A role for the substantia nigra pars reticulata in the gaze palsy of progressive supranuclear palsy

G. M. Halliday,1 C. D. Hardman,1 N. J. Cordato,1,2 M. A. Hely2 and J. G. L. Morris2

1Prince of Wales Medical Research Institute, Randwick and 2Department of Neurology, Westmead Hospital, Westmead, Australia

Correspondence to: Dr G. M. Halliday, Prince of Wales Medical Research Institute, High Street, Randwick, 2031 Australia
E-mail: G.Halliday@unsw.edu.au

Summary

We examined the topography and degree of cell loss within basal ganglia structures commonly involved in progressive supranuclear palsy in order to identify any relationship between degeneration in these nuclei and gaze palsy. Serial section analyses and unbiased quantitative techniques were applied to brain tissue from six cases with progressive supranuclear palsy (four with gaze palsy and two without) and six controls with no neurological or neuropathological abnormalities. The total number of nucleolated neurons within the substantia nigra pars compacta (SNC) and reticulata (SNr), the subthalamic nucleus, and the internal and external segments of the globus pallidus was determined for all subjects and the data expressed as percentages of control values to compare degeneration across these basal ganglia structures. The density of neurofibrillary tangles was also evaluated within these structures. Despite significant subcortical neurofibrillary tangle formation in all cases, there was considerable variability in the degree of neuronal cell loss in all basal ganglia regions, except the SNC which was consistently affected. There was no correlation between the ranked density of neurofibrillary tangles and the degree of neuronal cell loss in any basal ganglia region. Comparisons between cases with and without gaze palsy revealed a 40% greater decrease in the number of SNr neurons in cases with gaze palsy (75 ± 8% loss) compared with those without (35 ± 14% loss). This was the largest difference between these cases. As the SNr projects to the superior colliculus, degeneration of this basal ganglia structure may disrupt eye movements in progressive supranuclear palsy.

Keywords: globus pallidus; neuronal cell loss; substantia nigra; subthalamic nucleus; gaze palsy

Abbreviations: GPe = external globus pallidus; GPi = internal globus pallidus; NFT = neurofibrillary tangles; PSP = progressive supranuclear palsy; SNC = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; STN = subthalamic nucleus

Introduction

Progressive supranuclear palsy (PSP) is named after its most distinctive clinical sign, vertical supranuclear gaze palsy. Despite this, eye movements are usually normal at presentation and gaze palsy is occasionally absent throughout the disease (Litvan et al., 1996b; Santacruz et al., 1998). Progressive degeneration of supranuclear oculomotor pathways in the brainstem is thought to underlie this sign (Daniel et al., 1995; Litvan, 1998). Immunohistochemical evaluation of the burst neurons in the rostral interstitial nucleus of the medial longitudinal fasciculus in three cases (Juncos et al., 1991) suggested that these neurons regulating vertical gaze are significantly affected. Direct evaluation of the omnipause neurons in the pontine raphe interpositus show an average 35% loss of these gating neurons for vertical gaze in cases with gaze palsy compared with those without (Revesz et al., 1996). These burst and omnipause neurons receive afferent control from the superior colliculi (Goldberg et al., 1991; Büttner-Ennever and Horn, 1997), where, in PSP, some neurons have reduced choline acetyltransferase (Juncos et al., 1991) and neurofibrillary tangles (Daniel et al., 1995). However, PSP cases with and without gaze palsies are not distinguished by these pathologies (Daniel et al., 1995). Motor commands from the frontal cortex influence this system via a direct projection as well as through the basal ganglia (Goldberg et al., 1991; Stell and Bronstein, 1994; Büttner-Ennever and Horn, 1997; Sharpe, 1998).

To meet criteria for the neuropathological diagnosis of PSP (Litvan et al., 1996a), abundant pathology must be present in three of the four following brain regions: pons, pallidum, subthalamus and substantia nigra, but most commonly in the latter three basal ganglia structures (Agid et al., 1987; Daniel et al., 1995; Verny et al., 1996). As the

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greatest density of pathology is found in the basal ganglia (Agid et al., 1987; Daniel et al., 1995; Feany et al., 1996; Litvan et al., 1996a; Verny et al., 1996), these regions might be expected to be involved in the major clinical symptoms and signs of the disease. However, there have been no studies evaluating the relationship between the degree of cell loss in basal ganglia structures and gaze palsy in PSP. The aim of the present study is to determine whether the presence of gaze palsy correlates with the site and extent of basal ganglia neuronal loss in six patients with pathologically confirmed diagnosis of PSP, four cases with and two cases without gaze palsy.

