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MORPHOMETRIC ANALYSIS OF AGE-RELATED CHANGES IN THE PYRAMIDAL NEURONS OF THE HUMAN PREFRONTAL CORTEX FROM 8 TO 21 YEARS

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ABSTRACT — The article is devoted to the study of age-related changes in the size of pyramidal neurons of the human prefrontal cortex in postnatal ontogenesis. Histological material was obtained from 42 left cerebral hemispheres of males aged 8 to 21 years who died as a result of injuries without brain damage. The material was divided into three age groups: 1 — children aged 8 to 12 years (the age period of the second childhood, 15 observations), 2 — adolescents aged 13 to 16 years (12 observations), young men aged 17 to 21 years (15 observations). Morphometric analysis of pyramidal neuron sizes was performed in the III3 sublayer of the prefrontal cortex in the frontal eye field 8, the speech field 45 (Broca zone) and in the field 10 on the lateral surface of the frontal pole. For this purpose, we used virtual images of frontal paraffin sections of the prefrontal cortex 10 microns thick, stained with cresyl violet on Nissl. In each field of each of the three age groups we measured the height and width of the basal part of bodies at least 3000 neurons. The body volume of each neuron was calculated using a formula to calculate the cone volume. In each field of the prefrontal cortex we also analyzed age-related changes in the percentage of small, medium and large neurons. For indicators of different age groups and different individuals we calculated the average, the error of the average and the confidence interval with the level of significance P=95% ($p<0.05$). It was found that the largest individual differences in the size of pyramid neurons were characteristic of children whose individual indicators differed from the average indicators of the whole age group by 18–26%. We found a significant increase in the average volume of pyramid neurons in the field of 10 in adolescents compared to children. We also showed that in young men the change in the number of neurons in the classes of large cells led to the appearance of single neurons of the largest size in the range from 6101 to 8100 μm^3, which accounted for 5–7% of the total number of neurons studied. The obtained results demonstrate significant age-related changes in the size of pyramid neurons of the external pyramidal layer in the fields of 8, 45 and 10 prefrontal cortex in men aged from 8 to 21 years.

KEYWORDS — human prefrontal cortex, pyramidal neurons, morphometry, postnatal ontogenesis.

INTRODUCTION

The study of changes in the human cerebral cortex during ascending ontogenesis continues to be a topical problem in age-related neuromorphology. It is important to determine the degree and nature of age-related changes affecting the control systems of the brain. The leading role in these control systems is played by the prefrontal cortex, which participates in the implementation and control of the most complex forms of cognitive activity [1]. It is known that modern brain imaging methods do not yet allow studying the microstructural components of the cerebral cortex in living people so that it would be possible to trace intracortical structural changes at different stages of life. Histological examination of the human cerebral cortex using morphometric techniques provides this opportunity. Modern morphometric data indicate that in the prefrontal cortex of the left hemisphere the largest changes in pyramid neuron size are observed from birth to 2–3 years. By the age of 7, pyramid neuron sizes in the cerebral cortex of girls are approaching those of adult women, while in boys neuron sizes are still changing [2]. When studying the size of neurons, as a rule, the size of the profile field (median section) of neuronal bodies is analyzed, but not their volume [3]. However it is known that at growth of the sizes of three-dimensional object the size of its volume changes to a greater extent than the area of median section [4]. Body volume is an important quantitative characteristic of the metabolic and functional potential of the neuron.

OBJECTIVE

The aim of the study was to study the age-related dynamics of changes in body volumes of pyramid neurons in the human prefrontal cortex at late stages of ascending ontogenesis.

