показників є досить складною, то виникає необхідність проведення кореляційного аналізу, де наявність залежностей між морфометричними показниками визначали за допомогою коефіцієнту Браве- Пирсона Встановлено що діагностичними критеріями для оцінки функціонального стану піднижньощелепних залоз щурів після дії етанолу є залежність зовнішнього діаметру, діаметру просвіту та висоти епітеліоцитів на 12 добу у всіх паренхіматозних компонентах що відповідає формуванню хронічної алкогольної залежності у щурів.

Ключові слова: піднижньощелепні залози, щури, морфометрія, кореляційний аналіз.

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показателей есть достаточно сложным, то возникает необходимость проведения корреляционного анализа, где наличие зависимостей между морфометрическими показателям определяли с помощью коэффициента Браве – Пирсона. Установлено что диагностическими критериями для оценки функционального состояния поднижнечелюстных желез крыс после действия этанола проявляется зависимостью внешнего диаметра, диаметра просвета и высоты эпителиоцитов на 12 сутки во всех паренхиматозных компонентах, что соответствует формированию хронической алкогольной зависимости у крыс.

Ключевые слова: поднижнечелюстные железы, крысы, морфометрия, кореляционный анализ.

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THE MORPHOLOGY OF RADIAL GLIAL SPINAL CORD OF EMBRYOS AND HUMAN FETUSES

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The study of radial glia morphology and processes of targeted migration of neural stem cells in humans remains far from being resolved. The purpose of this study is to establish the morphological aspects of radial glia of spinal cord of human in prenatal period. Morphological examination of the spinal cord of human embryos and fetuses of 6-7 weeks up to 39-40 weeks was performed. Using anatomical, histological, immunohistochemical, and morphometric morphological aspects of the radial glia of the spinal cord were established. The results showed that strong expression of vimentin and CDX-2 radial glia fibers was observed up to 8-9 weeks. From 11-12 weeks, radial glia fibers retain a radial direction only in the middle part of the segments, which, in our view, is associated with the gradual involution of radial glia, which correlates with the formation of nuclear-neural complexes of gray matter. Until the moment of birth, vimentin-positive structures of radial glia gradually disappear and can be traced only in the neuroepithelium of segments.

Key words: prenatal period, spinal cord, radial glia, neuroepitelium, immunohistochemical markers.

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

Neurogenic fetal brain cells generate major cell types of the nervous system during the prenatal ontogeny, which lasts from fertilization to birth. Such neurogenic cells include neural stem cells (NSC), neural progenitor cells (NPC), and linear-specific progenitors and precursors [2]. NSC are known to be polypotent cells, which are characterized by the proliferation and formation of several cell pools simultaneously: neuroblasts or glioblasts [4, 10, 15]. To date, there is no doubt that the presence of permanent neurogenesis of some areas of the brain performs due to colonies of NSC [9, 14]. Rybachuk O.A. and Pivneva T.A. (2013) emphasize that during the embryonic ontogeny period NSC cranial nerve tubes showed greater proliferative activity than neural cells of the caudal compartments [3]. Thus, it can be predicted that the above processes are inherent in the spinal cord, which precede the formation of neuronal complexes, which in turn requires further study and refinement.

It should also be noted that the identification of NSC in vivo is traditionally based on the analysis of the morphology of these cells, their mitotic activity, and the expression of certain genes and protein synthesis. The most commonly identified NSC markers are Nestin, Sox2, Msashi 1, 2, Oct 4, Nanog, etc., but none can be used as the sole criterion for NSC identification [11].

The next step is to study the mechanisms of migration of differentiated unipotent neural cells that have just formed in the paraventricular zone.

It has been established that the most extensive migration of neural cells occurs during the process of laying the cerebral cortex [5]. At the same time, Tsymbaluk V. I. and Medvedev V. V. (2010) indicate that radial glial (RG) plays a key role in ensuring nerve cell migration during the development of other neural tube compartments and cell migration. The authors have shown that immature neurons migrate along the processes of RG cells in the centripetal direction [5].

