DOI 10.26724/2079-8334-2022-1-79-249-254
UDC 611.817.1:612.646/.647:572.7

V.S. Shkolnikov, L.L. Zalevsky, T.Yu. Shkolnikova
National Pirogov Memorial Medical University, Vinnytsya

MORPHOLOGICAL FEATURES OF EMBRYOS' AND HUMAN FETUSES' CEREBELLUM

e-mail: v.shkolnikov@gmail.com

The purpose of the study was to establish macro- and morphometric parameters of cerebellum and cytoarchitectonics of its structures. Morphometric, anatomical-histological, and immunohistochemical studies of cerebellum of 116 human embryos and fetuses aged from 6–7 weeks up to 39–40 weeks were performed. There were no congenital anomalies of central nervous system. During intrauterine development, there is a tendency to gradually increase in thickness of cerebellum intermediate layer due to development of neuronal glial complexes. While ventricular layer is gradually thinned by reducing the intensity of neural cell proliferation. The highest intensity of neural cell proliferation in the embryonic period occurred in the ventricular and outer granular layers, and the lowest intensity – in the intermediate layer. In the radial direction, radial glia fibers passed through all cerebellum layers and ended in the outer granular layer.

Key words: intrauterine development, cerebellar hemispheres, cerebellar vermis morphometric parameters, neurons, radial glia.

В.С. Школьніков, Л.Л. Залевський, Т.Ю. Школьнікова

МОРФОЛОГІЧНІ ОСОБЛИВОСТІ МОЗОЧКА ЕМБРІОНІВ ТА ПЛОДІВ ЛЮДИНИ

Метою дослідження було встановити макро- і морфометричні параметри мозочка і цитоархітектоніки його структур. Проводили морфометричне, анатомо-гістологічне, а також імуногістохімічне дослідження мозочка 116 ембріонів і плодів людини віком від 6–7 тиж. до 39–40 тиж. Вроджених аномалій центральної нервової системи не було. Протягом внутрішньоутробного розвитку спостерігається тенденція до поступового збільшення товщини проміжного шару мозочка у зв'язку з розвитком нейроно-гліальних комплексів. Тоді, як вентрикулярний шар поступово витончується за рахунок зменшення інтенсивності проліферації нейральних клітин. Найвища інтенсивність проліферації нейральних клітин у ембріональному періоді відбувалась у вентрикулярному та зовнішньому зернистому шарах, а найменш інтенсивна – у проміжному шарі. У радіальному напрямку через усі шари мозочка проходили волокна радіалоної глії та закінчувались у зовнішньому зернистому шарі.

Ключові слова: внутрішньоутробний розвиток, півкулі мозочка, черв'як мозочка, морфометричні параметри, нейрони, радіальна глія.

The study is a fragment of the research project "Determination of morphological changes of the human central nervous system during the prenatal period of ontogeny (macroscopic, histological, morphometric, immunohistochemical study)", state registration No. 0118U001043.

The structural and functional development of the central nervous system depends on neurogenesis, neuronal migration and the formation of chains of neurons themselves. This is a complex sequence of events that include a variety of molecular pathways. Neuronal migration is important for normal development because it brings cells into appropriate spatial interconnections with other cells [3, 6].

In the early embryonic stage, neural stem cells are represented as matrix cells, or neuroepithelial cells, that will be symmetrically divided in the neural tube. According to Rakic P., at the beginning of neurogenesis, they are represented as radial glia in the ventricular layer of the neural tube [6]. Studies have shown that the neurogenesis of neural stem cells continues in adults and mammals [1]. The results of these studies allowed us to suggest a therapeutic strategy using neural stem cells to repair damaged central nervous system tissues [5].

Detection of neural stem cells in the brains of embryos and adults has changed the perception of central nervous system regeneration. Instead of the assumption that nerve cells do not divide and are not restored, it was thought that due to neural stem cells in the adult body, the functions of damaged neurons are restored. Scientists around the world have recently been intensively studying neural stem cells. To date, enough clinical and experimental material has been studied with their effective use in various diseases [7].

The purpose of the study was to establish the macro- and morphometric parameters of the cerebellum and the cytoarchitectonics of its structures.

