ISSN 2079-0570, Advances in Gerontology, 2015, Vol. 5, No. 2, pp. 117–120. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © T.A. Ishunina, 2015, published in Uspekhi Gerontologii, 2015, Vol. 28, No. 1, pp. 37–41.

Sizes of Neuronal Nuclei and Pericarya in the Nucleus Basalis of Meynert and the Posterior Hypothalamus in Different Age Groups

T. A. Ishunina

Kursk State Medical University, ul. K. Marksa 3, Kursk, 305041 Russia
e-mail: ishunina@gmail.com

Received October 13, 2014

Abstract—The article examines the morphometric parameters (the area of neuronal pericarya and their nuclei, as well as the nucleocytoplasmic ratio) describing the metabolic activity of the nucleus basalis of Meynert (NBM), tuberomamillary (TMN) nucleus, and medial mammillary (MMN) nucleus of the human hypothalamus in four age groups. A reliable enlargement has been found in the size of neurons and their nuclei in elderly people. Increases in the metabolic activity of neurons in the NBM begin earlier than in the TMN and MMN and are morphologically manifested in middle-aged persons. The age-related metabolic activation of neurons in the studied human brain structures, which participate in the regulation of memory and other cognitive functions, can be attributed to defensive and adaptive and/or compensatory mechanisms in aging that target prevention of the development of Alzheimer's disease.

Keywords: area of neuronal nuclei, area of neuronal pericarya, the nucleus basalis of Meynert, tuberomamillary nucleus, medial mamillary nucleus, hypothalamus, aging, WHO age classification

DOI: 10.1134/S207905701502006X

INTRODUCTION

According to the recent data, disorders in cognitive functions due to aging are associated not with neuronal death, which accounts for less than 10% in the neocortex, but with a reduction in the number of synapses [1]. At the same time, disorders in synaptic connections may be caused by a toxic effect of the oligomeric form of β-amyloid (Aβ), which accumulates due to damage to the intracellular signaling cascades of γ-secretase. The worsening of cognitive functions is also associated with age-related reduction in the gray matter volume and the loss of white matter integrity, which are most prominent in the prefrontal and medial temporal cortices. The degree of structural alterations in the brain due to aging has been shown to correlate with the value of functional (behavioral) disorders.

For example, some MRT data showed the functional activation of some brain structures with aging, which was associated by researchers with certain improvements in the cognitive sphere. Other authors, on the contrary, associate the activation of brain structures in elderly people with the ineffective switch of additional resources, in fact, of a destructive character, which worsens cognitive functions. At the same time, a lower level of activity in various brain formations is always associated with a worsening in the cognitive sphere [5, 9]. Apart from the aforementioned electrical activity, neurons demonstrate a sufficiently high level of metabolic and synthetic processes, the intensity of which can be evaluated according to certain morphometric criteria, such as the sizes of neuronal nuclei and pericarya [2, 11].

The goal of this research was to study the aforementioned metabolic activity parameters of neurons in some structures of the basal forebrain and hypothalamus participating in the regulation of memory and other cognitive functions in aging. The NBM is the main source of cholinergic projections into the neocortex. The TMN serves as the main source of histamine, a neurotransmitter in the brain. The MMN is connected with the hippocampus through the fornix and participates in providing memories about recent events. Aggregates of neurofibrillary tangles representing the hyperphosphorylated τ protein and amyloid plaques containing toxic β-amyloid have been found in all indicated structures in Alzheimer's disease [2].

MATERIALS AND METHODS

The human brain tissue material was kindly provided by the Netherlands Brain Bank. There was written consent to use brain tissue for scientific research in all cases. Our morphometric analysis was performed on paraffin sections containing NBM (29 cases), TMN (43 cases), and MMN (26 cases) stained with polyclonal antibodies recognizing aromatase [6]. The contours of nuclei and pericarya are quite clearly identifiable due to this staining. The studied cases were

divided into four age groups according to the WHO age classification: the first group included young people (25–44 years old), the second group included the middle-aged (45–59 years old), the third included the elderly (60–74 years old), and the fourth was composed of old people (75–89 years old). Using ImageJ software, researchers manually drew the contours of neuronal pericarya and their nuclei, subsequently calculating their areas on microphotographies of the preparations. The nucleocytoplasmic ratio (NCR) was calculated by dividing the area of the nuclei by the area of neuronal pericarya. The reliability of differences among the investigated groups was assessed by Student's *t*-test. Pearson's coefficient was used to determine the correlation between the studied parameters and age.

