigra is thought to be pivotal to these diseases, while the role of the pedunculo-pontine tegmental nucleus is as yet unclear. The involvement of the caudal intralaminar nucleus can be inferred from its anatomical connectivity and may help explain certain disease features. The thalamic nuclei are major regulators of the caudate nucleus and putamen, as is the dopaminergic substantia nigra. In fact, it has been proposed that the glutamatergic caudal intralaminar nuclei are as important as dopamine for modulating information processing through the caudate nucleus and putamen (Percheron *et al.*, 1994). The CM nucleus in particular is strategically positioned to influence sensorimotor processing (Groenewegen and Berendse, 1994) via a distinctive (Nauta–Mehler) loop involving the putamen and internal globus pallidus projections (Nauta and Mehler, 1966; Sidibé and Smith, 1996). In contrast, the Pf nucleus has more widespread diffuse striatal projections (Nakano *et al.*, 1990). Both the caudal intralaminar thalamic nuclei and the dopaminergic substantia nigra receive dense innervation from the cholinergic pedunculopontine tegmental nucleus (Hallanger *et al.*, 1987; Parent, 1990; Groenewegen and Berendse, 1994; Oakman *et al.*, 1995). This may explain the degeneration of this cholinergic brainstem region in both PSP and Parkinson's disease via a retrograde mechanism.

In assessing the clinical correlates of thalamic degeneration in both diseases, we found no relationship between the degree of neurodegeneration of the caudal intralaminar nuclei and the age of disease onset, disease duration, the presence of tremor or the clinical severity of parkinsonism. Thus, these thalamic nuclei are affected to a similar degree at all disease stages in the two disease groups. This contrasts with the progressive loss of dopamine over a long period that is thought to begin some 5–10 years before symptom onset (Fearnley and Lees, 1991; Morrish *et al.*, 1998). For patients with Parkinson's disease, the loss of thalamic glutamatergic regulation may help explain some of the problems with dopamine replacement therapies, particularly over time. For patients with PSP, more widespread degeneration of basal ganglion structures would contribute to

poor treatment outcomes in the initial stages of the disease. Together, these data suggest that the caudal intralaminar nuclei may be affected towards the beginning of the clinical course of each disease.

In conclusion, we have identified substantial degeneration of the caudal intralaminar nuclei in both PSP and Parkinson's disease. While the characteristic pathologies specific to these diseases are observed within the thalamus, the proportion of remaining neurons affected by these pathologies is low. This may indicate additional (possibly common) cellular mechanisms responsible for the cell loss in these regions in both disorders. Loss of the caudal intralaminar nuclei together with the dopaminergic substantia nigra could contribute to the development of parkinsonism by disruption of striatal outputs and sensorimotor processing through the Nauta–Mehler loop (Nauta and Mehler, 1966; Sadikot *et al.*, 1992). Our work suggests that non-dopaminergic mechanisms are also important in the evolution of PSP and Parkinson's disease.

Acknowledgements

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