The Subthalamic Nucleus in Parkinson's Disease and Progressive Supranuclear Palsy

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Abstract. The subthalamic nucleus has become a promising target for the neurosurgical treatment of parkinsonian symptoms. We have used unbiased counting techniques to quantify the neuronal populations of the subthalamic nucleus in patients with idiopathic Parkinson's disease and progressive supranuclear palsy. In addition, the type of calcium binding proteins contained within these subthalamic neurons was established using immunohistochemistry. Most of the 250,000 subthalamic neurons contain either parvalbumin or calretinin calcium binding proteins, and patients with idiopathic Parkinson's disease sustained no damage to this nucleus. This is consistent with current theories of basal ganglia circuitry, which postulate that overstimulation of this excitatory nucleus contributes to the inhibition of the motor thalamus via the activation of inhibitory relays. In contrast, we found that there was substantial cell loss in the subthalamic nucleus in progressive supranuclear palsy (45–85% neuronal reduction) and that both cell types were equally affected. Extracellular neurofibrillary tangles as well as tau-positive glia were observed in the subthalamic nuclei of these cases. As the patients with Parkinson's disease and progressive supranuclear palsy all had overlapping parkinsonian symptoms, the loss of subthalamic simulation within the basal ganglia of progressive supranuclear palsy cases is puzzling, unless their parkinsonian symptoms were generated by an alternate mechanism.

Key Words: Calretinin; Immunohistochemistry; Parkinson's disease; Parvalbumin; Progressive supranuclear palsy; Subthalamic nucleus; Tau protein.

INTRODUCTION

The human subthalamic nucleus (STN) is a bilateral disc-shaped structure located within the transitional zone between the thalamus and midbrain. It lies ventral to the zona incerta and dorsomedial to the substantia nigra and cerebral peduncles. It is composed predominantly of Goli
gi type I projection neurons (1, 2), which in nonhuman primates are excitatory (3), projecting mainly to the glo
bus pallidus, substantia nigra and neostriatum (4, 5).

Hyperstimulation of the STN is a new corrective procedure for the symptoms of Parkinson's disease (PD) (6, 7). In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonian monkey lesions (8, 9) and hyperstimulation (10) of the STN successfully suppresses overactivity and ameliorates parkinsonian symptoms. It is hypothesized that dopaminergic cell loss from the substantia nigra is primarily responsible for STN overactivity and the ensuing parkinsonian symptoms (8). However, in progressive supranuclear palsy (PSP; also known as Steele-Richardson-Olszewski syndrome [11]) comparable symptoms occur (rigidity, akinesia and postural instability [12–14]) with significant pathology in the STN, sub
stantia nigra, and globus pallidus (12, 15). While PSP and PD are frequently separated on the basis of disease duration (shorter in PSP) and characteristic additional symptoms (resting tremor in PD and supranuclear gaze palsy in PSP [12, 14]), misdiagnosis of patients is not uncommon (16–18), and in some cases these diseases coexist (17).

Depigmentation and gliosis within the substantia nigra are consistently found in all PD and PSP patients. In PD such cell loss is accompanied by Lewy body formation (19), while in PSP neurofibrillary tangles (NFT), neur
topil threads (NT) and tau-immunoreactive (tau+) glia are not only found within the substantia nigra, but are widely distributed throughout basal ganglia structures including the STN (12, 15, 20, 21). While it is tempting to suggest that the common loss of dopaminergic neurons in these syndromes underlies their parkinsonian features, this theory relies on other basal ganglia pathways remaining intact. The present study aims to test this hypothesis by analyzing the STN in cases of PD and PSP in comparison with aged controls. In nonhuman primates the presence of different calcium binding proteins (parvalbumin [PV], calbindin [CB] and calretinin [CR]) has been used to distingui
sh different neuronal populations within the STN (22, 23). We have therefore used quantification and imm
unohistochemistry for PV, CB, CR, and tau to establish the extent of pathological damage to the STN in these parkinsonian disorders. Such an analysis may shed light on the current theories for the successful treatment and management of parkinsonism.

MATERIALS AND METHODS

Patients

Brain tissue samples from 6 patients with idiopathic PD, 5 patients with classical PSP, and 1 case with coexisting idiopathic PD and clinically silent PSP were used in this study.

