A comparison of degeneration in motor thalamus and cortex between progressive supranuclear palsy and Parkinson’s disease

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Changes in motor cortical activation are associated with the major symptoms observed in both Parkinson’s disease and progressive supranuclear palsy (PSP). While research has concentrated on basal ganglia abnormalities as central to these cortical changes, several studies in both disorders have shown pathology in the thalamus and motor cortices. In particular, we recently reported an 88% loss of corticocortical projection neurones in the pre-supplementary motor (pre-SMA) cortex in Parkinson’s disease. Further analysis of the degree of neuronal loss and pathology in motor cortices and their thalamocortical relays in Parkinson’s disease and PSP is warranted. Six cases with PSP, nine cases with Parkinson’s disease and nine controls were selected from a prospectively studied brain donor cohort. α-Synuclein, ubiquitin and tau immunohistochemistry were used to identify pathological lesions. Unbiased stereological methods were used to analyse atrophy and neuronal loss in the motor thalamus [ventral anterior, ventrolateral anterior and ventrolateral posterior (VLP) nuclei] and motor cortices (primary motor, dorsolateral premotor and pre-SMA cortices). Analysis of variance and post hoc testing was used to determine differences between groups. In Parkinson’s disease, the motor thalamus and motor cortices (apart from the pre-SMA) were preserved containing only rare α-synuclein-positive and ubiquitin-positive Lewy bodies. In contrast, patients with PSP had significant atrophy and neuronal loss in VLP (22 and 30%, respectively), pre-SMA (21 and 51%, respectively) and primary motor cortices (33 and 54%, respectively). In the primary motor cortex of PSP cases, neuronal loss was confined to inhibitory interneurones, whereas in the pre-SMA both interneurones (reduced by 26%) and corticocortical projection neurones (reduced by 82%) were affected. Tau-positive neurofibrillary and glial tangles were observed throughout the motor thalamus and motor cortices in PSP. These non-dopaminergic lesions in motor circuits are likely to contribute to the pathogenesis of both PSP and Parkinson’s disease. The selective involvement of the VLP and primary motor cortex in PSP implicates these cerebellothalamicocortical pathways as differentiating this disease, possibly contributing to the early falls.

Keywords: α-synuclein; motor cortex; stereology; tau; thalamus

Abbreviations: CM = centromedian thalamic nucleus; DPC = dorsolateral premotor cortex; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NFT = neurofibrillary tangles; PSP = progressive supranuclear palsy; SMA = supplementary motor area; VA = ventral anterior thalamic nucleus; VLA = ventrolateral anterior thalamic nucleus; VLP = ventrolateral posterior thalamic nucleus

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Introduction

Changes in motor cortical activation are associated with the common clinical features of bradykinesia, rigidity and postural instability in both Parkinson’s disease and progressive supranuclear palsy (PSP). Following either 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment or the development of severe parkinsonism, neurones in the primary motor cortex fire in long synchronized bursts (Goldberg et al., 2002, 2004; Brown, 2003). Current theories...
about the pathogenesis of these parkinsonian symptoms in both Parkinson’s disease and PSP emphasize changes in basal ganglia output to motor thalamus and the resultant change in motor cortex (Brown, 2003; Foffani et al., 2003; Devos et al., 2004). Recent studies have focused on the discharge rate and synchronization of basal ganglia output, with lower frequency oscillations facilitating the slow synchronized cortical activity observed in Parkinson’s disease and high frequency basal ganglia output restoring dynamic task-related cortical activity (Brown et al., 2001; Williams et al., 2002; Buhmann et al., 2003; Foffani et al., 2003; Hamani et al., 2003; Hashimoto et al., 2003; Devos et al., 2004; Vaillancourt et al., 2004). However, the concept that pathology in the basal ganglia is entirely responsible for changes in cortical output incorrectly assumes an absence of pathology in both the motor cortices and thalamus in Parkinson’s disease and PSP.