Over time, RG cells lose the ability to express nestin and vimentin [8]. However, the authors do not specify a term when such a property is lost. In contrast, Kirik O. V. and Korzhevskii D. E. (2012) indicate that the RG cell population is heterogeneous: some cells contain neural markers (they subsequently

become neuroblasts) and some glial (become glial cells). In the early stages of the development of the spinal cord, the protein of intermediate filaments – vimentin is expressed by cells of the neuroepithelium (subpendicular zone), as well as in RG cells [1].

At present, it is impossible to consider the issues of genealogical relationships between populations of neurogenic cells of the ventricular and subventricular zones and cells of radial glia completely solved [5]. In addition, according to Ostrem B. et al. (2014) study of RG morphology and processes of targeted NSC migration in humans remains relevant and has not been fully resolved to date [13].

The purpose of the study was to establish the morphological aspects of radial glia segments of the spinal cord of human embryos and fetuses using specific immunohistochemical markers.

Materials and methods. The study was performed at the Department of Human Anatomy of the National Pirogov Memorial Medical University, Vinnytsya and the Research Laboratory of Functional Morphology and Genetics of Development of the National Pirogov Memorial Medical University, Vinnytsya (certificate of accreditation: CDL No. 050/15, 03/02/2015 – 01/03/2020).

According to the 2017 Agreement on Joint Scientific and Practical Activities between National Pirogov Memorial Medical University, Vinnytsya and Vinnitsa Regional Anatomical Pathology Bureau, the material was examined directly by the Vinnitsa Regional Anatomical Pathology Bureau, and protocols of pathoanatomical examination were drawn up in accordance with Form No. 013-2/o approved by the order of the Ministry of Health of Ukraine of 14.08.2004 No. 417. According to the opinion of the Commission on Biomedical Ethics of National Pirogov Memorial Medical University, Vinnytsya (protocol No. 10 of 06.12.2018), the work was done in compliance with the basic provisions of the GCP (1996), Council of Europe Convention on Human Rights and Biomedicine (1997) and the study materials do not contradict the basic bioethical standards of the Declaration of Helsinki on the ethical principles of scientific and medical research with the participation of a person adopted by the 59th General Assembly of the World Medical Association in 2008.

This study was performed on 127 human embryos and fetuses between 6-7 and 39-40 weeks of prenatal development in the uterus in the absence of overtly harmful external and internal environmental factors. Serial sections of preparations of human embryos were made entirely (histologic "tomography"), since the extraction of the spinal cord from embryos is difficult and there is a risk of not maintaining its integrity. The method of micropreparation – under the control of binocular magnifier or by the method of thin preparation (in the fetus), the spinal cord was removed together with the membranes. During the removal of the spinal cord in the pre-fetus was used our own technique (Innovative proposal № 17 from 25.04.2002). Examination preparations of the spinal cord were stained with hematoxylin and eosin, toluidine blue (in the Nissl modification), by Van Gieson, and impregnated with silver according to Bilshovskyy.

Immunohistochemical study – «DacoCytomation» (Denmark) diagnostic monoclonal antibodies were used: vimentin, CDX-2, Ki-67 and synaptophysin. We used Vimentin and CDX-2 to investigate the morphology of radial glia, Ki-67 to evaluate the proliferative activity of NSC in the neuroepithelial layer, and synaptophysin – to determine the development of synaptic ligaments and to evaluate myelination of the fibers of the leading pathways. Photo M 1.21 (computer histometry) was used in the morphometric study of a series of sections of spinal cord segments. We studied indices of neuroepithelium values, length and thickness of radial glial fiber, area and linear dimensions of neural stem cells of neuroepithelium, neuroblasts and glioblasts.

Statistical processing of the obtained morphometric parameters was carried out using the standard software package "Statistica 6.1" by StatSoft (license No. BXXR901E246022FA) using parametric and non-parametric criteria for evaluating the obtained results. The differences between the samples were determined using the Mana-Whitney U-test and the Student's t-test, and the mean values for each trait and their standard deviations were determined.

