FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol.10, No 3, 2003, pp. 116 - 119 UC 612.822:612.67

SIZE AND NUMBER OF NEURONS IN THE AGING INDUSIUM GRISEUM

Snežana Pavlović, Natalija Stefanović, Rade Čukuranović, Svetlana Antić, Vesna Stojanović

Institute of Anatomy, Faculty of Medicine, Niš, Serbia
E-mail: nmp01@ptt.yu

Summary. *Neurons of indusium griseum, part of the allocortex, were investigated. The aim of the study was to establish the size of the neurons and their number across the age range. Specimens of the indusium griseum were taken from adult human brains. The size of the neurons was calculated from linear measurements. Stereological investigation was performed using M_{42} grid, and the numerical density was calculated from the equation of Floderus. The numerical density of the neurons was determined on three spots of the indusium griseum. The largest was above the trunk whereas the smallest was above the splenium of the callosal body. The average diameter of the neurons significantly decreased from 34.21 μm in the first age group up to 27.85 μm in the fourth age group. Numerical density of the neurons also significantly decreased in all the three spots of indusium griseum during aging. The reasons for here recorded findings might be degenerative changes and loss of cellular liquids that normally happen across during aging. Numerical density showed prominent neuronal loss which may have been a consequence of the aging processes which appear earlier in the allocortex.*

Key words: *Neurons, indusium griseum, aging*

Introduction

The Indusium griseum is a tiny gyrus of telencephalon, which belongs to the limbic system. It is also a part of the archicortex, located deep on the floor of the longitudinal fissure of the telencephalon, on the dorsal aspect of the callosal body. The structure of the cerebral cortex, specifically isocortex, was always an interesting topic for researchers who explore the brain (1,2,3). Information about these topics enables understanding of the complex harmony of functions, which are the basis of mental activities and behavior. In the last two decades, changes in the cortex that happen with aging are even more interesting (4,5,6,1,7,9,10). It is not the same case with the archicortex. While the hippocampus is the most often investigated part of archicortex, the indusium griseum is rarely a subject of interest.

Regarding the fact that the indusium griseum is a seldom investigated part of the human brain, we decided to explore it, and to shed some more light on the details of its composition.

Material and methods

The examination was performed on 25 adult human brains (both sexes, 20-83 years of age), with no signs of visible neuropathological changes. The brains were divided in four age groups. The first group (I), up to 25 years of age, was also used as a control. The second group (II) 26-50 years, the third (III) 51-70 years of age and the fourth (IV) group, 71 years and over. The tissue blocks for the histological procedure included the indusium griseum and a part of the underlying callosal body. They were systematically taken from three characteristic spots per brain: on the genu of the callosal body (G), in the middle of the trunk (Tr) and just above the splenium of the callosal body (S). The specimens were fixed in 10% neutral buffered formaline solution, and submitted to the routine histological procedure. 10 μm tick slices were stained by Golgy and crasyl violet techniques. The diameters of neurons were measured by linear measurements, using the ocular micrometer, on 100 casual cell profiles in each case. The average diameter was calculated using the following equation: $D = 4/\pi \times d$ ("d" was the measured diameter). The stereological analysis was performed using the M_{42} grid. The numerical density (Nv) of neurons was calculated using the equation of Floderus: $N_V = N_A/t + D - 2h$. N_A was the number of hit points on the test system's area, "t" was thickness of the slices, "D" was an average cell's diameter and "h" was a correcting factor calculated from the formula: $h = R - (R - r)$. ("R" was the largest diameter, "r" the smallest measured diameter). For the statistical analysis of numerical data Student's T-test was employed.

Results

The average calculated diameters of neurons of indusium griseum were between 26,98 μm and 37.46 μm (Figures 1 and 2). The calculated diameters of neurons are presented on the Table 1 and Figure 3. Among the groups, the average diameter was the largest in the first (I) age group, 34.21 μm. The diameters decreased to-

wards the fourth (IV) age group, reaching a significant level in the third (III) group of approximately 28.06 μm ($p<0.05$). In the fourth (IV) age group there was further slight decrease of average diameters (27.85 μm), without changing the level of significance.