The motor cortices are composed of a number of specialized areas with each area receiving different cortical and thalamic inputs (Geyer et al., 2000; Naidich et al., 2001; Picard and Strick, 2001; Dum and Strick, 2002). The primary (1°) motor cortex contains ~40% of spino-projection cortical neurones and receives thalamic input from the ventrolateral posterior (VLP) nucleus and cortical input from the supplementary motor area (SMA), dorsolateral premotor region (DPC) and sensory cortices (Geyer et al., 2000; Naidich et al., 2001). It has a somatotopically organized and controls kinematic and dynamic parameters of voluntary movements (Geyer et al., 2000; Naidich et al., 2001). The non-1° motor cortices contain the majority of the remaining spino-projecting neurones. These are the SMA proper and pre-SMA, the DPC, and the ventrolateral premotor cortex. The SMA proper plays a role in the initiation of and the correct performance of movements, while the pre-SMA cortex is concerned with the selection and preparation of specific movements required (Geyer et al., 2000; Naidich et al., 2001; Picard and Strick, 2001; Dum and Strick, 2002). The pre-SMA differs from the other motor regions because it does not project either to the 1° motor cortex or directly to the spinal cord (Geyer et al., 2000; Naidich et al., 2001).

All neocortical areas receive thalamic inputs and large ensembles of cortical and thalamic neurones discharge synchronously at stereotyped frequencies associated with different conscious states and events (Jones, 2001; Guillery and Sherman, 2002). Excitatory driver inputs to the thalamus are from ascending pathways (like the periphery or cerebellum) and/or from layer 5 cortical neurones, with many thalamic regions relaying only higher order information important for corticocortical communication and higher cortical functions (Sherman and Guillery, 1998; Rouiller and Welker, 2000; Sherman and Guillery, 2002). Thalamic modulators include inhibitory GABAergic inputs from the basal ganglia and excitatory glutaminergic input from neurones in cortical layer 6 as well as monoaminergic and cholinergic inputs (Sherman and Guillery, 1998, 2002; Rouiller and Welker, 2000; Guillery and Sherman, 2002) which in turn dynamically alter information received by cortical regions (Jones, 1998; Sherman and Guillery, 1998, 2002; Rouiller and Welker, 2000; Haber and McFarland, 2001; Jones, 2001). The thalamic relay neurones to the motor cortices are located in the ventral, laminar and medial cell masses (Jones, 1985) with neurosurgeons and neuroanatomists using different thalamic nomenclature and delineations for the primate motor thalamus [see reviews by Macchi and Jones (1997); Krack et al. (2002)]. We have chosen the terminology of Jones as this system is based on comparative anatomy of homologous regions between the monkey and human (Jones, 1985; Hirai and Jones, 1989; Macchi and Jones, 1997). Using this classification system the motor (ventral) thalamus has several subdivisions including the ventral anterior (VA), ventrolateral anterior (VLa) and VLP nuclei (Schell and Strick, 1984; Jones, 1985).

Feedback excitatory drive from the cerebellum and 1° motor cortex is relayed through the VLP to the 1° motor cortex (as described above), modulated by SMA proper and DPC corticothalamic afferents (Rouiller and Welker, 2000; McFarland and Haber, 2002). Excitatory drive from the pre-SMA is relayed through the VLa, dorsal VA and the centromedian nuclei of the thalamus to the SMA proper and DPC, modulated by the internal globus pallidus and 1° motor, SMA and DPC corticothalamic afferents (Jones, 1985; Percheron et al., 1996; Rouiller and Welker, 2000; McFarland and Haber, 2002). Feedback excitatory drive from the SMA proper and DPC is relayed through the anterior VA, ventromedial posterior and mediodorsal nuclei of the thalamus to the pre-SMA, frontal and cingulate cortices, modulated by the substantia nigra and SMA, DPC, frontal and cingulate corticothalamic afferents (Jones, 1985; Percheron et al., 1996; Rouiller and Welker, 2000; McFarland and Haber, 2002).

