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Age- Related Changes of Quantitative Parameters of Neurons in Extraocular Motor Nuclei

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ABSTRACT

Introduction: Extraocular motor nuclei are located in the midbrain (principal oculomotor and trochlear nucleus) and in the pons (abducens nucleus). With aging, there are significant changes in eyeball mobility.

Aim of the Study: The aim was to determine whether the quantitative parameters of neurons (volume and surface density, and the absolute number per mm^2 of the surface) in these nuclei significantly change with aging.

Patients and Methods: The study was done on 30 adult brainstems, both male and female, without diagnosed neurological disturbances. Three-millimeter-thick strata were taken in transversal plane and cut in 0.3 micrometer semi-serial sections stained with Mallory method. The images of studied nuclei were taken by „Leica" DM 1000 microscope and „Leica" EC3 digital camera under 400x magnification and analyzed by ImageJ software with A100 grid. The statistical analysis was performed by Statistical Package for the Social Sciences software using Pearson's correlation coefficient with 5% level of significance.

Results: The volume density of neurons had highly statistically significantly increased with age in principal oculomotor nucleus ($r = 0.571$, $p = 0.001$) and trochlear nucleus ($r = 0.581$, $p = 0.001$), while abducens nucleus showed no change in neuron volume. Changes of values of surface density and absolute number of neurons per mm^2 with age did not reach statistical significance.

Conclusion: Volume of neurons of extraocular motor nuclei located in the midbrain increase with age, while their surfaces and absolute number do not change significantly. These changes are not observed in the nucleus located in the pons.

Key words: Aging; oculomotor nuclear complex; trochlear nerve/anatomy and histology; abducens nucleus.

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Introduction

The first morphological signs of aging in the brain are found in the white mass at an early age (20- 40 years) and later (40-50 years) in the gray matter. As a result of these changes, the sensory-motor and cognitive skills will weaken.[1] The age-related changes in brain volume, weight

and the number of nerve cells vary in different parts of the brain. Macrostructural cerebral alterations progress with age without visible pathological changes,[2] while the microstructural changes, such as iron accumulation and loss of neurons, occur much earlier.

Extraocular motor nuclei (principal oculomotor, trochlear and abducens nucleus) belong to the group of general somatic efferent nuclei and contain motoneurons and internuclear neurons, which are a part of the circuit which coordinates eye movements.[3,4] Research of Clark and Isenberg has shown that with aging, between 30 and 90 years of age, there is the largest decline in the eyeball elevation, to an intermediate degree adduction and abduction, and the version least affected by age is depression.[5] A possible reason for these changes is the reduction in the number and size of myelinated axons, which leads to paralysis or neuropathy. Morphological study of Moriyama and associates showed that with age came a significant reduction in the total number of myelinated axons in the oculomotor nerve, while this change was not observed in the abducens nerve.[6] Stereological study of Sharma and associates showed a slight reduction in the total number of myelinated fibers and the surface of the axon with aging, whereby there was a significant increase in the thickness of the myelin sheath.[7] Reductions of the total number of myelinated fibers and surface of axons were not observed in the trochlear and abducens nerve with age, but even in their case there was an increase in the thickness of the myelin sheath.[8] In the available literature, we did not find any data on quantitative changes in extraocular motor nuclei with aging.

Aim of the Study

The aim was to determine whether quantitative parameters of neurons in extraocular motor nuclei (volume and surface density, and an absolute number per mm^2 of the surface) change significantly with aging.

Patients and Methods

The research was done with the permission of the Ethics Committee of the University Clinical Center of the Republic of Srpska, on samples of 30 adult brains, both sexes (11 female and 19 male), aged 21 to 83 years (average age 57.07 years) who died without diagnosed neurological diseases. Using conventional autopsy technique brains were extracted from the cranial cavity, and then immersed in a 10% formalin solution for fixation. After the fixation, brainstems were separated from forebrain, by cutting brain masses at the level of the posterior edge of mammillary bodies and from the cerebellum, by cutting cerebellar pedicles. After fixation, brainstems were cut in 3 mm thick strata in the transverse plane (stratified sampling), going caudally from the level of:

1. the middle of the superior mesencephalic colliculus (principal oculomotor nucleus),
2. caudal border of the superior mesencephalic colliculus (trochlear nucleus), and
3. caudal border of the facial colliculus (abducens nucleus).[9]

Obtained strata were used to make semi-serial sections (for principal oculomotor and abducens nuclei: 5,10,...,100; and for trochlear nucleus: 5,10,...,120), 0.3 μm thick, which were stained by the Mallory method. Referent space of the research in all cases was extraocular motor nuclei, and the study phase was nerve cells.

