Depression and Nigral Neuron Density in Lewy Body Spectrum Diseases

Laura Saari, MD [1,2]
Lauri Heiskanen, MD,[1,2]
Maria Gardberg, MD, PhD,[3] and
Valtteri Kaasinen, MD, PhD [1,2]

Parkinson's disease and other Lewy body spectrum diseases (LBDs) are associated with a specific risk for clinical depression. In the present clinicopathological study with 73 patients with LBD, we observed that the substantia nigra pars compacta dopamine neuron density was markedly lower in patients who had comorbid depression antemortem than in nondepressed patients (1.52 vs 2.32 n/mm^2, p = 0.004). There were no differences in cognition, motor disease severity, antiparkinsonian medications, or disease duration between groups. The results implicate the substantia nigra as an important psychomotor modulatory area of mood in patients with Lewy body disorders.

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Clinical depression is a common complication of Parkinson's disease (PD) and other Lewy body spectrum diseases (LBDs). It has been estimated that the prevalence of depression among patients with PD ranges from 20 to 50%,[1] and depression may be one of the premotor symptoms of the disease with onset several years before the emergence of motor symptoms.[2] Other synucleinopathies seem to similarly carry a specific risk for mood disorders, as patients with dementia with Lewy bodies (DLB) have been reported to have a nearly 3-fold higher risk for depression than Alzheimer's disease,[3] and 2 out of 3 patients with multiple system atrophy have depressive symptoms.[4] Although secondary reactive depression is possible in a chronic neurodegenerative disease, expert opinion and epidemiological, pathophysiological, and therapeutic data favor the hypothesis that PD depression is a specific clinical entity.[5,6] Regarding the mechanism, functional neuroimaging has suggested that depressive symptoms in PD may be due to not only a serotonergic defect but also a reduction of noradrenalin and dopamine innervation in the limbic system.[7] In line with this, depression in LBD appears to respond to dopaminergic drugs, such as pramipexole.[8]

The primary loss of dopaminergic neurons in PD occurs in the substantia nigra (SN) pars compacta,[9] but the nonmotor symptoms of PD have been considered to be mainly due to Lewy body pathology in other regions.[10,11] Importantly, however, depressive symptoms in nondemented elderly subjects without PD have been reported to correlate with α-synuclein deposits, neurofibrillary tangles, and tyrosine-hydroxylase (TH)-positive neuron counts in the brain stem.[12] Moreover, a previous study of patients with LBD suggested that a higher burden of alpha-synuclein pathology in the SN may be associated with depressive symptoms.[13]

In the present study, we focused on clinical depression and SN dopaminergic neuron degeneration, a key neuropathological event in PD. The primary aim was to investigate whether an association between depression and a lower density of dopaminergic neurons, previously demonstrated in nondemented elderly individuals, is present in patients with LBD who have specific nigral neuron loss and an increased risk for depression.

Methods

Neuropathology and Neuronal Counting

Using records from the Department of Pathology at Turku University Hospital, Finland, we identified 73 postmortem patients who had undergone neuropathological examination from 2002 to 2016. The patients were selected from a database of 168 postmortem cases with parkinsonism causing neurodegenerative diseases based on confirmed Lewy body pathology and sufficient antemortem clinical information, including neurological and psychiatric histories and medications until death. The minimum requirement for clinical information was sufficient details of symptoms and signs for phenotypical categorization (see below, Clinical records). The patients fulfilled neuropathological criteria for LBD (minimum Braak stage 3).[14,15] Patients were processed by a similar protocol, as described previously,[16] with minor variation

From the [1]Clinical Neurosciences, University of Turku, Turku, Finland; [2]Neurocenter, Turku University Hospital, Turku, Finland; and [3]Department of Pathology, Laboratory Division, Turku University Hospital and Institute of Biomedicine, University of Turku, Turku, Finland

Address correspondence to Dr Laura Saari, Neurocenter, Turku University Hospital, POB 52, 20521 Turku, Finland. E-mail: laasaa@utu.fi

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in delay times. The median time from death to autopsy was 5 days (range = 1–18 days), and the median time from autopsy to neuropathological examination was 23 days (range = 10–91 days; missing data for 2 patients).