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SUBTHALAMIC NUCLEUS IN PARKINSONIAN DISORDERS

TABLE 1
Summary of Clinical Details and Clinicopathological Diagnoses

<table>
<thead>
<tr>
<th>Case</th>
<th>Presenting clinical symptoms</th>
<th>Age at onset</th>
<th>Drug response</th>
<th>Age at death</th>
<th>Cause of death</th>
<th>Clinicopathological diagnosis</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>61</td>
<td>Renal failure</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>70</td>
<td>Pneumonia</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>74</td>
<td>Ruptured aorta</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>77</td>
<td>Pneumonia</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>82</td>
<td>Cardiac failure</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
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<td>—</td>
<td>—</td>
<td>84</td>
<td>Pulmonary edema</td>
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<tr>
<td>7</td>
<td>T, R, A, D</td>
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<td>67</td>
<td>Pneumonia</td>
<td>PD</td>
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<tr>
<td>8</td>
<td>R, A</td>
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<td>72</td>
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<td>9</td>
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<td>78</td>
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<td>PD</td>
</tr>
<tr>
<td>10</td>
<td>B, PI, T, D</td>
<td>64</td>
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<td>81</td>
<td>Pulmonary emboli</td>
<td>PD</td>
</tr>
<tr>
<td>11</td>
<td>T, R, D, B, A</td>
<td>70</td>
<td>Good</td>
<td>81</td>
<td>Cardiac &amp; Renal failure</td>
<td>PD</td>
</tr>
<tr>
<td>12</td>
<td>D, B, R, T, PI, dG, A</td>
<td>74</td>
<td>Poor</td>
<td>82</td>
<td>Pneumonia</td>
<td>PD</td>
</tr>
<tr>
<td>13</td>
<td>T, R, PI, B, A</td>
<td>61</td>
<td>Good</td>
<td>70</td>
<td>Ovarian cancer</td>
<td>PD/PSP</td>
</tr>
<tr>
<td>14</td>
<td>R, PI</td>
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<td>62</td>
<td>Pneumonia</td>
<td>PSP</td>
</tr>
<tr>
<td>15</td>
<td>PI, D, SGP</td>
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<td>Poor</td>
<td>68</td>
<td>Cardiac failure</td>
<td>PSP</td>
</tr>
<tr>
<td>16</td>
<td>daA, PI, D, SGP, R, B, dG, A</td>
<td>67</td>
<td>Poor</td>
<td>74</td>
<td>Pneumonia</td>
<td>PSP</td>
</tr>
<tr>
<td>17</td>
<td>PI, D, SGP, R, B, T, dG, A</td>
<td>75</td>
<td>Poor</td>
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<td>66</td>
<td>Poor</td>
<td>82</td>
<td>Pneumonia</td>
<td>PSP</td>
</tr>
</tbody>
</table>

Abbreviations used: A, akinesia; B, bradykinesia; D, dementia; daA, dystasia; dG, dysphagia; PI, postural instability; R, rigidity; SGP, supranuclear gaze palsy; T, resting tremor; C, control; PD, Parkinson’s disease; PSP, progressive supranuclear palsy.

(Table 1). Six age-matched controls free from neurological and neuropathological disease were used for comparison. With written consent for autopsy, diagnoses were confirmed neuropathologically following brain removal within 36 hours (h) of death. The prevalence of misdiagnosis and need for robust standardizer diagnostic protocols justifies the strict exclusion criteria employed in this study (14, 15, 18, 21, 24). Cases were excluded if other neurodegenerative conditions were present, including Alzheimer disease diagnosed according to established consensus criteria (25–27). The presenting clinical symptoms, age at onset, response to dopaminergic replacement therapy, age at death, cause of death, and clinicopathological diagnosis for each patient are listed in Table 1. The project was 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.

The PD cases were prospectively studied (28, 29) and their clinicopathological details were described previously in studies quantifying the cellular changes within the substantia nigra (30–32). All PD cases had cell loss and Lewy body formation within the substantia nigra.