Images of objects of the research were taken by the camera "Leica EC3" (Leica Microsystems CMS GmbH, Wetzlar, Germany) in JPEG format, 2048 x 1536 pixels resolution, under a 400x magnification of light microscope „Leica" DM 1000 (Leica Microsystems CMS GmbH, Wetzlar, Germany) and 0.7 magnification of the camera's c-mount. In the sample selection procedure, we have picked up every second field, and the sample sizes, i.e. the required number of measurements for each variable and for each group was determined according to the De Hoff's formula: $n = (200 / y \bullet s / x)^2$

n– number of visual fields that should be analyzed, x – mean of the orientation sample, s– standard deviation of the orientation sample, y– allowed mean tolerance.

Quantitative analyses were done using ImageJ software, version 1.49 j (National Institutes of Health, Bethesda, USA). Prior to analysis, the spatial calibration with objective micrometer was done, and the parameters of the test system A100 were determined. Based on these parameters and software option "grid" we have formed the grid of test system A100 (table 1)..

Table 1. Basic parameters of the test system

A-100	Objective 40x
Pt	100
d	0.020386 mm
Lt	4.0772 mm
At	0.04156 mm^2

Pt - total number of points of the test system; d - length on one line of the test system; Lt - length of all test lines; At - area of the test system; Lt=Pt•d•2; At=Pt•d²

After grid superimposing, images were analyzed with the cell counter tool. For the analysis of neurons, the following variables were determined: volume density, surface density and an absolute number of nerve cells per mm² of the surface were determined. A total number of analyzed test fields was: for principal oculomotor nucleus 2060, for trochlear nucleus 2354 and for abducens nucleus 2080.

For calculation of volume density (Vv), which represents the amount of space occupied by analyzed phase, the following formula was used: Vv (mm⁰)= Pf / Pt (Pf is the number of points of the test system falling on the studied phase; Pt – total number of points within the test system A100).[10]

The second analyzed parameter, surface density (Sv), indicated the size of a certain inner or outer surface in a volume unit. It was determined by the formula: Sv (mm⁻¹)= 2•If/Lt (If- number of intersections of test system lines with studies phase; Lt- total length of test lines).[10]

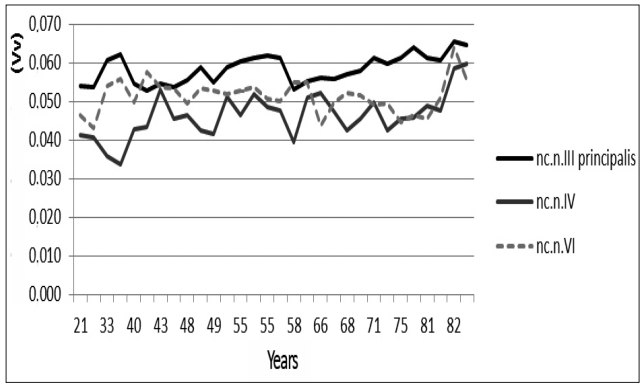
The absolute number of neurons per mm² of the surface was calculated by the formula: Nf= N/At (N- the number of neurons within test system; At- area of the test system).

Statistical analysis of the results was performed using SPSS software (SPSS Inc, Chicago, USA), version 16.0, using methods of descriptive statistics and Pearson's correlation coefficient. Statistical significance was tested for the level of statistical significance of 5%.

Results

The impact of aging on the volume density of neurons has differed in the investigated nuclei. There was a strong, positive correlation between the volume density of neurons and age in two extraocular motor nuclei- principal oculomotor nucleus (r = 0.571, p = 0.001) and trochlear nucleus (r = 0.581, p = 0.001). For the abducens nucleus, the correlation was weak and positive (r = 0.032, p = 0.865). Changes in values of volume density with aging are shown in Figure 1.

