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AGEING AND PARKINSON'S DISEASE: SUBSTANTIA NIGRA REGIONAL SELECTIVITY

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SUMMARY

The micro-architecture of the substantia nigra was studied in control cases of varying age and patients with parkinsonism. A single 7 μ section stained with haematoxylin and eosin was examined at a specific level within the caudal nigra using strict criteria. The pars compacta was divided into a ventral and a dorsal tier, and each tier was further subdivided into 3 regions. In 36 control cases there was a linear fallout of pigmented neurons with advancing age in the pars compacta of the caudal substantia nigra at a rate of 4.7% per decade. Regionally, the lateral ventral tier was relatively spared (2.1% loss per decade) compared with the medial ventral tier (5.4%) and the dorsal tier (6.9%). In 20 Parkinson's disease (PD) cases of varying disease duration there was an exponential loss of pigmented neurons with a 45% loss in the first decade. Regionally, the pattern was opposite to ageing. Loss was greatest in the lateral ventral tier (average loss 91%) followed by the medial ventral tier (71%) and the dorsal tier (56%). The presymptomatic phase of PD from the onset of neuronal loss was estimated to be about 5 yrs. This phase is represented by incidental Lewy body cases: individuals who die without clinical signs of PD or dementia, but who are found to have Lewy bodies at post-mortem. In 7 cases cell loss was confined to the lateral ventral tier (average loss 52%) congruent with the lateral ventral selectivity of symptomatic PD. It was calculated that at the onset of symptoms there was a 68% cell loss in the lateral ventral tier and a 48% loss in the caudal nigra as a whole. The regional selectivity of PD is relatively specific. In 15 cases of striatonigral degeneration the distribution of cell loss was similar, but the loss in the dorsal tier was greater than PD by 21%. In 14 cases of Steele-Richardson-Olszewski syndrome (SRO) there was no predilection for the lateral ventral tier, but a tendency to involve the medial nigra and spare the lateral. These findings suggest that age-related attrition of pigmented nigral cells is not an important factor in the pathogenesis of PD.

INTRODUCTION

The concept of neuronal loss occurring with advancing age was first proposed by Hodge (1894). For the substantia nigra this was confirmed by three morphometric studies, which found losses of 38%, 48% and 36% between the age of 20 and 90 yrs (Hirai, 1968; McGeer *et al.*, 1977; Mann *et al.*, 1984). This has also been verified by biochemical studies. Riederer and Wuketich (1976) found a 13% reduction of caudate dopamine per decade; Carlsson *et al.* (1984) observed a non-linear decline of striatal dopamine with little loss until the age of 60 yrs followed by a dramatic fall thereafter; Scherman *et al.* (1989) assessed striatal dopamine levels by measuring the binding of alpha-dihydro-tetrabenazine, a ligand of the vesicular monoamine transporter and found a linear decline of under 10% per decade. Neuronal loss in normal ageing is not sufficient to cause PD. In PD, there is a presymptomatic phase and clinical signs do not appear until 50% of nigral neurons and 80% of striatal dopamine is lost (Marsden, 1990).

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The Lewy body is a hyaline neuronal inclusion body, which is always present in PD (Gibb and Lees, 1988). Forno (1969) has proposed that incidental Lewy body cases are representative of the presymptomatic phase of PD. These are individuals who die without a history of parkinsonism or dementia, but who are found to have Lewy bodies at autopsy. However, it has been suggested that Lewy bodies as an incidental finding are a feature of normal ageing without any additional nigral cell loss (Hansen *et al.*, 1990).

Similarities have been drawn between senility and PD on the grounds of shared clinical features (Teräväinen and Calne, 1983; Evarts *et al.*, 1981) and enhancement of the long latency stretch reflex (Tatton and Lee, 1975; Evarts *et al.*, 1979). Moreover, ageing has been considered to play an important role in the pathogenesis of PD. It has been suggested that PD is a form of accelerated ageing (Mann and Yates, 1983; Barbeau, 1984) or alternatively that PD is a biphasic illness (Riederer and Wuketich, 1976; Calne *et al.*, 1986; Mattock *et al.*, 1988). The first phase, an acute illness early in life sharply depletes neuronal reserves, but not enough to cause clinical parkinsonism. This deficit is then unmasked by further losses due to normal ageing over the following 20 to 30 yrs. Evidence cited for this theory is the finding of subclinical nigral damage in methyl-phenyl-tetrahydropyridine (MPTP) toxicity (Calne *et al.*, 1985) and the frequent lag between encephalitis lethargica and the onset of parkonsonism (Duvoisin and Yahr, 1965).

The substantia nigra is an anatomically heterogeneous nucleus with regional variations in striatal projections and distribution of histochemical markers. Evidence suggests that degenerative diseases of the nigra demonstrate regional selectivity and this may be important in the pathogenesis of disease. In PD, neuronal loss is most severe in its caudal and ventrolateral parts (Hassler, 1938). In striatonigral degeneration (SND) the pattern of cell loss has been variously described as medial (Andrews *et al.*, 1970), lateral (Jellinger and Danielczyk, 1968; Sharpe *et al.*, 1973; Trotter, 1973; Buonanno *et al.*, 1975; Bannister *et al.*, 1981), central (Takei and Mirra, 1973; Sung *et al.*, 1979), dorsolateral (Borit *et al.*, 1975) and affecting the ventral and middle parts (Rajput *et al.*, 1972). The nigral distribution of Steele-Richardson-Olszewski syndrome (SRO) has been described as lateral (Steele *et al.*, 1964), ventromedial (Jellinger, 1971) and non-selective (Anzil, 1969). In ageing, the distribution of nigral cell loss has not been studied.

We present the first regional semi-quantitative study to be based on division of the substantia nigra by defined cell groups. Using this technique we studied the regional selectivity of normal ageing, incidental Lewy body cases, PD, SND and SRO.

