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COMPARATIVE MORPHOMETRIC ANALYSIS OF AGE-RELATED CHANGES IN THE PYRAMIDAL NEURONS OF THE HUMAN PREFRONTAL AND POSTERIOR ASSOCIATIVE CORTEX FROM BIRTH TO 7 YEARS

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Sami Omar[1], Tatiana Tsekhmistrenko[1,2]✉, Valentina Vasilyeva[2], Vadim Astashov[1], Dmitry Obukhov[3], Patimat Magomedova[1]

[1] Peoples' Friendship University of Russia (RUDN University), Moscow;
[2] Institute of developmental physiology, Russian Academy of Education, Moscow;
[3] St. Petersburg State University, St. Petersburg, Russia

✉ *tsekhmistrenko-ta@pfur.ru*

ABSTRACT — Aim: This article is devoted to the study of age-related changes in the body volume of pyramidal neurons (VP) of the human prefrontal (PFC) and posterior associative cortex (PAC) in children from birth to 7 years of age.
Methods: The material consisted of left cerebral hemispheres obtained from 60 male individuals. We studied the areas 8, 45 and 10 in the PFC and area 37 in subareas 37ac, 37a, 37d in the PAC. For morphometry of pyramidal neurons in cortical sublayer III3, we used virtual images of serial frontal paraffin sections of cortex 10 thick stained by Nissl. We determined the mean value of VP (mean VP), the standard error of the mean, and the confidence interval for each age group.
Results: The most significant changes in the mean VP in all studied cortical areas occurred during the first 6 months of life. The greatest increase in the VP was detected in the PFC from 3.5 to 7 years, and in the PAC from 1.5 to 3 years of age. There are close positive correlations between age-related changes in the size of neurons in the PFC and in the PAC.
Conclusion: In children, the development of pyramidal neurons of the external pyramidal plate in the PAC occurs earlier and at a more intensive pace compared to the PFC. We suggest that VP is an important quantitative indicator that allows us to assess the timing and rate of age-related structural changes in the cerebral cortex.

KEYWORDS — cerebral cortex in children, prefrontal cortex, posterior associative cortex, body volume of pyramidal cells, morphometry, postnatal ontogenesis.

INTRODUCTION

The study of neuronal growth and development in the human cerebral cortex is of considerable interest [9]. However, this issue has not been sufficiently studied to determine how neuronal growth processes in different cortical areas differ from each other during the early stages of postnatal ontogenesis. Pyramidal neurons in the outer pyramidal plate of the associative cortical zones — prefrontal cortex (PFC) and posterior associative cortex (PAC) — attract special attention. They play an important role in perception, attention, speech activity, auditory-motor and visual-motor coordination, mechanisms of working and long-term memory, cognitive activity, and behavior control in general [1, 5, 11]. Recently, researchers have been paying more and more attention to the quantitative analysis of the structural organization of the human cerebral cortex and nervous system during ontogenesis [14, 18]. One of the most important indicators of microstructural transformations in the cerebral cortex is the change in body volume of pyramidal cells (VP). The increase in nerve cell body volume correlates with the growth and development of their dendritic arborizations, complication of axon branching and synaptic apparatus, which serves as a significant indicator of subtle rearrangements in the brain structure underlying the improvement of its functions with age [6, 12].

The aim of the present study was to identify the timing and quantitative characteristics of age-related changes in the pyramidal neurons of the prefrontal cortex and posterior associative cortex in children aged from birth to 7 years.

MATERIALS AND METHODS

The material included the left cerebral hemispheres of 60 boys aged from birth to 7 years who died from injuries without brain damage. The Ethics Committee in the Institute of Developmental Physiology of the Russian Academy of Education allowed the collection of the necessary sectional material (protocol No. 3 of 23.05.1996) in forensic morgues in Moscow and the Moscow region.

The histological material was divided into 5 age groups, including: newborns (n = 11), 6 months (n = 13), 12 months (n = 10), from 1.5 to 3 years (n = 10) and from 3.5 to 7 years (n = 16).