Primary motor cortical neurones in both Parkinson’s disease and PSP contain intracellular inclusions. In Parkinson’s disease Lewy bodies occur in some corticospinal Betz cells (Wakabayashi et al., 2002), whereas in PSP these as well as other smaller neurones in the 1° motor cortex contain neurofibrillary tangles (NFT) (Hauw et al., 1990; Verny et al., 1996b). Afferent input to the 1° motor cortex is also affected in both Parkinson’s disease and PSP. In Parkinson’s disease there is a reduction in dopaminergic innervation to layer 1 (but not layers 3 or 5) (Gaspar et al., 1991) consistent with deficits in proton magnetic resonance spectra (Lucetti et al., 2001). In PSP there is a more significant loss of synapses in this region (Bigio et al., 2001), consistent with perfusion deficits (Salmon et al., 1997; Okuda et al., 2000; Piccini et al., 2001). In PSP neurones in both the cerebellum and thalamus contain NFT (Hauw et al., 1994; Dickson, 1999) implicating them directly in the disease process. The ventrolateral motor thalamus is structurally changed in tremor-dominant patients with Parkinson’s disease (Kassubek et al., 2002). In both Parkinson’s disease and PSP there is significant cell loss in the thalamic centromedian nucleus (Xuereb et al., 1991; Henderson et al., 2000a, b), while in Parkinson’s disease there is an 88% loss of corticocortical projection neurones in the pre-SMA (Macdonald and Halliday, 2002) consistent
with perfusion deficits in the cortex (Sabatini et al., 2000; Thobois et al., 2000; Fukuda et al., 2001) and VLA (Kassubek et al., 2001).

Overall, these studies suggest that different intrinsic changes in the motor thalamus and motor cortices (in concert with the dopaminergic deficits) could significantly influence motor cortical excitability propagating the symptoms observed in Parkinson’s disease and PSP. The present study examines these important motor neuronal populations in order to determine the full extent of neurodegeneration in Parkinson’s disease and PSP. Differences in the type and degree of thalamic and cortical damage may assist in explaining some of the major differences observed between these disorders.

**Methods**

**Case details**

Cases were obtained via our brain donation programme (Prince of Wales Medical Research Institute, Sydney, Australia), which has relevant institutional ethical approval. The study was conducted in accordance with the Declaration of Helsinki and National Health and Medical Research Council of Australia guidelines on human experimentation. Informed consent was obtained from the donor and next of kin. All 24 cases died between 1990 and 1996 (see Table 1 for demographic and clinical features) with their brain tissue used to study regional tissue atrophy (Cordato et al., 2000) and the degree of basal ganglia and medial and intralaminar thalamic cell loss (Hardman et al., 1996, 1997a, b; Hardman and Halliday, 1999a, b; Henderson et al., 2000a, b). All patients had clinical and pathological diagnoses based on current NINDS-SPSP criteria for the clinical and pathological diagnosis of PSP (Litvan et al., 1996) or current clinical and pathological diagnostic criteria for Parkinson’s disease (Gelb et al., 1999). The level of parkinsonian disability measured on levodopa medication by the Hoehn and Yahr scale (Hoehn and Yahr, 1967) was similar between the Parkinson’s disease and the PSP groups (Table 1). None of the cases had a clinical history of any other neurological or neuropsychiatric disorder or evidence of any other neurodegenerative disorder. Controls did not have any neuropsychiatric, neurological or neurodegenerative disease. Causes of death (mainly pneumonia, cardiovascular disease and cancer) and post-mortem delay (Table 1) were similar between the three groups.