MATERIALS AND METHOD

Two hundred and five control cases without a history of parkinsonism or dementia were collected prospectively over a 5 yr period by several hospital pathology departments through the auspices of the UK Parkinson's Disease Society Brain Bank and for 1 yr by the Department of Pathology, Innsbruck, Austria. All cases were used to calculate the prevalence of incidental Lewy bodies, but only a proportion were suitable for morphometric study. During the same period, 50 PD cases, 22 SND cases and 6 SRO cases were also collected. The brains were divided midsagittally: one-half immersion fixed in 10% buffered formalin for a period of 5 wks and the other frozen at −70°C. Multiple blocks were taken for microscopic examination. Pathological diagnosis of PD, SND and SRO was made on the distribution of cell loss and in the case of PD and SRO the presence and distribution of Lewy bodies and neurofibrillary tangles, respectively. A number of control cases had Lewy bodies in a distribution similar to PD and formed the incidental Lewy body group.

The anatomy of the substantia nigra was comprehensively studied by Hassler (1937) who identified around

50 neuronal groups. Hassler used the coronal plane perpendicular to the axis of Forel rather than the more contemporary transverse plane perpendicular to the axis of Meynert. For this study, nigral anatomy was reappraised in 5 control cases by examining 20 μ sections spaced every 250 μ to 500 μ throughout the nigra and stained with luxol fast blue/cresyl violet and cresyl violet alone. Blocks were taken in the transverse plane, but in one whole brain, one side of the nigra was blocked in the coronal plane for comparison.

Unlike Hassler who divided the nigra into separate oral and caudal parts, we found that the nigra is organized into three tiers: pars reticulata, ventral pars compacta and dorsal pars compacta, *see* Fig. 1. The tiers are staggered and the ventral tier is mainly confined to the caudal nigra arising orally within the pars reticulata. Within the two tiers of the pars compacta we were able to confirm Hassler's groups. However, we found that the oral and caudal groups were continuous forming parallel columns along the length of the tier, *see* Fig. 2. As a result of this parallel arrangement there was little difference in the cross-sectional configuration of the neuronal groups between the coronal and transverse planes, which are angled by 30° – 40°. However, the levels of sampling within the nigra were different. In the transverse plane compared with the coronal plane, the medial groups were sampled at a higher level and the lateral and dorsal groups at a lower level.

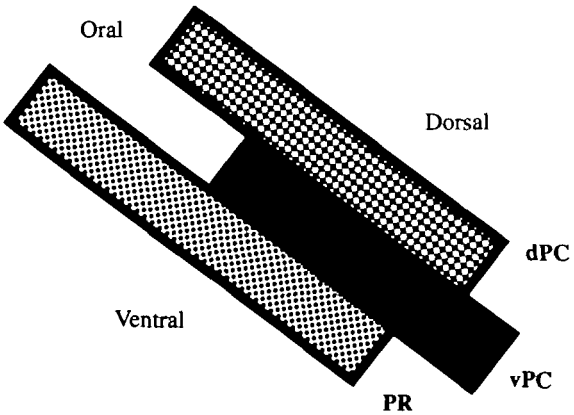

FIG. 1. *Substantia nigra tiers.* Sagittal representation of the substantia nigra. PR = pars reticulata (dots). vPC = ventral tier pars compacta (black). dPC = dorsal tier pars compacta (crosses).

An alternative system of classification of the pars compacta groups is proposed taking into account the concept of tiering and Hassler's original work. Each group is abbreviated to two letters corresponding to the tier and division and one letter in brackets corresponding to the group. The pars compacta is divided into two tiers: ventral – v, and dorsal – d. The dorsal tier has three divisions: medial – dm, lateral – dl and pars lateralis – pl. The ventral tier has two divisions: medial – vm, and lateral – vl. Each division has 1 to 4 groups: medial – (m), intermediate – (i), lateral – (l) and eutopic – (e). This classification is compared with Hassler's in Table 1. The group vl(e) corresponds to Hassler's eutopic group eJ and has been included with vl for the sake of simplicity. A number of Hassler's nigral groups correspond to the ventral tegmental area and the retrorubral area and have not been included.

A single 7 μ section of the caudal nigra stained with haematoxylin and eosin was used for morphometry. At this level, the IIIrd nerve fascicles demarcate the ventral tegmental area from the nigra (Olszewski and Baxter, 1954). Criteria for selecting a consistent level were defined on the basis of neuronal groups and their complementary nerve fibre fields as described by Hassler and are as follows: *medially,* where the IIIrd nerve fascicles divide the nigra from the ventral tegmental area above the decussation of the superior cerebellar peduncle between the main bulk of the Hassler's oral nerve fibre field rab, and the caudal field v; *centrally,* at the level of Hassler's neuronal group hj and its distinctive nerve fibre field; *laterally,* at the point where the striatonigral pathway (Hassler's nerve fibre field K) is well formed and oral enough to be distant from the medial lemniscus and caudal enough to be below neuronal group vl(e).

In the pathological cases, it was still possible to recognize neuronal groups by the remaining nerve cells,

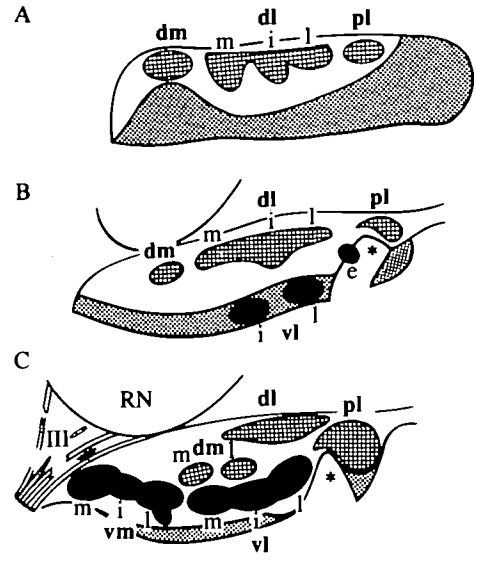

FIG. 2. *Substantia nigra groups.* The substantia nigra in the transverse plane perpendicular to the axis of Meynert: A, oral nigra; B, mid nigra; C, caudal nigra. Medial nigra left of page and lateral nigra right of page. Tiers coded according to Fig. 1. Bold letters correspond to the tier and the division and regular letters to group, *see* text for explanation. IIIrd nerve fascicles. RN = red nucleus. *Putaminonigral pathway (Hassler's nerve fibre field K).