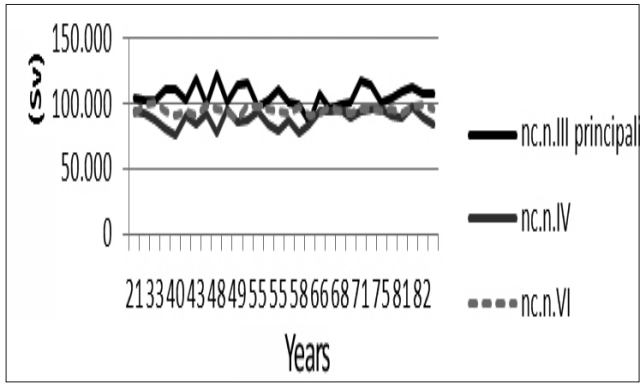
Figure 1. **Age-related changes in volume density (Vv) of extraocular motor nuclei**



Nc.n.III principalis- principal oculomotor nucleus; nc.n.IV- trochlear nucleus; nc.n.VI- abducens nucleus

The second tested parameter, surface density, did not significantly change with age, and correlations had a low intensity and were positive- for the principal oculomotor nucleus r = 0.086, p = 0.650, trochlear nucleus r = 0.081, p = 0.671, and for abducens nucleus r = 0.005, p = 0.979. Changes in values of the surface density with aging are shown in Figure 2.

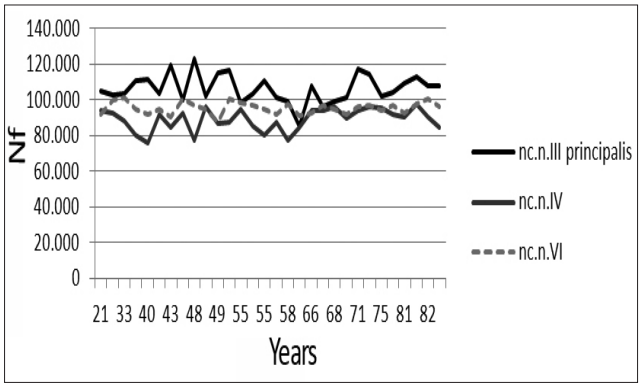
Figure 2. **Age-related changes in surface density (Sv) of extraocular motor nuclei**



Nc.n.III principalis- principal oculomotor nucleus; nc.n.IV- trochlear nucleus; nc.n.VI- abducens nucleus

Similar to the surface density, the absolute number of neurons per mm2 of surface with aging in all three extraocular motor nuclei did not significantly change, and correlations had a weak intensity- for principal oculomotor nucleus r = 0.010, p = 0.956, for trochlear nucleus r = 0.275, p = 0.142, and for abducens nucleus r = 0.043, p = 0.820. Changes in values of the absolute number of neurons of the extraocular motor nuclei per mm2 of surface with aging are shown in Figure 3.

Figure 3. Age-related changes in absolute number of neurons per mm2 of surface (Nf) of extraocular motor nuclei



Nc.n.III principalis- principal oculomotor nucleus;
nc.n.IV- trochlear nucleus;
nc.n.VI- abducens nucleus

Discussion

In the available literature, there is a little amount of quantitative data on changes in neurons of extraocular motor nuclei with aging. Available data are mainly related to the dimensions of the investigated nuclei and cells that are building them,[11-15] while changes of quantitative indicators with aging were not found. On the other hand, there are a number of diseases caused by changes in the cells of the extraocular motor nuclei, leading to certain clinical symptoms.[16-18]

In the present study, we have analyzed the changes of quantitative parameters of nerve cells of extraocular motor nuclei with aging. It was observed that neurons in the principal oculomotor nucleus and trochlear nucleus increase in volume with age, while this change was not observed in abducens nucleus. The mechanism related to this neuron hypertrophy has not been directly investigated in the present study, but observed increase in volume can be explained by the accumulation of neuropigments with aging, which occupy an increasing cell surface, which is particularly pronounced in large neurons and in brain regions involved with motor function.[19]