### Table 1 Comparison of demographic and clinical features between groups

<table>
<thead>
<tr>
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<th>Parkinson’s disease</th>
<th>Control</th>
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<tbody>
<tr>
<td>Number (sex)</td>
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<td>9 (2F, 7M)</td>
<td>9 (6F, 3M)</td>
</tr>
<tr>
<td>Age</td>
<td>70 ± 3</td>
<td>79 ± 1</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>Post-mortem delay (h)</td>
<td>18 ± 6</td>
<td>31 ± 4</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Disease duration</td>
<td>7 ± 2</td>
<td>9 ± 1</td>
<td>–</td>
</tr>
<tr>
<td>L-dopa responsive</td>
<td>0</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>HY Stage 2–3</td>
<td>2</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>HY Stage 4–5</td>
<td>4</td>
<td>7</td>
<td>–</td>
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</tbody>
</table>

HY = Hoehn and Yahr staging of disease severity; Stage 0 = normal; Stage 1 = early disease; Stage 2–3 = mild-moderate disease; Stage 4–5 = advanced-end stage disease.

**Preparation of brains and identification of cortical regions for sampling and analysis**

The preparation of the brains has been described previously. Briefly, the brains were fixed in 15% buffered formalin for 2 weeks, weighed, the length measured and the fixed volume determined using water displacement. Formalin-induced shrinkage was negligible (Cordato et al., 2000; Macdonald and Halliday, 2002), with brains showing an average decrease in volume of 0.15%. The external features of the brain were examined and any abnormalities noted. The cerebellum and brainstem were separated from the cerebrum and for each the weight and volume determined. For five of the cases from each group, gyri of interest (1st motor, DPC and pre-SMA) were identified and painted with waterproof dyes as previously described (Macdonald and Halliday, 2002). The brains were then embedded in 3% agarose and sliced coronally at 3 mm on a rotary slicer. Each slice was photographed at ×1 magnification for volumetric assessment using a point counting method (Cordato et al., 2000; Macdonald and Halliday, 2002).

**Tissue preparation**

The thalamus was blocked in all 24 cases, and tissue samples of the 1st motor, DPC and pre-SMA were taken from 15 cases (five from each group) and placed in 30% buffered sucrose for cryoprotection. The 1st motor block was taken from the most superiomedial gyrus at the level of the mammillary bodies. The DPC block was taken from a midpoint location on the lateral surface of the most superior gyrus corresponding to the coronal level containing the nucleus accumbens. The pre-SMA tissue block was taken directly superior to the cingulate gyrus at the coronal level containing the anterior commissure on the mesial surface of the cerebrum. Following cryoprotection, the tissue blocks were frozen in mounting medium at −80°C, and sectioned at 50 μm on a cryostat.

Serial sections were stained to identify different neuronal populations as previously described (Henderson et al., 2000a, b; Macdonald and Halliday, 2002). For the thalamus and cortex, adjacent parallel series of sections spaced 750 μm apart were stained with aqueous cresyl violet (0.5%) to identify all Nissl substance and therefore the entire neuronal population (neurones with nucleoli within their nuclei). Subsequent sections were stained for a variety of proteins using routine peroxidase immunohistochemistry (Henderson et al., 2000a, b; Macdonald and Halliday, 2002). Tau antibody was used (T5350, Sigma, St Louis, USA, diluted 1 : 10000) to show various pathologies typical of PSP including coiled bodies, NFT and tufted astocytes, while ubiquitin (Z0458, Dako, Glostrup, Denmark, diluted 1 : 500), and α-synuclein (18-0215, Zymed, USA, diluted 1 : 3000) antibodies were used to identify Lewy bodies and neurites. A subpopulation of cortical pyramidal neurones, including Betz cells, contains the non-phosphorylated 200 kDa neurofilament protein and can be visualized using immunohistochemistry (SMI32, Sternberger, Baltimore, USA, diluted 1 : 3000). Different populations of cortical Interneurones contain the EF-hand calcium binding proteins with virtually all interneurones identified using immunohistochemistry for calbindin (C8666, Sigma, St Louis, USA, diluted 1 : 20000) and parvalbumin (P3171, Sigma, St Louis, USA, diluted 1 : 10000).