TABLE 1. COMPARISON PRELIMINARY ANATOMICAL STUDY WITH THE WORK OF HASSLER (1937)

Fearnley and Lees			*Hassler's groups*	
Tier	*Division*	*Group*	*Oral*	*Caudal*
v	vm	(m)		Spvm
		(i)		Spvi
		(l)		Spvl
	vl	(m)		Spev
		(i)	Saivz	Spez
		(l)	Saivl	Sped, Spedd
		(e)		eJ
d	dm	(m)	Sam alpha*1 ak*2	Spzv*3
		(l)		Spzz
	dl	(m)	Saim	Spdm
		(i)	Saiz	Spdi
		(l)	Sail	Spdd
	pl	–	Sal	Spcd

Nomenclature (Fearnley and Lees): v = ventral, d = dorsal, m = medial, i = intermediate, l = lateral, e = eutopic. * Order of appearance from oral (1) to caudal (3).

nerve fibre fields, and condensation of glia. For example, even when vl has no pigmented neurons its outline is recognizable by a strip of closely packed astrocytes.

As there was no landmark laterally when cutting the midbrain many of the blocks were tilted and unsatisfactory for morphometry. The proportion of cases that were suitable was: 36/192 controls without LBs, 6/13 controls with LBs (incidental LB cases), 20/50 PD cases, 15/22 SND cases and 4/6 SRO cases.

Discrepancies between these proportions arose from chance and the fact that the brains had been cut by five different persons. Archival material at the Institute of Psychiatry, London, yielded a further 10 SRO cases and 1 incidental Lewy body case suitable for morphometry.

The substantia nigra of each case was divided into 6 morphometric regions (Fig. 3), by marking the coverslip of the slide with a 0.13 mm Rotring pen under 40 and 100× magnification. The morphometric regions (capital letters) differed from the anatomical groups in two instances. vl(m) was divided off from

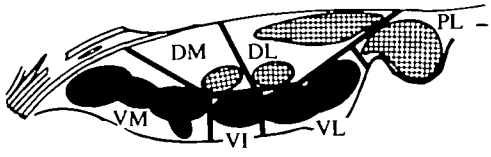

FIG. 3. *Morphometric regions.* Taken at the level of the caudal nigra, Fig. 2c. Dorsal tier: medial part (DM), lateral part (DL) and pars lateralis (PL). Ventral tier: medial part (VM) and lateral part (VI-intermediate and VL).

vl and counted separately as VI. The purpose in selecting this area for particular attention was to test further whether pathological processes respected the boundaries of the major nigral divisions. dl(l) was included in DM for ease of marking. Slides were coded so that the morphometrist was unaware of the patient's age. Pigmented neurons in each region were counted twice using alternately the cell body and nucleolus as the counting unit for the purposes of comparison and validation. The nucleolus has the advantages of good contrast with the rest of the cell, small size and uniform shape. However, a potential disadvantage in the substantia nigra is obscuration by pigment. The total count (number of pigmented neurons in a single section of the caudal nigra) was calculated by summating the regional counts. The slide was then remarked and recounted. Counting was performed under 400× magnification using an eyepiece graticule and parallel sweeps of the microscope stage. No corrections were made for split cell error. The following areas were not counted: pars reticulata; ventral tegmental area; retrorubral area and nucleus peripeduncularis.

Regression equations were calculated for the control regional and total counts vs age. The pathological cases including the incidental Lewy body cases varied in age. In order to assess the neuronal loss due to the pathological process separate from losses due to ageing it was necessary to correct the regional and total counts for age. For each of the pathological cases predicted control regional and total counts were calculated from the corresponding regression equations (Table 4). The pathological count was then expressed as a percentage of the predicted control count. Alternatively, cell loss was estimated by expressing the pathological count as a percentage of an age-matched control count. Significance was tested using Student's t test. Figures preceded by ± represent one standard deviation.

RESULTS

The results of the nucleolus and cell body counts were comparable except that the cell body counts reached a greater level of statistical significance due to the larger numbers counted and only data from cell body counting will be presented. Obscuration of the nucleolus by pigment was not a problem at a section thickness of 7 μ and using a magnification of 400×. Variation between consecutive counts (including re-marking of the slide) was $<5\%$. The average neuronal loss in the incidental Lewy body and parkinsonian groups was estimated by using age-adjusted counts expressed as a percentage of the predicted control and alternatively by comparing uncorrected counts directly with age-matched controls. Both methods gave similar results with no significant difference. No difference was found between VI and VL in normal ageing or disease. Uncorrected and age-adjusted cell body counts are listed in Tables 2 and 3, respectively.

TABLE 2. AVERAGE CELL BODY COUNTS

Age/sex	*Dur.*	*VM*	*VI*	*VL*	*DM*	*DL*	*PL*	*Total*
(A) Control								
21M		206	45	200	74	76	91	692
22F		184	54	216	93	108	137	692
29M		192	47	198	72	82	104	695
31M		171	46	178	83	88	91	657
44M		146	44	206	64	58	115	633
47M		176	44	200	48	50	65	583
53F		136	43	188	60	94	92	613
54M		184	45	181	87	84	111	692
55M		188	41	178	84	90	72	653
56F		172	47	188	75	88	88	658
58M		140	37	184	71	69	87	588
58M		155	37	167	60	58	67	544
60F		152	42	215	61	76	96	642
61M		150	40	177	69	65	86	587
61F		177	46	173	50	58	81	585
65F		88	34	147	32	53	49	403
65M		131	22	201	50	50	64	518
69F		188	51	187	68	96	112	702
70F		72	20	146	32	72	64	406
70M		152	48	200	71	44	100	615
71M		158	41	190	75	48	46	558
75F		124	39	170	50	37	73	493
75M		109	46	182	45	57	65	504
77M		85	40	160	30	29	46	390
78M		104	36	183	37	58	85	503
79M		132	49	180	58	44	57	520
80F		148	40	179	60	63	66	556
81F		139	42	152	68	83	59	543
81M		116	33	175	39	55	30	448
84F		193	40	190	61	62	102	648
85F		168	40	199	64	62	83	616
86M		120	37	176	45	39	54	471
87M		114	46	206	42	48	84	540
89M		139	35	192	50	59	103	578
91F		122	41	159	32	39	33	426
91M		90	42	167	30	35	30	394
(B) Incidental Lewy bodies								
65F		152	18	99	55	55	64	443
75M		156	22	113	55	48	65	459
78M		118	11	76	50	48	56	359
80F		132	20	94	51	47	57	401
80M		114	17	63	41	44	29	308
81F		143	24	72	53	51	61	404
82F		127	17	81	50	41	48	364
(C) Parkinson's disease								
68M	1½	133	24	78	67	56	45	403
62F	2	67	5	30	34	32	66	234
74M	6	36	8	22	39	38	49	192
81M	6	123	17	49	43	32	31	295
73M	10	70	16	23	38	31	34	212
73F	–	132	5	13	54	34	53	291
73M	11	8	2	11	20	23	33	97
83F	12	21	2	3	29	23	14	92

