AGE-RELATED STRUCTURAL CHANGES IN THE COMPACT PART OF THE SUBSTANTIA NIGRA IN HUMAN BRAIN

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The study of various structural and functional changes in human brain caused by normal aging and natural environmental factors (infectious diseases, toxic agrochemicals, increased background radiation, etc.) is an actual task of developmental biology. Environmental factors have a significant influence on the age involution. Their combination with genetic factors may contribute to the pathology that occurs in the elderly [2]. One of the most common diseases associated with old age is Parkinson's disease (PD) [1]. Structural changes in the brain during aging and PD are similar in many aspects because in both cases there is a death of dopamine neurons. Nerve cells reduction in PD occurs primary in the ventral region of compact part of substantia nigra (SNc) and reachs up to 98% [4]. Neuronal death in the substantia nigra by physiological aging is not exactly localized and its scale is not fully elucidated: according to some authors the number of nerve cells decreases about 10% [7], according to other [5] - 33%.

Objective: To study age-related morphometric properties of neurons in the compact part of the substantia nigra in human brain during physiological aging.

Methods

Brain autopsy of neurologically healthy patients (12 cases) who died of intercurrent disease in age 52 -87 years. Brain autopsy material was grouped on age factor (mature age - 3 men and 1 woman, older - 2 men and 2 women, and old age - 2 males and 2 females). Study samples of the brain were subjected to standard histological processing and enclosed in paraffin blocks and were laid on SNc level in 10 microns thickness frontal sections. For morphological control the sections were stained with cresyl violet, tyrosine hydroxylase (a marker for dopamine neurons) revealed by immunohistochemical reaction (ABC method) using reagents «Sigma-Aldrich» (Germany) following the recommendations of the manufacturer. The specimens were studied on a microscope «Leica» (model «DMLB», Germany) equipped with a digital video camera and a computer video analysis system «Leica QWin».

The overall density of neuronal structures in SNc was determined by

counting the number of nerve cells in microscope field of view (X40, X10). Then the number was calculated on 0.01 mm2. Dopamine neuronal density was determined similar. There were examined at least 25 fields of view in each case.

The area of neurons bodies and nuclei were measured in a microscope field of view, which was displayed on a monitor. In each case we examined at least 100 cells.

Neurons morphometry was performed in selected segments (nerve cells clusters) in ventral and dorsal SNc regions in accordance with histological nuclei topography [5]. The ventral region was divided into medial (VM), intermediate (I) and lateral (VL) segment and in the dorsal - into medial (DM), lateral (DL) segments and lateral subregion (LS). Due to the fact that the intermediate and lateral ventral segments of SNc were not clearly divided one from each other the calculations were performed like into one segment (VL+ I).

For statistical analysis of the data was used «SigmaPlot-12.0» application. Comparison of neurons quantitative parameters considering the age factor was performed by ANOVA, the Fischer-Snedecor test was used. To clarify the differences between the studied parameters in the age groups were used the Student's t-test and Bonferroni test. The correlation between age and neurons quantitative parameters changes was characterized by matching between the studied age groups rank Spearman correlation coefficients (r) of the nerve cells in a similar performance SNc segments.

Results and Discussion

Comparison revealed SNc neurons morphometric characteristics in elderly and maturity ages has shown that in old age the overall density of the location of the nerve cells decreases in VM and LS segments and density of the location of the dopamine neurons - in VM and (VL+ I) segments (table A 1). At the same age area of increased nerve cell bodies in the VM and DM segments and area of neurons nuclei was not significant changed (except segments VL+I, where they were reduced by 14%). The most significant cell parameters changes in the elderly compared to the maturity age were identified in the segment VM: total neuronal density decreased by 34%, dopamine neuronal density - 20% and the area of nerve cell bodies was increased to 16%.

Table 1. Quantitative parameters of neurons in the in maturity, elderly and senile human brain[1].

Main neurons parameters	segments of compact part of the substantia nigra				
	VM	VL+I	DM	DL	PL
maturity age					
M_{neur}	20,0±1,3	17,7±0,92	10,8±0,86	13,1±1,32	15,9±1,54
$M_{neur\ TH+}$	12,8±0,64	12,6±0,68	6,6±0,31	7,1±0,54	8,4±0,39

S_{neur}, mcm^2	480,2±15,6	472,6±22,81	399,6±22,62	448,6±21,35	472,6±22,81
S_{nu}, mcm^2	222,8±6,75	240,5±6,29	168,3±8,23	190,7±5,29	186,2±5,9
Elderly age					
M_{neur}	13,2±0,76*	15,5±0,75	10,6±0,63	12,6±0,52	10,7±0,75*
$M_{neur\ TH+}$	10,2±0,26*	9,9±0,2*	6,6±0,25	6,6±0,29	7,5±0,22
S_{neur}, mcm^2	558,2±14,57*	500,6±19,77	491,7±22,74*	514,6±17,97	500,6±19,77
S_{nu}, mcm^2	228,8±6,51	207,6±5,87*	159,2±6,34	195,9±7,0	187,6±8,12
senile age					
M_{neur}	13,4±1,17*	13,6±1,07*	7,1±0,9*#	11,9±0,75	9,3±0,59*
$M_{neur\ TH+}$	9,2±0,38*	9,6±0,27*	6±0,34	6,3±0,27	7,4±0,24
S_{neur}, mcm^2	603,1±17,97*	522±22,28	501,6±23,57*	543,8±17,08*	522±22,28
S_{nu}, mcm^2	185,5±6,92*#	204,8±4,63*	155,7±7,53	163,1±8,05*#	160,1±5,89*#

Note: 1 – there paired two-sample Student t-test was used: M - the average value, m - the average error. * - P <0.05 for parameters in maturity and old age compared with mature age; # - P <0.05 for parameters in senile age compared with elderly age. Here and in the Table. 2 : VM - medial segment, VL + I - lateral and intermediate segments of the ventral region; DM - medial, DL - lateral, LS - lateral subregion in the dorsal region of SNc. M_{neur}- density of neurons, $M_{neur\ TH+}$ - density of tyrosine hydroxylase contained neurons, S_{neur} - area of neurons bodies, S_{nu} - area of neuronal nuclei.