Methods
Case selection
The neuropathology and clinical features of the cases analysed in the present study have been described previously (Hardman et al., 1997a, b; Hardman and Halliday, 1999a, b). Written consent for autopsy was given in all cases. The project is approved by the Human Ethics Committee of the University of New South Wales under the Human Tissue Act of the State of New South Wales and complies with the Declaration of Helsinki on human experimentation. The brains were fixed in buffered formalin for 2 weeks. The cerebellum and brainstem were separated from the cerebrum and the volume of the cerebrum determined. The length of each hemisphere and the length of the brainstem were measured prior to being embedded in 3% agar in a square mould with the longest anteroposterior axis square to the mould surfaces. Thin slices, in the coronal plane for the cerebrum or the transverse plane for the brainstem, were cut using a rotary slicer. For each case the mean slice thickness was determined by dividing the lengths measured by the number of slices cut. The average slice thickness for the cases examined was 3.35 mm (SD = 0.25 mm).

Tissue preparation has been previously described in detail (Hardman et al., 1997a, b). For the cellular quantitation, coronal blocks of the basal ganglia and transverse slices of the midbrain were cryoprotected in 30% sucrose solution prior to freezing on a cryostat and serially sectioned at 50 μm. The accuracy of the cryostat to cut sections at 50 μm was evaluated by dividing the known thickness of the slice (see above) by the number of sections cut from each slice (average section thickness 50 μm, SD = 0.5 μm). The serially cut sections were arranged into 15 sequential series (sections spaced 750 μm apart). Sections from the first series were mounted on to slides and stained with cresyl violet for anatomical localization and cellular quantitation. Subsequent series of sections were stained with haematoxylin/eosin, modified Bielschowsky silver and immunohistochemistry using several antibodies: tau II (T5530, Sigma, St Louis, Mo., USA; diluted 1 : 10 000) and ubiquitin (Z0458, Dako, Glostrup, Denmark; diluted 1 : 200) both counterstained with cresyl violet, GFAP (Z334, Dako; diluted 1 : 500) counterstained with luxol fast blue, as well as substance P (MAS055, Seralab, Leicestershire, UK; diluted 1 : 1000), encephalin (MAS083, Seralab; diluted 1 : 500) and parvalbumin (P3171, Sigma; diluted 1 : 10 000) for the delineation of the basal ganglia structures, as described previously (Hardman et al., 1997a, b; Hardman and Halliday, 1999a, b). Peroxidase visualization was used for the immunohistochemistry (Halliday et al., 1995; Hardman et al., 1997b).

For this study, standardized pathological diagnostic procedures were applied (Feany and Dickson, 1995; Litvan et al., 1996a). Microscopic examination was performed on the sections prepared for quantitation as well as representative areas sampled in a standardized way. Samples of the precentral gyrus [Brodmann area (BA) 4], frontal (BA 9), temporal (BA 20), parietal (BA 39), occipital (BA 17 and 18) and cingular (BA 24) cortices, hippocampus (at the level of the lateral geniculate nucleus), amygdala, cerebellar vermis and lateral lobe (including the dentate nucleus), pons and medulla oblongata were paraffin-embedded, sectioned at 10 μm and stained using the same staining protocols as those described above.

Eight cases of PSP were diagnosed between 1990 and 1996. Two PSP cases were excluded because of coexisting large cerebral infarctions leaving six cases with PSP for detailed examination (Table 1). No PSP case fulfilled either plaque or tangle based criteria for Alzheimer’s disease (Braak and Braak, 1991; Mirra et al., 1991). Six age-matched controls (cases free from neurological and neuropathological disease) were selected for comparison as published previously (Hardman et al., 1997a, b; Hardman and Halliday, 1999a, b).

