

**ПРОСТРАНСТВЕННАЯ РЕКОНСТРУКЦИЯ КОМПАКТНОЙ ЧАСТИ
ЧЕРНОГО ВЕЩЕСТВА ГОЛОВНОГО МОЗГА ЧЕЛОВЕКА**

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Обоснование. До настоящего времени нет общепринятой схемы пространственной организации групп нейронов компактной части черного вещества (ЧВ, *substantia nigra*) среднего мозга человека. Детальное исследование цитоархитектоники этого образования необходимо для патоморфологического анализа изменений, происходящих в нервной ткани с возрастом, и развивающихся при этом нейродегенеративных заболеваний, сопровождающихся избирательной гибелью дофаминовых нейронов. **Цель.** Уточнить особенности морфохимической организации ЧВ мозга человека и провести пространственную реконструкцию структур его компактной части. **Материалы и методы.** На аутопсийном материале мозга людей без неврологической патологии (n=10, возраст от 52 до 84 лет) методом компьютерной морфометрии произвели пространственную реконструкцию компактной части ЧВ, используя срезы среднего мозга, окрашенные по Нисслию и иммуногистохимически – для локализации тирозингидроксилазы – маркера дофамина. **Результаты.** Выявили в компактной части ЧВ скопления нейронов в форме 9 тяжей, ориентированных в rostro-каудальном направлении, которые представили в виде 4 областей: медиальной, латеральной, дорсальной и вентральной. Морфометрический анализ обнаружил значимые различия в плотности расположения нейронов и показателях экспрессии тирозингидроксилазы между областями ЧВ. **Заключение.** Схема клеточной организации компактной части ЧВ, предложенная нами на основании трехмерной реконструкции, отличается высокой детализацией по сравнению с подобными работами и демонстрирует выраженную пространственную дифференцировку групп нейронов ЧВ, что необходимо учитывать при патоморфологических исследованиях.

Ключевые слова: мозг человека, черное вещество, средний мозг, дофаминовые нейроны, трехмерная реконструкция.

**THREE-DIMENSIONAL RECONSTRUCTION OF SUBSTANTIA NIGRA PARS
COMPACTA OF HUMAN BRAIN**

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Background. Up to the moment there is no universally accepted scheme of spatial organization of the groups of neurons of *substantia nigra pars compacta* of the human midbrain. A detailed study of the architectonics of this structure is necessary for pathomorphological analysis of age-related changes in the nervous tissue and the associated neurodegenerative diseases with selective death of dopamine neurons. **Aim.** To clarify the peculiarities of the morphochemical organization of the substantia nigra (SN) of a human brain and to create a



three-dimensional model of pars compacta. **Materials and methods.** Three-dimensional reconstruction of substantia nigra pars compacta was performed on the brain autopsy material of individuals without neurological pathology (n=10, between 52 to 84 years of age) using a method of computed morphometry. Sections of the midbrain were stained by Nissl method and by an immunohistochemical method for localization of tyrosine hydroxylase – a marker of dopamine. **Results.** In the SN pars compacta accumulations of neurons were identified in the form of 9 bands oriented in the rostro-caudal direction and including four areas: medial, lateral, dorsal and ventral. Morphometric analysis detected significant differences in the density of neurons and in expression of tyrosine hydroxylase between the areas of SN. **Conclusion.** A model of cellular organization of SN pars compacta proposed by us on the basis of three-dimensional reconstruction is characterized by a high degree of detalization as compared to similar works, and shows expressed spatial differentiation of the groups of neurons of SN which should be taken into consideration in pathomorphological examinations.

Keywords: human brain, substantia nigra, midbrain, dopamine neurons, three-dimensional reconstruction.

It is known that physiological ageing and Parkinson disease (PD) are manifested by morphological alterations of the substantia nigra (SN) of the brain. Here, age-related involution of SN is characterized by natural reduction in the amount of neurons [1], while Parkinson disease is associated with a selective death of dopamine neurons in pars compacta [2]. Intensity and uniformity of quantitative changes of neurons in the structures of SN both in physiological ageing and in pathology – PD – can be evaluated by a morphometric examination. Here, in-depth evaluation of quantitative changes of cell structures of SN is based on examination of the respective parameters of separate aggregations of neurons that make up this structure of the brain. In this context, different variants of division of SN to separate cellular structures were proposed. In one variant SN was divided to two zones – black (pars compacta) represented by 21 groups of pigmented neurons, and red (pars reticulata), consisting of poorly pigmented cells [3]. Other authors combined dopamine neurons located in the region of the midbrain into 6 separate groups [4], and arranged dopamine neurons of substantia nigra into groups according to nigrosomes – zones of immunoreactivity of calbindin (calcium-binding protein present in nigrostriatal afferent fibers and in cell neuropil) [5]. Besides, there were proposed schemes for division of

SN pars compacta of a human brain into regions accessible for studying by morphometric methods [6].