RESULTS AND DISCUSSION

The areas of pericarya and neuronal nuclei in the NBM were enlarged in the second group (middle age) and remained at the same level in the third (elderly) and fourth (old age) groups (Fig. 1). A reliable enlargement was marked in the area of neuronal nuclei in the third and 4th groups, compared with the first one (young), $p = 0.018$ and $p = 0.049$, respectively. The area of neuronal pericarya was also higher in the third and fourth groups, compared with the first one, and the difference between the fourth and first groups appeared statistically reliable ($p = 0.033$). A positive correlation between the sizes of neuronal pericarya ($r = 0.362$) and, to a lesser degree, between the sizes of their nuclei ($r = 0.298$) and the age was observed in all investigated groups, which was reflected in some decrease of the NCR ($r = -0.321$). However, these correlations cannot be considered statistically reliable. It should be noted that no reliable NCR intergroup differences have been revealed. In total, the obtained data confirm the metabolic activity in NBM neurons increasing with aging, which is manifested in middle-aged persons, coinciding with the onset of menopause in women and andropause in men.

The neuronal nuclei area in TMN was reliably larger in the second (middle age) and third (elderly) groups than in the first ($p = 0.028$ and $p = 0.013$). The pericarya area gradually became larger and reached its maximum in the third (elderly) group as compared with the first (young) and the second (middle age) groups, $p = 0.016$ and $p = 0.025$, respectively. In the fourth group (old age), a certain reduction was observed in the sizes of neuronal nuclei and pericarya as compared with the third group, but both parameters remained higher than in the first group (Fig. 1). An insignificant positive correlation was marked between the neuronal pericarya area ($r = 0.240$) and, to a lesser extent, between the areas of their nuclei ($p = 0.115$) and the age when all age groups were considered. Due to opposite changes in the sizes of nuclei and pericarya, NCR somewhat decreased with aging ($r = -0.227$). Similar to those of the NBM, the aforementioned correlations were not statistically reliable. The highest NCR value was revealed in the second age group (as compared with the first $p = 0.022$, the third $p = 0.006$, and the fourth groups $p = 0.003$). Thus, as with the NBM, an age-related increase was observed in the metabolic activity of neurons in the TMN with its maximum in the third group.

The area of neuronal nuclei and their pericarya in the MMN gradually increased from the first to the third group and reached its highest values in elderly people ($p = 0.042$ for the neuronal nuclei area and $p = 0.013$ for the neuronal pericarya area when comparing the values of the third and first groups). The values of the fourth group remained at approximately the same level as in the third (Fig. 1). A positive but unreliable correlation with age was, to a greater extent, observed in the entire group for neuronal nuclei size ($r = 0.339$) and, to a lesser extent, for neuronal pericarya size ($r = 0.244$). Due to the more intensive growth in nuclei size, the NCR increased insignificantly ($r = 0.235$) with aging and reached its highest values in the fourth group. Thus, by analogy with the NBM and TMN, an increase in the metabolic activity of neurons in aging was observed in the MMN, reaching its maximum in the third group.

Therefore, this study demonstrates that increases in the metabolic secretory activity of neurons are observed in the human basal forebrain and the posterior hypothalamus with aging, reaching their maximum in elderly people. The onset of neuronal activation was marked in the NBM at the age of 45–59 years, whereas this takes place in the hypothalamic TMN and MMN at elderly ages (60–74 years). Neuronal metabolism activation can be considered a trigger of defensive and adaptive mechanisms with aging. This is confirmed by the results obtained by M. Riudavets et al. [10], which show that enlargements in neuronal nuclei size is a defensive response that provides resistance to Alzheimer's disease. In particular, hypertrophy of the neuronal nuclei in the presence of amyloid plaques was observed in individuals with unaltered cognitive status, i.e., with asymptomatic course of Alzheimer's disease. This allowed the authors to make the conclusion that enlarged neuronal nuclei may represent a defensive and adaptive phenomenon of aging. The adaptive nature of enlargements in neuronal nuclei size was noted earlier by C. Navarro et al. [8]. Given the reduction in brain matter with aging, we also cannot exclude a compensatory mechanism for the increased metabolic activity of neurons in the basal forebrain and hypothalamus.

Some authors of scientific studies in the first half of the 20th century noted that the cell's cytoplasmic mass became more significantly enlarged relative to its nucleus with aging. As a consequence of such disproportional changes, the NCR reduces [7]. According to V.N. Nikitin and Zh.A. Medvedev, the control of the