The clinicopathological details for most of the PSP cases have been described previously in a study of the substantia nigra (32). Diagnosis of PSP was based on clinical presentation and the presence of numerous NFT in the basal ganglia and brainstem in accordance with the diagnostic criteria for PSP proposed by the National Institute of Neurological Disorders and Stroke (15, 24). PSP cases 15 to 18 (Table 1) died with end-stage disease and were bedridden and completely immobile prior to death. Case 14 died of complications resulting from an assault by intruders while sleeping at home at night. He had been diagnosed by a neurologist as having corticobasal degeneration because of his asymmetrical rigidity, particularly affecting the right hand, and his poor response to dopa therapy. He did not have end-stage disease and was able to independently perform daily activities, living alone at the time of death. He had had Parkinsonian features for 4 years without further clinical signs indicative of the PSP found post mortem. For all PSP cases a significant number of silver- and tau-positive NFT were seen in multiple subcortical sites including the globus pallidus, STN, substantia nigra and pons, with more variable amounts within the neostriatum, oculomotor complex, medulla, and dentate nuclei (32). Although case 14 was initially given a clinical diagnosis of corticobasal degeneration, this diagnosis could not be confirmed on neuropathological examination. NFT formation was restricted to only the hand area of the left motor cortex. In this case no NFT or other cellular pathologies were noted in any other cortical regions, including the more medial regions of the left motor cortex and the entire right motor cortex. The definitive diagnosis of PSP was given in this case because there was severe subcortical pathology in comparison with only focal cortical pathology in the absence of any balloononed neurons. Corticobasal degeneration is the most prevalent misdiagnosis of PSP by specialist clinicians (15, 21), and the asymmetrical involvement of the hand region of the motor cortex in this case is likely to have contributed to this clinical diagnosis.
Only one PSP case also had Lewy body pathology. Case 13 died with ovarian cancer and had dopa-responsive parkinsonism without the differentiating symptoms of PSP. While the disease duration was 9 years, this case did not exhibit signs of either end-stage PD or PSP despite having the pathology for both diseases post mortem (NFT in the globus pallidus and STN, and both NFT and Lewy bodies within the substantia nigra). Because of her successful response to dopa therapy, we feel that this case was in the earliest stages of PSP and that the PD pathology was largely responsible for her clinical profile.

Tissue Collection and Immunohistochemistry

All brains were immersion fixed in formalin for 2 weeks. For each case the brainstem was dissected from the cerebrum at the level of the rostral midbrain and the cerebellum dissected from the brainstem. The cerebrum and brainstem were then embedded separately in agar and cut on a rotary slicer into 3 mm coronal and transverse sections, respectively. Tissue samples from the neocortex (prefrontal, motor, temporal and anterior cingulate), hippocampus, neostriatum, globus pallidus, dentate nucleus, and vermis were chosen, paraffin-embedded, sectioned at 10 µm and stained with hematoxylin and eosin, Bielschowsky silver, cresyl violet, and ubiquitin- and tau-immunohistochemistry as previously published (33). In addition, one block each of the subthalamus, midbrain,pons and medulla oblongata were frozen and the first 20 µm and 30 µm sections cut, mounted on slides and stained as above. The diagnostic evaluation of these tissue samples was performed using the criteria outlined above.

All blocks of the left and right subthalamus, which were taken from the coronal sections of the cerebrum and rostral transverse sections of the midbrain, were cut on a Leica cryostat into serial 50-µm sections. Every fifteenth section was mounted onto slides and stained with cresyl violet. Six additional series of 750-µm spaced consecutive sections were collected and stained with hematoxylin and eosin, nickel peroxidase (33), and stained immunohistochemically for PV, CB, CR or tau. Furthermore, to discern the cellular specificity of tau in each case, the mid-antero-posterior coronal section labeled for tau was counterstained with cresyl violet. For the immunohistochemistry, free floating sections were washed in 50% ethanol, a solution of 3% H2O2 in 50% ethanol, and 10% normal horse serum in 0.1% azide in 0.1M Tris (hydroxymethyl) methylamine buffered saline (tris) solution (pH=7.4) to expose binding sites and block endogenous peroxidase activity and nonspecific binding, respectively. Following these treatments, sections were incubated in either mouse monoclonal PV (P3171, Sigma USA, diluted 1:10,000), CB (C8666, Sigma USA, diluted 1:2,000), or tau (T2530, Sigma USA, diluted 1:10,000) antibodies or in rabbit polyclonal CR (7696, Swant Switzerland, diluted 1:2,000) antiserum in 0.1% azide, 0.1M tris solution for 48 h at 4°C. Sections were subsequently incubated in solutions of biotinylated horse anti-mouse IgG or goat anti-rabbit IgG (BA2000 and BA1000, respectively, Vector Laboratories, CA, USA, diluted 1:200 in 0.1M tris) and streptavidin-biotin-horseradish peroxidase complex (PK6100 Elite, Vector Laboratories, CA, USA, diluted 1:500 in 0.1M tris) for 1 h each at room temperature with 0.1M tris washes in between. Each series of sections was subsequently incubated in 5 mL of 0.6 mg/ml diaminobenzidine tetrahydrochloride (DAB) in 0.1M tris for 10 minutes (min), with addition of 25 mL of 10% H2O2 in 0.1M tris for 5 min (final concentration of H2O2 0.05%) for visualization of the tertiary complex. Following washes in 0.1M tris solution, sections were mounted onto slides, allowed to dry overnight, dehydrated in graded ethanol and xylene, and coverslipped with DPX. The subset of sections labeled for tau (see above) were rehydrated, counterstained with cresyl violet, and dehydrated prior to coverslipping. The specificity of the immunohistochemical reaction was tested by omitting the primary antiserum as outlined previously (31), and no peroxidase reaction was seen in these test sections.