The cortical areas for the study were selected according to K. Brodmann's cytoarchitectonic map. After fixation in 10% neutral formalin, tissue fragments were excised in the anterior part of area 8 (frontal eye field, BA 8), in the speech-motor area 45 (Broca's area, BA 45), on the lateral surface of the frontal pole in area 10 (BA 10) and in area 37 (BA 37) of the PAC. In area 37, we selected subareas 37ac, 37a, and 37d for the study in accordance with the Atlas of cytoarchitectonics of the human cerebral cortex [4]. In the upper section of the 37ac subarea (SA 37ac), a fragment was isolated in the hMT/V5 multimodal region, which is involved in the processing of tactile and visual information about the direction of moving objects [17]. On the inferior medial surface of the temporal lobe, we investigated subarea 37a (SA 37a) in the fusiform face area (FFA) [8], and on the medial surface, subarea 37d (SA 37d, or V8 zone). V8 zone is included in a system of anatomically related structures that are essential for declarative memory (conscious memory for facts and events) [15].

We carried out a morphometric analysis of pyramidal neurons bodies volume (VP) in sublayer III3 of the cortex on virtual images of frontal paraffin sections 10 μm thick, stained with Nissl cresyl violet. For this purpose, we used Image Tools technology (National Institutes of Health, USA) and ImageExpert™ Gauge geometric measurement software for microobjects (NEXSYS, Russia), as well as a Biolam-15 LOMO microscope with a built-in USB camera UCMOS01300KPA (Altami, Russia). In each area in 5 age groups, we measured the height of 800-1000 pyramidal neurons and the width of the basal part of their bodies, then the volume of the cell body of each neuron was calculated using the cone volume formula. Statistical processing of the obtained data included checking the normality of the distribution of values in the compared samples, and analyzing the probability distribution of quantitative signs [10]. We calculated mean body volume of pyramidal cells (mean VP), standard error and confidence interval using SigmaPlot software package (SYSTAT Software, USA) for different age groups. Significance of differences between mean values of different age groups was determined using two-sample t-criterion at P>95% ($p<0.05$).

To study the relationship between age-related changes in the size of pyramidal neuron bodies in the compared cortical areas, the study material was grouped into 9 age groups: newborns, children 6 months, children 12 months, and then the age groups were followed in annual intervals from 2 to 7 years. To assess the relationship between age-related changes in mean body volumes of pyramidal neurons in different cortical areas, we calculated Spearman's rank correlation coefficient (Rs) and its statistical significance using Student's criticality table.

RESULTS

It was found that in newborns in the PFC the largest volume of pyramidal neurons was observed in BA 8. In this area, the sizes of pyramidal neurons were 1.2 times larger than in BA 10 and BA 45 (Table 1).

Growth rates mean VP in different areas of the PFC were different during the first months. By 6 months in BA 8 and BA 10, this indicator increased 1.5 times, and in field 45 — 2.7 times compared with newborns. As a result, in boys of 6 months, the sizes of pyramidal neurons in BA 45 were on average 1.6–1.8 times larger than in BA 8 and BA 10. In the second half of the year, mean VP in BA 8 and BA 10 continued to grow, increasing on average 1.6 times as compared with the indicators of children 6 months old. By the end of the first year of life in BA 45, the average somal volume did not change, the sizes of the pyramidal neurons of the PFC did not differ between areas. During 2 and 3 years of life, mean VP in BA 8 and BA 45 remained stable, and in BA 10 it increased 1.2 times compared with one-year-old children. In children 2–3 years old, we did not observe significant differences in mean VP between the areas of the PFC. In the group of children from 4 to 7 years old, the size of pyramidal neurons in the PFC increased in comparison with children 2–3 years old: in BA 8 — 1.8 times, in BA 10 — 1.6 times, and in BA 45 — in 1.4 times. Pyramidal neurons in BA 8, on average, had 1.3 times the volume in comparison with BA 45.