Results of the study and their discussion. The data on the course of NSC proliferation processes in human embryos 6-7 weeks which was obtained indicate that they occur more intensively in the dorsal part of the neuroepithelium than in the ventral (fig. 1).

The intensity of Ki-67 expression in the dorsal part of the neuroepithelium of segments along the spinal cord of human embryos of 6-7 weeks can be estimated as strong, since cell staining was 92 %. In the ventral part, 12 % of cells reacted. However, this pattern is characteristic of all segments of the spinal cord. Due to the high proliferative activity of cells of the dorsal part of the neuroepithelium, a higher density of neuro- and glioblasts locations within the posterior horns was observed.

From the neuroepithelium, NSC migrate along the RG fibers into the mantle layer, where further differentiation of neuroblasts and proliferation of glioblasts is carried out. Neuroblast proliferation in the

mantle layer has not been established. RG fibers that express vimentin protein penetrate into the boundary layer. We also found that, in addition to vimentin, in this gestational term, RG fibers express the CDX-2 protein (fig. 1). NSC, which are located in the mantle layer have an elliptical or spherical shape. The morphology and size of these cells are virtually identical in all parts of the spinal cord. There are larger cells with light cytoplasm that already have axonal tubercles – these are neuroblasts. Their average area was 46.7±1.4 μm^2. The area of the nuclei of the neuroblasts was 22.6±1.2 μm^2. Smaller cells with dark cytoplasm and accompanying neuroblasts are glioblasts. The area of this cell averaged 31.5±1.5 μm^2 and the area of the nucleus of glioblasts was 10.8±1.0 μm^2.

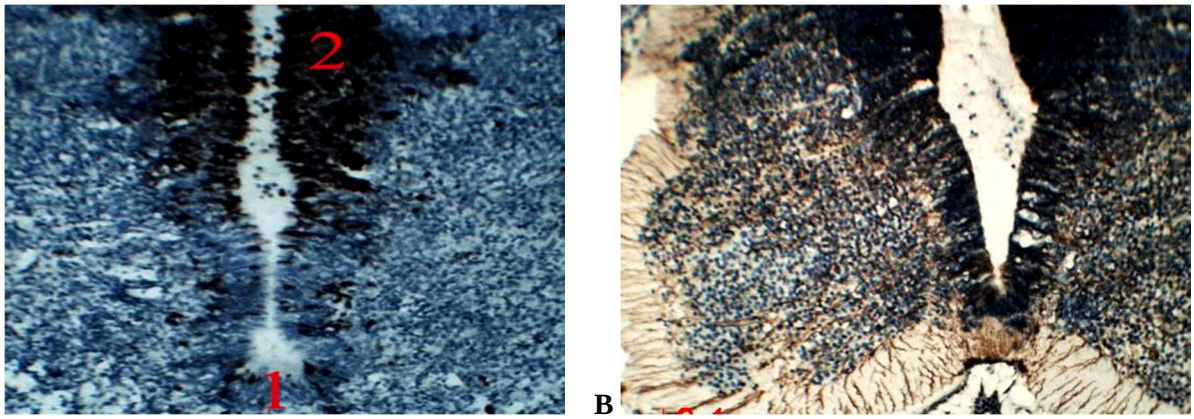


Fig. 1. Spinal cord of human embryo 6-7 weeks. A – neuroepithelium: 1 – ventral part; 2 – dorsal part. Ki-67; ×100. B – expression of CDX-2 in segments. CDX-2; ×100.

The structural basis of the boundary layer is RG fibers (see Fig. 1). Preferably, in the cervical and lumbar segments, the concentration of RG fibers involved in the formation of the anterior roots is noted (fig. 1). Such fibers start from the basement membrane of the neuroepithelium and penetrate the marginal layer. In the course of the study we found that the intensity of synaptophysin expression in segments of spinal cord of embryos of 6-7 weeks was determined in the boundary layer and within the anterior and posterior spinal commissure, which can obviously be associated with the establishment of synaptic connections and the initiation of myelination of the leading pathways. In the mantle layer, synaptophysin expression is mediocre. Thus argue that the first stage of differentiation of post-mitotic cells occurs during their migration, after which maturation continues and ends with the establishment of a synaptic linkage network. Fedorkovskaya B. O. (2013) adheres to the idea that when myelination of nerve fibers begins, then synapses begin to form. According to the chronology, the author attributes this process to the 5th month of intrauterine period [6].