Anatomical Analysis

Outlines of the left and right STN in every second cresyl violet-stained coronal and transverse section (total distance separating sections thus 1.5 mm) were drawn directly into the Neurolu- cida software (MicroBrightField, USA) using a microscope/computer analysis system. The area of each drawing was then calculated and using Cavaliere’s principle (34, 35), the volume of the STN calculated by multiplying the sum of these areas by the distance separating each drawing (i.e., 1.5 mm). All cresyl violet-stained neurons within these 1.5 mm consecutively separated sections and all PV immunoreactive (PV+) and CR immunoreactive (CR+) neurons within adjacent and equally spaced sections were then plotted onto these drawings. Using the unbiased fractionator method for neuronal estimation (34, 35), the total number of neurons was estimated by multiplying the total numbers of plotted neurons by the reciprocal of the fraction sampled (i.e., 30/1). Statistical analysis of PD and PSP vs control data was performed using analysis of variance with Fisher’s protected least significant difference (PLSD) tests. Regression analysis was utilized to determine whether age of onset, age at death, disease duration or postmortem delay correlated with any variable. A p-value of less than 0.05 indicated a significant finding.

Pathological Analysis

Computer-assisted quantitative procedures were also used to analyze the distribution and amount of cellular damage. The distribution of pathology within the STN was compared across PSP, PD, and control cases in coronal sections taken at the same mid-antero-posterior level. Cresyl violet, PV+ and CR+ neurons and tau+ NFT were plotted at this level and the diameter of each measured using the Neurolucida software. The number of neurons and NFT were compared between diagnoses using analysis of variance with Fisher’s PLSD tests and the mean values calculated. In addition, neuronal and NFT sizes were compared within and between cases using two-way analysis of variance with Fisher’s PLSD tests, and the mean values were calculated. Tau+ glia were also plotted at the same levels. The ratio of tau+ glia to cresyl violet neurons in each case was then calculated.

RESULTS

The STN in the control cases was 254±41 mm² in total volume (left plus right; Table 2) and comprised numerous densely packed round and ellipsoid-shaped neurons (Figs. 1A, B) with a mean diameter of 22 µm. Many STN neurons displayed intense immunoreactivity for PV and CR

TABLE 2
Volume of the Subthalamic Nucleus and Estimated Total Neuronal Numbers

<table>
<thead>
<tr>
<th>Case</th>
<th>Volume of the STN</th>
<th>Total number of CV STN neurons</th>
<th>Total number of PV+ STN neurons</th>
<th>Total number of CR+ STN neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>254 ± 41</td>
<td>554,595 ± 52,885</td>
<td>301,560 ± 78,280</td>
<td>333,180 ± 112,335</td>
</tr>
<tr>
<td>PD</td>
<td>265 ± 38</td>
<td>584,190 ± 57,052</td>
<td>313,390 ± 74,817</td>
<td>346,495 ± 56,782</td>
</tr>
<tr>
<td>13</td>
<td>184</td>
<td>355,500</td>
<td>179,970</td>
<td>190,740</td>
</tr>
<tr>
<td>14</td>
<td>146</td>
<td>306,750</td>
<td>69,300</td>
<td>112,020</td>
</tr>
<tr>
<td>15</td>
<td>104</td>
<td>229,200</td>
<td>79,140</td>
<td>32,640</td>
</tr>
<tr>
<td>16</td>
<td>146</td>
<td>264,600</td>
<td>66,750</td>
<td>101,430</td>
</tr>
<tr>
<td>17</td>
<td>116</td>
<td>179,820</td>
<td>44,640</td>
<td>41,460</td>
</tr>
<tr>
<td>18</td>
<td>73</td>
<td>80,400</td>
<td>28,170</td>
<td>28,050</td>
</tr>
<tr>
<td>PSP</td>
<td>*117 ± 31</td>
<td>*212,154 ± 87,170</td>
<td>*57,600 ± 20,728</td>
<td>*63,120 ± 40,271</td>
</tr>
</tbody>
</table>