On the basis of similar schemes and classifications, distribution of cholinergic [7] and dopaminergic [8] neurons in SN of the brain was analyzed in laboratory animals, and models of spatial structural organization of SN were described. In the meanwhile, we did not find any information concerning similar three-dimensional models of organization of neuronal structures of SN pars compacta in a human brain in the available literature.

The aim of work was to clarify peculiarities of morphochemical organization of SN of a human brain and to obtain a spatial reconstruction of the structures of its pars compacta.

Materials and Methods

Neuronal structures of SN were examined on the autopsy brain of individuals without neurological pathology in the anamnesis who died from intercurrent diseases at the age from 52 to 84 (10 cases). The average age was 71 years old. The material was obtained from the collection of the Laboratory of functional morphochemistry of the Federal State Budget Scientific Institution “Research Center of Neurology” (FSBSI RCN). The protocol of the research was approved by Local Ethical Committee of FSBSI RSCN (Protocol №6-3/17 of 25.05.2017).

Specimens of the midbrain selected for research were fixed in 4% formalin solution, subject to standard histological processing and embedded into paraffin blocks, which were arranged into successive series of frontal sections 10 μ in thickness. A part of sections was stained with 1% solution of cresyl violet by Nissl method, the other part was used for immunohistochemical reactions. Dopamine neurons were identified by immunohistochemical methods by localization of tyrosine hydroxylase (TH) using polyclonal rabbit's antibodies to tyrosine hydroxylase (Sigma, № T8700) in 1:1500 dilution. Binding of antibodies was determined using Thermo Fisher Ultra Vision set on the basis of the polymer system of detection with alkaline phosphatase. Staining was performed according to the protocols of antibody manufacturers. Preparations were studied and documented using microscope Leica DMLB (Germany) equipped with digital camera Leica DC300 (Germany).

For three-dimensional reconstruction and morphometry, series of 25-35 sections were selected from the caudal two thirds of SN with 0.2 mm interval between series. Stained brain sections were scanned using Plustek Opticfilm 8200i slide-scanner (China) with 3600 dpi resolution. For three-dimensional reconstruction, the series of sections were aligned according to their anatomical landmarks and processed using Image J program (free software, National Institutes of Health, USA). The obtained images were used for localization of groups of cells in the examined structures. From the images of sections stained with cresyl violet, maps of distribution of neurons were obtained, with manual marking of neuron somas using Wacom graphic pad. Images of sections stained for tyrosine hydroxylase, were segmented by brightness for identification of boundaries of dopaminergic structures. Using Free-D program (ex-commercial software, Institut Jean-Pierre Bourgin, France) [9], the areas with the highest local density of neurons in SN and boundaries of other structures of the midbrain were marked in the images, after

which three-dimensional reconstruction was conducted on the basis of the obtained contours of cell aggregations. The obtained three-dimensional model of SN was smoothed and its final image was created in Blender program (free software, Blender Foundation, the Netherlands). The applied methods were described by us in more detail earlier [10].

Morphometric examination was carried out using Leica QWin Standart v.2.6 program (licensed software, Leica Microsystems Imaging Solution, Serial №4563, Great Britain). The density of localization of neurons in the structures of pars compacta was determined on sections stained by Nissl method (objective x40, ocular x10). For this, neurons were counted in the field of microscope through the entire depth of the section, with recalculation of their number was per unit volume (0.1 mm³). Besides, in the same structures the intensity of immunostaining for TH was evaluated in standard units (from 0 to 225). The work was carried out on 8-bit images obtained with x4 magnification of the objective, taking into account the background staining, with calculation of median values in the group at different levels along the rostro-caudal axis.

The obtained results were processed in GNU PSPP v.1.01 program (free software, Free Software Foundation, USA). The density of arrangement of neurons in the structures of pars compacta of SN was compared using Student test for comparison of groups with pairwise independent variants.

Results and Discussion

In unstained macropreparations – frontal sections of the midbrain – structures of SN were identified as a separate band of dark brown color (Fig. 1,a). With Nissl method of staining two zones of aggregations of neurons were distinguished (Fig. 1,b): one adjoined the cerebral peduncle and was more densely packed with cells, the other was located downward and laterally from the red nucleus. The boundaries of SN were determined by localization of dopamine neurons and their processes (Fig. 1,c), and position of SN in relation to other structures of the brainstem

was determined by three-dimensional reconstruction on the basis of sections of the mid-brain stained for TH (Fig. 2,a).