In the ventral part of the neuroepithelium of human fetuses 8-9 weeks. there are 5-6 (4 %) mitotic or post-mitotic NSC. In the dorsal part of such cells – 10-11 (10 %). In general, expression of Ki-67 in the neuroepithelial segments can be assessed as in the ventral and dorsal part as weak.

After mitosis, NSC along the RG fibers migrate into the mantle layer, which extend in the radial direction, starting from the basal membrane of the neuroepithelium, penetrating the mantle layer and ending at the marginal (fig. 2). In the middle part of the mantle layer, the RG fibers sharply change their direction and extend back to the horn region. Short fibers are contained mainly within the neuroepithelium and make up its thickness (fig. 2).

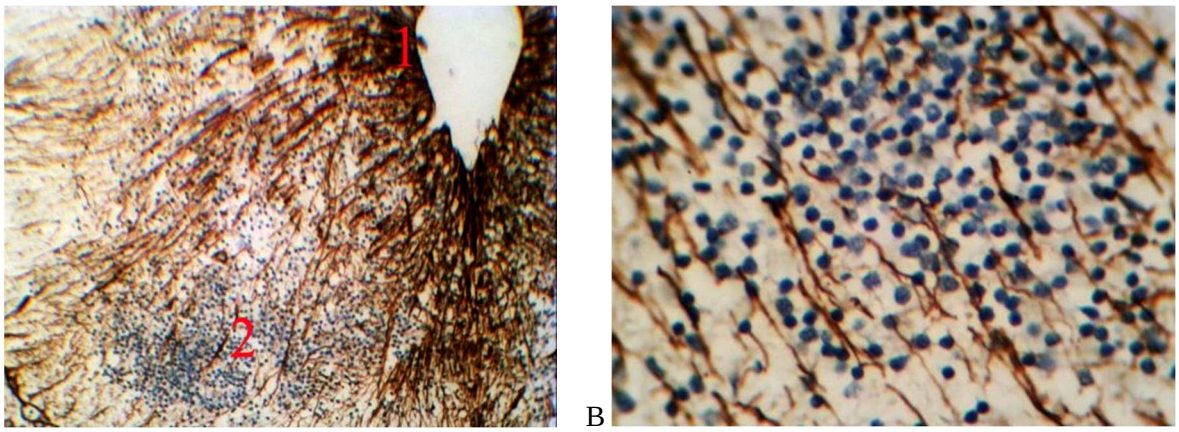
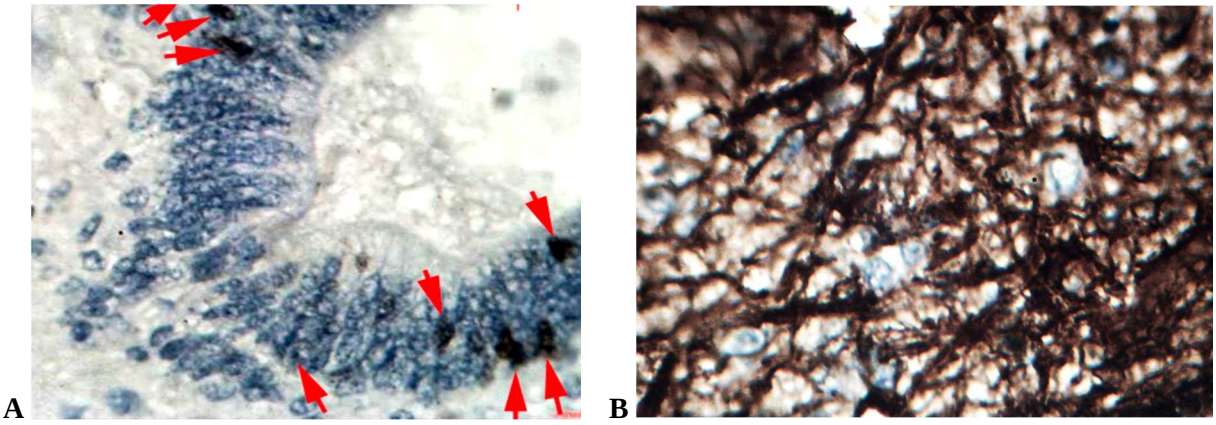


