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Morphometric Characteristics of Cell Structures in the Substantia Nigra in Humans

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Neurons and glial cells were studied by computerized morphometry in segments of the ventral and dorsal areas of the compact zone of the substantia nigra (CZSN) of the brain in autopsy material obtained from six men and three women aged 52–87 years. Neuron body and nuclear sizes were found to be greater in segments of the ventral area of the CZSN than in segments of the dorsal area. Neuron and glial cell densities in the ventrolateral segment were higher than in segments of the dorsal area. The glial index in the ventromedial segment was lower than in segments of the dorsal area. These morphometric differences between CZSN segments need to be considered for assessment of morphological changes in the substantia nigra associated with both age-related involution and pathological processes.

Keywords: human brain, substantia nigra, morphometry, neurons, neuroglia.

Considerable attention is currently being paid to studies of different aspects of of ageing and diseases linked with old age, among which an important place is held by Parkinson's disease, whose clinical manifestations result from the selective death of dopaminergic neurons in the substantia nigra (SN) of the brain [7, 8]. Thus, investigations of the structural-functional heterogeneity of the SN in the human brain are of interest [1, 12], and in particular the quantitative assessment of cellular structures of the SN in both healthy people and those with Parkinson's disease to provide a basic understanding of the location and distribution of the pathological process. The main quantitative indicators of nerve cells and glial elements in human SN structures have thus far been presented in only a few separate studies [5].

The aim of the present work was to investigate the morphometric parameters of nerve and glial elements in structures of the compact zone of the substantia nigra (CZSN) of the human brain.

Materials and Methods

Autopsy brain specimens were obtained from neurologically healthy patients (six men and three women) wo died from intercurrent diseases at age 52–87 tears; specimens were fixed in 10% formalin, dehydrated, and embedded in paraffin, and frontal sections of thickness 10 μm were cut at the level of the CZSN and stained with cresyl violet. SN structures were examined under a Motic SMZ-161 stereo microscope (Hong Kong); cellular elements were studied under a Leica DMLB microscope (Leica Microsystems, Germany) fitted with a digital video camera and analyzed with the Leica QWin computer video image analysis system.

The number of neurons and the total number of glial cells were determined in individual segments (groups of neurons) in the ventral and dorsal areas of the CZSN [6]. The ventral area included the ventromedial (VMS), ventrolateral (VLS), and intermediate (IS) segments, while the dorsal area included the dorsomedial (DMS) and dorsolateral (DLS) segments, as well as the lateral subarea (LS) (Fig. 1). As the ventral area of the VLS and the IS were not clearly demarcated from each other, they were combined into a single segment (VLS + IS).

Neurons and glial cells in CZSN structures were counted per microscope field (objective ×40, ocular ×10) and numbers per unit area (0.01 mm^2) were then calculated, i.e., cell densities were determined. At least 25 fields were examined in each case. The glial index, i.e., the gliocyte:neuron density ratio, was calculated. The cross-sectional areas

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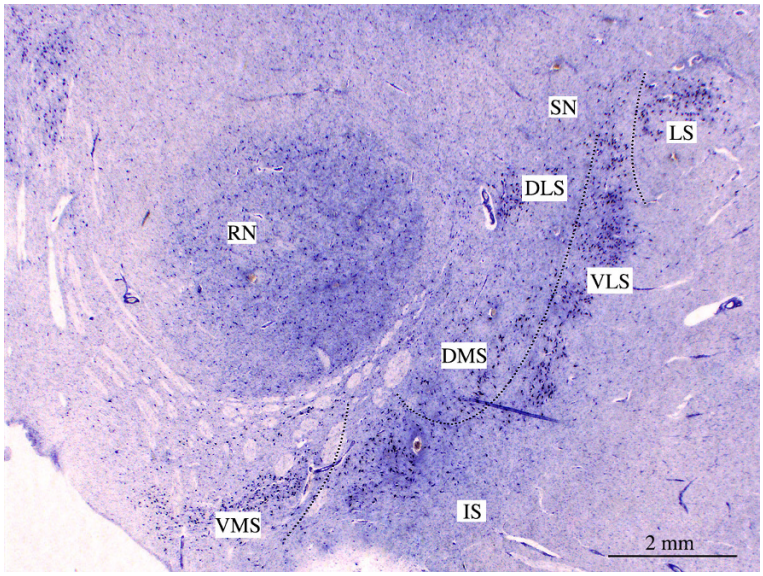

Fig. 1. Distribution of groups of neurons in the compact zone of the substantia nigra of the human brain. RN – red nucleus; SN – substantia nigra; VMS – ventromedial, IS – intermediate, VLS – ventrolateral, DMS – dorsomedial and DLS – dorsolateral segments; LS – lateral subarea. Stained with cresyl violet.