MATERIALS AND METHODS

42 left hemispheres of men aged 8 to 21 years who died from injuries unrelated to brain damage were investigated. Collection of sectional material was authorized by the Ethical Commission of the Institute of Developmental Physiology of the Russian Academy of Education (Protocol No. 3 of 23.05.1996) and was carried out in the forensic mortuaries of Moscow and

Moscow region. The material was divided into three age groups: 1 — children aged 8 to 12 years (the age period of the second childhood, 15 observations), 2 — adolescents aged 13 to 16 years (12 observations), young men aged 17 to 21 years (15 observations). The pieces of the prefrontal cortex for histological examination were taken in the frontal eye field 8, the speech field 45 (Brocae zone) and on the lateral surface of the frontal pole in field 10 according to the Atlas of cytoarchitectonics [5]. The material was fixed in 10% neutral formalin, dehydrated in alcohols of ascending concentration and poured into paraffin according to standard methods. Each 40th frontal section 10 μm thick was stained with Nissl cresyl violet. We used a computer morphometry method with Image Tools technology (National Institutes of Health, USA) and ImageExpert™ Gauge microobject geometric measurement program (NEXSYS, Russia), as well as a Biolam-15 LOMO® microscope with an integrated USB camera UCMOS01300KPA (Altami, Russia) for measurements in the external pyramidal layer of the cortex (III^3 sublayer) of the height (H), as well as the width of the basal part (a) bodies of neurons with clearly visible nucleus, nucleolus and cytoplasm. The volume of the conical cell body (V) for each pyramidal neuron was calculated by the formula:

$$V = \frac{1}{3}\pi H[\frac{a}{2}]^2$$

The sample size for each section was not less than 10 neurons, for each histological preparation — not less than 4 sections, for each individual — not less than 5 preparations, for each annual age interval - not less than 3 individuals, for each age group — not less than 3000 neurons in each of the studied fields. We determined 8 size groups to calculate the percentage of pyramidal neurons of different sizes. Two groups were assigned to small neurons: 1 — from 101 to 1100 μm^3, 2 — from 1101 to 2100 μm^3. Three groups were assigned to middle neurons: 3 — from 2101 to 3100 μm^3, 4 — from 3101 to 4100 μm^3, 5 — from 4101 to 5100 μm^3. The largest groups also included 3 groups: 6 — from 5101 to 6100 μm^3, 7 — from 6101 to 7100 μm^3 and 8 — from 7101 to 8100 μm^3. For indicators of different age groups and different individuals, the average value, the error of the average and the confidence interval with a significance level of P = 95% ($p < 0.05$) were calculated.

RESULTS

In boys aged 8–12 years in the III^3 sublayer of the external pyramidal layer, the mean group indices of pyramid neuron volumes in fields 8 and 45 did not have significant differences (Table 1). In comparison with these fields in the field 10 of frontal pole the size of pyramid neurons was on the average 1.2 times less. Individual averages for different children within the same age group ranged in the field 8 from 1,646.5±88.7 to 2,681.4±142.9 μm^3, in the field 45 — from 1,696.9±84.2 to 2,610.5±126.6 μm^3, in the field 10 — from 1,503.3±78.6 to 2,488.4±118.5 μm^3. In adolescents 13–16 years old, the volume of pyramid neurons in fields 8 and 45 did not change on average in comparison with children of the second childhood period. In field 10, the sizes of neurons increased 1.1 times, however, despite a significant increase, they were 1.1 times smaller than in other fields of the same age group. Minimum average individual indices of pyramid neuron volumes in all investigated fields increased by 1.1–1.2 times in comparison with children aged 8–12 years. As a whole, individual indicators within the group of adolescents ranged in the field of 8 from 1910.6±95.4 to 2607.3±137.8 μm^3, in the field of 45 — from 1921.5±112.2 to 2344.8± 104.1 μm^3, in the field of 10 from 1832.0±89.3 to 2421.3±132.7 μm^3. In young men aged 17 to 21, the average size of pyramid neurons in all investigated fields of the prefrontal cortex remained stable and did not differ from those found in children and adolescents. The differences between the volumes of pyramidal neurons in field 10 and the remaining fields in young men were the same as in adolescents. Individual indicators within the group of young men ranged in the field of 8 from 2057.5±107.2 to 2714.0±152.6 μm^3, in the field of 45 — from 1930.19±91.8 to 2558.09±125.0 μm^3, in the field of 10 from 1838.0±96.4 to 2410.0±130.6 μm^3. The study of pyramid neuron distribution by size classes showed that in all age groups small neurons prevailed, the total content of which varied in children from 56 to 73%, in adolescents — 66–68%, in young men — 54–58%. In adolescents in the field 8 the redistribution of neuron sizes towards their increase occurred within the small cell classes, while the content of medium-sized neurons increased insignificantly (Fig. 1). In fields 45 and 10, the content of medium-sized neurons increased compared to children (Fig. 2, 3). The content of size classes 4 and 5 was 12–22% of the total neuron population. In young men, the content of small neurons in all fields changed insignificantly. However, the redistribution of the number of neurons within large cell classes led to the appearance of single neurons of the largest size in the range from 6101 to 8100 μm^3, which accounted for 5–7% of the total number of neurons.