Fig. 2. Spinal cord of human fetus 8-9 weeks. A – RG fiber architectonics in segments: 1 – neuroepithelium; 2 – posterior horns. Vimentin; ×100. B – NSC migration along radial glial fibers. Vimentin; ×400.

The average minimum fiber length was 179.7±7.1 μm. The average maximum fiber length of the RG was 688.2±21.4 μm. Strong expression of vimentin was observed in the RG fibers of the neuroepithelium and along the anterior and posterior median septa (fig. 2). In other segments of segments, the expression of vimentin is mediocre. Cells with relatively large nuclei migrate along the RG fibers, these are neuroblasts and cells with small nuclei, these are glioblasts (fig. 2). The nuclei of the neuroblasts have a spherical shape and the nuclei of the glioblasts are elliptical. The average area of the nucleus of the neuroblast was 66.1±3.2 μm^2, and the average area of the nucleus of the glioblast was 38.9±1.9 μm^2.

Medium expression in the anterior and posterior median septa of the segments and poor expression in the posterior cords were observed with the use of CDX-2.

In fetus of 11-12 weeks NSC proliferation processes occur relatively more intensely in the ventral part of the neuroepithelium than in the dorsal one (fig. 3). This trend persists in all segments of the spinal cord. In the ventral part of the neuroepithelium there are 11-12 (13 %) mitotic or post-mitotic NSC, in the dorsal part – 6-7 (7 %) cells. In the ventral and dorsal parts, Ki-67 expression is weak.



A **B**

Fig. 3. Spinal cord of human fetus 11-12 weeks. A – nature of NSC proliferation in neuroepithelium (indicated by arrows). Ki-67; ×400. B – formation of RG mesh structures within neural complexes. Vimentin; ×400.

RG fibers retain their intrinsic direction only in the middle part of the segments, between the anterior and posterior horns. The average maximum fiber length of the RG was 963.9±28.7 μm. The average minimum fiber length is 104.0±4.4 μm. Relatively strong expression of vimentin was observed in the RG fibers around the neuroepithelium and along the anterior and posterior median septa. In other segments of the segment, the expression of vimentin is mediocre. Within the neural complexes, RG fibers form reticulated structures (fig. 3). We will associate this phenomenon with the formation of the neural complexes themselves. The expression of CDX-2 in segmental structures was absent at this age.

The nuclei of migrating neuroblasts have a spherical shape and the nuclei of the glioblasts are elliptical. The average area of the nucleus of the neuroblast was 71.6±2.9 μm^2, and the average area of the nucleus of the glioblast was 44.1±2.6 μm^2.

Synaptophysin expression in segments along the spinal cord was observed to be relatively strong in the mantle layer. However, the expression of synaptophysin in the neuroepithelium itself is absent.

In human fetus of 17-18 weeks in the ventral part of the neuroepithelium of segments there are 8-9 (10 %) mitotic or post-mitotic cells, and in the dorsal part – 5-6 (5 %) of cells.

RG fibers stored radial direction only in the middle part of the segments and around the neuroepithelium. In the mantle layer, the RG fibers are intermittent and are stored mainly near the vessels. The average minimum fiber length was 59.5±4.6 μm. The average maximum fiber length of the RG was 633.3±33.0 μm. Strong expression of vimentin was observed in the RG fibers of the neuroepithelium and along the anterior and posterior median septa. In other segments of the segments, the expression of vimentin is weak. The average nuclei area of migrating neuroblasts was 73.9±2.7 μm^2, and the average nuclei area of glioblasts was 48.2±2.8 μm^2.