Age/sex	*Dur.*	*VM*	*VI*	*VL*	*DM*	*DL*	*PL*	*Total*
76F	13	16	0	0	22	16	24	78
80M	15	19	0	10	14	12	27	83
78M	20	30	0	0	27	41	26	124
80M	–	33	0	8	34	30	35	140
78M	15	33	0	2	16	13	4	68
71F	17	8	1	13	18	19	7	66
71M	18	9	0	4	18	22	25	78
87F	25	3	0	0	31	17	19	70
82F	29	2	0	0	12	11	12	37
64F	38	12	0	0	18	9	8	47
74M	–	12	0	0	15	15	15	57
77M	–	6	2	7	25	17	18	75
(D) Striatonigral degeneration								
68M	2	14	0	0	10	9	3	36
67F	3	66	5	11	25	15	16	138
51M	4	26	1	2	20	12	15	76
56F	5	10	0	0	4	2	2	18
61M	5	42	0	3	27	27	8	107
66M	5	44	3	4	33	11	24	119
72F	5	82	17	14	39	19	18	189
43M	6	13	0	2	4	5	8	32
62M	6	48	4	1	26	5	4	88
74F	6	19	0	1	16	20	13	69
65M	7	8	0	11	14	34	30	97
64F	8	57	5	3	17	10	13	105
78M	8	74	2	4	31	29	34	174
59F	10	14	0	0	5	4	3	26
61F	10	3	0	1	7	5	4	20
(E) Steele-Richardson-Olszewski syndrome								
70M	2	32	8	46	15	14	37	152
59F	4	34	9	66	11	24	34	178
59M	4	14	8	92	10	12	16	152
68M	4	32	10	39	11	15	20	127
79F	4	15	6	22	4	7	25	79
66F	5	8	2	20	9	8	2	49
75M	5	31	5	20	11	3	26	96
79F	5	3	2	13	3	5	5	31
55F	6	3	2	15	6	8	15	49
67F	6	18	10	32	7	6	29	102
71M	6	10	4	37	10	14	25	100
72F	8	11	10	63	7	12	32	128
66F	9	28	11	22	12	8	4	85
68F	–	9	4	17	15	9	28	82

Control cases and ageing

The 36 control brains suitable for morphometry ranged in age from 21 to 91 yrs. Regression equations were calculated for the regional and total neuronal counts vs age, *see* Table 4. There was a linear decline with advancing age in both the total count of the caudal nigra (Fig. 4) and the regional counts. The regression equations were then used to calculate the neuronal loss between the age of 20 and 90 yrs for the caudal nigra and each region. The total count fell 33% (4.7% per decade). Regionally there was sparing of VI/VL with 2.5 to 3 times greater loss in the other regions (Fig. 5B). The

TABLE 3. AVERAGE AGE-ADJUSTED CELL BODY COUNTS (PERCENT OF PREDICTED CONTROL)

Age/sex	*Dur.*	*VM*	*VI*	*VL*	*DM*	*DL*	*PL*	*Total*
(A) Incidental Lewy bodies								
65F	−1.6	104	44	54	94	87	82	78
75M	−2.8	115	55	63	104	86	92	86
78M	0.1	89	28	43	78	87	82	68
80F	−1.4	102	51	53	102	87	85	77
80M	1.9	88	43	36	82	81	43	59
81F	−1.7	111	61	41	108	97	92	79
82F	−0.4	99	43	46	103	78	73	71
(B) Parkinson's disease								
Mild cases								
68M	1½	93	59	42	118	92	60	72
62F	2	45	12	16	57	49	83	40
74M	6	26	20	12	73	67	69	36
81M	6	95	43	28	87	61	47	57
73M	10	51	40	13	71	54	48	39
73F	–	96	12	7	100	59	74	54
Moderate cases								
73M	11	6	5	6	37	40	46	18
83F	12	17	5	2	60	45	22	18
76F	13	24	0	0	40	29	35	15
80M	15	15	0	6	28	23	41	16
78M	20	23	0	0	53	75	38	24
80M	–	25	0	5	68	56	53	27
Severe cases								
78M	15	25	0	1	31	24	6	13
71F	17	6	2	7	33	32	10	12
71M	18	6	0	2	31	37	34	14
87F	25	2	0	0	68	35	31	14
82F	29	2	0	0	25	21	19	7
64F	38	8	0	0	31	14	10	8
74M	–	9	0	0	28	26	21	11
77M	–	5	5	4	46	31	26	14
(C) Striatonigral degeneration								
68M	2	10	0	0	18	15	4	6
67F	3	46	12	6	44	23	20	25
51M	4	16	2	1	30	17	17	12
56F	5	6	0	0	6	3	2	3
61M	5	28	0	2	44	41	10	18
66M	5	30	7	2	57	18	31	21
72F	5	61	42	8	70	32	25	35
43M	6	8	0	1	6	7	9	5
62M	6	32	10	0	43	8	5	15
74F	6	14	0	1	30	35	18	13
65M	7	6	0	6	24	54	39	17
64F	8	39	12	2	29	16	17	18
78M	8	56	5	2	60	53	50	33
59F	10	9	0	0	8	6	4	4
61F	10	2	0	0	12	8	5	4
(D) Steele-Richardson-Olszewski syndrome								
70M	2	23	20	25	27	25	50	27
59F	4	22	22	36	18	36	42	30