Abbreviations used: C, mean control values; PD, mean Parkinson’s disease values; PSP, mean progressive supranuclear palsy values; STN, subthalamic nucleus; CR+, calretinin immunoreactive; CV, cresyl violet-stained; PV+, parvalbumin immunoreactive. Values for volume are in millimeters cubed + SD. Other values are estimated total neuronal numbers + SD. *p < 0.0001, ANOVA with Fisher’s PLSD tests.

(Fig. 1C–G), with the size and morphology of these neurons not significantly different to each other or to the cresyl violet-stained neurons (ANOVA p value = 0.68; Figs. 1B, D, F). When quantified, those STN neurons immunoreactive for PV and CR represented approximately 54% and 60% of the cresyl violet-stained neurons, respectively (Table 2). Neurons immunoreactive for PV were concentrated contralaterally and posteriorly within the STN, while CR+ neurons concentrated ventromedially and anteriorly (Figs. 1C, E, G, 2A). In addition to PV+ and CR+ somata within the STN, numerous thick, intensely labeled PV+ fibers and terminals (Figs. 1C, D) and a small number of fine CR+ fibers and terminals were seen (Figs. 1E, F). CB+ neurons were not found in the human STN, in contrast to nearby structures (36).

Overall, the STN in the cases of PD was similar to the STN of controls in both size (Fisher’s PLSD p value = 0.62; Table 2) and neuronal complement (Fisher’s PLSD p values for cresyl violet neuron number = 0.45, for PV+ neuron number = 0.76 and for CR+ neuron number = 0.78; Table 2; Figs. 2B, 3A–C). In contrast, the STN in the PSP cases was approximately half the size (Fisher’s PLSD p value < 0.0001; Table 2) and there were significantly less cresyl violet-stained neurons (38% of control values, Table 2; Figs. 2B, 3E), PV+ neurons and CR+ neurons (both 19% of controls; Table 2, Figs. 2F, G; Fisher’s PLSD p values all <0.0001). The PSP case with the smallest STN volume and the most cell loss had the longest disease duration (case 18; Tables 1, 2), while the case with the largest STN volume and the least cell loss did not progress to end-stage PSP (case 14; Table 2). Neuronal loss occurred uniformly throughout the STN in all cases of PSP. This is supported by the equal reduction in the number of PV+ and CR+ neurons, despite the distinctive distribution of these neuronal populations within the nucleus (Figs. 1, 2A). The STN of the case with concomitant PD and PSP (case 13) was smaller than controls and contained significantly fewer neurons (64% of CV control values, 60% of PV+ control values, 57% of CR control values; Table 2) although it was not affected as much as other PSP cases. The mean diameters and morphologies of STN neurons were not significantly different between the PD and PSP cases (Fisher’s PLSD p value = 0.94; Fig. 3A–C, E–G), although they were significantly smaller in both groups compared with controls (21 µm vs 22 µm, Fisher’s PLSD p values = 0.03).

In both the control and PD cases very little or no tau immunoreactivity was seen (Fig. 3D). However, in all PSP cases (including case 13) intense labeling of NFT, glia and other structures within the STN was seen (Fig. 3H). These pathologies were evenly distributed throughout the nucleus. Tau+ NFT appeared extracellular as no nucleus or plasma cell membrane was evident in sections counterstained with cresyl violet (not shown) and the NFT were significantly larger (mean diameter 26 µm) than the remaining neurons (paired t-test p values < 0.0001). When quantified, tau+ NFT in the PSP cases represented approximately 6% of control neuronal number (Fig. 2B), 10% of the number of neurons missing (if all NFT are extracellular), and 16% of the number of neurons remaining (if all NFT are intracellular). The number of tau+ NFT within the STN of the PSP cases did not correlate with either the age at disease onset, age at death, duration of disease, or the stage of disease at death.