In the obtained three-dimensional model of spatial organization of SN constructed on the basis of sections of the midbrain stained by Nissl method, pars compacta of SN (Fig. 2,b) was represented by 9 bands consisting of aggregations of groups of neurons and

oriented in rostro-caudal direction. In projection of this model on a plane, four areas in SN pars compacta were identified: medial, lateral, dorsal and ventral (Fig. 2,b).

Morphometric examination of the density of neurons in the areas of SN pars compacta identified by us, showed higher density in the medial and ventral areas than in lateral and dorsal ones (Fig. 3,a).

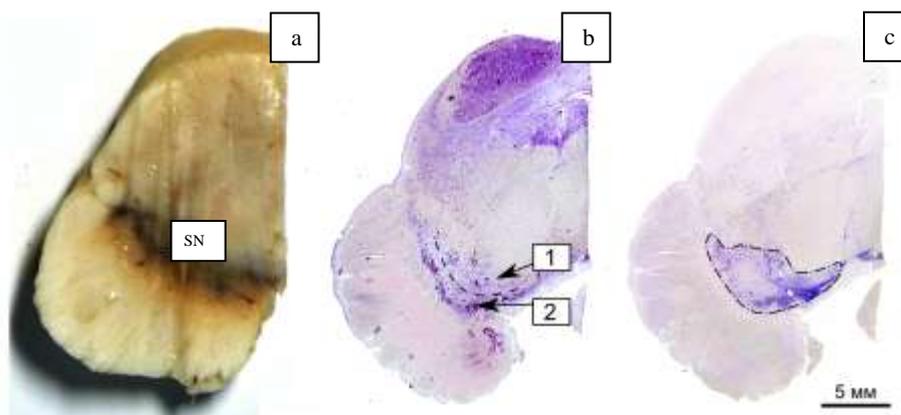


Fig. 1. Localization of substantia nigra in the cross section of a human midbrain.
 a – a macropreparation fixed with formalin; b – staining by Nissl method;
 c – immunohistochemical identification of tyrosine hydroxylase; SN – substantia nigra;
 1 – ventral area with sparsely packed neurons; 2 – dorsal area with densely packed neurons;
 dotted line – the boundaries of substantia nigra pars compacta

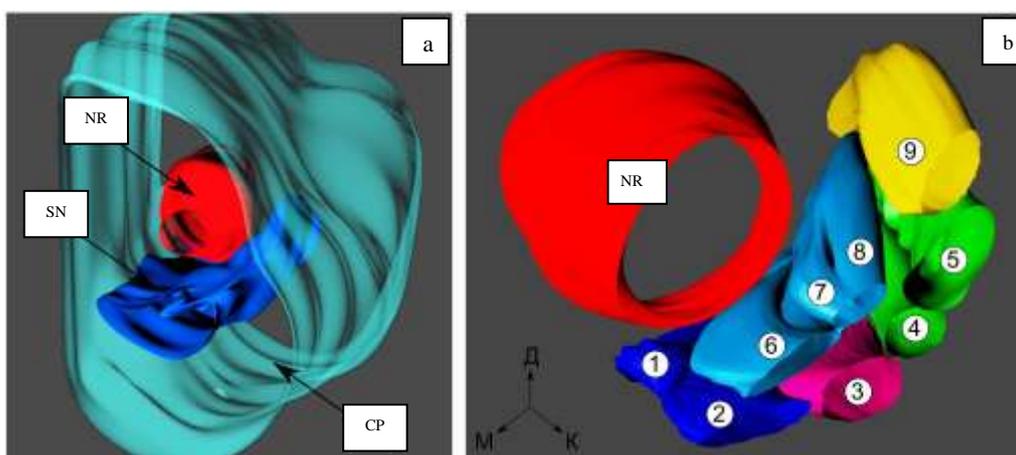


Fig. 2. Three-dimensional reconstruction of substantia nigra of a human midbrain.
 a – boundaries and relative arrangement of dopaminergic structures of substantia nigra in the midbrain by localization of tyrosine hydroxylase (blue); b – spatial arrangement of aggregations of neurons in the caudal two thirds of substantia nigra stained with cresyl violet. SN – substantia nigra; CP – cerebral peduncle; NR – nucleus ruber. Groups of neurons of substantia nigra (1-9):
 medial area – 1,2; dorsal area – 6-8; ventral area – 3-5; lateral area – 9