Age/sex	*Dur.*	*VM*	*VI*	*VL*	*DM*	*DL*	*PL*	*Total*
59M	4	9	19	49	16	18	20	26
68M	4	23	25	21	20	25	27	23
79F	4	11	15	12	8	13	38	15
66F	5	6	5	11	16	13	3	9
75M	5	23	13	11	21	5	37	18
79F	5	2	5	7	6	9	7	6
55F	6	2	5	8	9	12	18	8
67F	6	12	25	17	12	10	38	18
71M	6	7	10	20	18	24	34	18
72F	8	8	25	35	17	21	45	24
66F	9	19	27	12	21	13	5	15
68F	–	6	10	9	26	15	37	15

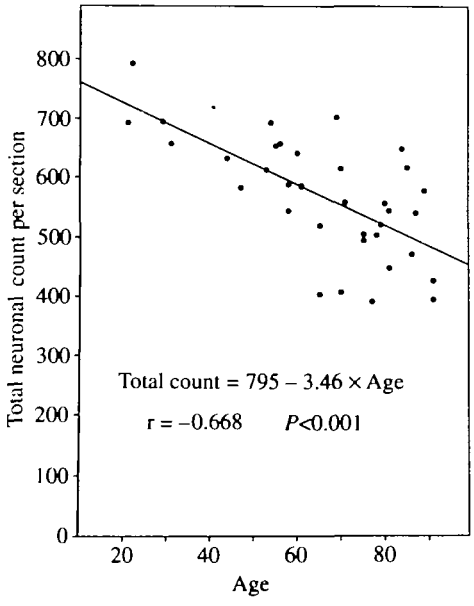

FIG. 4. *Ageing and the substantia nigra.* The total neuronal count equals the number of pigmented cell bodies in one section of the caudal substantia nigra.

range of counts within each decade increased with consecutive decades suggesting variability in the effects of ageing. Qualitatively, DM, DL, PL and less frequently VM were the most heavily pigmented regions within the nigra.

Incidental Lewy body cases

Incidental Lewy bodies were found in 13 of the 205 control cases. In no case was there a history of parkinsonism or dementia. The incidental cases ranged in age from 46 to 90 yrs with a mean age of 76 ± 12. The age specific prevalence increased with age to 12% over 80 yrs (Table 5).

There was a significant reduction in the total count ($P < 0.01$) of the 7 incidental cases suitable for morphometry compared with age-matched controls. Regionally, there was only significant loss in VI/VL ($P < 0.001$). The average reduction in the total count was $27 \pm 17\%$ and in VI/VL $52 \pm 9\%$ (Fig. 5c).

TABLE 4. CONTROL CASES: AGE-RELATED NEURONAL ATTRITION BETWEEN THE AGE OF 20 AND 90 YRS

Counts Region*	Attrition** between 20 and 90 yrs	Regression equation (count = b−m·age) b	m	Correlation r	P<
VM	38%	214.0	1.050	−0.580	0.001
VI	18%	49.0	0.122	−0.339	0.05
VL	14%	206.4	0.356	−0.387	0.02
DM	48%	95.6	0.572	−0.630	0.001
DL	48%	104.6	0.632	−0.619	0.001
PL	46%	125.5	0.735	−0.552	0.001
Total***	33%	794.8	3.462	−0.668	0.001

*See Fig. 3 for the location of the region within caudal nigra. **Calculated from regression equation:

$$\text{attrition} = (\text{count}_{20} - \text{count}_{90})/\text{count}_{20} \cdot 100\% = (70 \cdot m)/(b - 20 \cdot m) \cdot 100\%$$

b = intercept; m = slope; r = correlation coefficient.

***Caudal substantia nigra.

Parkinson's disease cases

There was a significant reduction in both the total and regional counts of the PD cases compared with controls ($P < 0.001$). The average percent losses were maximal in VI/VL followed by VM and then the dorsal regions DM, DL and PL, *see* Fig. 5D.

The 20 PD cases ranged in age from 62 to 87 yrs (mean 75 ± 6). In 16 cases the onset of symptoms was well documented with an average age of 60 ± 11 yrs and an average duration of 15 ± 10 yrs. There was no correlation between age and symptom duration or between age and neuronal count. However, there was good correlation between symptom duration and neuronal loss ($P < 0.001$). There was an exponential decline in the total and regional age-adjusted counts with increasing symptom duration (Figs. 6, 7), so that losses were greatest in the beginning of the disease and then tailed off as it progressed. In the first decade from the onset of neuronal loss, there was a 45% fall in the age-adjusted total count, ten times greater than the rate of loss that could be accounted for by ageing. Similar results were found using uncorrected counts.

Presymptomatic phase of Parkinson's disease

The presymptomatic phase was taken as the period from the onset of neuronal loss to the onset of symptoms. It was found to be 4.7 yrs using the equation from Fig. 6 for the total age-adjusted count in PD. During this period neuronal loss due to PD, excluding age-related loss, was 31% for the total count and 64% for the regional VL count. The loss due to ageing was calculated from the control regression equations of Table 4 over the interval from both birth to onset of neuronal loss (mean age 55 yrs) and birth to the onset of symptoms (mean age 60 yrs). The average loss due to ageing was 24−26% for the total count and 10−11% for the regional VL count. The average combined loss at the onset of symptoms was 48% for the total count and 68% for the VL regional count.