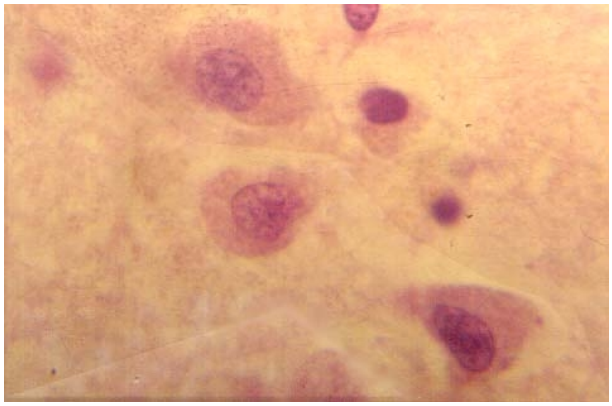

Fig. 1. Neurons of indusium griseum (HE x 600)

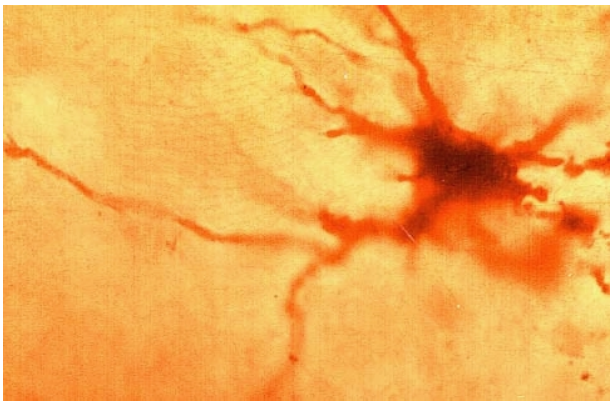

Fig. 2. Pyramidal cell of indusium griseum (Golgy x 800)

Table 1. The average diameters of neurons of indusium griseum (μm). * $p<0.05$

Statistical parameter	I (up to 25 years)	II (26-50 years)	III (51-70 years)	IV (>70)
x	34.21	30.84	28.06	27.85
SD	4.05	1.57	0.75	0.97
X_{max}	37.46	32.09	28.75	28.89
x_{min}	28.33	28.75	27.07	26.98
SEM	2.03	0.79	0.38	0.56
T-test	2.03	0.79	2.98*	3.02*

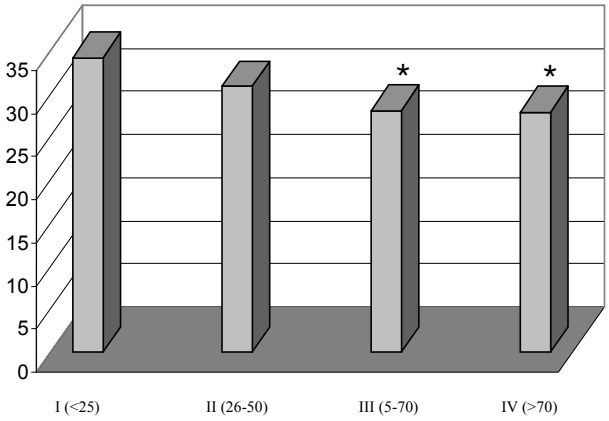

Fig. 3. The average diameters of neurons of indusium griseum (μm). $p<0.05$

The numerical density was calculated using the equation of Floderus. The data are presented in the Table 2 and Figure 4. In 60% of cases Nv was the largest in Tr position compared with G and S position (in 33.33% of the cases the least in the G and in 66.66% the least in the S position). In 26.7% of the cases Nv was the largest in S position (in 50% of the cases the least in the G and in the another 50% of the cases the least is in the Tr position). In 13.33% of the cases Nv was the largest in the G position (in 50% cases the least in the Tr and in 50% the least in the G position). If observed according to the age, Nv of the pyramidal cells was the largest in the first (I) age group, between 34540 mm^{-3} on the G position and 37220 mm^{-3} in Tr position. In the second (II) age group Nv was between 32185mm^{-3} on the G and 39207 mm^{-3} on the Tr position. In the third (III) age group Nv was between 23769 mm^{-3} on the S and 25577 mm^{-3} on the Tr position. And finally, in the fourth (IV) age group Nv was between 13362 mm^{-3} on the G and 13670 mm^{-3} on the S position.

The numeric data show that Nv decreases with age. The decrease on the G position reaches a significant level in the third (III) age group ($p<0.05$). There is a further decrease which becomes very significant ($p<0.001$) in the fourth (IV) age group. On the Tr position the decrease of the Nv values reaches statistical significance in the third (III) age group ($p<0.001$), while in the fourth (IV) age group it is highly significant ($p<0.0001$). The decrease of the Nv on the S position reaches significant level also in the third (III) age group ($p<0.001$), and shows a further decrease in the same level of significance.