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Pathology and cellular quantitation

The densities of various pathologies characteristic of PSP were quantified. At ×200 magnification the numbers of NFT, coiled bodies and tufted astrocytes were counted in the different thalamic regions and in four cortical fields from each cortical region. Five levels of pathological involvement were used: 0 = absent, + = 1 or 2, ++ = 3–5, +++ = 6–20 and ++++ = 20 or more. The same index was used for all the different pathologies.

Three dimensional unbiased counting techniques were used for cellular quantitation, as previously published (Henderson et al., 2000a, b; Macdonald and Halliday, 2002). The volumes of the cortical regions of interest were estimated (Cordato et al., 2000; Macdonald and Halliday, 2002). The boundaries of the thalamic nuclei (VA, VLa and VLp) were plotted using an integrated computerized microscope system (Neurolucida, MicroBrightField, USA) which calculated cross-sectional area for each thalamic nucleus in each 50 μm serial section analysed. Volume was subsequently determined by multiplying the sum of the areas by the interval between sections (750 μm). Repeated measures gave <5% intra-rater variation and <10% inter-rater variation, attesting to the reproducibility and reliability of the boundaries and techniques employed.

The total numbers of neurones within VA, VLa and VLp were estimated using the unbiased optical disector technique with a disector counting frame of 200 μm × 200 μm at ×400 magnification and a grid array separated by 2 mm. Each nucleus was sampled at three evenly spaced anteroposterior levels with an average of six sections (for the three nuclei) being sampled through the entire anteroposterior extent of the thalamus in each case. The number of disector frames sampled varied from 24–90 for VA, 20–45 for VLa and 41–88 for VLp. The number of cresyl-violet stained neurones counted varied between 76–273 for the VA, 42–157 for the VLa and 157–445 for the VLp. Similar results were obtained from repeat measures in multiple sections from multiple cases. Neuronal density was estimated by dividing the total number of neurones counted by total sample volume. Total neuronal number for each nucleus was estimated by multiplying neuronal density (coefficient of variation range 0.15–0.24) by volume.

The number of cortical neurones was counted within 3D samples of the cortex from each region of interest. Data for the pre-SMA and DPC in the Parkinson’s disease and control cases have been previously published (Macdonald and Halliday, 2002). In our previous study, serially sectioning the entire precentral gyrus, we determined that a volume of between 0.15 and 0.25 mm³ was necessary to study, serially sectioning the entire precentral gyri, we determined DPC in the Parkinson’s disease and control cases have been previ-

representations. The density of neurones within each region was calculated and the total number of neurones estimated for the region of interest by multiplying the density of neurones by the cortical volume. The number of neurones immunoreactive for SMI32 or the different calcium binding proteins was calculated in the same way to give the number of interneurones and SMI32-positive pyramidal neurones in each motor cortical region. The total number of pyramidal neurones was calculated from the total Nissl number by subtracting the number of interneurones. The total number of non-SMI32-immunoreactive pyramidal neurones was evaluated by subtracting the total number of SMI32-immunoreactive neurones from this number.

The SPSS program (Software MacKiev, CA) was used for statistical analysis. Data are expressed as mean ± standard error with P < 0.05 accepted as significant. Owing to the number of cases analysed, non-parametric Kruskal–Wallis tests were used to analyse group differences in the estimated volume and total neuronal number of each region studied. Post hoc analysis using Mann–Whitney U-tests determined which groups were affected if significant differences were found. Common factor analyses investigated relationships between variables and standard coefficient loadings <0.70 were not considered significant.

