The substantia nigra of the human brain
II. Patterns of loss of dopamine-containing neurons in Parkinson’s disease

P. Damier, E. C. Hirsch, Y. Agid and A. M. Graybiel

1INSERM U289, Hôpital de la Salpêtrière, Paris, France and 2Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Correspondence to: Dr P. Damier, INSERM U289, Hôpital de la Salpêtrière, 47, boulevard de l’hôpital, 75013 Paris, France
E-mail: cic.salpetriere@psl.ap-hop-paris.fr

Summary
To achieve accuracy in studying the patterns of loss of midbrain dopamine-containing neurons in Parkinson’s disease, we used compartmental patterns of calbindin D28K immunostaining to subdivide the substantia nigra with landmarks independent of the degenerative process. Within the substantia nigra pars compacta, we identified dopamine-containing neurons in the calbindin-rich regions (‘matrix’) and in five calbindin-poor pockets (‘nigrosomes’) defined by analysis of the three-dimensional networks formed by the calbindin-poor zones. These zones were recognizable in all of the brains, despite severe loss of dopamine-containing neurons. The degree of loss of dopamine-containing neurons in the substantia nigra pars compacta was related to the duration of the disease, and the cell loss followed a strict order. The degree of neuronal loss was significantly higher in the nigrosomes than in the matrix. Depletion was maximum (98%) in the main pocket (nigrosome 1), located in the caudal and mediolateral part of the substantia nigra pars compacta. Progressively less cell loss was detectable in more medial and more rostral nigrosomes, following the stereotyped order of nigrosome 1 > nigrosome 2 > nigrosome 4 > nigrosome 3 > nigrosome 5. A parallel, but lesser, caudorostral gradient of cell loss was observed for dopamine-containing neurons included in the matrix. This pattern of neuronal loss was consistent from one parkinsonian substantia nigra pars compacta to another. The spatiotemporal progression of neuronal loss related to disease duration can thus be drawn in the substantia nigra pars compacta for each Parkinson’s disease patient: depletion begins in the main pocket (nigrosome 1) and then spreads to other nigrosomes and the matrix along rostral, medial and dorsal axes of progression.

Keywords: Parkinson’s disease; dopamine; calbindin; substantia nigra; basal ganglia

Abbreviation: TH = tyrosine hydroxylase

Introduction
In Parkinson’s disease, the loss of dopamine-containing neurons in the midbrain is progressive and affects different parts of the nigral complex to different degrees, the most severe loss occurring in the ventrolateral part of the substantia nigra pars compacta (Hassler, 1938; Fearnley and Lees, 1991). Other distributions of maximal neuronal loss in the nigral complex occur with the normal ageing process and in other degenerative disorders affecting the substantia nigra (Fearnley and Lees, 1991). These differences suggest that the regional selectivity of the lesions is specific to the disease process and is thus related to the mechanisms underlying the neurodegenerative changes. Precise knowledge of the patterns of depletion of dopamine-containing neurons in Parkinson’s disease may thus be crucial to understanding its pathogenesis.

We have identified such patterns by applying a new calbindin D28K-based analysis that allowed us to plot the distributions of nigral neurons in consistently identified nigral compartments, the nigrosomes and the surrounding matrix (Damier et al., 1999). This method permitted the first quantitative study of cell loss in individually distinct subdivisions of the substantia nigra pars compacta in Parkinson’s disease. Our results suggest that cell loss is consistently greater in the nigrosomes than in the matrix, and that, within the system of nigrosomes, there is an ordered
pattern of loss consistent across Parkinson’s disease patients. This analytical method has great promise as a diagnostic and investigatory tool.