The most numerous tau+ structures within the STN were glial cells (Fig. 3H). These glia were either tufted, shaped with numerous irregular processes, or round or coil shaped, and approximately 12 to 13 µm in diameter with 1 to 3 curved processes (Fig. 3H). We determined the proportion of glia to the total complement of STN neurons by directly comparing their number with control neuronal numbers. The number of tau+ glia was by far
Fig. 1. Photomicrographs of cresyl violet-stained (CV; A, B), parvalbumin-immunoreactive (PV+; C, D), and calretinin-immunoreactive neurons (CR+; E, F) and cell plots of their distributions in the coronal plane of the left subthalamic nucleus (STN) (G). The STN is ellipsoid shaped in the coronal plane lying dorsomedial to the cerebral peduncle (cp) and ventrolateral to the zona incerta (ZI) (A, C). It is made up of numerous densely packed round or oval shaped neurons (A, B), with large
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Fig. 2. A: Graph of the mean anteroposterior distribution of cresyl violet-stained (CV; unshaded area), parvalbumin-immunoreactive (PV+; pale shaded area), and calretinin-immunoreactive neurons (CR+; dark shaded area) in the subthalamic nucleus (STN) of controls. CR+ neurons concentrate in the anterior and have substantial anteroposterior overlap (moderate shaded area) with the PV+ neurons, which concentrate toward the posterior. Most neurons within the STN contain either one of the calcium binding proteins. B: Graph of the mean number of cresyl violet-stained neurons (left) and tau-immunoreactive neurofibrillary tangles (tau+ NFT; right) within the STN of Parkinson’s disease (PD; unshaded bar) and progressive supranuclear palsy cases (PSP; shaded bars) expressed as percentages of the average number of cresyl violet-stained neurons in controls. Triangles, circles and crosses show individual case values with bars of the standard deviation. The unbroken line on the left indicates the control mean and dashed lines indicate ± 1 standard deviation.

the greatest in case 14, who died prior to reaching end-stage PSP. When this case was excluded from the analysis there was on average one tau+ glia for every 5 to 6 STN neurons. The number of tau+ glia did not correlate with either the age of disease onset, age at death, duration of the disease or any other factor. If degeneration is taken into account, there was on average 1 tau+ glia for every 3 missing neurons, or 1 for every 2 living neurons in these PSP cases. On the other hand, in case 14 there was 1 tau+ glia for every STN neuron or two tau+ glia for every missing or living neuron.

DISCUSSION

There have been few anatomical or pathological studies of the STN in humans. Our detailed quantification has shown that the STN usually contains approximately 550,000 neurons bilaterally. Large numbers of these neurons are immunoreactive for the calcium binding proteins PV (54% of STN neurons) and CR (60% of STN neurons). The number and distribution of STN neurons expressing PV in humans is similar to that reported in other primates (22). However, there appear to be species differences in the complement of CR+ and CB+ neurons. In nonhuman primates, only a small number of STN neurons express CR (23), while in rodents the STN is completely devoid of CR+ neurons (37, 38). This suggests that the number of STN neurons expressing CR is species dependent, increasing from rodents to man. However, we found that the STN was devoid of CB+ neurons, which is similar to reports for rodents (39). In contrast, the STN of nonhuman primates contains more CB+ than CR neurons (22, 23). The absence of CB immunoreactivity that occurred in our study clearly was not a false negative finding, as many neurons lying within adjacent dopaminergic nuclei were intensely immunoreactive for CB (36) as they are in other species (22, 36, 39). Overall, the present findings suggest that humans have a unique complement of calcium binding proteins with STN neurons. PV+ and CR+ fibers and terminals seen within the STN of humans in this study most likely represent the efferent projections of external pallidal neurons or the intranuclear projections of STN neurons.

The only other quantitative study of the STN has been performed in rats, where the total number of neurons was recently estimated at 27,200 (40). The expansion in neuron number in humans is similar to that seen for the dopaminergic substantia nigra (21,000 in rats vs 550,000 in