In the ventral part of the neuroepithelium segments of the spinal cord of human fetus 22-23 weeks there are 7-8 (9 %) mitotic or post-mitotic NSC and 5-6 (5 %) similar cells in the dorsal part. Ki-67 expression in neuroepithelia is generally weak.

After mitosis, NSC from the neuroepithelium migrate into the mantle layer along the RG fibers, which does not retain radial direction even in the middle part of the segments around the neuroepithelium. The average minimum fiber length is 51.1±3.7 μm. The relatively long RG fibers extend away from the basement membrane of the dorsal part of the neuroepithelium and extend along the posterior median septum. The average maximum fiber length of the RG was 346.7±11.5 μm. Relatively strong expression

of vimentin was observed in the neuroepithelium and in the middle part of the segments at some distance from the neuroepithelium itself. In the mantle layer, the expression of vimentin was weak and persisted mainly along the vessels and at the site of spinal cord formation, that is, it had a focal character. Also, relatively weak expression of vimentin persisted within the boundary layer. The nuclei of migrating neuroblasts have a spherical shape and the nuclei of the glioblasts are elliptical. The average area of the nucleus of the neuroblast was 84.0±2.9 μm^2. The average nuclei area of the glioblast was 55.4±1.6 μm^2.

A relatively strong expression of synaptophysin in the spinal cord segments was observed within the mantle layer. Medium expression of synaptophysin occurred within the posterior horns and in fasciculus gracilis, weak - in the anterior and lateral cords.

The processes of proliferation of NSC neuroepithelium of segments of the spinal cord in fetuses 35-36 weeks occur relatively more intensely in the ventral part than in the dorsal one. We found that in the ventral part of the neuroepithelium of segments there are 4-5 (5 %) mitotic or post-mitotic NSC. In contrast, in the dorsal part there are 2-3 (3 %) such cells.

Remains of RG fibers retain radial direction only within the neuroepithelium. The average minimum fiber length was 35.8±1.5 μm. The relatively long RG fibers extend away from the basement membrane of the dorsal part of the neuroepithelium and extend along the posterior median septum. The average maximum fiber length of the RG is 122.1±2.2 μm. Relatively strong expression of vimentin was observed in the fibers of the RG of the neuroepithelial layer and along the posterior median septum. In the mantle layer, the expression of vimentin was weak and persisted mainly along vessels and at the site of spinal cord formation.

The nuclei of the neuroblasts have a spherical shape and the nuclei of the glioblasts are elliptical. The average area of the nucleus of the neuroblast was 90.0±2.4 μm^2. The average nuclei area of the glioblast was 58.3±1.7 μm^2.

Relatively strong synaptophysin expression in spinal cord segments was noted in the mantle layer.

In previous research, Barry D. et al. (2013) reported that in embryonic spinal cord, radial glial cells are defined as conductors for migrating neurons. The density of radial glial cells is maintained during development in the dorsal, lateral and ventral parts of the neuroepithelium [7]. We consider the characteristic of the neuroepithelium given by the author to be incomplete, as not only does the relative density of radial glial cells in individual parts of the neuroepithelial segments remain during the prenatal period of ontogeny, but also changes its thickness and the intensity of the mitotic activity of the cells of the neuroepithelium in the in the ventral and dorsal parts. Up to 11-12 weeks the thickness and intensity of cell mitoses are greater in the dorsal part of the neuroepithelial segments than in the ventral. In this case, the relative density of the cells of the posterior horn dominates as such in the anterior horns. After 11-12 weeks the above indicators are already prevalent in the ventral part of the neuroepithelium and this tendency persists until birth. Thus, in our opinion, this phenomenon is connected with the fact that at first a person develops sensitivity and only after that motor functions are established.

Nowakowski T. et al. (2016), by developing a hypothesis for the role of RG in neurogenesis and describing its morphology in mammals, indicates that architectonically radial glia forms a "framework" of continuous fibers covering the thickness of any CNS formation. The difference between people is that the author believes that RG fibers have a discontinuous course [12]. Our research proved the fact that up to 8-9 weeks in human fetus, RG fibers have a radial direction from the central canal and permeate the entire thickness of the nerve tube and also participate in the formation of the spinal cord. By integrating the findings from the research on the expression of vimentin and RG architectonics in segmental structures, we conclude that a decrease in the intensity of vimentin expression before the birth of a child is associated with its gradual involution. In our opinion, the statements of Nowakowski T. et al. (2016) should be supplemented by the fact that, in the early fetus period, RG fibers form reticulated structures, which is a prerequisite for the "terminal stop" of neurons and the structuring of neural complexes.