**Material and methods**

**Brain samples**

Five brains from patients who died with a diagnosis of idiopathic Parkinson’s disease [age ± SEM = 72 ± 4 years] were analysed and compared with brains from five control patients (84 ± 4 years) who had no clinical or pathological history of neuropsychiatric disease. Disease duration was defined as the interval between first diagnosis and death. The clinical diagnosis of Parkinson’s disease was confirmed by histopathological examination performed on one hemisphere on the basis of severe loss of melanized neurons in the substantia nigra pars compacta and the presence of Lewy bodies. The five control brains were analysed in the study reported in the accompanying paper (Damier et al., 1999).

Midbrain blocks were fixed as described previously (Damier et al., 1999). Transverse 40-µm-thick serial sections were cut from the tissue blocks on a sliding microtome, immersed free-floating in 0.25 M Tris buffer containing 0.1% sodium azide, and stored at 4°C.

**Calbindin D<sub>28K</sub> and tyrosine hydroxylase immunohistochemistry**

For three parkinsonian and three control midbrains, every third section was processed for calbindin D<sub>28K</sub> immunostaining and every ninth for tyrosine hydroxylase (TH) immunostaining. Each of the TH-stained sections was immediately adjacent to one of the calbindin-stained sections. In two other parkinsonian and two other control midbrains added for the quantitative analysis, every ninth section was prepared by calbindin immunohistochemistry and every 36th for TH. The protocol for the calbindin and TH immunohistochemistry was carried out as described by Damier and colleagues (Damier et al., 1999).
Fig. 2 Regional and intranigral loss of dopamine-containing neurons in Parkinson’s disease. The colorimetric scale indicates the estimated amount of cell loss (least = blue; most = red). Cell losses in the different groups of the midbrain were calculated by comparing mean numbers of TH-positive neurons in each group of five parkinsonian midbrains (disease duration = 7–32 years) with corresponding means for five control midbrains. A8 = dopaminergic cell group A8; CGS = central grey substance; CP = cerebral peduncle; DBC = decussation of brachium conjunctivum; M = medial group; Mv = medioventral group; N = nigrosome; RN = red nucleus; SNpd = substantia nigra pars dorsalis; SNpl = substantia nigra pars lateralis; III = exiting fibres of the third cranial nerve.
Table 1: Quantitative analysis of dopamine-containing neurons and their loss in parkinsonian midbrain

<table>
<thead>
<tr>
<th></th>
<th>CGS</th>
<th>A8</th>
<th>M</th>
<th>Mv</th>
<th>SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>5303 ± 438</td>
<td>14315 ± 2693</td>
<td>9111 ± 1582</td>
<td>15182 ± 1916</td>
<td>28733 ± 1202</td>
</tr>
<tr>
<td>Cell loss (%)</td>
<td>9</td>
<td>31</td>
<td>7</td>
<td>46</td>
<td>79</td>
</tr>
</tbody>
</table>

Values indicate number of cells (mean ± standard error of the mean) with percentage reduction compared with controls indicated below. 

Table 2: Rostrocaudal variation in percentage loss of dopamine-containing neurons in parkinsonian midbrain

<table>
<thead>
<tr>
<th></th>
<th>CGS</th>
<th>A8</th>
<th>M</th>
<th>Mv</th>
<th>SN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rostral –</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17</td>
<td>34</td>
<td>24</td>
</tr>
<tr>
<td>Intermediate</td>
<td>6</td>
<td>–</td>
<td>9</td>
<td>56</td>
<td>81 (P &lt; 0.01)</td>
<td>65 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Caudal</td>
<td>27</td>
<td>51</td>
<td>7</td>
<td>43</td>
<td>89 (P &lt; 0.001)</td>
<td>72 (P &lt; 0.001)</td>
</tr>
</tbody>
</table>

Mapping and quantitative analysis of dopamine-containing neurons

Dopamine-containing neurons in the midbrain were identified as TH-positive neurons. Charts of the distributions of dopamine-containing neurons were constructed from every TH-stained section by plotting TH-positive neurons with a computer-assisted image analysis device (HistoRag, Biocom, Les Ulis, France). Five dopaminergic cell groups were identified in the midbrain (Hirsch et al., 1988), and the substantia nigra was subdivided into the substantia nigra pars compacta, formed by the matrix and five nigrosomes, the substantia nigra pars dorsalis and the substantia nigra pars lateralis, according to the patterns of calbindin immunostaining observed. This plan of subdivision is described in detail in the accompanying paper (Damier et al., 1999).