proportions of these neurons displaying intense immunoreactivity for PV and CR (C-G). Those neurons, which display immunoreactivity for PV, are concentrated centrolaterally within the STN (C, G), while CR+ neurons are concentrated ventromedially (E, G). In addition to immunoreactive neuronal somata, numerous PV+ thick fibers and terminals (C, D) and a small number of CR+ terminals (E, F) are present within the STN. Equivalent 1 mm scale bar for A, C and E in A, equivalent 100 μm scale bar for B, D and F in F; and 2 μm scale bar in G.
Fig. 3. Photomicrographs of cresyl violet (A, E), parvalbumin-immunoreactive (PV+; B, F) and calretinin-immunoreactive (CR+; C, G) neurons and tau-immunoreactive structures (tau+; D, H) within the subthalamic nucleus (STN) of PD case 10 (A–D) and PSP case 17 (E–H). The PD case shows a relatively large number of cresyl violet, PV+, and CR+ STN neurons (A–C),
humans [41-43]), and may reflect the expansion of the basal ganglia system in humans compared with rats. However, there are other substantial anatomical differences in the projections of the STN between rats and primates. The majority of STN neurons in rats have axons which bifurcate and project to both rostral (globus pallidus and neostriatum) and caudal (substantia nigra) target nuclei, while a small number also have intranuclear branches (44-47). In nonhuman primates, the majority of neurons have a principle axon that remains unbranched and supplies a single target nucleus (4, 5). Thus, 1 neuron in rodents most likely serves the function of 2 or 3 neurons in primates.

As predicted by the current theories for parkinsonian circuitry, we confirm that the STN is unaffected in PD. In PSP, however, almost two-thirds of STN neurons are lost on average (62%). This cell loss ranged from 45% in a patient who died prior to end-stage PSP (case 14) to 86% in a case with a 16-year disease duration (case 18). Despite this, the loss of STN neurons did not correlate with disease duration. From the above it thus appears that the extent of neurodegeneration is most likely related to the clinical stage of the disease rather than disease duration as the greatest cell loss occurred in those cases progressing to end-stage PSP. In addition to cell loss, the percentages of the remaining STN neurons with detectable levels of PV and CR were reduced by half when compared with control and PD cases. On average only 27% of the remaining STN neurons were immunoreactive for PV, while 30% were immunoreactive for CR. Both PV and CR are members of the family of high-affinity calcium binding proteins which are presently believed to act in buffering against abrupt changes in intracellular calcium (48, 49). This function is thought to be important in preventing cell dysfunction and excitotoxic damage due to increased intracytoplasmic concentrations of calcium (50-56). Furthermore, by sequestering calcium and increasing the decay in calcium signal, PV reduces calcium-activated potassium channels and the hyperpolar refractory period, enabling neurons to fire at a higher rate (39, 48, 57). These findings along with others (58) suggest that the expression of PV and CR is most likely proportional to the level of neuronal activity. As there is neither STN neuron loss nor changes in the relative percentages of STN neurons immunoreactive for PV and CR in PD, this suggests that the STN is functioning normally. In PSP, however, with 62% neuron loss and reductions in the percentages of remaining neurons containing detectable levels of PV and CR, the output of the STN is obviously reduced. Whether the reduction in PV and CR levels contributes to STN neuronal loss in PSP is questionable as similar reductions occur in the percentages of pars reticulata neurons immunoreactive for PV in PSP and PD with and without neuronal loss, respectively (31, 32). Reductions in PV and CR are more likely the result of the pathological process which leads to neuronal loss in PSP.

The STN of the PD and control cases contained very little or no immunoreactivity for the microtubule-associated protein tau. In cases with PSP (including case 13) intense immunoreactivity for tau was identified in intra- and extracellular NFT as well as in glial cells. In all cases the majority of NFT were extracellular. This was made evident by the absence of a neuronal cell membrane or nucleus associated with most tau+ NFT. In addition, the NFT were significantly larger in diameter than cresyl violet-stained STN neurons, suggesting some hypertrophy which may have contributed to their demise. The extracellular nature of the majority of NFT within the STN of PSP cases suggests that the formation of NFT and subsequent cell death is quite rapid while the breakdown and removal of extracellular debris occurs more slowly. When the NFT were quantified, they represented approximately 6% of the normal STN neuronal population regardless of disease stage or duration, or the presence of PD. This finding could suggest that the rates of NFT formation and breakdown are constant throughout the disease. However, if this were true, neurodegeneration should also be constant and a correlation between disease duration and cell loss should have occurred. Because of the lack of such a correlation, we suggest that STN neurodegeneration occurs via more than one mechanism. The coexistence of two neurodegenerative mechanisms may account better for the variability in cell loss observed between cases and would explain why substantially more cell loss than NFT occur at all disease stages.