Table 2. Numerical densities of neurons of indusium griseum (mm^{-3}). (mm^{-3}). *$p<0.05$, **$p<0.01$, ***$p<0.0001$

	Age groups											
Statistical	I (up to 25 years)			II (26-50 years)			III (51-70 years)			IV (> 70 years)		
parameter	G	Tr	S	G	Tr	S	G	Tr	S	G	Tr	S
x	34540	37220	36763	32185	39207	35837	23780*	25577**	23769**	13742**	13362***	13670**
SD	992.4	1082	667.2	7788	18772	11825	4339	768.3	4928	1809	3708	4722
x_{max}	35890	38629	37592	38750	61609	50584	28891	26518	29804	16300	17337	19414
x_{min}	33500	36014	36001	21052	15669	21670	18284	24636	17733	12406	9989	7918
SEM	496.2	541.4	338.6	3894	1042	5912	2552	548.8	2898	1064	1854	2361

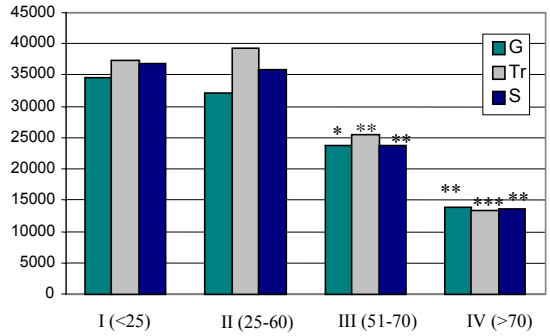

Fig. 4. Numerical densities of neurons of indusium griseum (mm^{-3}). *p<0.05, **p<0.01, ***p<0.0001

Discussion

Neurons of the human cortex were often the subject of investigations for many researchers. They were mostly examined in different parts of neocortex, but also in some parts of allocortex such as the hippocampus. Neurons differ in size according to the location from around 10 μm even up to 80 μm (11). Neurons of the indusium griseum had the average diameter of 30.24 μm. The average diameter is the largest in the first age group, 34.21 μm, and tends to decrease in the older age groups. The decrease reaches a significant level in the third age group ($p<0.05$). There is further decrease in size in the fourth age group, at the same level of significance. The decrease in size with age is a consequence of general hypotrophy, which develops in all the tissues and organs in the body. The hypotrophy is caused by the intra and extra cellular liquid loss and increasing degenerative changes on the cell's organelle (7).

Number of neurons and their loss during normal and pathological aging were of special interest for researchers. There were though some difficulties in determining their total number. The most serious one is individual variation in the cortical neuron number, which can differ up to 100% (6). Besides that, the neuron number is also determined by sex and age (2). The problem is overcome using a large number of samples and appropriate statistical method for very variable samples.

In our study the numerical density (Nv) of the neurons of the indusium griseum was determined in three characteristic spots: on the genu of the callosal body, above the middle of the trunk and on the splenium. Nv was the greatest above the trunk of the callosal body, where the thickness of the indusium griseum was the largest (8), except for the eldest age group. In the first age group, which was also taken as a control, it was 36174 mm^{-3}. Nv decreases with age, highly significantly from around 50 years of age (24375 mm^{-3}). There is a further decrease in number of pyramidal cells up to 13591 mm^{-3} in the fourth age group. The decrease of the pyramidal cells is not equal in all the parts of the indusium griseum. The largest decrease is in the Tr position, where pyramidal cells are the most numerous, the smallest in the G position where they are the least numerous.

The assessment of whether neurons are lost from the cortex during normal aging would be the most accurate if it was possible to count neurons of the same person in two different points in time. Since it is not possible the neuronal loss is assessed comparing the number of neurons of older individuals with those of younger individuals, which makes the result approximate (3). We couldn't compare our findings of significant decrease of neurons in the indusium griseum after 50 years of age because we didn't find any data regarding that part of the cortex in the literature, but they were in accordance with results of many researchers found on the basis of cell counting in different parts of the neocortex (5,1,4). Recent studies of number of neurons have been performed using the dissector method to estimate the total number of neurons (2). They state that cortical neurons are largely preserved. Actually, they found an overall loss of neurons from the cerebral cortex with age, but not more than 10%. They also found no change in neuronal density with age. One possible explanation for the discrepancies in repports of neuron loss with age may be the shrinkage of brain tissue during the histological procedure, which happens in younger brains more than in older ones (15%). Consequently, in cell counts, the sections from younger brains give higher neuronal densities than those from older brains.