The total numbers of dopamine-containing neurons in each cell group were calculated for each patient by integrating values for individual sections over the entire length of the midbrain. The percentage of cell loss in Parkinson’s disease was calculated from these values by comparing means for parkinsonian and control midbrains. Split-cell counting errors were corrected by applying the formula of Abercrombie (Abercrombie, 1946). The correction factor was 0.61; no significant differences were found between the sizes of neuronal nuclei in control and Parkinson’s disease brains.

Statistical analysis

Statistical analysis was done by analysis of variance (general linear model from SAS software, SAS Institute Inc., Cary, NC, USA) based on three factors: (i) disease status (Parkinson’s disease versus controls); (ii) midbrain subdivisions [central grey substance, dopaminergic cell group A8, medial group, medioventral group and the substantia nigra (Hirsch et al., 1988)] or subdivisions of the substantia nigra [substantia nigra pars lateralis, substantia nigra pars dorsalis, and the matrix and nigrosomes 1–5 of the substantia nigra pars compacta (Damier et al., 1999)]; and (iii) rostrocaudal location (rostral = anterior to the level of exit of third cranial nerve fibres; intermediate = at the level of their exit; caudal = posterior to the level of their exit). Interactions among these factors were added to the model. For post hoc comparisons, we used the Tukey method to correct for multiple test effects.

Results

Compartmental pattern of calbindin immunostaining in the parkinsonian midbrain

The conspicuous three-dimensional compartmental organization of calbindin immunostaining characteristic of the control midbrains was preserved in the parkinsonian midbrain. Although the calbindin-poor zones were shrunken, all five nigrosomes found in the control midbrains were identifiable in the parkinsonian midbrain (Fig. 1). This preservation of the nigrosoe/matrix organization of the substantia nigra pars compacta was the fundamental basis for the quantitative study undertaken. Because we were able to identify these nigral compartments and the associated calbindin-rich matrix, despite shrinkage and loss of landmark patterns based on TH immunostaining, we were able to identify subgroups of dopamine-containing neurons and to quantify the cell loss characteristic of each.

Global analysis of the loss of dopamine-containing neurons in the parkinsonian midbrain

There was a mean reduction of 64% (range 57–72%) in the total count of dopamine-containing neurons in the five cases.
Dopaminergic lesions in parkinsonian midbrain

Heterogeneous patterns of cell loss in the substantia nigra in Parkinson’s disease

There were highly significant differences \((P < 0.00001)\) in the extent of cell loss in the different subgroups of the nigral complex. Loss was higher in the substantia nigra pars compacta \((86\%, \text{range } 75–95\%)\) than in the substantia nigra pars dorsalis \((57\%, \text{range } 44–67\%)\), and there was a small loss \((21\%, \text{range } 0–72\%)\), which did not reach statistical significance, in the substantia nigra par lateralis \(\text{(Table 3)}.\)

The calbindin-based definition of compartments within the substantia nigra pars compacta demonstrated further heterogeneity in neurodegenerative patterns. First, as shown in Table 3, the cell loss was significantly \((P < 0.01)\) greater in the nigrosomes \((95\%, \text{range } 87–99\%)\) than in the matrix \((80\%, \text{range } 67–91\%)\). This was true for each rostrocaudal level for all nigrosomes, excepting only the caudal nigral levels in patient P974, in which cell loss was nearly total in both nigrosomes and matrix \(\text{(Fig. 5)}.\)

Secondly, there were clear differences in the loss of dopamine-containing neurons in the different nigrosomes \(\text{(Table 3)}.\) The mean cell loss was maximal in nigrosome 1 \((98\%, \text{range } 93–100\%)\). The few TH-positive neurons that did survive in nigrosome 1 did not appear to have characteristic locations \(\text{(e.g. in its dorsal or its ventral part)}.\) Nigrosome 2 and nigrosome 4 were the next most affected, and nigrosome 3 and nigrosome 5 were considerably less affected. The degree of loss of dopamine-containing neurons in the different nigrosomes was strictly ordered: nigrosome 1 > nigrosome 2 > nigrosome 4 > nigrosome 3 > nigrosome 5.