Comparison of the main parameters of SNc neurons in senile and maturity patients revealed that in senile age general nerve cell density decreases in all segments (except for the DL segment), while the location density of dopamine neurons - only VM and the VL+I segments (Table. 1). By senile age (compared with the maturity age) area of neuron body in the VM, DM and DL segments increases and the area of their nuclei decreases in all segments (except for the segment DM). Also area of neuron nuclei decreases in senile age not only compared to the mature age, but also in comparison with elderly age (in VM, DL, PL segments).

The most significant cell parameters changes in senile age, as compared to the mature, were found in the ventral region, VM and VL+ I segments of SNc. Overall neurons density decreases with the aging of 33% in VM segment and in VL+I segments - of 23%. Dopamine neurons density in these segments reduces by 28% and 24% respectively. At the same time in VM segment the area of neurons bodies in senile age increased by 26%, but there is no significantly differences in VL+I segments. Area of neurons nuclei decreases by 17% in VM and by 15% in VL+I segments.

Table 2. Correlation coefficients comparison of quantitative parameters of neurons in the compact part of the substantia nigra in the in maturity, elderly and senile human brain[1].

Main neurons parameters	segments of compact part of the substantia nigra				
	VM	VL+I	VM	DL	VM
maturity age / elderly age					

M_{neur}	- 0,614*	- 0,316	- 0,054	- 0,735*	- 0,469
$M_{neur\ TH+}$	- 0,131	- 0,292	- 0,028	- 0,072	- 0,248
S_{neur}, mcm^2	0,148	0,065	0,314*	0,054	0,027
S_{nu}, mcm^2	0,105	0,286*	0,141	0,19	0,179*
maturity age / senile age					
M_{neur}	- 0,461*	- 0,061	- 0,067	- 0,002	- 0,192
$M_{neur\ TH+}$	- 0,024	- 0,148	- 0,095	- 0,043	- 0,096
S_{neur}, mcm^2	0,086	0,059	0,275*	0,09	0,351
S_{nu}, mcm^2	0,048	0,044	0,053	0,214*	- 0,127
elderly age / senile age					
M_{neur}	0,683*	- 0,17	- 0,233	- 0,153	- 0,143
$M_{neur\ TH+}$	- 0,165	- 0,099	- 0,45*	- 0,06	- 0,315
S_{neur}, mcm^2	0,263*	0,213*	0,032	0,092	0,025
S_{nu}, mcm^2	0,149	- 0,021	0,009	- 0,006	- 0,122

Note: 1 – there Spearman (r) rank correlation coefficient was used. * - P <0.05 for values of r by comparing the respective age groups.

By comparison between study age groups morphometric neurons parameters correlation coefficients in similar SNc segments was found that there is a correlation between age and neuronal density change particularly in VM segment in elderly and senile age as compared to the mature. This correlation is reversed - aging leads to nerve cells density decrease. (Table. 2). Furthermore, in the same segment (VM) is found (Table. 2) a direct correlation between age and the area of the nerve cells. A similar correlation is found between these parameters in the DM segment by comparing the elderly and senile with the mature age.

The study demonstrated the dynamics of age morphometric

parameters changes of cellular structures in SNc caused by age-related involution: total neuronal density, dopamine neurons density, areas of neuronal bodies and nuclei. Involutory changes were primarily associated with reduction of total neuronal density (in all segments except DL) and a decrease in size of their nuclei (in all segments except DM). The general density reduction of nerve cells was correlated with patients age increasing. That have been shown by other authors who studied morphometric parameters of SNc cellular structures in aging [9]. Furthermore, as it was shown in our research, there is a reduction in area of cells nuclei in most SNc segments during aging. The nucleus is the central element of cellular metabolism and its size reflect the functional and metabolic activity in the nerve cell. Therefore, it can be supposed that the reduction in nuclei size is determined by decreased functional activity of neurons during aging and may indicate the development of atrophic process, which is one of the components in age involution.

Age differences in SNc neurons basic parameters were more expressed

in VM and VL+I segments in the ventral region. As well as total neurons reduction, dopaminergic cells number reduction during aging in these segments indicates that the age involution proceeds more rapidly than in other segments of SNc. At the same time, the senile loss of dopamine neurons in the VM and VL+I segments is comparable to the total loss of nerve cells in these segments. That characterizes the natural aging process and allows us to differentiate it from the neurodegenerative process in which the number of dopamine neurons is reduced more rapidly than the total number of nerve cells [6]. Increase in the size of neurons that we found in the elderly may be caused by neuromelanin accumulation [3], which has a natural neuroprotective properties [8].

Our SNc morphometric structures study revealed specifics changes in quantitative parameters of neurons associated with the natural aging (total neurons reducing, neurons enlargement and size reduction of the cell nuclei). It was demonstrated that the involutive process occurs more rapidly in VM and VL+I segments of the ventral region than in other segments of the substantia nigra in human brain.

Age-related structural changes in the compact part of the substantia nigra in human brain should be considered for an objective estimation of involution in this structure and discriminated from neurodegenerative process in Parkinson's disease.

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