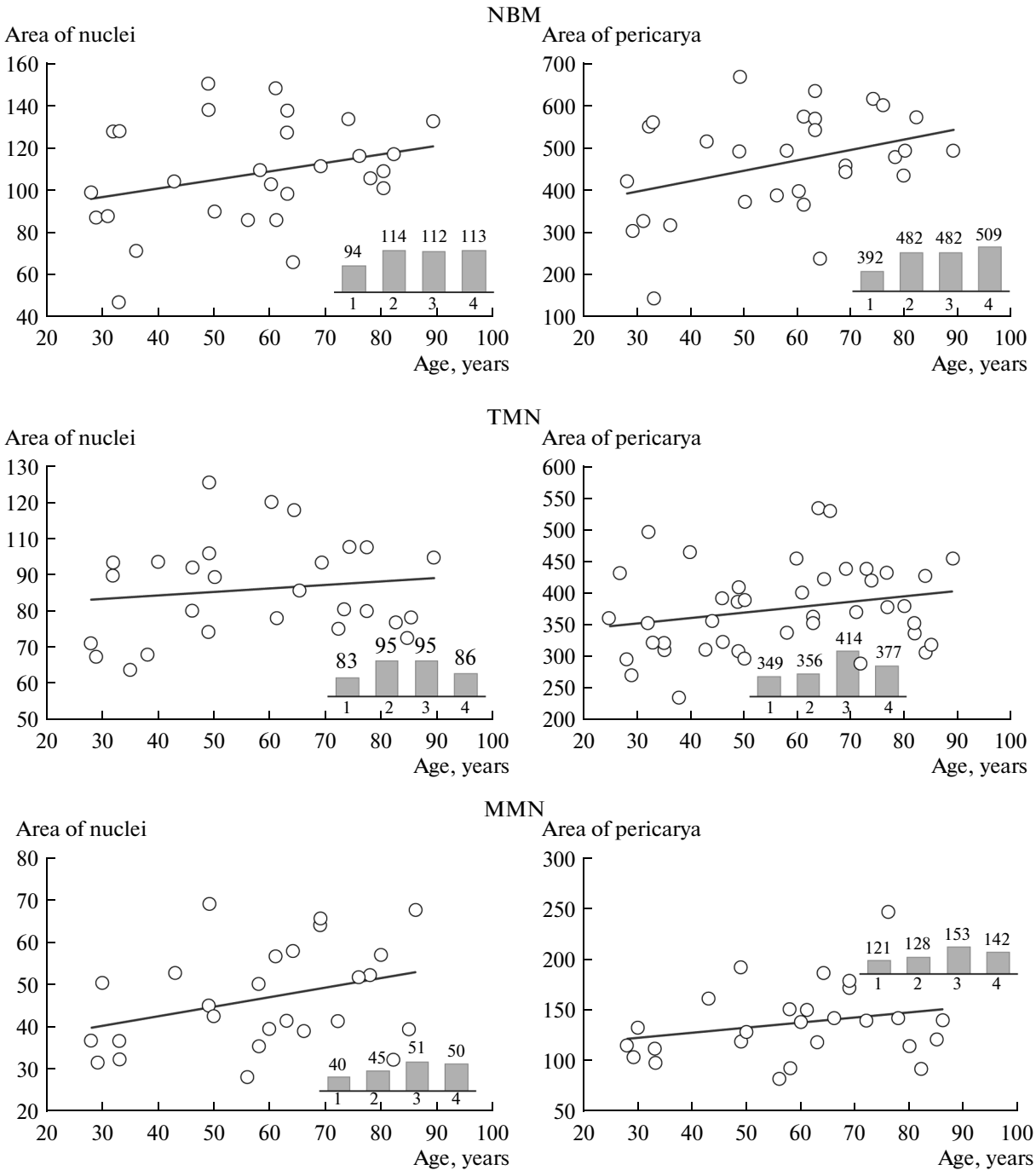

Fig. 1. The area (square μm) of neuronal nuclei (the left panel of the graphs) and pericarya (the right panel of the graphs) in the nucleus basalis of Meynert (NBM), tuberomamillary (TMN), and medial mamillary (MMN) nuclei of the human hypothalamus. Individual values for each case are indicated by circles; the lines indicate the direction and significance of correlations between the morphometric parameters with aging; the inset histograms demonstrate the mean morphometric alues (indicated above each column) of neurons in four age groups (the first group—young, 25–44 years old; the second group—middle aged, 45–59 years old; the third—elderly, 60–74 years old; and the fourth—old age, 75–89 years old).

nucleus over the cytoplasm weakens [3, 4]. M.S. Milman's theory (1926) suggested that the location of organelles and their interrelations were disturbed as a result of uneven growth in individual cell structures, which can lead to a cellular hunger.

According to the results of our study, no significant changes in the NCR take place with aging in the studied brain structures.