Clinical evaluations
Each case was prospectively studied by a neurologist, from presentation until death, with detailed records of history, as well as physical and mental examination. Data from clinical records and standardized forms were used to assess the following clinical features: bradykinesia and rigidity, rest tremor, levodopa response, early falls, eye movement abnormalities and dementia. Early falls were defined as occurring within the first 3 years of clinical disease onset. All cases received at least one standardized assessment.

All six PSP cases exhibited bilateral limb bradykinesia and rigidity as well as unsteadiness of gait with associated falls. Prior to death, four of the six cases were either completely immobile or unable to stand unassisted. The remaining two cases (cases 4 and 6) were still independently mobile but were experiencing frequent falls. The duration of gait instability with falls varied considerably between PSP cases (mean 3.7 years, range 1–12 years; Table 1). Four PSP cases (cases 1, 2, 3 and 5) developed supranuclear gaze palsy during life. In contrast to falls, the duration of gaze palsy was relatively constant (mean 3.5 years, range 3–5 years; Table 1). Preceding death, the severity of eye movement disorder ranged from moderate limitation of voluntary vertical saccades and pursuit eye movements (cases 1 and 5) to complete loss of all eye movements (case 2). Clinical
dementia was absent in all six PSP cases as assessed using the Clinical Dementia Rating Scale (Hughes et al., 1982).

Quantitation of basal ganglia neurofibrillary tangles and cell loss

Basal ganglia nuclei where the neuropathological changes are known to be maximal in PSP (Daniel et al., 1995; Litvan et al., 1996a; Verny et al., 1996) were selected for quantitation: the substantia nigra pars compacta (SNc) and pars reticulata (SNr), the internal (GPi) and external (GPe) segments of the globus pallidus, and the subthalamic nucleus (STN). The substantia nigra was identified as a convex disc-shaped structure located within the ventrolateral portion of the midbrain tegmentum directly dorsomedial to the cerebral peduncles. It was divided into a more dorsally located dopaminergic and pigmented SNc and a more ventrorostally located GABAergic SNr. The globus pallidus was identified as a wedge-shaped structure ventrolateral to the internal capsule, dorsolateral to the optic chiasm and tract, and medial to the external medullary lamina and putamen. It was segmented posteriorly into the small, medially located GABAergic GPi and the larger, laterally located GABAergic GPe. The substantia nigra and GPi were defined by their dense substance P innervation from the caudate and putamen, as previously described (McRitchie et al., 1995, 1996; Hardman and Halliday, 1999b). The GPe was defined by its dense enkephalin innervation from the caudate and putamen, as described previously (Hardman and Halliday, 1999a). The STN was defined as a ventromedial to dorsolaterally oriented disc-shaped structure located ventrolateral to the diencephalon, dorsolateral to the internal capsule and rostral to the substantia nigra. The STN was defined by its dense staining for the calcium binding protein parvalbumin, as described previously (Hardman et al., 1997b). The anatomical boundaries for each region were plotted using a microscope/computer analysis system (Neurolucida, MicroBrightField, Colchester, Vt., USA) and then superimposed on the sections undergoing analysis through a camera lucida.

Neuronal loss within the SNc was analysed using an areal fractionator technique, as previously published (Hardman et al., 1997b). Briefly, for each case a transverse section through the midbrain at the level of the exiting third nerve fibres was taken and stained with cresyl violet. This level was chosen as it contains the most consistent sample of the largest number of SNc cell groups in transverse midbrain sections (McRitchie et al., 1995). Using Neurolucida software the cross-sectional area of individual pigmented SNc cell clusters was calculated (<2% variation on repeated measures). For each cell cluster a central sample of neurons was point counted within a 10 × 10 eye-piece graticule at 400× magnification giving the cellular areal fraction occupied by the pigmented neurons (<10% variation with multiple grid placements). The cellular areal fraction was multiplied by the cross-sectional area of the cell cluster to give the overall cell area within each cluster and these values summed to give the total area occupied by pigmented dopaminergic neurons for each case. The value for each case is expressed as the percentage of the mean control value (control SD = 1%).