To test whether any of the incidental Lewy body cases might have had undetected

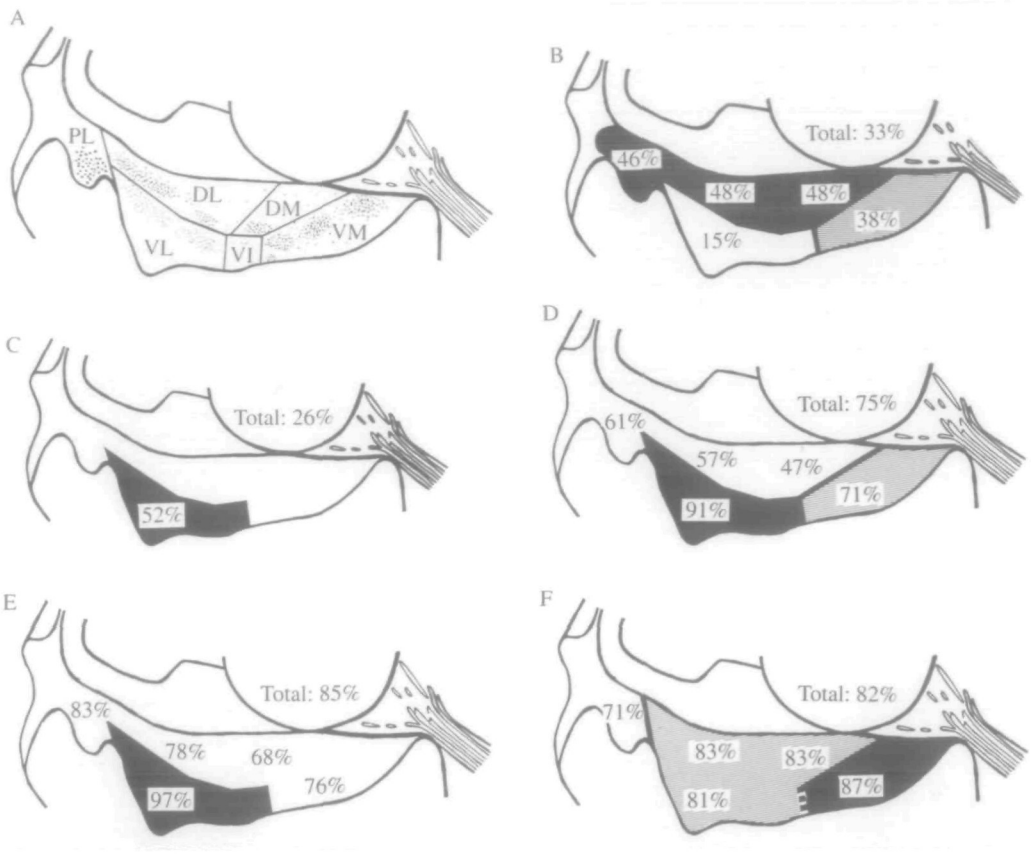

FIG. 5. *Substantia nigra regional losses in ageing and disease.* A, normal anatomy; B, ageing*; C, incidental Lewy body cases**; D, Parkinson's disease**; E, striatonigral degeneration**; F, Steele-Richardson-Olszewski syndrome**. A continuous border between two regions indicates a significant difference in the percent neuronal loss. *Percentage loss between the age of 20 and 90 yrs. **Average percentage loss in cases studied.

TABLE 5. AGE SPECIFIC PREVALENCE OF INCIDENTAL LEWY BODIES

Age	*n*	*Percent*
<20	0/2	
20–29	0/6	
30–39	0/6	
40–49	1/7	3.7
50–59	0/20	
60–69	2/51	3.9
70–79	3/56	5.4
80–89	6/49	12.2
90–99	1/8	12.5

parkinsonism during life individual total counts were scrutinized. From each the symptom duration was calculated using the equation of Fig. 6. Using this analysis, 1 case might well have been symptomatic for a duration of 2 yrs. Another case may have been

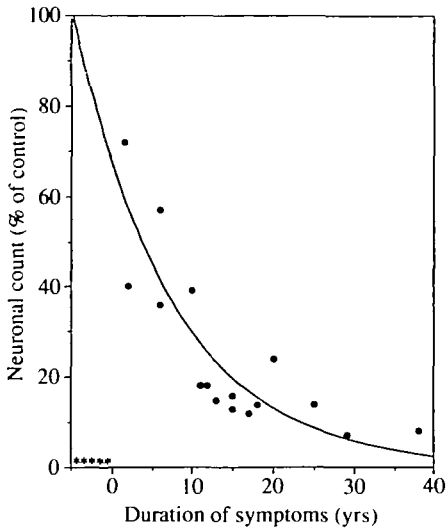

FIG. 6. *Parkinson's disease.* Total age adjusted count vs symptom duration.

*****Presymptomatic phase: duration 4.7 yrs.

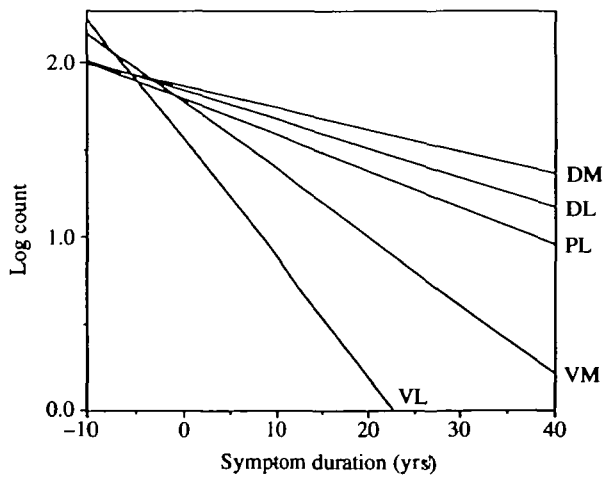

FIG. 7. *Parkinson's disease.* Logarithm of regional age adjusted counts vs symptom duration.

Region	*Regression equation*	*r*	*P*
VM	Log (count) = 1.78−0.0393 duration	0.740	< 0.01
VL	Log (count) = 1.57−0.0685 duration	0.787	< 0.001
DM	Log (count) = 1.87−0.0128 duration	0.618	< 0.02
DL	Log (count) = 1.84−0.0169 duration	0.742	< 0.001
PL	Log (count) = 1.80−0.0211 duration	0.637	< 0.01

marginally symptomatic with a symptom duration of 1 mth. Both these cases would have been aged 78 yrs at the onset of symptoms.

Topographical progression of Parkinson's disease

The topographical progression in the nigra of PD was studied by including the incidental Lewy body cases, as representative of the presymptomatic phase, and dividing the PD

TABLE 6. PARKINSON'S DISEASE CASES

Group	No.	Total count (age adjusted)	Clinical details	
			Duration**	Stage*,**
+ mild	6	> 35%	5±3.5	3.4
++ moderate	6	< 35%	14±3.6	4.4
+++ severe	8	< 15%	24±8.8	4.8
Significance of differences				
Mild−moderate		$P < 0.001$	$P < 0.01$	$P < 0.05$***
Moderate−severe		$P < 0.02$	$P < 0.05$	n.s.