The values of Pearson's correlation coefficient showed that there was no statistically significant change in the number of neurons in extraocular motor nuclei. These results correspond to the results of Vijayashankar and Brody, who studied changes in the number of trochlear nucleus neurons and noted that from newborns to 87 years of age, there is no statistically significant change in the number of neurons in this nucleus. The only difference observed by these authors was that the neurons are more densely arranged in a newborn and that the adult nuclei were slightly longer (0.2 to 1.3 mm).[20] The same authors have studied the changes in abducens nucleus with aging.[21] In addition to individual variations, no significant reduction in the number of nerve cells in this nucleus was observed as well, but the length of adult nuclei was almost doubled. In studies on age changes in the number of neurons of all three extraocular motor nuclei in mice, Sturock also noted that there was no statistically significant difference in the number of neurons. However, the author states that in the neurons of these nuclei there is no significant loss of Nissl's substance with aging and that there is a very little accumulation of lipofuscin, and no variation in the diameter of the nucleus of neurons with aging.[22,23] Given the fact that extraocular muscles are very active throughout life, the nuclei of the nerves which innervate these muscles also have huge activity. It is assumed that muscle activity prevents loss of neurons in these nuclei, since activity can delay the occurrence of deterioration in certain nerve cells groups.[24]

Although principal oculomotor nucleus is not functionally directly related to the red nucleus and substantia nigra, because of the close topographic position in the same transverse plane, the research results were compared with the values of quantitative parameters of age- changes of neurons in aforementioned structures. Analyzed quantitative parameters of neurons in the magnocellular part of the red nucleus decrease with age, but the decrease does not reach statistical significance.[25] Quantitative measurements of substantia nigra show decrease in the value of quantitative parameters of neurons with age, with the exception that the analysis of histogram of the frequency distribution has shown that the increase of the average volume of substantia nigra neurons in older subjects is not the result of a selective loss of small size neurons, but a real hypertrophy of bodies of pigmented nerve cells.[26]

Conclusion

With aging, there is an increase of the volume of cells in principal oculomotor and trochlear nucleus, while this enlargement is not seen in abducens nucleus cells. Surface density and an absolute number of neurons do not change significantly with aging.

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Promjene kvantitativnih parametara neurona ekstraokularnih motornih jedara sa starenjem

SAŽETAK

Uvod: Ekstraokularna motorna jedra se nalaze u srednjem mozgu (nucleus nervi oculomotorii principalis, nucleus nervi trochlearis) i u moždanom mostu (nucleus nervi abducentis). Starenjem dolazi do značajnih promjena u pokretljivosti očne jabučice.

Cilj rada: Cilj rada je da se odredi da li se kvantitativni parametri (volumenska i površinska gustina, i apsolutni broj po mm^2 površine) neurona ovih jedara značajnije mijenjaju sa starenjem.

Ispitanici i metode: Istraživanje je obavljeno na 30 preparata moždanih stabala odraslih lica, oba pola, bez dijagnostikovanih neuroloških oboljenja. Stratume debljine 3 milimetra smo uzimali u transferzalnoj ravni i rezali u semiserijske rezove debljine 0,3 mikrometra koji su bojeni Mallory metodom. Fotografije istraživanih jedara su slikane pomoću mikroskopa Leica DM1000 i digitalne kamere Leica EC3 pod uvećanjem 400x, i analizirane softverom ImageJ uz korišćenje mrežice A 100. Statistička analiza je obavljena programom SPSS korišćenjem Pearson-ovog koeficijenta korelacije uz nivo značajnosti razlike od 5%.

Rezultati: Volumenska gustina neurona se visoko statistički značajno povećavala sa godinama života kod nucleus nervi oculomotorii principalis (r=0,571, p=0,001) i nucleus nervi trochlearis (r= 0,581, p=0.001), dok kod nucleus nervi abducentis nije bilo promjene u volumenu neurona. Promjene vrijednosti površinske gustine i apsolutnog broja neurona po mm^2 sa starenjem nisu dostigle statističku značajnost.

Zaključak: Volu Neuroni ekstraokularnih motornih jedara locirani u srednjem mozgu se volumenski povećavaju sa starenjem, dok im se površina i apsolutni broj značajnije ne mijenjaju. Ova promjena se ne uočava u jedru lociranom u moždanom mostu.

Ključne riječi: Starenje; okulomotorni jedarni kompleks; trohlearni nerv/anatomija i histologija; abducensno jedro.