A fractionator technique was used to quantify neuronal loss in all other basal ganglia nuclei, as previously described (Hardman et al., 1997a, b; Hardman and Halliday, 1999a, b). Because of the variations in the size and anatomical boundaries of each region within the sections, the sampling for each region varied. For the smallest structure (SNr), every 15th section was used for the estimation of neuronal number (every 50 μm Nissl-stained section spaced 750 μm apart) while every 60th section was used for the largest structure (GPe) (every fourth Nissl-stained section spaced 3 mm apart). For the GPe and STN, every 30th section was sampled (every second Nissl-stained section spaced 1.5 mm apart). Estimates of the total number of neurons for each region were calculated by multiplying the total number of nucleolated neurons counted within the samples by the reciprocal of the fraction sampled. The value for each case is expressed as the

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at death (years)</td>
<td>74</td>
<td>80</td>
<td>51</td>
<td>62</td>
<td>82</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Duration of falling (years)</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Duration of gaze palsy (years)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Cause of death</td>
<td>PSP</td>
<td>Pneumonia</td>
<td>Pneumonia</td>
<td>Assault*</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Brain weight (g)</td>
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<td>1334</td>
<td>1053</td>
<td>1349</td>
<td>1338</td>
</tr>
<tr>
<td>SNc NFT</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>SNr NFT</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Gpi NFT</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Gpe NFT</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>STN NFT</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Cortical NFT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

F = female; M = male; + = 1 or 2 NFT per 200× field; ++ = 2–5 NFT per 200× field; +++ = 6 or more NFT per 200× field.

*Not end-stage disease.
Fig. 1 Comparison of the tau pathology in the GPi of cases 6 (A) and 4 (B). In the present study, case 4 had the most extensive tau pathology while case 6 had the least. In both cases extensive tau-positive glia (open arrowheads) and neurofibrillary tangles (arrows) were found, consistent with a diagnosis of PSP. Occasional neurons were filled with tau-immunoreactivity (closed arrowheads), with some apparently devoid of tangle formation (A) while others had additional fibrillar condensation (B).

percentage of the mean control value (control SNr SD = 7%, GPi SD = 11%, GPe SD = 11%, STN SD = 7%).

Both the modified Bielschowsky silver stained sections as well as the sections immunoreactive for tau II were used to grade the degree of neurofibrillary pathology in the basal ganglia, as previously described (Verny et al., 1996). The neurofibrillary tangles in the basal ganglia have a distinctive globose shape which is easily differentiated from the prevalent small, irregularly-shaped glial inclusions (Fig. 1). The density of neurofibrillary tangles was quantified to obtain a severity index on a four level scale (at 200× magnification): 0 = absent, + = one or two neurofibrillary tangles, ++ = two to five neurofibrillary tangles, +++ = six or more neurofibrillary tangles. Both staining protocols gave equivalent scores for each case. Spearman rank correlations were used to identify any relationship between the density of neurofibrillary tangles and the degree of neuronal cell loss, and P values of <0.05 accepted as significant.
Table 2 The number of neurons in each basal ganglia region studied for each PSP case

<table>
<thead>
<tr>
<th>%SNc*</th>
<th>SNr</th>
<th>STN</th>
<th>GPi</th>
<th>GPe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>100 ± 13</td>
<td>49,095 ± 3390</td>
<td>554,595 ± 52,885</td>
<td>352,360 ± 25,730</td>
</tr>
<tr>
<td>Case 1</td>
<td>28</td>
<td>6,873</td>
<td>229,200</td>
<td>194,720</td>
</tr>
<tr>
<td>Case 2</td>
<td>29</td>
<td>13,747</td>
<td>179,820</td>
<td>183,042</td>
</tr>
<tr>
<td>Case 3</td>
<td>23</td>
<td>16,201</td>
<td>216,292</td>
<td>264,268</td>
</tr>
<tr>
<td>Case 4</td>
<td>34</td>
<td>27,002</td>
<td>306,750</td>
<td>250,141</td>
</tr>
<tr>
<td>Case 5</td>
<td>9</td>
<td>12,274</td>
<td>80,400</td>
<td>131,400</td>
</tr>
<tr>
<td>Case 6</td>
<td>11</td>
<td>36,821</td>
<td>355,500</td>
<td>299,780</td>
</tr>
</tbody>
</table>

Mean control values and the standard deviation are given for comparison. For most cases these values have been published previously (Hardman et al., 1997a, b; Hardman and Halliday, 1999a, b). *An areal fractionator technique was used to determine the degree of neurodegeneration in the SNc. This technique gives the proportional cell area occupied by pigmented dopaminergic neurons rather than the estimated number of neurons. For all other basal ganglia nuclei a numerical fractionator technique was used to estimate the total number of neurons.