In the matrix of the substantia nigra pars compacta, the decreases in dopamine-containing neurons seemed mainly to follow a rostrocaudal gradient \(\text{(Table 4}; \text{Figs 2, 5 and 6)}.\) Cell loss in the medial part of the matrix \((77\%, \text{range } 57–91\%)\) was slightly, but not significantly, lower than that in the lateral part \((86\%, \text{range } 79–93\%)\).

Individual differences in the loss of midbrain dopamine-containing neurons

Cell losses in the different dopamine-containing cell groups of the midbrain were different from one patient to another \(\text{(Figs 5 and 7)}.\) The extent of depletion showed no apparent relation to disease duration in any of the cell groups except the substantia nigra pars compacta. For example, the medial cell group was severely affected in a patient with a 7-year
Distributions of dopamine-containing neurons in the different groups of dopamine-containing neurons in the control (filled circles) and parkinsonian (filled diamonds) midbrain. The plots show the mean numbers of dopamine-containing neurons; bars indicate the standard error of the mean calculated from three control and three parkinsonian midbrains studied extensively. The abscissa indicates distance (mm) to the rostral point of exit of third cranial nerve fibres (defined as level 0). Values on the ordinate indicate the number of TH-positive neurons. Areas under the curves indicate total numbers of neurons in the different regions; the indicator box below shows the area corresponding to 5500 neurons. A8 = dopaminergic cell group A8; CGS = central grey substance; M = medial group; Mv = medioventral group; SN = substantia nigra.

disease duration (P1008) and almost unaffected in another patient with a 25-year disease duration (P1062). An inverse relationship with disease duration did not account for the cell loss in this cell group. For example, in patient P974, with a 32-year history of Parkinson’s disease, the medial group was nearly depleted of dopamine-containing neurons. There was also no obvious relationship between the degree of cell loss within those groups and the age of the patients.

In striking contrast, in the substantia nigra pars compacta the degree of cell loss appeared to be closely related to disease duration. The values were 75, 85, 88, 89 and 95% loss, respectively, in the five patients with Parkinson’s disease ordered according to the duration of their disease, from 7 to 32 years (Figs 5 and 7). Moreover, despite the different total amounts of cell loss in the substantia nigra pars compacta for each subject, the degree of degeneration was similarly ordered by compartment, as were the overall means of cell loss: nigrosome > matrix for each rostrocaudal level, and nigrosome 1 > nigrosome 2 > nigrosome 4 > nigrosome 3 > nigrosome 5. Figure 7 shows this consistency in diagrammatic form. In Fig. 7A, the data for cell loss in the five nigrosomes and the matrix are ordered according to the disease duration indicated in years for the different patients, and the compartments are ranked for each patient from least to most affected. The clear patterning of loss by duration and compartment stands in sharp contrast to the lack of patterning by age or subdivision for the dopamine-containing cell groups outside the substantia nigra pars compacta, shown in Fig. 7B.