In addition to NFT, tau+ glia characterize PSP (15, 20, 24, 59-64). The tau+ glia seen within the STN in our PSP cases were similar to those previously described as “tufted astrocytes” and “coiled bodies” (see 20 for review). When quantified, the number of tau+ glia was more varied than the number of NFT. This finding was due to the presence of a great deal more tau+ glia in the non-end stage case (case 14), suggesting a possible role

with very few structures labeled for tau (D). In the PSP case, on the other hand, the STN is very gliotic with few cresyl violet, PV+ or CR+ neurons (E-G) and a high density of large tau+ neurofibrillary tangles (straight arrows) and small tau+ glia either tufted (curved arrow), round or coil shaped (unfilled arrowheads) (H). Equivalent 200-μm scale bar for all in D.

in the degenerative process itself. In this case there was a one-to-one relationship between the number of reactive glia and the number of neurons normally found within the STN. In the other PSP cases this relationship was 1:5 and did not correlate with either the age of disease onset, age at death, duration of PSP or any other factor. Interestingly, the amount of both tau+ glia and NFT within the substantia nigra was also greatest, although asymmetrical, in the case with non-end-stage PSP (32). Cell loss within the substantia nigra pars reticulata (but not the substantia nigra pars compacta) was also greatest on the left side with a correspondingly greater symptom severity on the right. The number of tau+ glia and NFT and the amount of cell loss within the STN, however, was not asymmetrical in this case (data not shown). From these findings it would appear that in this case of PSP the asymmetrical progression of the symptoms is more related to tau accumulation, NFT formation, and cell loss within the pars reticulata and cortex while the pars compacta and STN were affected bilaterally.

As mentioned above, we have now analyzed 3 important basal ganglia regions in the cases presented, the STN (present study), the substantia nigra pars compacta (30, 32) and the substantia nigra pars reticulata (31, 32). Comparing the amount of degeneration over these three regions shows that the PD cases had an average 76% loss of pars compacta neurons with no loss of either the pars reticulata or STN. In the cases with non-end-stage PSP (case 13 and 14), 75% of pars compacta neurons, 35% of pars reticulata neurons and 41% of STN neurons degenerated. In the end-stage PSP cases, 74% of pars compacta, 78% of pars reticulata, and 66% of STN neurons degenerated. In general, the end-stage PSP cases had significantly more symptoms over their disease course compared with the PD and non-end-stage PSP cases. In particular, supranuclear gaze palsy was seen in all end-stage PSP cases, but was not observed in either the PD or non-end stage PSP cases. Cell loss within the pars reticulata may correlate with the supranuclear gaze palsy as the nucleus has a large projection to the superior colliculus (65–69). Interestingly, recent studies have also correlated supranuclear gaze palsy with superior colliculus cell loss (70), as well as with cell loss in brainstem nuclei known to be involved in ocular motility (70, 71). The most prominent overlapping symptom was rigidity (found in all cases), which is likely to occur because of the common neurodegeneration in the pars compacta (also seen in all cases). However, it is not clear what the clinical correlate of STN cell loss would be in the PSP cases. As the STN is primarily involved in regulating SN and globus pallidus activity (see below), the clinical symptoms associated with pathology in these latter two regions may dominate the disorder.

In PD it is hypothesized that loss of pars compacta neurons and their input to the neostriatum results in increased neostriatal inhibition of the external pallidum and decreased inhibition of the internal pallidum and pars reticulata (8). Decreased output from inhibitory external pallidal neurons results in disinhibition and overactivation of excitatory STN neurons. This overstimulation coupled with decreased internal pallidal and pars reticulata inhibition further activates the internal pallidum and pars reticulata inhibitory neurons projecting to the motor thalamus. Increased inhibition of the motor thalamus and subsequent suppression of cortical activation is thus believed to account for the clinical manifestations of PD (8). In PSP we have shown that, in addition to pars compacta loss (32), both the pars reticulata (32) and the STN (present study) are substantially damaged. In addition, the globus pallidus is affected in PSP containing significant amounts of tau immunoreactivity (15, 24). The output of all these regions would be substantially decreased in PSP. As most PSP cases have pronounced parkinsonian features, it is difficult to resolve the theoretical model for parkinsonism in cases of PSP. When these findings are taken into account, however, one would assume that inactivating the STN via lesioning procedures or hyperstimulation (6, 72) should make PD cases more like PSP. Further studies are obviously needed, in which the state of other brain structures involved in motor circuitry are investigated in PD and PSP, so that the underlying neural circuitry can be resolved and more effective treatment procedures for both diseases postulated.

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