Results

The motor thalamus and motor cortices in Parkinson’s disease

Compared with the substantia nigra, little pathology was seen in the motor thalamus or motor cortices in Parkinson’s disease. There were rare isolated intraneuronal Lewy bodies in both the motor thalamus and cortices [see also Macdonald and Halliday (2002); Wakabayashi et al. (2002) for cortical pathology and (Rub et al., 2002) for thalamic pathology]. The motor thalamus, DPC and 1st motor cortex were spared significant neurodegeneration in Parkinson’s disease (Table 2). As previously described (Macdonald and Halliday, 2002), there was a selective loss of corticocortical non-SMI32-positive pyramidal neurones in the pre-SMA in Parkinson’s disease (Table 2).

A factor analysis was performed to determine whether the variables measured related to each other. Factor analysis is a statistical approach used to analyse interrelationships among a large number of variables and to explain these variables in terms of their common underlying dimensions (factors). For the Parkinson’s disease cases factor analysis revealed that three major factors accounted for 41, 27 and 21% of the total variance in the data collected. The first factor (41% of the variance) shows that the volume and neurone number for connected structures are related, as may have been expected (loadings >0.70 were considered significant). The size and number of neurones in the 1st motor cortex (volume 0.92, neurones 0.93, interneurones 0.87) are negatively correlated with the size and number of neurones in its thalamic relay, the VLp (volume −0.82, neurones −0.75). These measures are in
turn correlated with corticobulbar and interneurone populations in the cortical regulator of the VLp, the pre-SMA (SMI32-positive neurones −0.77, interneurones 0.84). There is also a positive correlation with the size and number of neurones in VA (volume 0.97, neurones 0.95), the thalamic relay for premotor information feedback to the pre-SMA. The relationships identified in factor 1 suggest subtle changes in pre-SMA corticobulbar and VLp relays may be important in Parkinson’s disease. The second factor (27% of the variance) shows that there is a flow on effect from the significant loss of pre-SMA corticocortical connections (non-SMI32-positive neurones −0.86) to the DPC (volume 0.71, SMI32-positive neurones 0.71, interneurones 0.91) and 1° motor cortex (SMI32-positive neurones −0.78). The third factor (21% of the variance) was related to disease duration (−0.70) and suggests there are changes over time in the pre-SMA relay VL (neurones 0.91), DPC (all neurones 0.76) and 1° motor cortex (non-SMI32-positive neurones −0.73).

### The motor thalamus and motor cortices in PSP

In contrast to Parkinson’s disease and controls, significant tau-positive lesions were observed in both the motor thalamus and motor cortices in PSP (Fig. 1). As seen in nearby medial and intralaminar thalamic nuclei (Henderson et al., 2000b), the motor thalamus contained tau-positive NFT, coiled bodies, tufted astrocytes and threads (Fig. 1A–C) to variable degrees depending on the case (Table 3). In the motor cortices, pyramidal neurones had varied tau immunostaining, in some neurones appearing definitely fibrillar while in others appearing throughout the ballooned neurones (Fig. 1D and F). Similar to the motor thalamus, significant tau immunoreactivity was also present in oligodenodendroglia (as coiled bodies) and astrocytes (as tufted astrocytes) of the motor cortices (Fig. 1D and E). Cases with significant tau-positive pathologies in one motor region tended to have significant pathology in other motor regions (Table 3). The motor thalamus tended to have more pathology than the motor cortices in most but not all cases (Table 3).

Immunostaining for calcium binding proteins in the motor cortices revealed laminar patterns and cell morphology as in other published studies (Macdonald and Halliday, 2002). Typical large and small cortical pyramidal neurones contained non-phosphorylated neurofilament protein (SMI32) immunoreactivity, as expected. These neurones concentrated in layers III and V and did not differ in morphology between control and PSP cases (Fig. 1G). The location of tau-immunoreactive cells was variable between cases. In some cases tau immunostaining was seen across cortical laminae (Fig. 1H) but in others it was only seen in the small neurones of layer II.