* Hoehn and Yahr (1967). ** Where available. *** Wilcoxon rank sum test.

cases into mild, moderate and severe groups depending on the total count (*see* Table 6). Fig. 8 illustrates the staggering of regional neuronal loss, starting in VI/VL and then spreading to the other regions and in particular VM.

Striatonigral degeneration cases

The 15 SND cases ranged in age from 43 to 78 yrs (mean 63±9) and the average symptom duration was 6±2 yrs. Compared with age-matched controls there was a significant reduction in both the total and regional counts ($P < 0.001$). The average percent neuronal loss is shown in Fig. 5E. There was no significant difference between the average total count of the SND cases and the PD cases. However, loss in the dorsal tier (DM, DL and PL combined) was greater than PD by 21% ($P < 0.01$). When compared with the moderate/severe cases of PD (average total loss 85%), the SND cases had a smaller loss in VM of around 12% ($P < 0.05$).

Steele-Richardson-Olszewski syndrome

The 14 SRO cases ranged in age from 55 to 79 yrs (mean 68±7) and the average symptom duration was 5±2 yrs. Compared with age-matched controls there was a significant reduction ($P < 0.001$) in both the total and regional counts. The average

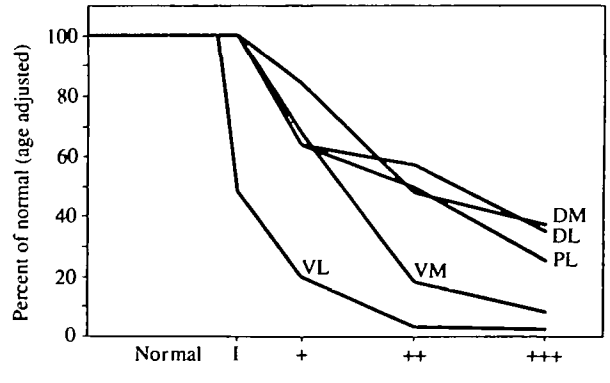

FIG. 8. *Topographical progression of Parkinson's disease.* Horizontal axis: phase of disease from no neuronal loss (normal) through presymptomatic disease (I: incidental Lewy body cases) to mild (+), moderate (++) and severe (+++) symptomatic Parkinson's disease. Vertical axis: average age adjusted regional counts for each group.

percent loss is shown in Fig. 5F. The difference between regions is not as distinct as occurs in PD and SND. There was no significant difference between the divisions of the ventral tier, VM, VI and VL. VM sustained greater losses than the dorsal tier divisions DM ($P < 0.05$), DL and PL ($P < 0.001$). There was no difference between the middle groups VI, VL, DM and DL. PL was relatively spared compared with all the other regions ($P < 0.02$). Compared with PD, losses in the dorsal tier were greater by 30% ($P < 0.001$). Losses in the lateral ventral tier (VI and VL combined) were smaller than in PD by 10% ($P < 0.01$) and smaller than moderate/severe PD by 17% ($P < 0.001$).

DISCUSSION

The anatomy of the substantia nigra may be crucial to the understanding of its pathology. The pars compacta is divided into a ventral and dorsal tier. Gerfen *et al.* (1987) found that in the rat, the ventral tier projects to the striatal striosomes and the dorsal tier to the matrix. The striosomes project back to the cell bodies and proximal dendrites of the ventral tier and the matrix projects back to the pars reticulata and distal dendrites of the ventral tier. The striatal striosomes and matrix are histochemically distinct and have separate dendritic fields. The striosomal projecting nigral neurons of the cat have a high concentration of sigma receptors (Graybiel *et al.*, 1989) and the dorsal tier is marked by the presence of calcium binding protein (Gerfen *et al.*, 1985). Also there are developmental differences, the ventral tier forms connections with the striatum before the dorsal tier (Olson *et al.*, 1972). In PD, the ventral tier and especially its lateral part bears the brunt of neuronal loss, which suggests that the cellular characteristics or connections of the ventral tier may be important in providing clues to the pathogenesis of PD.

Each tier of the pars compacta has several neuronal groups. In the monkey, Parent *et al.* (1983) demonstrated alternating clusters of putamen and caudate projecting neurons forming columns through the nigra. It is possible that the anatomical groupings seen in the human correspond to these clusters and that there may be associated functional divisions.

Topographical studies of the nigrostriatal projection in the monkey have established that the oral nigra projects mainly to the head of the caudate and the caudal nigra projects to the putamen and the body of the caudate (Carpenter and Peter, 1972; Szabo, 1980). The lateral ventral nigra projects to the dorsal putamen. In PD, neuronal loss begins in the lateral ventral tier and throughout the illness this remains the region most severely affected. Kish *et al.* (1988) in a post-mortem study of PD found severe dopamine depletion in the putamen with relative sparing of the caudate. Within the putamen, the dorsal and intermediate zones were more depleted than the ventral, but severe dopamine reduction occurred in all regions.

The correlation between the regional selectivity of PD and its clinical expression is uncertain. The putamen is considered to be predominantly involved in motor programming and the caudate in cognition (DeLong *et al.*, 1983). The motor cortex input to the putamen is somatotopically organized. The leg area of the motor cortex projects to the dorsal zone of the putamen in non-human primates, the arm area to the intermediate zone and the head area to the ventral zone (Künzle, 1975). In PD, cell loss starting in the lateral

ventral nigra suggests that dopamine depletion starts in the dorsal putamen. If striatal function mirrors the motor cortex input then symptoms would be expected to start in the leg. However, it is more common for symptoms to start in the arm (Schelosky and Poewe, 1990). It can be argued that a small deficit of hand function is soon noted by the patient, but the same deficit in the leg goes unnoticed. Furthermore, the bipedal gait of the human may explain the difference. In contrast, dystonia in young onset PD usually begins in the leg and often does not involve the arm.