Mean control values and the standard deviation are given for comparison. For most cases these values have been published previously (Hardman et al., 1997a, b; Hardman and Halliday, 1999a, b). *An areal fractionator technique was used to determine the degree of neurodegeneration in the SNc. This technique gives the proportional cell area occupied by pigmented dopaminergic neurons rather than the estimated number of neurons. For all other basal ganglia nuclei a numerical fractionator technique was used to estimate the total number of neurons.

Fig. 2 Graph of the proportional reduction in neuronal number in each of the basal ganglia regions evaluated in PSP cases with and without gaze palsy. The number of neurons in each basal ganglia region is expressed as a percentage of the control mean for each region (control SNc SD = 13%, SNr SD = 7%, GPi SD = 11%, GPe SD = 11%, STN SD = 7%). With the exception of the GPe in one case, there was significant neuronal loss in all basal ganglia regions in PSP cases compared with controls. With the exception of degeneration in the SNc, there was considerable variability in the degree of neuronal loss within the basal ganglia structures evaluated. Comparison between PSP cases with and without gaze palsy suggests that significant degeneration of the SNr differentiates these cases (asterisks) with an average 40% more cell loss found in the SNr of PSP cases with gaze palsy. Mean and SEM are indicated for each group.

Results

By definition, all PSP cases had significant subcortical neurofibrillary tangle (NFT) and glial pathology with limited cortical involvement (Table 1). As has been previously described (Hauw et al., 1994; Daniel et al., 1995; Feany et al., 1996; Litvan et al., 1996a; Verny et al., 1996), the pattern of NFT formation in the different basal ganglia structures was not consistent across cases (Table 1). The pattern of neuronal cell loss also varied considerably across the PSP cases (Table 2), although substantial loss of pigmented SNc neurons was universal (Fig. 2). The impact of the disease on these basal ganglia structures was greatest in the SNc, followed by the STN, SNr and then the GPi (Fig. 2). The GPe was the least affected basal ganglia region in all cases with no GPe cell loss in one case (Fig. 2; Table 2).

No correlation between the degree of neuronal cell loss and the density of NFT could be identified (r < 0.80, P > 0.05 for all structures). Within the GPi, more than six NFT per field at 200× magnification were found in all cases (Fig. 1), although the degree of neurodegeneration varied greatly in this nucleus (Fig. 2). The basal ganglia structure with the most variable density of NFT pathology was the GPe (Table 1). The case with the greatest reduction in GPe neurons (case 5, Table 2) had the smallest number of NFT in this nucleus (case 5, Table 1). The case without significant degeneration of the globus pallidus or SNr (case 4, Table 2) had the greatest density of NFT pathology (Fig. 1B) in all basal ganglia regions (case 4, Table 1). This data suggests that the degree of neurodegeneration within the basal ganglia is unrelated to the density of NFT. Fibrillar pathology was also evident throughout the neuropil in the form of tufted astrocytes, coiled bodies and neuropil threads (Fig. 1), as described previously (Probst et al., 1988; Chin and Goldman, 1996; Ikeda et al., 1998). The density of these fibrillar structures within the basal ganglia appeared proportional to the density of NFTs (Table 1).

Four cases had gaze palsy (Table 1). At the time of death
these cases all had moderate to severe limitations in voluntary vertical saccades and pursuit eye movements. The duration of gaze palsy was similar in all cases (Table 1). In the other two cases there was no evidence of gaze palsy throughout the course of their disease (cases 4 and 6, Table 1). Case 4 (Table 1) had detailed examinations by a neurologist 1 year after the onset of symptoms as well as 3 years later, 3 months prior to an untimely death during an assault. Case 6 (Table 1) presented 18 months after the onset of symptoms and was examined by a neurologist every 2 years. Eight years into the disease, intercurrent ovarian carcinoma was diagnosed. Examination by a neurologist revealed normal eye movements at this time. Case 6 died 6 months later from tumour-related complications.