The numeric density of neurons in the indusium griseum is a relative category, counted in volume units, and also in the relative specific area. Some authors think that neuronal loss with age may be regionally specific and limited. In this view, neurons could be lost from one architectonic area, but not from the area next to it, or they may be lost from a specific cortical layer. For this reason the total number of neurons may be the appropriate category for estimation of neuronal loss (2).

The neuron loss, which we found in the indusium griseum in not such old age may be a consequence of an earlier appearance of degenerative changes in the phylogenetically older cortices as the indusium griseum. This supposition may be supported by abundant corpora amylacea, found in the older age groups (III and IV) (8, 9, 12).

References

1. Henderson G, Tomlinson BC, Gibson PH. Cell counts in human cerebral cortex in normal adults through life, using an image analysis computer. J Neural Sci 1980; 16: 113-136.
2. Parkenberg BG, Gundersen HJG. Neurocortical neuron number in humans: effect of sex and age. J Comp Neurol 1997; 381: 312-320.
3. Peters A, Morrison JH, Rosene DL, et al. Cerebral cortex. 1998; 8: 295-300.

4. Anderson JM, Hubbard BM, Coghill GR, et al. Neurophysiological changes in healthy adults across the age range. Neurobiol Aging 1983; 14: 623-625.
5. Devaney KO, Johnson HA. Neuron loss in the aging visual cortex of man. J Gerontol 1980; 35: 811-836.
6. Hang H. Are neurons of the human cerebral cortex really lost during aging? A morphometric evaluation. In: Senile dementia of the Alzheimer type. Traber J, Gispen WH (eds.). Springer, Berlin, 1985: 150-163.
7. Kostiok PG. Opšti principi starenja. U: Biologija starenja. Nauka, Leningrad, 1982.
8. Pavlović S. Morfološka, histološka i stereološka studija induzium grizeuma čoveka. Doktorska disertacija. Niš, 1999.
9. Pavlović S, Stefanović N, Čukuranović R, Antić S, Jovanović I. Some anatomical changes in human indusium griseum associated with non-pathological aging. Scripta Scientifica Medica 2002: 34 Suppl.1 p.87.
10. Peinado MA. Histology and histochemistry of the aging cerebral cortex: an overview. J Microsc Res Tech. 1998; 43:1-7.
11. Williams Gray's anatomy. Churchill Livingstone. London, 1995.
12. Sternberg SS. Histology for pathologists. Lippincott-Raven. New York, 1997.

VELIČINA I BROJ NEURONA INDUZIUM GRIZEUMA TOKOM STARENJA

Snežana Pavlović, Natalija Stefanović, Rade Čukuranović, Svetlana Antić, Vesna Stojanović

Institut za anatomiju, Medicinski fakultet Niš

Kratak sadržaj. *Istraživani su neuroni induzium grizeuma, koji je deo alokorteksa. Cilj istraživanja je bio da se odredi veličina neurona i njihov broj tokom starenja. Uzorci induzium grizeuma uzeti su sa mozgova obdukovanih odraslih osoba. Veličina neurona određivana je pomoću linearnih merenja. Stereološka istraživanja su izvedena korišćenjam M42 mrežice, a numerička gustina neurona određena je na tri karakteristična mesta induzium grizeuma. Najveća vrednost numeričke gustine bila je iznad tela, dok je najmanja iznad splenijuma kaloznog tela. Prosečan prečnik neurona značajno se smanjivao na svim mestima induzium grizeuma od 34,21 μm u prvoj starosnoj grupi do 27,85 μm u četvrtoj grupi. Numerička gustina neurona se takođe značajno smanjila na sva tri mesta induzium grizeuma tokom starenja. Razlozi ovih nalaza bi mogli biti u degenerativnim promenama i gubitku ćelijske tečnosti koji normalno nastaju tokom godina. Numerička gustina je pokazala gubitak neurona koji je možda nastao kao posledica procesa starenja koji u alokorteksu počinje ranije.*

Ključne reči: *Neuroni, induzium grizeum, starenje*