In the past ageing has been considered to play an important role in the pathogenesis of PD, because of the considerable neuronal loss with advancing age in the substantia nigra. This study confirmed a linear fallout of pigmented neurons at a rate of 4.7% per decade in the caudal pars compacta.

In the biphasic theory of ageing and PD, the rate of neuronal loss in the second phase including the period of symptomatic PD is equivalent to the rate of neuronal loss seen in normal ageing. However, the rate of loss in cases of established PD is not linear but exponential and in the first decade there is a 45% loss, 10 times greater than is seen in normal ageing. McGeer *et al.* (1988) have also found that the rate of neuronal loss in PD is greater than could be accounted for by ageing by estimating the numbers of degenerating neurons at the time of death.

The alternative notion of PD being a form of accelerated ageing also seems improbable. Neuronal loss due to ageing shows marked regional variation in the nigra. Losses are greatest in the dorsal tier (6.9% per decade) followed by the medial ventral tier (5.4%) and least in the lateral ventral tier (2.1%). If PD is due to an acceleration of normal ageing then the pattern of cell loss should be similar to that seen as a result of normal ageing. In fact, the complete opposite applies. Neuronal loss in PD is greatest in the lateral ventral tier (average loss 91% excluding ageing) followed by the medial ventral tier (71%) and the dorsal tier (56%). These findings are inconsistent with the accelerated ageing theory.

It seems likely that PD is a relatively acute monophasic illness. The exponential loss of neurons and greater losses early in the disease means that the presymptomatic phase is considerably shorter than previously suspected, lasting around 5 yrs. At the onset of symptoms, striatal dopamine is reduced by 80%, but the total caudal nigral count is only reduced by about 50% (lateral ventral tier by 70%). This suggests that the remaining neurons are dysfunctioning and Hirsch *et al.* (1988) have found that 17% of pigmented nigral neurons in PD failed to stain for tyrosine hydroxylase.

It has been proposed that the presymptomatic phase of PD is represented by incidental Lewy body cases, which exhibit neuronal loss intermediate between control and PD cases (Forno, 1969). Hansen *et al.* (1990) have recently challenged this concept by suggesting that Lewy bodies may be a feature of normal ageing. They found in a study of 13 patients with dementia and Lewy bodies at autopsy that 4 had no cell loss in the nigra. However, this report was not supported by any quantitative evidence and no regional morphometry was carried out. In this study of 7 incidental cases, there was an average 27% loss of nigral neurons in excess of ageing ($P < 0.01$). Regionally, this loss was confined to the lateral ventral tier with an average loss of 52% ($P < 0.001$) similar to the pattern of cell loss seen in PD. This suggests that incidental Lewy bodies are indicative of presymptomatic PD rather than a feature of normal ageing. Furthermore in PD, neuronal loss appears to be confined to the lateral ventral tier early in the disease,

but by the time symptoms appear it has spread to the other regions and in particular the medial ventral tier.

There is a discrepancy between the prevalence of incidental Lewy body cases and the prevalence of clinical PD in the community. This could be explained by a long presymptomatic phase with the majority of incidental cases dying before the onset of parkinsonism (Gibb and Lees, 1988). However, if there is indeed as our results suggest a relatively short presymptomatic phase this explanation is untenable. It might be that PD is considerably under-diagnosed in the elderly. The incidence of new PD cases has been reported to fall after the age of 75 (Schoenberg, 1986). Two of the 7 incidental Lewy body cases in this study might have had undiagnosed PD with an onset of parkinsonism at the age of 78 yrs. Therefore, the difference between the prevalence of PD and incidental cases might be spurious, because of an underestimation of PD in the elderly and a consequent overestimation of the number of true incidental Lewy body cases.

PD regional nigral selectivity is relatively specific when compared with SND and SRO. Both PD and SND share a predilection for the lateral ventral tier, which is not seen in SRO. Differences were seen in the involvement of the dorsal tier, which was greatest in SRO followed by SND and least in PD. Neuronal loss in the dorsal tier was greater in SND and SRO than in PD by approximately 21% and 30%, respectively. In the monkey, Parent *et al.* (1983) found that putaminal projecting clusters were more frequent in the caudal ventral nigra and that caudate clusters were more frequent in the oral dorsal nigra probably corresponding to the ventral and dorsal tiers, respectively. This would suggest that greater involvement of the dorsal tier would result in greater reduction of nigral terminals in the caudate. This would explain the differences in ^{18}F-dopa striatal uptake between PD, SND and SRO on positron emission tomography (Brooks *et al.*, 1990). Reduction of uptake in the caudate was greatest in SRO followed by SND and least in PD.

Since the advent of MPTP induced parkinsonism it has been mooted that PD is due to one or several environmental neurotoxins (Davis *et al.*, 1978). However, epidemiological studies have failed to unearth any single conclusive exogenous factor although some association with exposure to herbicides and residence in rural areas has been implicated (Barbeau *et al.*, 1986). Furthermore, despite close similarity between the MPTP lesion and that seen in PD, differences in the pathological lesion exist. Although the nigral lesion has a ventral predilection (German *et al.*, 1988), the caudate and putamen are equally depleted of dopamine, unlike PD in which there is some sparing of the caudate (Pifl *et al.*, 1988). There is also some question that MPTP in the human spares the nucleus basalis of Meynert and that Lewy bodies do not occur (Burns *et al.*, 1984). However, it is possible that some of these histological differences are due to the acute administration of the toxin.

Neuromelanin has been implicated in the pathogenesis of PD (Hirsch *et al.*, 1988), but non-pigmented regions can be equally affected (Marsden, 1983). It has been speculated that because MPP^+ has a high affinity for neuromelanin it may be slowly released after the initial exposure and result in chronic toxicity (D'Amato *et al.*, 1986). However, we found that the neuromelanin-containing cells with the least pigment to be the most affected in PD suggesting that neuromelanin accumulation is not a pathogenetic factor (Gibb *et al.*, 1990). However, neuromelanin may be important in the pathogenesis of

age-related neuronal attrition as ageing seems to be confined to the most heavily pigmented regions. It is known that the formation of neuromelanin is associated with the generation of free radicals and toxic quinones (Graham, 1978), which may be responsible for age-related cell death.

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