Severe neurodegeneration of the SNc occurred in all cases (Fig. 2). In general, neuronal loss within other basal ganglia regions was more marked in the cases with gaze palsy (Fig. 2). The largest difference between cases with and without gaze palsy was the loss of SNr neurons (Fig. 2). The SNr had an average of 75 ± 8% cell loss in cases with gaze palsy compared with only 35 ± 14% cell loss in the cases without. In this region the variation in the degree of cell loss in cases with gaze palsy was less than that observed in any other basal ganglia region (Fig. 2). This is consistent with the limited variation in the duration of gaze palsy in these cases. However, the density of NFT was similar in cases with or without gaze palsy (Table 1) (Daniel et al., 1995).

Discussion

This is the first study to evaluate the degree of degeneration across several basal ganglia structures in PSP with respect to associated gaze palsy. Unbiased stereological techniques were used in cases with the typical pathology of PSP (Litvan et al., 1996a). The number of cases analysed is small due to the low incidence of the disease (1.5–3 cases per million population (Golbe, 1994; Lantos, 1994; Bower et al., 1997), the presence of additional confounding pathologies in a proportion of cases (Gearing et al., 1994; Hauw et al., 1994; Lantos, 1994) and the lack of available tissue for adequate stereological sampling from regions required for the diagnosis of the disease (no additional cases are available for serial section analysis of these basal ganglia structures from our inquiries to other brain banks). Despite this, with the exception of the work by Revesz and colleagues (Revesz et al., 1996), the number of cases quantitatively analysed in the present study is greater than that reported in the majority of previous publications analysing neuronal degeneration in association with gaze palsy (Juncos et al., 1991; Malessa et al., 1991, 1994). In addition, by sequentially sampling throughout the basal ganglia, biases due to regional concentrations of pathology are eliminated and correction estimates not required. The use of unbiased quantitative methods and strict inclusion criteria has allowed us to make several observations concerning the disease process.

Gaze palsy in PSP is associated with substantial degeneration of the GABAergic SNr. The SNr is one of the major output nuclei of the basal ganglia and has two major GABAergic projections: one to the superior colliculus and the other to the motor thalamus (Francois et al., 1984; Tokuno et al., 1993). The frontal eye fields have a powerful excitatory influence on neurons of the superior colliculus both directly and through the basal ganglia (Sharpe, 1998). GABAergic SNr neurons inhibit saccade burst neurons in the superior colliculus, thereby stabilizing fixation by preventing unwanted extraneous saccades and reflexive saccades (Sharpe, 1998). GABAergic caudate neurons inhibit SNr neurons with the frontal eye fields exciting the superior colliculus by way of this double inhibition. These visuomotor pathways are concerned with complex saccadic behaviours, including maintaining fixation, disengaging fixation so that a saccade can be made to a new target, and making saccades to remembered target locations (Goldberg et al., 1991; Stell and Bronstein, 1994; Büttner-Ennever and Horn, 1997; Kawagoe et al., 1998; Sharpe, 1998). Substantial SNr degeneration as well as the loss of dopaminergic regulation of the frontal eye field relay through the caudate nucleus would certainly disrupt basal ganglia pathways influencing visuomotor processing in the superior colliculus in PSP.

Degeneration of basal ganglia structures has not been evaluated in relation to gaze palsy in PSP because of the considerable emphasis placed on cell loss in other non-basal ganglia brainstem regions (Daniel et al., 1995; Litvan, 1998). While degeneration is maximal within the basal ganglia in PSP, considerable variability in neuronal vulnerability was observed in these structures in the present study (see also Daniel et al., 1995) and evidence for a relationship between a site of basal ganglia degeneration and gaze palsy is presented. Only two other research groups have evaluated regional cell loss in relation to gaze palsy: one comparing three, then later four PSP cases with gaze palsy with four controls without neurological impairment (Juncos et al., 1991; Malessa et al., 1991, 1994) and the other comparing eight PSP cases with gaze palsy with five PSP cases without gaze palsy and six controls (Revesz et al., 1996). The first group used immunohistochemistry to evaluate cell loss (Juncos et al., 1991; Malessa et al., 1991). However, we have subsequently shown that a reduction in immunohistochemical markers does not necessarily equate with cell attrition (Hardman et al., 1996, 1997a, b). Therefore, further work is required to substantiate the theory that degeneration of the burst neurons in the rostral interstitial nucleus of the medial longitudinal fasciculus (Juncos et al., 1991) underlies vertical gaze palsy in PSP.