Materials and methods. Morphometric, anatomical-histological, and immunohistochemical studies of the cerebellum of 116 human embryos and fetuses aged from 6–7 weeks up to 39–40 weeks were performed. There were no congenital anomalies of the CNS.

During the work, morphometric anatomical and histological studies, as well as immunohistochemical analysis of the cerebellum of 116 human embryos and fetuses aged 6–7 weeks up to 39–40 weeks were performed, which were received as a result of abortion in Vinnytsia Regional Pathological Bureau. Congenital CNS anormalies were not detected.

Fixation of cerebellar drugs was performed according to the own methods [9]. With the help of a sledge microtome, serial sections 10–12 mm thick were made, after which the preparations were stained with toluidine blue, hematoxylin and eosin, and by the method of Van Gieson. Diagnostic monoclonal antibodies were used during the immunohistochemical study by "DACO" (Denmark): synaptophysin, Ki-67 and vimentin. Morphometric study was performed using the computer software ToupView. Processing of statistical digital data was performed using the software package "Statistica 6.0" from Statsoft (licensed №BXXR901E246122FA) using non-parametric and parametric criteria for assessing the settled results. Differences between traits were determined using the U-test of Mann-Whitney and t-test.

Results of the study and their discussion. During the study of the neural tube wall of embryos aged 6–7 weeks – the thickness in the area of the fourth ventricle was different: in the dorsal part – 24.7±1.3 mm, in the ventral – 199.8±9.8 mm. The cavity of the mesencephalon is surrounded by a wall of the neural tube, which consisted of three layers: neuroepithelial, mantle and marginal, but a clear boundary between these layers is not detected (fig. 1).

The wall thickness, in the area of the fourth ventricle, of the neural tube at the level of the basal plate was – 429.8±22.3 mm, the rhombic lip was – 300.5±14.2 mm, and the pterygoid plate was – 486.9±23.4 mm. The neural tube at the level of the basal plate had the following layer thickness: neuroepithelial layer – 250.1±12.0 mm, mantle layer – 101.3±4.7 mm and the marginal layer – 70.1±3.6 mm. Morphometric parameters at the level of the pterygoid plate had the following parameters: neuroepithelial layer – 340.0±17.0 mm, mantle layer – 110.6±5.2 mm and the marginal layer – 40.3±1.8 mm. The neural tube at the level of the rhombic lip had the following layer thickness: neuroepithelial – 250.7±11.1 mm and mantle – 30.1±1.4 mm.

We found that the formation of the cerebellum occurs due to the growth of the dorso-lateral wall of the neural tube within the future hindbrain. NSC migrate to the area of the rudiment of the cerebellum separated from the ependymal layer, where they form the outer germinal layer. Hereinafter they are differentiated. The direction of NSC movement is controlled by radial glia. In our opinion, the neuroblasts of the matrix zone migrate in the first weeks of development and lead to the aniage of neuro-glial complexes of the cerebellum and Purkinje cells.

In the process of cerebellar examination of 8–9 weeks, we obtained the following parameters: the right cerebellar hemisphere (hemisphaerium cerebellare) had a total thickness of all layers – 1675.7±79.2 mm, the externally granular layer (granuloso strato exteriore) – 28.2±1.2 mm, the molecular layer (molecular strato) – 20.6±1.0 mm, the intermediate layer – 1590.3±74.7 mm, the ventricular layer – 36.30±1.70 mm. The left hemisphaerium cerebellare had a total thickness of all layers – 1643.8±76.9 mm, granuloso strato exteriore – 28.0±1.2 mm, molecular strato – 20.1±1.0 mm, intermediate layer – 1559.4±75.5 mm and the ventricular layer – 36.2±1.8 mm.

We found that for 8–9 weeks NSC migration in all layers occurs in the radial direction, and in the externally granular layer occurs in the tangential direction. In the radial direction, RG fibers passed through all layers of the cerebellum and ended in the externally granular layer. In RG fibers, the expression of vimentin in the intermediate layer was observed to be relatively moderate and relatively strong in the ventricular and outer granular layers. The density of NSC in the ventricular layer of the cerebellum was – 134.0±3.9 cells per 0.01 mm^2, in the intermediate – 74.0±3.0 cells per 0.01 mm^2 in the externally granular – 151.0±4.1 cells by 0.01 mm^2, and in the molecular – 22.0±0.8 cells per 0.01 mm^2, which had the lowest cell density.