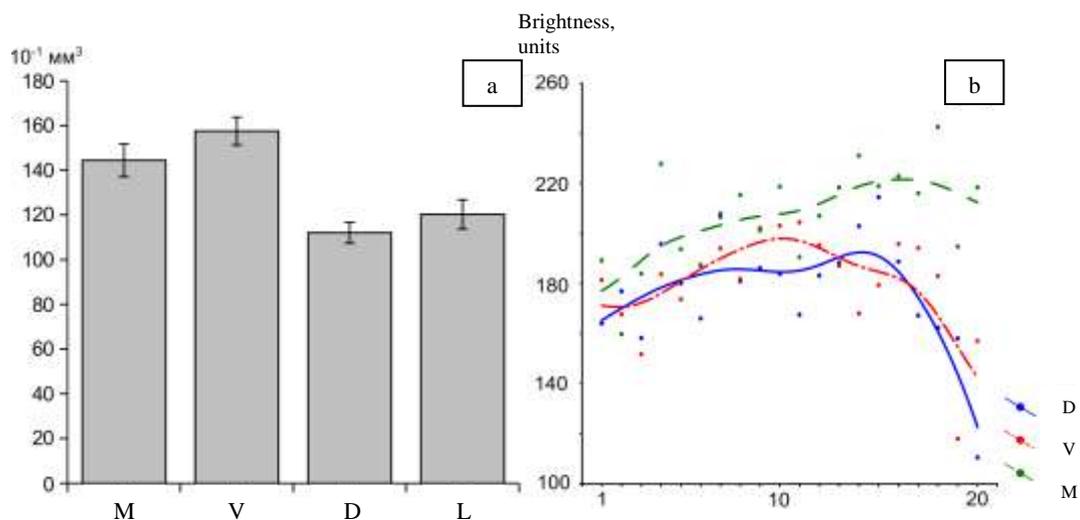


Fig. 3. Quantitative parameters of substantia nigra areas.

a – distribution of density of neurons per 0.1 mm³; b – distribution of intensity of staining for tyrosine hydroxylase (in standard units of brightness) in the caudal-rostral direction (the abscissa shows the numbers of sections with 0.2 mm interval). D – dorsal area; V – ventral area; M – medial area; L – lateral area

Evaluation of the intensity of immunohistochemical staining for TH in these areas (except for lateral area) revealed the highest expression of TH in the medial area of SN (Fig. 3,b).

The conducted research permitted to clarify spatial localization of aggregations of groups of neurons in SN of the brain of elder individuals and to divide SN pars compacta to separate areas accessible for studying by methods of mathematical morphology. Three-dimensional reconstruction of substantia nigra of the brain of rats and mice demonstrated similar cytoarchitectonic division of SN pars compacta of rodents into three or four regions depending on if authors identify the medial area [8,11]. Here, groups of dopamine receptors of SN of rodents were probably less differentiated in comparison to humans that was manifested by the absence of clearly discernable segmental division of parts of SN and was confirmed by the given work and by other authors [12].

According to the results of our work, the pars compacta of SN of humans consists of 9 bands (aggregations of groups of neu-

rons) which, being compared with the literature data [13], correspond to the following segments: bands 1 and 2 – to ventromedial segment, band 3 – to intermediate segment, bands 4 and 5 – to ventrolateral segment, band 6 – to dorsomedial segment, bands 7 and 8 – to dorsolateral segment. Band 9 corresponds to the lateral part of the dorsal area. Three-dimensional model of SN of the brain proposed by us does not show complete correspondence with other variants of SN organization [14,15] which is manifested by a higher specification of structures of this part of the brain identified by us. This discrepancy is probably due to the fact that one of the above mentioned variants of division of SN suggested study of immunoreactivity zones of calbindin, and both variants did not suggest use of methods of three-dimensional reconstruction.

Morphometric examination of SN pars compacta showed the higher density of neurons in the medial area than in other areas of SN pars compacta (except for ventral area), and intensity of staining for TH was highest in the medial area which agrees with the results obtained by other authors [13]. In view

of this it can be suggested that in elderly individuals involuntal alterations to a lesser extent involve the medial area of this brain structure. According to literature data, different groups of dopamine neurons of the mid-brain differ not only in location and connections, but also in vulnerability to damaging actions in experiment, in neurodegenerative pathology and in physiological ageing [16]. The causes of this are not completely clear, but it is suggested that the basis of selective resistance of separate groups of dopamine neurons is their different neurochemical pro-

file that preconditions their susceptibility to oxidative stress [17].

Conclusion

Thus, the spatial structural morphochemical organization of SN of a human brain is characterized by aggregations of neurons along the full length of SN forming 9 bands oriented in rostro-caudal direction, which, being projected onto a horizontal plane, forms 4 areas: medial, lateral, dorsal and ventral. Morphometric examination of these areas found that the medial area of SN is least susceptible to age-related involution.

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