Discussion

The selectivity and temporospatial progression of neuronal loss in Parkinson’s disease should provide critical clues to understanding the degenerative process underlying this
Table 3  Quantitative analysis of dopamine-containing neurons and their loss in parkinsonian nigral complex

|                  | SNpl          | SNpd          | Snpc
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Matrix</td>
</tr>
<tr>
<td>Number</td>
<td>1692 ± 479</td>
<td>12937 ± 1116</td>
<td>11842 ± 2344</td>
</tr>
<tr>
<td>Cell loss (%)</td>
<td>21</td>
<td>57 (P &lt; 0.05)</td>
<td>80 (P &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total SNpc: 14 04 ± 3213</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>541± 335</td>
<td>483 ±256</td>
<td>338 ±106</td>
<td>387 ±72</td>
<td>513 ± 250</td>
</tr>
<tr>
<td>Cell loss (%)</td>
<td>98 (P &lt; 0.001)</td>
<td>94</td>
<td>78</td>
<td>92</td>
<td>76</td>
</tr>
</tbody>
</table>

Values indicate number of cells (mean ± standard error of the mean) with percentage reduction compared with controls indicated below. P values indicate significance of the loss (Tukey’s post hoc comparison). SNpl = substantia nigra pars lateralis; SNpd = substantia nigra pars dorsalis; SNpc = substantia nigra pars compacta; N = nigrosome.

Table 4  Rostrocaudal variation in neuronal loss in the different dopamine-containing cells of the midbrain groups

<table>
<thead>
<tr>
<th></th>
<th>SNpl</th>
<th>SNpd</th>
<th>Matrix</th>
<th>Nigrosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rostral</td>
<td>–</td>
<td>–</td>
<td>53</td>
<td>69</td>
</tr>
<tr>
<td>Intermediate</td>
<td>27</td>
<td>62</td>
<td>84 (P&lt; 0.01)</td>
<td>94 (P&lt; 0.05)</td>
</tr>
<tr>
<td>Caudal</td>
<td>56</td>
<td>76</td>
<td>90 (P&lt; 0.05)</td>
<td>98 (P&lt; 0.01)</td>
</tr>
</tbody>
</table>

Values indicate percentage reduction in cell number relative to control counts. P indicates significance level of loss (Tukey’s post hoc comparison). SNpl = substantia nigra pars lateralis; SNpd = substantia nigra pars dorsalis.

Dopaminergic lesions in parkinsonian midbrain

Disorder. To investigate these factors reliably, it is essential to have a way to assess the selectivity for different subpopulations of dopamine-containing neurons degenerating during the course of disease. Landmarks permitting such analysis have been defined at a regional level (Hirsch et al., 1988), but they have been missing for the most vulnerable part of the midbrain nigral complex, the large and complex pars compacta of the substantia nigra. In the first paper of this series, we demonstrated that the compartmental pattern of calbindin D28K immunostaining can be used to delineate subgroups of dopamine-containing neurons in the substantia nigra pars compacta in a reliable way from one brain to another (Damier et al., 1999). Here we confirm the observations of Ito and colleagues (Ito et al., 1992) that the calbindin-positive neuropil survives in the substantia nigra in Parkinson’s disease, and we further show that the distinctive compartmental patterns of calbindin immunostaining defining the nigrosomes and the matrix are preserved in the midbrains of patients with Parkinson’s disease for as long as 30 years after diagnosis. These patterns of calbindin staining, probably corresponding to the distribution patterns of striatonigral afferent fibres, provided landmarks apparently independent of the degenerative process in Parkinson’s disease with which we could recognize consistently different subgroups of dopamine-containing neurons in the parkinsonian midbrain despite massive neuronal degeneration. The differential loss of dopamine-containing neurons in Parkinson’s disease could therefore be calculated with great accuracy for the different parts of the substantia nigra pars compacta as well as for the midbrain as a whole.

Dopamine-containing cell groups in the midbrain show different patterns of neurodegeneration in Parkinson’s disease

Neuronal loss was uneven across the different dopamine-containing cell groups of the midbrain, both within individual cases of Parkinson’s disease and across cases. These observations confirm the earlier reports (Hirsch et al., 1988; German et al., 1989). In cell groups outside the substantia nigra pars compacta, the degree of neuronal loss did not seem to be related to the duration of disease, and hence may not reflect disease progression in Parkinson’s disease. Other factors related to ageing or to the associated degenerative processes may be important in the aetiology of these lesions. For example, age-related loss of dopamine-containing neurons has been found to be high in the dorsal part of the substantia nigra (Fearnley and Lees, 1991), and loss in the medial and medioventral midbrain has been shown in Alzheimer’s disease (Mann et al., 1987).