In future studies, it is promising to use immunohistochemical markers to elucidate the role of radial glia in the formation of nuclear neural complexes of the spinal cord during the prenatal period of human ontogeny and to study the morphology of neural stem cells; as well as comparing the results with similar results in malformations.

Conclusion

The results showed that by 8-9 weeks there was a strong expression of radial glial fibers of both vimentin and CDX-2, which penetrated all layers of the spinal cord. In 8-9 weeks CDX-2 is poorly expressed only in the anterior and posterior median septa of the segments. At 11-12 weeks, radial glia fibers retain radial direction in the middle part of the segments and around the neuroepithelium. In the mantle

layer, the radial glial fibers are intermittent and are predominantly located near the vessels. By birth, the vimentin-positive structures of radial glia gradually disappear and are only observed in the neuroepithelial of segments.

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Реферати

МОРФОЛОГІЯ РАДІАЛЬНОЇ ГЛІЇ СПИННОГО МОЗКУ ЕМБРІОНІВ ТА ПЛОДІВ ЛЮДИНИ

Школьніков В.С., Приходько С.О., Поліщук С.С., Кривов'яз О.В., Галунко Г.М.

Вивчення морфології радіальної глії та процесів адресної міграції нейральних стовбурових клітин у людини лишається далеко не вирішеним до кінця питанням. Метою дослідження є встановлення морфологічних аспектів радіальної глії спинного мозку людини у пренатальному періоді. Проведене морфологічне дослідження спинного мозку ембріонів та плодів людини від 6-7 тиж. до 39-40 тиж. При використанні анатомічних, гістологічних, імуногістохімічних, морфометричних методик були встановлені морфологічні аспекти радіальної глії спинного мозку. Отримані результати показали, що сильна експресія віментину та CDX-2 волокон радіальної глії спостерігалась до 8-9 тижня. З 11-12 тижня волокна радіальної глії зберігають радіальний напрямок тільки у середній частині сегментів, що на наш погляд пов'язано з поступовою інволюцією радіальної глії, яка корелює із формуванням ядерно-нейронних комплексів. До народження віментин-позитивні структури радіальної глії поступово зникають і простежуються тільки у нейроепітелії сегментів.

Ключові слова: пренатальний період, спинний мозок, радіальна глія, нероепітелій, імуногістохімічні маркери.

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МОРФОЛОГИЯ РАДИАЛЬНОЙ ГЛИИ СПИННОГО МОЗГА ЭМБРИОНОВ И ПЛОДОВ ЧЕЛОВЕКА

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Изучение морфологии радиальной глии и процессов адресной миграции нейральных стволовых клеток у человека остается далеко не решенным до конца вопросом. Целью данного исследования является установление морфологических аспектов радиальной глии спинного мозга человека в пренатальном периоде. Проведенное морфологическое исследование спинного мозга эмбрионов и плодов человека от 6-7 нед. до 39-40 нед. При использовании анатомических, гистологических, иммуногистохимических, морфометрических были установлены морфологические аспекты радиальной глии спинного мозга. Полученные результаты показали, что сильная экспрессия виментина и CDX-2 волокон радиальной глии наблюдалась до 8-9 недели. С 11-12 недели волокна радиальной глии сохраняют радиальное направление только в средней части сегментов, что на наш взгляд связано с постепенной инволюцией радиальной глии, которая коррелирует с формированием ядерно-нейронных комплексов. До момента рождения виментин-позитивные структуры радиальной глии постепенно исчезают и прослеживаются только в нейроэпителии сегментов.

Ключевые слова: пренатальный период, спинной мозг, радиальная глия, нейроэпителий, иммуногистохимические маркеры.

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