TABLE 1. Morphometric Characteristics of Cellular Structures in Different Segments of the Compact Zone of the Substantia Nigra of the Human Brain

Segments of the substantia nigra studied	Segment No.	Density, Me [LQ; HQ]		Glial index
		neurons	glial cells	
VMS	1	14 [10; 17] *3	81 [76; 85.25] *2, 3, 5	5.79
VLS + IS	2	15 [12.25; 19.75] *3, 4, 5	90 [86.5; 97.5] *3, 4	6
DMS	3	9 [7; 12] *4	71 [66; 77] *5	7.89
DLS	4	12 [10; 15]	77 [72.5; 82] *5	6.42
LS	5	11 [9; 14.25]	87,5 [83.25; 91.75]	7.95

*Significant differences compared with values in same-numbered segments, $p < 0.05$. Here and Table 2: VMS – medial segment; VLS + IS – lateral and intermediate segments of the ventral area; DMS – medial segment, DLS – lateral segment, LS – lateral subarea of the dorsal area. Variation parameters: Me – median, LQ = 1st quartile; HQ = 3rd quartile.

(μm^2) of neuron bodies and nuclei were determined in CZSN segments with a ×100 objective and a ×10 ocular (at least 100 cells in each segment).

Data were analyzed statistically in SigmaPlot-12.0. Statistical significance was assessed (except for the glial index) by unifactorial analysis of variance (ANOVA on ranks) using the Kruskal–Wallis H test. A posteriori analysis was with Dunn's Q test, comparing values for each parameter with the critical value (Q_k).

Results

These studies showed that the SN could be followed on serial frontal sections throughout the human midbrain. It was apparent as an extended cord, curved in the dorsolateral direction and located between the red nucleus and the base of the cerebral peduncles. Areas with high and low cell density were identified in the CZSN. The CZSN had a dorsal part and a wider ventral part. Neurons in both parts were gathered into groups which in turn formed segments, with three segments in each area (see Fig. 1).

Morphometric studies showed that the mean neuron density per 0.01 mm^2 was 13.2 ± 0.3 and the mean gliocyte density was 83.3 ± 0.8 cells.

Comparison of the group values for neuron distributions in CZSN segments revealed a significant difference ($H = 43.94, p < 0.001$, ANOVA on ranks). Neuron density in the VLS + IS of the ventral area (Table 1) was significantly

TABLE 2. Cross-Sectional Areas of Neuron Bodies and Nuclei in Different Segments in the Compact Zone of the Substantia Nigra of the Human Brain, Me [LQ; HQ]

Segments of the substantia nigra studied	Segment No.	Neuron body, μm²	Neuron nucleus, μm²
VMS	1	534.62 [398.55; 698.3] *3, 4, 5	201.95 [156.64; 262.01] *3, 4, 5
VLS + IS	2	583.46 [431.28; 715.76] *3, 4, 5	202.91 [162.76; 258.29] *3, 4, 5
DMS	3	389.57 [293.01; 632.61] *4	148.49 [111.5; 196.74] *4, 5
DLS	4	501.11 [339,39; 655,29]	176.84 [139.93; 221.84]
LS	5	481.5 [337.21; 660.42]	171.64 [133.98; 222.53]

greater than in segments of the dorsal area (compared with the DMS, Q = 6.18; with the DLS segment, Q = 3.86; with the LS, Q = 2.96; $Q_k = 2.81$; $p < 0.05$ for all comparisons, while density in the VMS of the ventral area was significantly greater than in the DMS segment of the dorsal area (Q = 4.68; $Q_k = 2.81$; $p < 0.05$). Comparison of this parameter in different segments in a given CZSN area in the ventral area revealed no differences, while in the dorsal area differences were seen only between the DLS and DMS: neuron density in the DLS was significantly higher than in the DMS (Q = 3.21; $Q_k = 2.81$; $p < 0.05$).

Comparison of group glial cell density values in CZSN segments revealed significant differences (H = 95.92, $p < 0.001$, ANOVA on ranks). Comparison of glial cell density by segment in different areas of the CZSN showed differently directed changes (see Table 1). Thus, glial cell density in the VMS of the ventral area was significantly greater than that in the DMS (Q = 3.17; $Q_k = 2.81$; $p < 0.05$) and significantly lower than that in the LS (Q = 3.23; $Q_k = 2.81$; $p < 0.05$), but not significantly different from the value in the DLS. Glial cell density in the other segment of the ventral area (the VLS + IS) was higher than in the DMS (Q = 8.36; $Q_k = 2.81$; $p < 0.05$) and the DLS (Q = 6.79; $Q_k = 2.81$; $p < 0.05$), but was not significantly different from that in the LS segment. Comparison of glial cell density in segments within each area showed that in the ventral area, this value was significantly greater in the VLS + IS than the VMS (Q = 5.26; $Q_k = 2.81$; $p < 0.05$), while in the dorsal area it was significantly greater in the LS than the DMS (Q = 6.31; $Q_k = 2.81$; $p < 0.05$) and the DLS (Q = 4.72; $Q_k = 2.81$; $p < 0.05$).