Formalin-fixed and paraffin-embedded tissue from the midbrain of each patient sampled routinely at the level of the third cranial nerve was consecutively sectioned at 8 micrometers for Luxol fast blue (LFB) staining and for TH immunohistochemical staining. The slides were scanned with a Pannoramic P25 Flash slide scanner and analyzed with CaseViewer software version 2.3.0.99276 (3D HISTECH Ltd, Budapest, Hungary). The margin of the SN pars compacta was annotated on the LFB stained slides within a 1.0 times magnification window to allow a general view of the sample. CaseViewer software was used to calculate the SN pars compacta surface area (mm^2). The annotated area outline was transferred to corresponding TH stains using histological landmarks such as blood vessels and section outline, after which TH-positive cells were calculated at high magnification, including only cells with visible nucleus (Fig A, B). Diameters of nuclei were used for Abercrombie correction as described.[17]

Clinical Records

The hospital records of the patients were examined to identify factors that could correlate with neuron counts (Table 1). Patients were classified as having clinical depression if they had a diagnosis of depression or had clinically significant symptoms of depression or chronic antidepressive selective serotonin reuptake inhibitor (SSRI) / serotonin-norepinephrine reuptake inhibitor (SNRI) medication over the course of neurological disease (Table 2). No severity threshold was used for depression. Patients were classified according to a clinical phenotype (parkinsonism dominant group, parkinsonism and dementia, or dementia dominant group; Supplementary Table S1).[18] Classification was based on phenotypical descriptions in case histories, such as clinically observed rest tremor, rigidity, bradykinesia, and problems with memory and orientation. Numeric data were collected if available, including Mini-Mental State Examination (MMSE) scores and Beck Depression Inventory (BDI) scores. Hoehn and Yahr scale scores were estimated using information in case histories related to symmetry of symptoms, balance, and mobility. To receive drug reimbursement for PD in Finland, the diagnosis must be made by a certified neurologist using either the UK Brain Bank criteria or the

TABLE 1. Demographic and Clinical Characteristics of Patients With and Without Depression

	Depression	No depression	p[a]
n	12	61	—
Age at death, yr	77.0 (6.6) [12]	79.8 (7.5) [61]	0.220
Sex, M/F	7/5	41/20	0.740
Parkinsonism symptom onset to death, yr	7.7 (5.8) [8]	8.6 (4.6) [39]	0.602
Diagnosis to death, yr	4.8 (5.0) [9]	6.3 (4.1) [46]	0.200
LEDD, mg	414 (294) [12]	377 (359) [54]	0.619
MMSE score	17.6 (2.7) [8]	20.4 (5.6) [33]	0.054
Hoehn and Yahr scale score	3.8 (1.1) [10]	3.9 (1.1) [47]	0.771
BMI, kg/m^2	25.4 (4.3) [5]	23.7 (5.1) [31]	0.467
Brain weight, g	1,398 (141) [11]	1,410 (166) [59]	0.800
Concurrent AD pathology, Y/N	6/6	37/24	0.534
SNc neuron count, n	52.0 (23.0) [12]	72.9 (42.1) [61]	0.022
SNc area, mm^2	35.0 (6.4) [12]	32.3 (7.9) [61]	0.220
SNc neuron density, n/mm^2	1.52 (0.65) [12]	2.32 (1.33) [61]	0.004

Values are the mean (SD) [n].

[a]Independent samples t test, Mann–Whitney U test, or Fisher exact test.

AD = Alzheimer's disease; BMI = body mass index; LEDD = levodopa equivalent daily dose of antiparkinsonian drugs; MMSE = Mini Mental State Examination; SNc = substantia nigra pars compacta.

Movement Disorder Society (MDS) clinical criteria on the basis of clinical examination. Thus, all clinical PD diagnoses in the present cohort were based on these criteria. In addition, other patients with parkinsonism phenotype were diagnosed by neurologists. Patients with a dementia dominant phenotype (n = 20) had been diagnosed and treated by neurologists, geriatricians, or general practitioners. The study was approved by the local ethics committee.

Statistical Analyses

SPSS Statistics 25 (IBM, Armonk, NY, USA) was used for statistical analyses. Differences between 2 groups were analyzed with independent samples *t* test (normal distribution) or Mann–Whitney *U* test (non-normal distribution) for continuous variables, as appropriate. Differences among 3 groups were analyzed with 1-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA; normal distribution), or Kruskal–Wallis test (non-normal), and dichotomic variables were analyzed by Fisher exact test or chi-square test. The normality of distribution was evaluated with histogram and Shapiro–Wilk test. The p values < 0.05 were considered statistically significant.

Results

Out of the 73 included patients, 12 were confirmed to have clinical depression. In patients without depression, the SN neuron density was 52.0% higher than that in patients with depression (p = 0.004; see Table 1, Fig.). There were no significant differences in other variables between groups (Table 1). The clinical characteristics of patients with depression are presented in Table 2. The difference in SN neuron density between patients with and without depression remained significant when patients without parkinsonism (dementia as the clinical syndrome) were excluded from the analysis (n = 10 vs 43, p = 0.02) and when MMSE scores were used as a covariate in the analysis (F = 5.18, p = 0.029). The total duration of depressive symptoms was unknown for most patients, but 9 out of 12 patients had recorded depression within 2 months before death (37, 35, and 47 months in the remaining 3 patients). The difference in nigral neuron density remained significant when the 3 patients with longer intervals were excluded from the analysis (p = 0.038). Clinical phenotypes included 23 patients with parkinsonism-predominant, 20 patients with dementia-predominant, and 30 mixed patients (Supplementary Table S1).