The NCR was stable in the NBM in four age groups. The NCR in TMN is the same in the first,

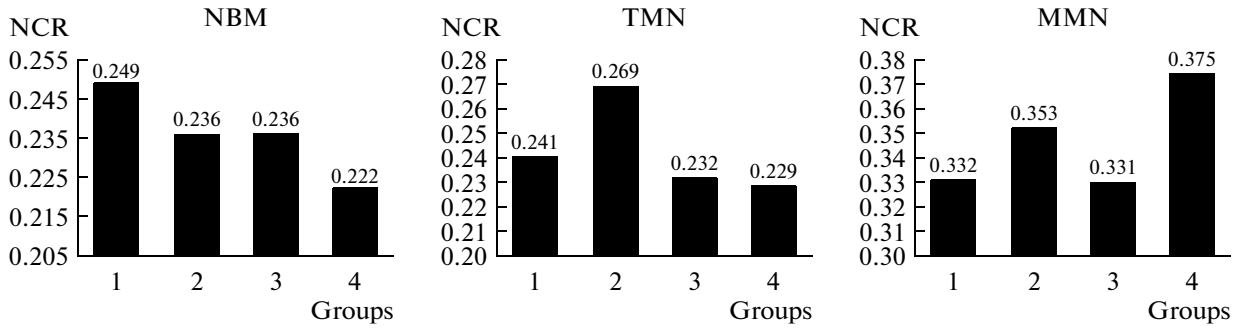

Fig. 2. Nucleocytoplasmic ratio (NCR) of neurons in nucleus basalis of Meynert (NBM), tuberomamillary (TMN), and medial mamillary (MMN) nuclei of human hypothalamus in four age groups (the first group—young, 25–44 years old; the second group—middle aged, 45–59 years old; the third—elderly, 60–74 years old; and the fourth—old age, 75–89 years old).

third, and the fourth groups, with the exception of an increase in the second age group. The NCR in MMN is the same in the first, second, and the third groups, and only insignificantly increases in the fourth group (Fig. 2). None of the mentioned correlations related to the NCR is statistically reliable.

CONCLUSIONS

Thus, this study showed an enlargement in the sizes of neuronal nuclei and pericarya in the nucleus basalis of Meynert, as well as in the tuberomamillary and medial mammillary nuclei in elderly people aged 60–74 years. This reflected an increased metabolic activity of neurons participating in the realization of cognitive functions. These results confirm the defensive and adaptive nature of the noted morphometric changes, which allow the development of neurodegenerative processes to be prevented or delayed. These defensive mechanisms switch on earlier in middle-aged persons (45–59 years) in the nucleus basalis of Meynert, which, to a greater extent, is affected in Alzheimer's disease (shrinkage of large neurons, the presence of a significant number of neurofibrillary tangles and amyloid plaques). It should be noted that the WHO periodization in this case not only reflects the age-related norms for the sizes of neuronal nuclei and pericarya but also indicates the particular age period in the onset of defensive and adaptive responses.

REFERENCES

1. Anisimov, V.N., *Molekulyarnye i fiziologicheskie mekhanizmy stareniya* (Molecular and Physiological Mechanisms of Aging), St. Petersburg: Nauka, 2008, vol. 1.
2. Ishunina, T.A., Changes in human cortical and subcortical brain structures at aging and Alzheimer's disease, *Morphol. Newslett.*, 2011, no. 4, pp. 83–97.
3. Medvedev, Zh.A., *Biosintez belkov i problemy ontogeneza* (Protein Biosynthesis and Problems of Ontogenesis), Moscow: Medgiz, 1963.
4. Nikitin, V.N., Recent theories of aging, in *Molekulyarnye i funktsional'nye osnovy ontogeneza* (Molecular and Functional Principles of Ontogenesis), Moscow: Meditsina, 1970, pp. 7–34.
5. Antonenko, D. and Floel, A., Healthy aging by staying selectively connected: a mini-review, *Gerontology*, 2014, vol. 60, pp. 3–9.
6. Ishunina, T.A., van Beurden, D., van der Meulen, G., et al., Diminished aromatase immunoreactivity in the hypothalamus, but not in the basal forebrain nuclei in Alzheimer's disease, *Neurobiol. Aging,* 2005, vol. 26, pp. 173–194.
7. Minot, C.S., The problem of age, growth, and death, *Pop. Sci. Month.*, 1907, vol. 71, pp. 97–120.
8. Navarro, C. and Gonzalo, L.M., Changes in the human amygdaloid complex due to age, *Rev. Med. Univ. Navarra*, 1991, vol. 35, pp. 7–12.
9. Park, D.C. and Reuter-Lorenz, P., The adaptive brain: aging and neurocognitive scaffolding, *Ann. Rev. Psychol.*, 2009, vol. 60, pp. 173–196.
10. Riudavets, M.A., Iacono, D., Resnick, S.M., et al., Resistance to Alzheimer's pathology is associated with nuclear hypertrophy in neurons, *Neurobiol. Aging*, 2007, vol. 28, pp. 1484–1492.
11. Swaab, D.F., Brain aging and Alzheimer's disease, "wear and tear" versus "use it or lose it," *Neurobiol. Aging.* 1991, vol. 12, pp. 317–324.

Translated by N. Tarasyuk