Cell proliferation occurs more intensively in the ventricular and externally granular layers, less intensively – in the intermediate layer of the cerebellum (fig. 2).

At 11–12 weeks three layers were identified: ventricular, intermediate layers and cortex. The cerebellar cortex is divided into externally granular, intermediate and inner granular layers.

The thickness in both hemispheres of all layers of the cerebellum varies. The left hemisphaerium cerebellare had a total thickness of all layers – 1569.1±75.3 mm. Cortex of the left hemisphere cerebellum had a total thickness of – 549.7±24.2 mm, which is formed by the following layers: granuloso strato exteriore – 13.7±5.3 mm, molecular strato – 15.9±5.4 mm, inner granular layer (granuloso strato interior) – 520.0±22.8 mm. The intermediate layer is – 985.3±47.3 mm, the ventricular layer is – 34.4±6.9 mm.

The right hemisphaerium cerebellare had a total thickness of all layers – 1985.1±91.3 mm. Cortex of the right hemisphere cerebellum had a total thickness of – 1133.3±53.3 mm, which is formed by the following layers: granuloso strato exteriore – 24.1±6.5 mm, molecular strato – 25.4±6.6 mm, granuloso strato interior – 1083.8±54.2 mm. The intermediate layer is – 817.9±34.3 mm, the ventricular layer is – 33.9±2.2 mm.

The highest cell density was observed in the ventricular layer – 260.0±11.4 cells per 0.01 mm^2, in the outer granular layer was – 235.0±10.2 cells per 0.01 mm^2, in the inner granular layer – 166.0±3.5 cells per 0.01 mm^2 in the molecular layer – 72.0±3.6 cells per 0.01 mm^2. In the intermediate layer, the density was – 55.0±2.5 cells per 0.01 mm^2, which corresponds to the lowest density.

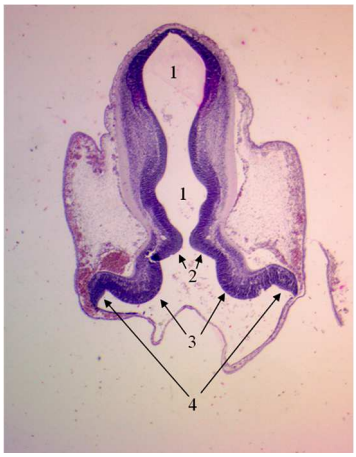

Fig. 1. Horizontal section of the brain of a human embryo aged 6–7 weeks at the level of the hindbrain: 1 – the cavity of the mesencephalon, 2 – basal plate, 3 – wing-shaped plate, 4 – rhombic lip. Hematoxylin-eosin; x 20.

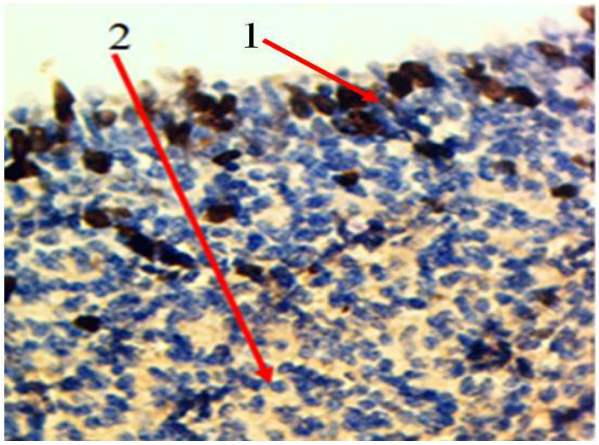

Fig. 2. Proliferation in the cerebellar hemispheres 8–9 weeks. 1 – ventricular layer, 2 – intermediate layer. Ki-67; x 400.