TABLE 2. Clinical Characteristics of Patients with Depression

No.	Age at death, yr	Sex	Clinical diagnosis	Clinical syndrome	Symptom onset to death, yr	HY	MMSE	Antiparkinsonian medication	LEDD, mg
1	68	M	PD	Parkinsonism	4.5	2	—	LDC, PRX	810
2	75	M	PD	Parkinsonism + dementia	20	4	19	LDCE	599
3	77	M	PD	Parkinsonism + dementia	—	4	—	LDC	400
4	68	F	Atypical parkinsonism	Parkinsonism + dementia	12	4	17	LDCE, PRX	906
5	74	M	Undetermined	Dementia	—	5	20	—	0
6	85	F	PD	Parkinsonism + dementia	5.25	—	—	LDC	150
7	81	F	PD	Parkinsonism + dementia	4	2	16	LDC	250
8	82	M	PD	Parkinsonism + dementia	8	5	18	LDC	600
9	86	F	PD	Parkinsonism + dementia	—	4	14	LDB	250
10	67	F	Undetermined	Dementia	—	5	22	—	0
11	75	M	PD	Parkinsonism	6	4	—	LDC	500
12	80	M	PD	Parkinsonism + dementia	2.5	3	15	LDC	500
Mean (SD)	77 (6.6)	—	—	—	7.7 (5.8)	4 (1.1)	18 (2.7)	—	414 (294)

Data are from the time of death or the last recorded visit before death.
HY = Hoehn and Yahr scale; LDB = levodopa + benserazide; LDC = levodopa + carbidopa; LDCE = levodopa + carbidopa + entacapone; LEDD = levodopa equivalent daily dose; MMSE = mini-mental state examination; PD = Parkinson's disease; PRX = pramipexole.