Cellular quantitation revealed no substantial neurodegeneration in the DPC or its thalamic relay the VA, compared with controls and Parkinson’s disease cases (Table 2). Similar to Parkinson’s disease, there was a loss of neurones in the pre-SMA with its thalamic relay, the VLp, being spared compared with controls (Table 2). In PSP the degree of degeneration in the pre-SMA corticocortical SMI32-negative neurones was equivalent to that observed in Parkinson’s disease (P = 0.91), although pre-SMA interneurones were also affected in PSP, significantly reducing the total number of neurones in this region in PSP compared with Parkinson’s disease and controls (Table 2). In contrast to Parkinson’s disease, both the 1° motor cortex and its thalamic relay, the VLp, had significantly reduced neuronal numbers in PSP compared with controls (Table 2). In the 1° motor cortex the interneurones were primarily affected in PSP (Table 2).

Factor analysis of the data from the PSP cases revealed three major factors accounting for 50, 28 and 22% of the total variance in the data collected. The first factor (50% of the variance) suggests that the degree of volume and cell loss in the VLp (volume −0.98, neurones −0.99) and VL (neurones −0.73) is related to tau deposition in both the motor thalami.
Fig. 1 Photomicrographs of histopathology in 50 μm thick sections of the motor thalamus (A–C) and motor cortices (D–H) in PSP. Scale in F is equivalent for A–E. Tau-immunohistochemistry in VLa (A) and VLp (B) of case 5 (Table 3) shows globose neurofibrillary tangles, coiled bodies and threads. A large tufted astrocyte can be seen in the VLp (B). (C) Tau-immunohistochemistry in VLp of case 4 (Table 3) shows a large, globose neurofibrillary tangle surrounded by threads and some coiled bodies. Tau-immunohistochemistry in the 1° motor cortex (D) and DPC (E) of case 5 (Table 3) shows many coiled bodies and threads as well as globose neurofibrillary tangles (D) and tufted astrocytes (E). (F) Tau-immunohistochemistry in the 1° motor cortex of case 2 (Table 3) shows a tau-positive ballooned neurone. (G) SMI32 immunohistochemistry of the 1° motor cortex of case 5 (Table 3) showing a normal distribution of pyramidal neurones in upper (left) and lower (right) cortical layers. (H) Low magnification photomicrograph of the tau-immunoreactive structures throughout the upper cortical layers of case 1 (Table 3).


**Table 3** Quantitation of typical tau-positive lesions found in the PSP cases

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
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<th>Case 5</th>
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</table>

+ = 1 or 2, ++ = 2–5, +++ = 6–20, ++++ = 20 or more.

(VLa NFT 0.97, VLp glia 0.86, VA NFT 0.97, VA glia 0.97, VLa NFT 0.79) and motor cortices (1° motor NFT 0.79, 1° motor glia 0.94, pre-SMA NFT 0.79, pre-SMA glia 0.79) and to the number of neurones in the DPC (all neurones −0.96, interneurones −0.71) and its thalamic relay, VA (0.96), with these changes increasing over time (disease duration −0.93). The second factor (28% of the variance) suggests that the cortical neurodegeneration is related (all pre-SMA neurones 0.98, pre-SMA SM132-negative neurones 0.85, 1° motor volume 0.91, all 1° motor neurone 0.89, 1° motor SM132-positive neurones 0.97, 1° motor SM132-negative neurones −0.94, 1° motor interneurones 0.76). The third factor (22% of the variance) suggests a relationship between DPC histopathology (NFT 0.86, glia 0.74) and corticobulbar neurones in the DPC (SM132-positive neurones 0.86) and pre-SMA (SM132-positive neurones 0.73), and the volume of the DPC relay, the VA (−0.99).