At 11–12 weeks more intense cell proliferation occurs in the ventricular and less intense – in the intermediate layers of the cerebellum. Synaptophysin marker expression was relatively weak in all layers of the cerebellum. From the ventricular layer of RG fibers, pass through all layers of the cerebellum to the outer granular layer in the radial direction. Expression of the marker vimentin in RG fibers was observed to be relatively moderate in the outer granular and intermediate layers and relatively strong in the ventricular and inner granular layers.

At 17–18 weeks we obtained the following morphometric parameters of the cerebellum. The right hemisphaerium cerebellare had a total thickness of all layers – 4189.7±234.6 microns. Cortex of the right hemisphere cerebellum had a total thickness of – 447.3±21.9 mm, which is formed by the following layers: granuloso strato exteriore – 28.3±1.3 mm, molecular strato – 56.0±2.7 mm, granuloso strato interior – 363.0±19.2 mm. The intermediate layer is – 3711.0±144.7 mm and the ventricular layer is – 31.4±1.8 mm.

The left hemisphaerium cerebellare had a total thickness of all layers – 4056.2±229.4 mm. The cortex of the left hemisphere of the cerebellum had a total thickness of – 441.1±20.8 mm, which is formed by the following layers: granuloso strato exteriore – 27.2±1.2 mm, molecular strato – 55.4±2.6 mm, interior granuloso strato – 358.5±18.7 mm. The intermediate layer is – 3584.0±193.5 mm, the ventricular layer is – 31.1±1.8 mm.

The highest cell density was observed in the ventricular layer and was – 18.3±8.4 cells per 0.01 mm^2, and in the outer granular – 14.0±0.5 cells per 0.01 mm^2, in the inner granular – 9.0±0.3 cells per 0.01 mm^2, and in the intermediate layer – 6.3±0.2 cells per 0.01 mm^2. In the molecular layer – 3.7±0.1 cells per 0.01 mm^2, which corresponds to the lowest cell density.

More intense cell proliferation took place in the ventricular layer and less intense - in the intermediate layer (fig. 3).

The expression of the vimentin marker in RG fibers was relatively moderate in the outer granular and intermediate layers, and relatively strong in the ventricular and inner granular layers.

During the study of the cerebellum of the fetus aged 20–21 weeks we established the following parameters: the right hemisphaerium cerebellare had a total thickness of all layers – 4588.7±238.6 mm. Cortex of the right hemisphere cerebellum had a total thickness of – 391.1±18.4 mm, which is formed by the following layers: granuloso strato exteriore – 34.9±1.7 mm, molecular strato – 55.1±2.8 mm, granuloso strato interior – 301.1±14.5 mm. The intermediate layer is – 4172.3±212.8 mm, the ventricular layer is – 25.2±1.2 mm.

The left hemisphaerium cerebellare had a total thickness of all layers – 4472.5±214.7 mm. Cortex of the right hemisphere cerebellum had a total thickness of – 389.8±19.1 mm, which is formed by the following layers: granuloso strato exteriore – 34.9±1.8 mm, molecular strato – 54.2±2.7 mm, granuloso strato interior – 300.7±14.0 mm. The intermediate layer is – 4058.1±194.8 mm, the ventricular layer is – 25.2±1.2 mm.

The highest cell density was observed in the ventricular layer and was – 21.0±0.9 cells per 0.01 mm^2, and in the outer granular – 16.0±0.7 cells per 0.01 mm^2, in the inner granular – 6.0±0.4 cells per 0.01 mm^2, in the intermediate – 8.0±0.5 cells per 0.01 mm^2. In the molecular layer – 4.0±0.3 cells per 0.01 mm^2, which corresponds to the lowest cell density. The highest expression of Ki-67 in this age period is also preserved in the ventricular and outer granular layers of the cerebellum and relatively less intense - in the intermediate layer. Expression of the marker vimentin of the fetus aged 20–21 weeks is relatively strong in the ventricular and inner granular layers and relatively moderate in the outer granular and intermediate layers.

Synaptophysin expression was observed in all layers of the cerebellum in human fetuses aged 22–23 weeks, and relatively strong expression was observed in the molecular layer (fig. 4).