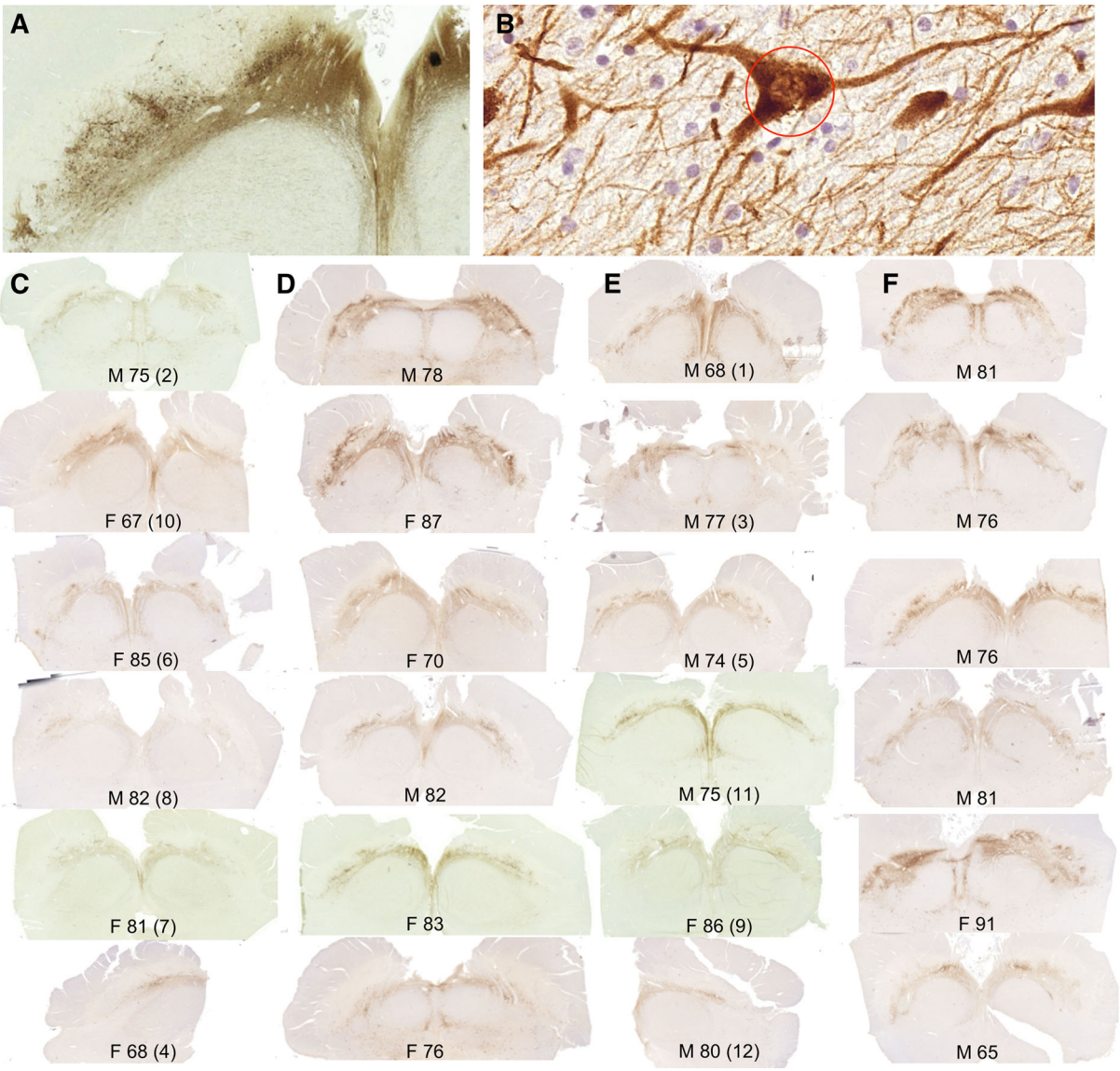

FIGURE: Cell counting protocol (A, B) and individual nigral sections in patients with depression and representative age and sex-matched patients without depression (C–F). (A) Substantia nigra (SN) pars compacta margin was delineated from the scanned tissue sample (tyrosine hydroxylase staining, 1.0 times magnification) using Luxol Fast Blue staining for myelin (not shown), (B) Each tyrosine hydroxylase positive neuron was counted from an 8 micrometer section using high magnification 40 to 63 times (level at the origin of the third cranial nerve). Only neurons with visible nucleus were counted (*red circle*) and diameters of nuclei were used for correction as described.[17] (C, E) Patients with depression. (D, F) Patients without depression. Sex (M/F) and age at death are shown for each patient. For patients with depression, the number refers to the patient number in Table 2.

Discussion

Our results show that patients with LBD with comorbid depression have lower dopaminergic neuron densities in the SN than nondepressed patients. The relative difference was large (> 50%), and it was not explained by differences in cognition, motor symptoms, antiparkinsonian medication doses, or disease duration.

A key function of the SN is to control motor behavior via nigrostriatal dopaminergic innervation. The view of the SN as a pure motor center may, however, be overly simplistic because there are multiple roles of dopamine in the modulation of nonmotor functions[19] and because there are specific neuronal interdependencies of the dopaminergic subcortical–cortical motor system with nonmotor cortical affective and cognitive systems.[20] Specifically, and relevant to the possible role of the SN in depression, there are complex interactions between raphe nucleus serotonin and SN dopamine.[21] Our results expand previously demonstrated dopaminergic changes in nondemented elderly individuals who died without clinically diagnosed LBD. Wilson and colleagues demonstrated in a sample of 124 elderly American subjects that the density of mesencephalic dopaminergic neurons correlated negatively with the level of depressive symptoms antemortem.[12] In our study, with a sample of 73 Finnish patients

with LBD, we observed that the dopaminergic neuronal density in the SN was clearly lower in patients who had depressive symptoms prior to death. These findings are also in line with results from a PD animal model, as bilateral dopamine loss within the SN has been reported to cause motivational deficits and affective impairments in 6-OHDA rats that resemble neuropsychiatric symptoms observed in PD.[22] A limitation of the present study is the retrospective nature of data collection, which resulted in missing data for some clinical variables, and the lack of more detailed disease severity measurements, such as the Unified Parkinson's Disease Rating Scale or quantitative depression measurements. As a retrospective clinicopathological investigation, the study is also limited by inevitable delays between clinical and neuropathological finding, and postmortem patients in a specialized university hospital institute do not necessarily fully represent common outpatient PD or DLB cases. Further prospective studies with more detailed clinical evaluations and stereological cell counting methods for both the SN and ventral tegmental area are needed.

To conclude, previous studies in nondemented non-PD elderly individuals and an animal model have suggested a relevant role of SN dopaminergic neurons in the modulation of affective symptoms. Our results in patients with LBD demonstrate that the degeneration of dopaminergic neurons is much more severe in those who suffer from depression during their neurological disease. The combined results point to a key role for the SN in the modulation of mood in patients with LBD.

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Author Contributions

V.K. contributed to the conception and design of the study. L.S., L.H., M.G., and V.K. contributed to the acquisition and analysis of data. L.S. and V.K. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

The authors declared no conflict of interest.

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