DISCUSSION

The results obtained show that significant changes in the size of pyramid neurons of the upper floor of the prefrontal cortex occur not only in the early stages

Table 1. *Volume of pyramid neurons in the III³ sublayer of the human prefrontal cortex at different stages of postnatal ontogenesis (M+m) (mkm³)*

Cortical area	Second childhood	Adolescents	Young men
Field 8	2243,9±45,2	2336,6±53,7	2351,2±56,8
Field 45	2216,4±39,9	2241,6±32,6	2259,9±54,4
Field 10	1889,6±34,3*	2083,4±45,4*#	2150,2±50,0^

Differences are significant (at p<0,05) in comparison with:
** other fields of the same age group; # same field previous age group; ^ field 8 in the same age group.*

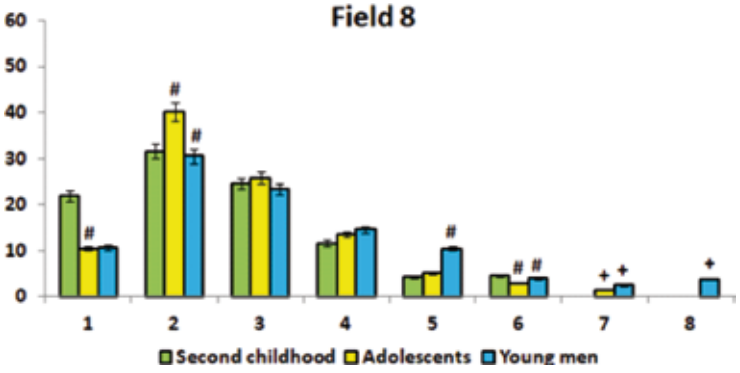

Fig. 1. *Percentage of pyramidal neurons from different size classes in sublayer III3 of field 8*

Here and on Fig. 2 and 3: *on the x-axis — neuronal size groups (1 — 101–1100 μm³, 2 — 1101–2100 μm³, 3 — 2101–3100 μm³, 4 — 3101–4100 μm³, 5 — 4101–5100 μm³, 6 — 5101–6100 μm³, 7 — 6101–7100 μm³ and 8 — 7101–8100 μm³); on the y-axis — neuronal percentage.*
Green bars — children 8–12 years old, yellow bars — adolescents, blue bars — young men. The vertical segments represent the error of the mean. # — differences are significant (at p<0,05) in comparison with same field previous age group; + — emergence of the largest neurons.

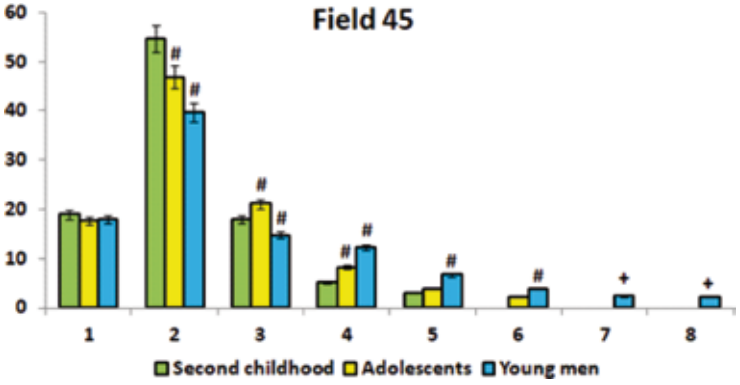

Fig. 2. *Percentage of pyramidal neurons from different size classes in sublayer III³ of field 45*