In contrast to this apparently sporadic distribution of neuronal loss in cell groups outside the substantia nigra pars compacta, loss of dopamine-containing neurons in the substantia nigra pars compacta, defined by its calbindin-positive neuropil, appeared to bear an orderly relationship to disease duration. This finding suggests that the loss of
Fig. 5 Individual patterns of depletion of dopamine-containing neurons in the five parkinsonian midbrains analysed. The data are presented in the order of disease duration (shown at left), ranging from 7 to 32 years. The colorimetric scale indicates the degree of cell loss (blue = least; red = most). DBC = decussation of brachium conjunctivum; CP = cerebral peduncle; RN = red nucleus; III = exiting fibres of third cranial nerve.
Dopaminergic lesions in parkinsonian midbrain

Dopamine-containing neurons in the substantia nigra pars compacta is more directly reflective of the degenerative process in Parkinson’s disease than is degeneration in other dopaminergic cell groups, and might even be solely a consequence of the process underlying Parkinson’s disease. In the two cases with the shortest survival after diagnosis (7 years), degeneration within the substantia nigra pars compacta was already severe (75 and 82% cell loss). Thus, our sample missed three-quarters of the disease progression, as measured by estimated amounts of cell death. The differences in amount of cell loss from the cases with a disease duration of 7 years to the next longer duration (21 years) also were small (75 and 82% versus 85% cell loss). Even so, there was a clear rank ordering in the amount of total cell loss across the cases, with a total range of 15%. These findings, similar to those of Fearnley and Lees (Fearnley and Lees, 1991), give one estimate of the overall lesion progression in Parkinson’s disease in terms of the cell death endpoint. Further, they underline the great potential of developing neuroprotective measures in Parkinson’s disease.

**Differential vulnerability of the nigral compartments, nigrosomes and matrix in Parkinson’s disease**

A major finding of this study is that, in every parkinsonian midbrain, dopamine-containing neurons in nigrosomes were more affected than dopamine-containing neurons of the matrix. Based on the subdivisions defined by calbindin patterns in neurologically normal midbrains (Damier et al., 1999), dopamine-containing neurons of the substantia nigra pars compacta can be divided into two types: sparsely distributed neurons included in a calbindin-rich matrix...
Our data suggest that this differential loss of neurons in nigrosomes may not solely reflect the high packing density of nigrosomal neurons. For example, the loss of dopamine-containing neurons was moderate (56%) in the medioventral group, in which neurons are densely packed at the level of exiting third cranial nerve fibres, in comparison with the 84% loss of neurons at this level in the matrix, in which neurons are sparsely distributed. Further, cell loss in different nigrosomes ranged from 76 to 98% despite dense cell packing in each of them. The greater loss of dopamine-containing neurons in the nigrosomes than in the matrix also did not appear to be related solely to the preferential topographical locations of these neurons in the midbrain. Whatever the rostrocaudal level analysed, the degree of cell loss in the nigrosomes was higher than that in the immediately surrounding matrix. Similarly, a preferential ventral location of many of the dopamine-containing neurons in the nigrosomes did not appear to account for the differential vulnerability. Within a given nigrosome, the loss of TH-positive neurons did not show a dorsoventral gradient, even in the large nigrosome 1, and neurons in more dorsal nigrosomes (e.g. nigrosome 4) were more affected than neurons in the matrix ventral to them at the same rostrocaudal level. The preferential lateral location of many dopamine-containing neurons belonging to nigrosomes was also not a sufficient correlate of the differential vulnerability of nigrosomal neurons. Dopamine-containing neurons included in the medially situated nigrosome 2, for example, were more affected (94%) than those of the medial matrix (77%) at the same rostrocaudal level.