A number of obvious trends were observed in the formation of pyramidal neurons in the PFC. In functionally different areas, a significant increase in the size of pyramidal neurons occurred synchronously during the first six months of life and heterochronously in subsequent years of ascending ontogenesis: in the BA 8 (frontal eye field) — by 1 and 7 years, in BA 10 of the frontal pole — by 1, 3, and 7 years, BA 45 (speech motor area) — by the age of 7. The increase in mean VP in different areas of the PFC occurred heterodynamically, that is, at different rates and with different intensities. During the first 6 months, the sizes of pyramidal neurons increased most intensively in the BA 45, in the second half of life — in the BA 8, over 1.5-3 years — in BA 10, and within 3.5–7 years — again in BA 8. At the stage of 3.5–7 years, a significant increase in mean VP occurred in all studied areas of the PFC, which signaled

Table 1. Age-related changes in the average body volume of pyramidal neurons in III3 sublayer of the prefrontal cortex in children (M+m) (mkm^3)

Age group	n	Area 8	Area 10	Area 45
Newborns	11	458,9±24,0	377,7±14,7	390,0±16,9
6 months	13	678,3±49,5*	581,4±46,5*	1053,7±118,9*
1 year	10	1112,4±72,2*	928,4±69,9*	983,6±81,1
1.5–3 years	10	1127,6±69,3	1071,1±55,8*	1080,5±64,1
3.5–7 years	16	2014,2±147,5*	1688,5±108,1*	1498,5±77,6*

*Note to Tables 1 and 2: * — the differences are significant (at p<0.05) in comparison with the same area of the previous age group*

an increase in the functional activity of distributed neural networks with its participation.

In the PAC in newborns, the highest mean VP was in SA 37d, located on the border with the limbic lobe, and the lowest in SA 37a in the FFA region. The difference in the average group indicators in these subfields was 1.2 times (Table 2).

The volume of pyramidal neurons in SA 37a was on average 1.3-fold greater than in SA 37ac. In children aged 4 to 7 years, the mean body volume of pyramidal cells in SA 37ac increased by 1.2 times in comparison with children 2–3 years old, and did not change in SA 37a and SA 37d. As a result, the differences in the sizes of pyramidal neurons between SA 37ac and SA 37a, which we noted in children 2–3 years old, smoothed out by the age of 7 years.

Table 2. Age-related changes in the average body volume of pyramidal neurons in III3 sublayer of the posterior associative cortex in children (M+m) (mkm^3)

Age group	n	Subarea 37ac	Subarea 37a	Subarea 37d
Newborns	11	446,3±30,9	392,0±22,4	488,4±14,3
6 months	13	1202,5±86,9*	975,5±51,6*	1019,8±44,5*
1 year	10	1369,9±51,9	1462,4±93,1*	1733,4±88,1*
1.5–3 years	10	1813,2±121,7*	2372,6±133,4*	2046,3±111,7*
3.5–7 years	16	2189,3±118,8*	2402,3±134,8	2081,9±116,3

By the end of the first half of the year, mean VP increased 2.6 times in SA 37ac and SA 37a, and 2.1 times in SA 37d compared with newborns. The sizes of SA 37ac pyramidal neurons were, on average, 1.2 times larger compared to SA 37a and did not differ significantly from SA 37d. Interestingly, in this segment of postnatal ontogenesis, the growth rates mean VP in the BA 37 subfields are similar to the growth rates of pyramidal neurons in BA 45 and are significantly higher compared to BA 8 and BA 10 of the PFC. In the second half of the first year of life, the size of pyramidal neurons did not change in SA 37ac, increased 1.5-fold in SA 37a, and 1.7-fold in SA 37d compared to 6-month-old children. By the end of the first year of life, the average volume of neuronal soma in SA 37d was 1.3 times larger compared to SA 37ac.

In 2–3-year-old boys, the mean VP in the PAC increased synchronously and heterodynamically compared to those in children aged 1 year: 1.3-fold in SA 37ac, 1.6-fold in SA 37a, and 1.2-fold in SA 37d.

In the PAC, we noted a synchronous increase in the size of pyramidal neurons in SA 37a and SA 37d during the first year of life, as well as in the age group of children 1.5–3 years old. In SA 37ac, synchronously with the rest of the fields of the PAC, the mean VP increased during the first 6 months of life and in the age group of children 2–3 years old. In the age group of children 3.5–7 years old, we noted an increase in mean VP in SA 37ac. The heterodynamic nature of the increase in the size of pyramidal neurons was illustrated by the fact that during the first 6 months the mean VP increased most intensively in SA 37ac and SA 37a, in the second half of the year — in SA 37d, over 2–3 years — in SA 37a, and during 4–7 years — in SA 37ac.