The glial index, which characterizes the ratio of glial cells to neurons, was greater in segments of the dorsal area than in segments of the ventral area (see Table 1). The greatest difference between values in segments in this area was by 37%, as, for example, between the VMS in the ventral area and the LS of the dorsal area, while the smallest difference, by 7%, was seen between values in the VLS + IS in the ventral area and the DLS in the dorsal area.

Comparison of neuron body cross-sectional area in CZSN segments by unifactorial analysis of variance revealed significant differences (H = 81.9, $p < 0.001$, ANOVA on ranks). Comparison of this parameter in segments in different areas of the CZSN showed that it was significantly greater in segments of the ventral area than the dorsal (Table 2). Segment-by-segment comparison within each area showed that differences were minor in the ventral area and were present only between the DLS and the DMS in the dorsal area: neuron body cross-sectional area was significantly greater in the DLS than the DMS (Q = 2.87; $Q_k = 2.81$; $p < 0.05$).

Comparison of neuron nucleus cross-sectional area in different areas of the CZSN by unifactorial ANOVA revealed significant differences between these (H = 81.9, $p < 0.001$, ANOVA on ranks). In the segments of the ventral area, this parameter was significantly greater than in the dorsal area (see Table 2). There were no differences between segments in the ventral area, though differences were seen in the dorsal area: neuron nucleus cross-sectional area was significantly greater in the DLS and LS than in the DMS (Q = 4.29 and 2.87, respectively; $Q_k = 2.81$; $p < 0.05$).

Discussion

These studies identified differences in parameters such as neuron and glial cell density, the glial index, and neuron body and nucleus cross-sectional areas, which were clearly apparent on comparison of segments in different areas of CZSN. Thus, the VLS + IS was significantly differemt from the other segments of the dorsal area (DMS, DLS, and LS) in terms of all study parameters. The only exception was the LS, for which there was no significant difference in glial cell density from the VLS + IS. The other segment of the ventral area – the VMS – was significantly different from the segment of the dorsal area – the DMS – in terms of each of the study parameters, while comparison with the LS identified a difference only in terms of neuron density. The VMS also differed significantly from the DLS – in terms of neuron body and nucleus cross-sectional area.

Thus, each of the segments of the ventral area differed significantly from each of the segments in the dorsal area, particularly by the large sizes of neuron bodies and nuclei.

Our results from studies of neuron density in individual segments of the CZSN were not significantly different from analogous measures in elderly people reported by other investigators [10]. At the same time, our data indicate that

neuron body cross-sectional area in the CZSN was greater than in brain specimens from Indians of both the elderly age group (60–89 years) and the adult age group (30–59 years) [2]. This noncorrespondence may, according to the data reported by these authors [2], be due to the way CZSN neuron structures are organized in Indians: neuron density in these structures was higher than in Europeans and North Americans, and like other basic morphometric indicators of CZSN neurons, did not change significantly with age. In Europeans [3] and North Americans [4], age is associated with decreases in the numbers of neurons in individual CZSN populations (the number of neuromelanin-containing neurons in Europeans and the number of dopamine-containing neurons in North Americans), while neuron size increased, which some authors link with their hypertrophy [3] and others with neuromelanin accumulation [11].

Published data indicate that CZSN neuron death in Parkinson's disease due to neurodegeneration is more characteristic of segments of the ventral area – the VMS [6] and the VLS [10] – than segments of the dorsal area.

Our results provide evidence that neuron body and nucleus sizes in neurologically healthy people are significantly larger in segments of the ventral area than in those of the dorsal area, i.e., the neurodegenerative process is, as noted above, more intense in segments of the ventral area. It is well known that the central component of cellular metabolism is the nucleus, and nucleus size significantly reflects the functional and metabolic activity of the neuron. In this situation, it can be suggested that neurons in the ventral area of the CZSN are characterized by high functional activity. At the same time, as shown by our studies, the number of glial cells per neuron (the glial index) in the ventral area is overall smaller than that in the dorsal area. Considering that the neuroglia support nerve tissue homeostasis and normal neuron functioning [9], the "deficit" in glial cells in segments of the ventral area seen here in normal conditions may, in pathology, promote selective loss of the functional activity of the neurons forming this area.

Thus, morphometric study of the CZSN of the human brain (autopsy material) at age 52–87 years showed quantitative differences between structures in its ventral and dorsal areas (segments) in terms of parameters such as neuron and glial cell density and the cross-sectional areas of neurons and their nuclei, which need to be considered for objective assessment of morphological changes in the SN of the brain, due to age-related involution and pathological processes.

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