Among the different nigrosomes, there were also consistent differences in the amount of cell loss, the greatest loss always being in nigrosome 1. These differential patterns of cell loss suggest that it is not only the environment common to all nigrosomes (e.g. low levels of calbindin-positive neuropil) that sets the threshold of vulnerability. The special vulnerability of nigrosome 1 is in good accord with the preferential loss found in the ventrolateral clusters by Hassler (Hassler, 1938) and by Fearnley and Lees (Fearnley and Lees, 1991). This strong bias indicates that, in addition to a generally greater vulnerability of neurons in nigrosomes than in adjacent parts of the matrix, there is heightened vulnerability of ventrocaudal nigral regions that are of unknown origin but reflecting a well-known general topography of the disease. What our analysis adds to this topographical analysis is the notion that factors contributing to neuronal loss interact in the substantia nigra pars compacta with dopamine-containing neurons of two types—those in the matrix and those in nigrosomes—with appreciably different vulnerabilities in Parkinson’s disease.

Evidence for stereotyped temporospatial progression of cell loss in Parkinson’s disease

One of the most interesting results of our nigrosome/matrix analysis is the indication of orderly lesion progression in
Parkinson’s disease. The lesions appeared to follow a stereotyped temporospatial progression, beginning in nigrosonme 1, extending to other nigrosonmes in the order nigrosonme 1, nigrosonme 2, nigrosonme 4, nigrosonme 3, nigrosonme 5, and then affecting the matrix, with a global caudal-to-rostral, lateral-to-medial and ventral-to-dorsal direction of progression. Our case material was limited to only five patients, but if this pattern appears in a larger population of parkinsonian cases it may prove a signature of the neuropathology in Parkinson’s disease.

The general gradient loss observed in the substantia nigra pars compacta is probably related to the gradient depletion of dopaminergic terminals observed post-mortem in the striatum of Parkinson’s disease patients (Kish et al., 1988) and in [18F]fluorodopa (Brooks et al., 1990) or 11C-Win 35 428 (Frost et al., 1993) PET scans, with a higher loss in dorsal and caudal parts of the putamen than in the caudate nucleus. In relation to the somatotopic projection of cortical afferents to the putamen, one might expect an initial symptomatology in the foot in Parkinson’s disease (Vidailhet et al., 1994), but other authors have found symptoms expressed first in the arm (Schelosky and Poewe, 1990).

We have no information about whether there are different nigrostriatal projections from matrix neurons that might add detail to this general mapping and that might therefore be of genuine significance in terms of clinical interpretation.

The particular temporospatial lesion progression that we observed probably reflects the pathogenesis of Parkinson’s disease. Because the nigrosonme/matrix analysis refers to compartmental subdivisions within the substantia nigra pars compacta, the most obvious conclusion would be that compartmental locality in the substantia nigra pars compacta itself is a key to differential vulnerability. If these different localities have, as suggested above, different striatal projection zones, then it is possible that the initial trigger for cell loss is at the level of the striatum, and that the effect moves retrogradely back to the different nigral compartments.

If the basis for the pathology is within the substantia nigra pars compacta, our results suggest three main types of hypothesis: (i) that dopamine-containing neurons are exposed in Parkinson’s disease to variable amount of exogenous or endogenous toxins whose amounts increase from nigrosonme 1 to other nigrosonmes and then to the matrix; (ii) that the dopamine-containing neurons are exposed to similar amounts of toxin, regardless of compartment, but that mechanisms of defence against such toxins vary in an orderly way from one compartment of dopamine-containing neurons to the next; and (iii) that neurons in the different compartments have different patterns of expression of genes implicated in the disease process. Our results point to the possibility that the nigrosonmes and matrix compartments of the substantia nigra differ in term of their content of growth factors and receptors, compounds related to excitotoxicity, agents involved in oxidative metabolism, and potentially predisposing genes such as those for α-synuclein (Polymeropoulos et al., 1997) and parkin (Kitada et al., 1998).

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