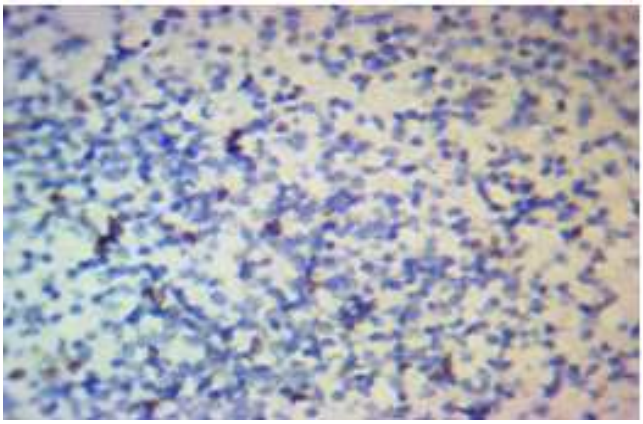

Fig. 3. The cerebellum of the human fetus aged 17–18 weeks. Proliferation of glioblasts in the cerebellar hemispheres. Ki-67; x 400.

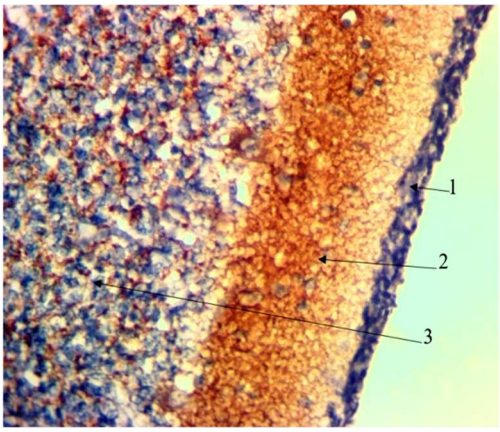

Fig. 4. Horizontal section of the cerebellum of a human fetus aged 22–23 weeks: 1 – outer granular layer, 2 – molecular layer, 3 – inner granular layer. Synaptophysin; x 400.

In the fetuses aged 30–31 weeks right hemisphaerium cerebellare had a total thickness of all layers – 6954.2±326.8 mm. Cortex of the right hemisphere cerebellum had a total thickness of – 1046.1±51.3 mm, which is formed by the following layers: granuloso strato exteriore – 56.1±2.4 mm, molecular strato – 307.9±15.4 mm, granuloso strato interior – 682.1±32.7 mm. The intermediate layer is – 5881.1±299.9 mm, the ventricular layer is – 27.0±1.2 mm.

The left hemisphaerium cerebellare had a total thickness of all layers – 6895.3±344.8 mm. The cortex of the left hemisphere of the cerebellum had a total thickness of – 1043.9±52.2 mm, which is formed by the following layers: granuloso strato exteriore – 55.7±2.7 mm, molecular strato – 307.2±15.1 mm, granuloso strato interior – 681.0±32.4 mm. The intermediate layer is – 5878.0±298.7 mm, the ventricular layer is – 27.0±1.3 mm.

The density of cells in the ventricular layer was – 21.1±1.1 cells per 0.01 mm^2, in the outer granular layer – 24.0±1.3 cells per 0.01 mm^2, in the inner granular – 16.0±0.7 cells per 0.01 mm^2, in the intermediate – 6.1±1.4 cells per 0.01 mm^2. In the molecular layer – 3.0±0.2 cells per 0.01 mm^2, which corresponds to the lowest cell density. More intense glioblast proliferation was observed in the inner granular and less intense in the intermediate layers of the cerebellum.

Expression of the marker vimentin in RG fibers was observed to be relatively moderate in the outer granular and intermediate layers, and relatively strong in the ventricular and inner granular layers. Synaptophysin expression was observed in the layers of the cerebellum.

In human fetuses aged 34–35 weeks the right hemisphaerium cerebellare had a total thickness of all layers – 7817.1±383.0 mm. Cortex of the right hemisphere cerebellum had a total thickness of – 1089.3±56.6 mm, which was formed: granuloso strato exteriore – 50.9±2.2 mm, molecular strato – 276.5±13.0 mm, granuloso strato interior – 761.9±38.9 mm. The intermediate layer is – 6702.7±328.4 mm, the ventricular layer is – 25.1±1.2 mm.