of life, but also in adolescents and young men. The field 10 of the prefrontal cortex is one of the areas most late maturation in postnatal ontogenesis [6]. The significant increase of neuron sizes detected by us in field 10 on the lateral surface of the hemisphere in adolescents in comparison with children of 8–12 years old serves as an argument that microstructural transformations of the prefrontal cortex and its cortical-cortical connections do not end with children and continue in the puberty period. In adolescents, the importance of the frontal pole cortex increases for the realization of such functions as coordination of the sequence of actions in the time continuum, maintenance of working memory, control of emotional aspects of behavior, change of attitude to a specific type of activity [7, 8]. In this regard, in our opinion, the body volume of pyramidal neurons can be considered as one of the structural descriptors that allow estimating the level of morphofunctional cortical development in adolescents and young men. Studying the average size of pyramid neurons in individuals has shown that significant individual differences are observed in each of the age groups. There is an opinion that individual differences in the complex of morphological parameters in the population increase as they grow older. This process is seen as a consequence of the influence of not only hereditary but also environmental factors [9]. However, when analyzing the average group indicators of pyramid neuron volumes in the upper floor of the prefrontal cortex included in complex associative neural networks, we found that the most variable in terms of the studied parameter are neurons in the cortex not in young men but in children aged 8-12 years. Individual differences of neuronal sizes in the studied fields in children vary by ±18–26% from the average value of the whole age group. In adolescents, the

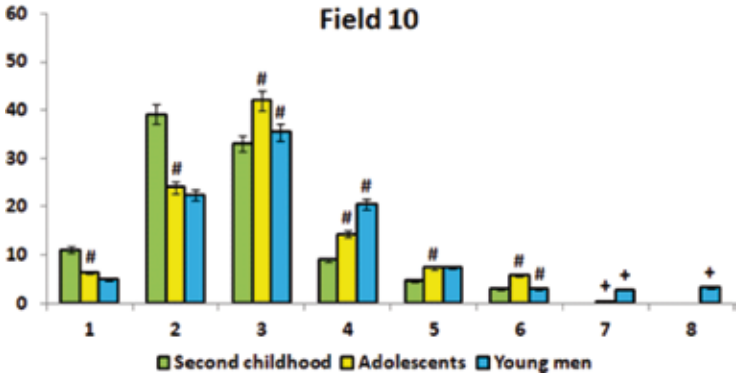

Fig. 3. Percentage of pyramidal neurons from different size classes in sublayer III³ of field 10

range of individual variability is narrowed compared to the second childhood period. The variability of average individual neuronal volume in adolescents is ±5–18% of the average of the whole age group. The largest range of variability is found in the frontal eye field 8, the smallest — in the speech field 45. In young men there is *stabilization* of the range of neuronal size fluctuations in individuals. The average volume of neurons individually varies in all investigated fields of the prefrontal cortex by ± 12–15% of the mean group size. The results showed that not only children, but also adolescents and young men have a redistribution of neurons in size classes. This redistribution in different fields of the prefrontal cortex has specific features. However, the appearance of the largest pyramid neurons occurs in all fields in young men, which in our opinion is typical for the prefrontal cortex. Such neurons have particularly extensive receptive fields and can probably be regarded as polysensory multifunctional neurons with mixed selectivity [10]. It can be assumed that they are able to effectively engage in the activity of various functionally specialized neural networks, ensuring the implementation of the most complex cognitive processes involving the prefrontal cortex.

CONCLUSION

The study demonstrates the possibilities of computational neuromorphology to determine age-specific features of the cerebral cortex microstructure at different stages of postnatal ontogenesis. Further perspective of applying quantitative approach to assessment of age-related changes and variants of structural organization of the cerebral cortex may be connected with improvement of noninvasive methods of human brain tissue visualization.

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