To assess the relationship between the rates of age-related changes mean VP in functionally different zones of the PFC and PAC in children, we used Spearman's rank correlation analysis. The number of rank pairs (n = 9) of the compared values corresponded to the number of age groups from birth to 7 years in annual intervals, taking into account the indicators of newborns and children at the age of 6 months. Significant correlation coefficients Rs between mean VP in BA 8, BA 10 and BA 45 and SA 37ac, SA 37a and SA 37d are presented in Table 3.

Table 3. *Areas with significant positive correlations of age-related changes in the mean body volume of pyramidal neurons in sublayer III3 in children from birth to 7 years*

n=9	Rs	t-stat
Area 8 – Area 10	0,95	0,34
Area 8 – Area 45	0,92	0,43
Area 8 –Subarea 37ac	0,75	0,71
Area 8 – Subarea 37a	0,82	0,62
Area 10 – Area 45	0,90	0,47
Area 10 – Subarea 37ac	0,90	0,47
Area 10 – Subarea 37a	0,77	0,69
Area 10 – Subarea 37d	0,83	0,59
Area 45 – Subarea 37a	0,85	0,57
Subarea 37a – Subarea 37d	0,95	0,34

Note: *n is the number of rank pairs of compared values; Rs is the Spearman's correlation coefficient; t-stat is Student's t-test. Two-sided level of significance (p-value) < 0.05.*

It follows from the table that a statistically significant strong and direct two-way relationship existed between age-related changes in the size of pyramidal neurons in all the studied areas of the PFC. In the PAC, we detected a strong and direct bilateral relationship between changes in the dimensional parameters of pyramidal neurons only in SA 37a and SA 37d, whereas SA 37ac, located on the lateral surface of the hemisphere, had no significant correlations with these subareas. We also found statistically significant strong and direct two-way correlations between changes in the mean VP in BA 10 and in all subfields of BA 37. Similar correlations existed between BA 8 and subareas 37ac and 37a, and between BA 45 and SA 37a.

DISCUSSION

Our study, carried out on a large histological material grouped into 5 age groups from birth to 7 years, showed that the most significant changes in mean VP in the areas of the PFC and PAC of the left cerebral hemisphere occurred in children during the first 6 months of life. During postnatal development, the greatest increase in mean body volume of pyramidal cells was found in the PFC at the age interval from 3.5 to 7 years, and in the PAC — from 1 to 3 years.

It should be noted that the increase in the size of pyramidal neurons continues after 7 years [2], which can be traced by analyzing the redistribution of the relative number of neurons in the cell population in the direction from small-cell to large-cell size classes [16]. An increase in pyramidal neuron body volume is accompanied by lengthening of dendrites, complication of their terminal branching and an increase in the number of synaptic contacts, which up to a certain limit expands the receptive fields of neurons, and also affects excitability and intracellular changes associated with learning and memory [13]. As a result, competitive conditions are created for the selective activity of neurons in the system of distributed neural networks and fine tuning of the previously established intercellular interaction [7]. In our opinion, it is this natural process that underlies the transition from generalized cortical reactivity at the early stages of postnatal ontogenesis to the gradual formation of specialized and locally structured responses to modally specific stimuli in children and adolescents.

The presence of close correlations between neuronal size changes in BA 10 of the PFC and the BA 37 subareas indicates the important role of the frontal pole cortex in controlling the processes of visual perception, attention and goal-directed behavior, which are based on signals received from the inferior temporal associative cortical areas [3]. The close selective relationship between neuronal size changes in areas 45 and 37a reflects the important role of visual facial perception for the development of speech articulation. Similar connections between oculomotor area 8 and subareas 37ac and 37a serve as the basis for recognition of moving objects and spatial visual memory.

CONCLUSIONS

Thus, we conclude that in children from birth to 7 years of age, the development of pyramidal neurons of the external pyramidal plate in the posterior associative cortex occurs earlier and at a more intensive pace compared to the PFC. We were also convinced that the pyramidal neuron body volume is one of the important indicators that allow us to evaluate not only the timing and rate of age-related structural changes in the cerebral cortex, but also to obtain informative data on the morphofunctional relationship between different cytoarchitectonic cortical areas at different stages of postnatal ontogenesis.

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