The triggering of burst neurons to make saccades is controlled by omnipause neurons in the pontine nucleus raphe interpositus (Goldberg et al., 1991; Büttner-Ennever and Horn, 1997). A detailed study of this region in PSP cases with gaze palsy (Revesz et al., 1996) showed a substantial loss of omnipause neurons (on average 50% loss compared with controls). This cell loss is highly variable in PSP patients (50 ± 19% loss) and there is considerable overlap between the PSP cases with and without gaze palsy.
This contrasts with our results in the SNr where there is a greater and less variable reduction in neuronal number in PSP cases with gaze palsy (average 75 ± 8% loss) and no overlap between the degree of cell loss in patients with and without this sign. The degree of degeneration in the SNr compared with the raphe interpositus supports an important role for the SNr in at least some PSP cases with gaze palsy. Our thesis is that considerable destruction of any of the neuronal groups involved in pathways regulating eye movements is likely to disrupt such behaviours. The more consistent pathological involvement of the basal ganglia in PSP patients with gaze palsy leads to greater probability of this region playing a primary role in the generation of this clinical feature. It will be important to examine more PSP cases with and without eye signs to identify the regions consistently associated with this clinical feature of the disease.

Our data also suggest that the STN may contribute to gaze palsy in PSP cases. The STN receives significant afferent input from the motor and premotor cortices, including the frontal eye fields and the supplementary eye fields (Künzle and Akert, 1977; Noda and Oka, 1993; Bevan et al., 1995; Nambu et al., 1996) and in turn provides excitatory regulation of the basal ganglia output nuclei, the GABAergic SNr and GPi (Parent and Hazrati, 1995). In humans the frontal eye fields are located in the lateral precentral gyrus (Luna et al., 1998), which is the most affected cortical region in cases with PSP (Daniel et al., 1995; Verny et al., 1996). Degeneration of this pathway could more directly influence gaze palsy by causing a reduction in the excitatory regulation of the GABAergic SNr by the STN. However, most of the PSP cases with gaze palsy had slightly more cell loss in the SNr compared with the STN, suggesting a similar involvement of both regions and the same outcome, that is, a decrease in the inhibitory output of the SNr.

We found that the ranked density of NFT pathology does not correlate with the degree of neuronal loss within the basal ganglia in PSP (see also Agid et al., 1987). This contrasts with previous findings comparing neuronal and NFT densities directly (Lantos, 1994; Revesz et al., 1996). A close relationship between these variables could suggest ongoing neurodegeneration at the time of evaluation. If so, the reported data suggest that there is ongoing degeneration in the raphe interpositus at the time of death (Revesz et al., 1996) but more variable involvement within the basal ganglia structures (present study). Surprisingly, our data show that the density of NFT pathology was lowest in regions with the greatest neuronal loss (e.g. the dopaminergic SNc) and in cases with the longest duration (case 5, Table 1). In contrast, NFT formation was greatest in cases with the most limited basal ganglia degeneration and shortest duration (case 4, Table 1). This suggests that degeneration is virtually complete in some basal ganglia regions in all cases (hence no significant relationship), but ongoing to various degrees in other regions (largely case dependent). It also suggests that NFT are more rapidly removed from the brain in PSP (within the time course of the disease), which is in contrast to NFT in Alzheimer’s disease where lesions can be seen for decades, even after the death of the cell (Braak and Braak, 1998).

We present novel results supporting a role for degeneration of the SNr in the development of gaze palsy in at least some cases with PSP. The pathological involvement of this basal ganglia structure in many PSP cases suggests that it may be a widespread phenomenon. We agree with the previous study by Revesz and colleagues, which suggested that careful morphometric evaluations of cases with PSP distinguish histological differences that relate to their disease symptoms and signs (Revesz et al., 1996). Thus, the findings presented in the present study add further to our understanding of the morphological basis of eye movement abnormalities in PSP.

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