During the study, the left hemisphaerium cerebellare had a total thickness of all layers – 7789.5±397.3 mm. The cortex of the left hemisphere of the cerebellum had a total thickness of – 1087.8±53.3 mm, which was formed: granuloso strato exteriore – 50.5±2.4 mm, molecular strato – 276.1±13.5 mm, granuloso strato interior – 761.2±38.8 mm. The intermediate layer is – 6676.6±307.1 mm, the ventricular layer is – 25.1±1.3 mm.

The density of cells in the ventricular layer was – 20.2±0.9 cells per 0.01 mm^2, in the intermediate – 5.0±0.2 cells per 0.01 mm^2, in the outer granular – 25.4±1.4 cells by 0.01 mm^2, in the inner granular – 17.1±0.8 cells by 0.01 mm^2. In the molecular layer – 2.9±0.1 cells per 0.01 mm^2, which corresponds to the

lowest density, while in the outer granular – 25.4±1.1 cells per 0.01 mm^2, which corresponds to the highest density of NSC.

Expression of the marker vimentin in RG fibers was observed to be relatively moderate in the outer granular and intermediate layers, and relatively strong in the ventricular and inner granular layers. Synaptophysin marker expression was present in all layers of the cerebellum.

At 39–40 weeks the right hemisphaerium cerebellare had a total thickness of all layers – 9689.3±436.2 mm. The cortex of the right hemisphere of the cerebellum had a total thickness of – 926.9±43.6 mm, which was formed: granuloso strato exteriore – 47.4±1.9 mm, molecular strato – 35.6±1.5 mm, granuloso strato interior – 843.9±38.8 mm. The intermediate layer is – 8762.4±420.6 mm.

The left hemisphaerium cerebellare had a total thickness of all layers – 9571.6±469.0 mm. The cortex of the left hemisphere of the cerebellum had a total thickness of – 889.5±40.9 mm, which was formed: granuloso strato exteriore – 46.1±1.8 mm, molecular strato – 34.2±1.4 mm, granuloso strato interior – 809.2±40.5 mm. The intermediate layer was – 8682.1±425.4 mm.

We observed the highest cell density in the outer granular layer of the cerebellar hemispheres, which was – 272.0±12.5 cells per 0.01 mm^2. In the intermediate layer – 40.0±1.9 cells per 0.01 mm^2, in the inner granular – 152.0±7.6 cells per 0.01 mm^2. In the molecular layer – 27.0±1.2 cells per 0.01 mm^2, which corresponds to the lowest cell density.

During the appliance of Ki-67 proliferation protein, more intense cell proliferation was observed in the inner granular, and less intense – in the intermediate layers. Vimentin expression was relatively mediocre in the outer granular and molecular layers. Synaptophysin marker expression was relatively strong in the cerebellar layers.

From 8–9 weeks, NSC proliferation occurs from the ventricular surface or near it in the radial direction (outward) in order to occupy the entire thickness of the cerebellum, except for the outer (cranial)granular layer, the proliferation occurs in it in the tangential direction. At the same time, Volpe J. points out that the proliferation of NSC in human fetuses occurs in the radial direction, and in human embryos is the formation of the outer granular layer and cell migration in the tangential direction (above the cerebellar surface) [10].

The formation of the cerebellum occurs as a result of the growth of the neural tube wall (cranial end) in the hindbrain [10]. In the period of 8–9 weeks, when examining histological specimens, we noted that the hemispheres are already formed above the fourth ventricle and the cerebellum worm is being formed. Cho K. describes that from 7–8 weeks there is an expansion of the pterygoid plate and rhomboid lip, to form the cerebellar hemispheres. Towards the 10th week, the cerebellar hemispheres merge along the midline to form the cerebellar worm [2].

At 11–12 weeks expression of the synaptophysin marker was relatively weak in all layers of the cerebellum. We associate this fact with weak establishment of synaptic circuits between neurons and the insufficient process of fiber myelination. According to Sarnat Harvey B., from the 13th week, the expression of the marker synaptophysin was detected in the layers of the cerebellum [8].