**Discussion**

As far as we are aware, this is the first comprehensive study of the thalamocortical motor pathways in Parkinson’s disease and PSP. Significant cortical changes were observed in both Parkinson’s disease and PSP, while degeneration of the motor thalamus was confined to PSP. Although the number of cases examined is relatively small, the degree of damage was substantial in select regions and the measurement of multiple variables in the same cases has revealed important interrelationships. We found a similar significant loss of pre-SMA corticocortical projection neurones in PSP as we previously documented in Parkinson’s disease (Macdonald and Halliday, 2002). The substantial loss of VLP in PSP is consistent with a previous descriptive study of the thalamus in PSP (Amano et al., 1992). Overall, these changes are likely to have a significant impact on motor cortex activity independently of any changes in basal ganglia functioning.

By studying related neuronal populations we have been able to determine the impact of the changes observed on associated motor pathways. In both Parkinson’s disease and PSP, the SMA proper and the DPC do not have significant resting perfusion deficits (Okuda et al., 2000; Cunnington et al., 2001; Haslinger et al., 2001; Piccini et al., 2001), although significant functional abnormalities occur during motor performance (Berardelli et al., 1998; Haslinger et al., 2001; Sabatini et al., 2000). These findings are consistent with the lack of overt neurodegeneration found in the DPC in the present study. As may have been predicted, the loss of pre-SMA corticocortical neurones had a subtle impact on the number of DPC corticobulbar neurones and interneurones in Parkinson’s disease. The reduced intracortical inhibition in Parkinson’s disease, even following adequate levodopa medication (Lewis and Byblow, 2002), may relate to the substantial loss of non-dopaminergic pre-SMA corticocortical neurones and the subtle changes in the DPC. In addition, there are further subtle changes over time in the DPC and 1° motor pyramidal neurones and pre-SMA relay neurones in the VLa in Parkinson’s disease. These subtle progressive changes may contribute to the shortened desynchronization latencies (Devos et al., 2004), the enhancement of motor cortex excitability (Tamburin et al., 2003) and decreased movement speed (Vaillancourt et al., 2004) observed over time in Parkinson’s disease, and therefore also contribute to the decreased levodopa response characteristic of this condition. Recent experimentation with extradural stimulation of the 1° motor cortex in Parkinson’s disease appears to alleviate these problems (Pagni et al., 2003).

In contrast to Parkinson’s disease, further overt cortical deficits were found in PSP. In PSP there was additional significant loss of cortical interneurones in the pre-SMA and 1° motor cortices, with factor analysis showing that the degree of cell loss was related across these regions. This, coupled with lack of inhibitory input to the thalamus due to pallidal cell loss (Hardman and Halliday, 1999b), suggests that cortical projection neurones are likely to be overactive in PSP owing to decreased pallido-thalamic inhibition and the lack of local intracortical inhibition from loss of interneurones. Indeed, enlarged cortical somatosensory evoked potentials and changes in long latency reflexes indicating intracortical disinhibition (Koller et al., 2000; Kuhn et al., 2004) occur in PSP patients. Considerable tau-immunoreactive pathology is commonly found in the motor cortices of patients with PSP (Hauw et al., 1990; Vermesch et al., 1994; Hanihara et al., 1995; Nishimura et al., 1995; Verny et al., 1996a; Li et al., 1998). In our study the severity of DPC tau histopathology was related to its corticobulbar projection pathways.
and subtle atrophy of its thalamic relay VA. These results indicate that abnormal tau accumulation does not necessarily cause substantial cell loss in the neocortex in PSP. However, the degeneration in the 1st motor and pre-SMA cortices in PSP appears to precipitate a significant tau reaction throughout the motor thalamus and motor cortices and the subsequent degeneration of the 1st motor relay the VLP over the disease course. The loss of VLP could explain the recent observation of loss of synaptophysin (a marker for synapses) in the 1st motor cortex in PSP (Bigio et al., 2001) and the progressive atrophy of the superior cerebellar peduncle in PSP (Tsuboi et al., 2003). Degeneration of these cerebellolothalamic pathways could contribute to the early falls in PSP patients (Litvan, 1997; Wenning et al., 1999).

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