As a result of our study, the highest expression of Ki-67 at 22–23 weeks is preserved in the ventricular and outer granular layers of the cerebellum and relatively less intense – in the intermediate layer, Abraham H. claims that the expression of Ki-67 in the cerebellar cortex of human fetus aged 24 weeks occurs more intensively in the outer granular layer [4].

Conclusions

1. In embryos aged 6–7 weeks. rhombic lip consisted of three layers: neuroepithelial, mantle and marginal layers. At 8–9 weeks. all layers of cerebellum are detected, except internal and external granular layers. The outer granular layer begins to appear at 9–10 weeks of fetal development. The inner granular layer begins to appear at 11–12 weeks.

2. During fetal development, there is a tendency to gradually increase the thickness of the intermediate layer of the cerebellum due to the development of neuro-glial complexes. Meanwhile the ventricular layer is gradually thinning by reducing the intensity of neural cell proliferation.

3. Up to 17–18 weeks, there was a gradual increase of the density of neutral cells in the layers of the cerebellum. The highest density of neural cells was observed in the ventricular layer from the 11th to the 14th week of the prenatal period of ontogenesis with a subsequent decrease. From 22 to 23 weeks, the lowest cell density was observed in the molecular layer and was present until birth.

4. The highest intensity of neural cell proliferation in the embryonic period occurred in the ventricular layer and the outer granular layer, and the least intense occured- in the intermediate layer. In the fetal period, the intensity of cell proliferation was relatively higher in the ventricular and inner granular layers.

5. Radial glia fibers penetrate all layers of the cerebellum in the radial direction and end in the outer granular layer. Relatively moderate expression of vimentin in fertile glia fibers was observed in white matter and relatively strong was observed in the molecular layer and the outer granular layer. Prior to birth, there is a focality of radial glia presence.

References

1. Bartheld CS, Bahney J, Herculano-Houzel S. The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. J Comp Neurol. 2016 Dec 15;524(18):3865–3895. doi: 10.1002/cne.24040.
2. Cho KH. Early fetal development of the human cerebellum. Surg Radiol Anat. 2011 Aug 33(6):523–530. doi: 10.1007/s00276-011-0796-8.
3. Hibi M, Shimizu T. Development of the cerebellum and cerebellar neural circuits. Dev Neurobiol. 2012 Mar 72:282–301. doi:10.1002/dneu.20875.
4. Marzban H, Del Bigio MR, Alizadeh J, Ghavami S, Zachariah RM, Rastegar M. Cellular commitment in the developing cerebellum Front. Cell. Neurosci. 2015 Jan 12;8:450. doi: 10.3389/fncel.2014.00450.
5. Rahimi-Balaei M, Bergen H, Kong J, and Marzban H. Neuronal Migration During Development of the Cerebellum. Front. Cell. Neurosci. 2018 Dec 12:484. doi: 10.3389/fncel.2018.00484.
6. Rakic P. Evolution of the neocortex: a perspective from developmental biology. Nature Reviews Neuroscience. 2009 10:724–735.
7. Rybachuk OA, Pivneva TA. The role of neural stem cells in the regeneration of the central nervous system. Physiology. 2013.59 (2): 111–121.
8. Sarnat HB, Flores-Sarnat L, Auer RN. Sequence of synaptogenesis in the fetal and neonatal cerebellar system - part 1: Guillain-Mollaret triangle (dentato-rubro-olivo-cerebellar circuit). Dev Neurosci. 2013 June; 35(1):69–81. doi: 10.1159/000350503.
9. Shkolnikov VS, Zalevsky LL, Stelmashchuk PO, Tykholaz VO, Zalevskaya IV, inventor; Vinnytsia Pirohov Memorial National Medical University. Method of fixing cerebellum in prenatal period of human ontogenesis for immunohistochemical research. Patent of Ukraine No. 117723. 2018 Sep 10.
10. Volpe J. Cerebellum of the premature infant: rapidly developing, vulnerable, clinically important. Child Neurol. 2009 Sep 24(9):1085–1104. doi:10.1177/0883073809338067.

Стаття надійшла 28.02.2021 р.