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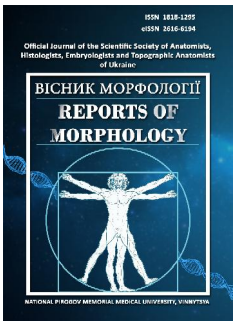


# Neuron-glial relations of the posterior horns of the spinal cord of human fetuses

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*Despite the relatively sufficient study of the structure and functioning of the nervous system, interest in the problem of neuron-glial relationships continues to grow steadily, as this parameter reflects the dynamics of the development of nervous tissue and can be used to assess the quality level of morphological changes. The purpose of the study: to establish the morphogenesis and neuron-glial relationships of the posterior horns of the human spinal cord in the fetal period of ontogenesis. This study was performed on the preparations of 104 human fetuses from 8-9 weeks to 39-40 weeks using anatomical, histological, immunohistochemical and morphometric methods. Statistical processing of the numerical data of the obtained results was carried out using the licensed software package "Statistica 6.1" of the StatSoft company using parametric and non-parametric methods. During the research, it was established that in the fetal period, the greater proliferative activity of the dorsal neuroepithelium is determined at 8-9 weeks: in the cervical segments - 10 % ($p<0.05$), in the thoracic, lumbar and sacral segments - 9 % ($p<0.05$). By 39-40 weeks, this indicator gradually becomes smaller: in the cervical and lumbar segments, 4 % of cells (2-3 cells reacted) ($p<0.05$) and in the thoracic and sacral segments - 3 % (1-2 cells reacted) ($p<0.05$). It was found that throughout the fetal period there is a tendency to a gradual decrease in the density of neurons and gliocytes. The glial index, on the contrary, up to 39-40 weeks increases, and at the time of birth it is equal to 2.1 in the cervical, thoracic and lumbar segments, and 2.0 in the sacral segments. It was found that at 11-12 weeks, radial glia fibers form mesh structures within the neuronal complexes, which coincides with the beginning of the formation of neuron-glial complexes of the posterior horns. At 17-18 weeks, the fibers of radial glia keep the radial direction only in the middle part of the posterior horns. At 34-35 weeks, vimentin expression was determined to be relatively moderate in the remnants of radial glia near the dorsal neuroepithelium and focal expression of vimentin around vessels within the posterior horns. Expression of vimentin in the neuroepithelium of fetuses of 39-40 weeks was absent. In this age period, the neuroepithelium is structured from ependymocytes and radial glia cells are absent, as there is a relatively strong expression of S-100 in the neuroepithelium. Relatively strong expression of synaptophysin occurred in the posterior horns of 8-9 week fetuses. This age period is the beginning of the establishment of synaptic connections.*
**Keywords:** *human fetuses, spinal cord, posterior horns, neuron-glial relations, radial glia, neural stem cells.*

## Introduction

Over the last decade, the study of the anatomy of the human fetus has grown in popularity among scientists around the world due to the development of modern imaging methods, as well as the technical complication of surgical interventions during the correction of malformations in the fetal period, which is required today [1].

It is known that the spinal cord is quite complex in terms of cytoarchitectonic and functional control system, and therefore plays a significant role in sensory-motor integration and in the implementation of control programs of the skeletal-muscular system [18]. In addition, over the past 2-3 years, the attention of scientists to the detailed study of the cellular composition of the posterior horns of

the spinal cord has significantly increased [16, 24]. This phenomenon is related to the fact that the rear horns perform a multifaceted functional load in the sensory integration of the entire body. Therefore, in understanding emerging pathological conditions [17, 28], a detailed study of the structuring and formation of the rear horns during ontogenesis, especially in the prenatal period, is important. Until now, there is no doubt about the presence of permanent neurogenesis of some areas of the brain due to colonies of neural stem cells (NSCs) [11, 20]. During the embryonic period of ontogenesis, NSCs of the cranial parts of the neural tube showed greater proliferative activity than neural cells of the caudal parts [21]. Thus, it is possible to predict that the above-mentioned processes are inherent in the spinal cord, which precede the formation of centers and cellular complexes, and this, in turn, requires further research and clarification.

According to Cedeno D. L. [10], the scientific world has an urgent problem of studying neuron-glial relations, since this parameter is a reflection of the dynamics of the development of the central nervous system and can be used to assess the quality level of morphological changes. As Stepanov A. S. noted [25], research in this direction is promising and certainly has practical significance.

Therefore, *the aim of this study* was to establish the morphogenesis and neuron-glial relationships of the posterior horns of the human spinal cord in the fetal period of ontogenesis.

## Materials and methods

This study was performed on preparations of human fetuses aged from 8-9 weeks to 39-40 weeks of the fetal period with a total number of 104 objects. During the development of fetuses in the uterus, there were no pronounced harmful factors of the external and internal environment and those obtained during medical abortions, or stillbirths in relatively healthy women in the Regional Pathological Bureau and in the maternity hospitals of Vinnytsia. There was no pathology of central nervous system formations. As a result of the expert opinion of the commission on biomedical ethics of the National Pirogov Memorial Medical University, Vinnytsya (protocol of the meeting of the Bioethics Committee № 10 of December 6, 2018), the work was performed in compliance with the main provisions of the GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine (1997) and research materials do not contradict the basic bioethical norms of the Helsinki Declaration on Ethical Principles of Scientific and Medical Research with Human Participation, adopted by the 59th General Assembly of the World Medical Association in 2008. Pursuant to the 2017 agreement on joint scientific and practical activities between the National Pirogov Memorial Medical University, Vinnytsya and VRPAB, pathological examination protocols were drawn up in accordance with form № 013-2/o approved by the order of the Ministry of Health of Ukraine dated August 14, 2004 № 417.

The preparations of the spinal cord were stained with hematoxylin and eosin, toluidine blue, and silver impregnation was carried out according to Bilzhovskyi.

Immunohistochemical technique: use of vimentin, CDX-2, Ki-67, S-100 and synaptophysin (diagnostic monoclonal antibodies of the company "DacoCytomation" (Denmark)). Vimentin and CDX-2 - to study the morphology of radial glia, Ki-67 - to evaluate the proliferative activity of neutral cells and synaptophysin - to study the development of synaptic connections and to evaluate the myelination of nerve fibers. The quality of protein expression was evaluated according to the scale of staining intensity: absent - absence of a positive reaction in cells; weak - up to 30 % cell reaction; average - 31-60 % and strong - 60 % and more [9].

Among the morphometric methods, histo- and karyometry were used. Histometry of the dorsal neuroepithelium and formations of the posterior horns of the spinal cord segments of human embryos and fetuses was performed using the PhotoM 1.21 software. Karyocytometry was performed using the software described above. Transverse and longitudinal dimensions were studied, and the area of cells and nuclei of radial glia, NSCs, neuroblasts and neurons of the marginal nucleus, gelatinous substance, nucleus of the posterior horn and thoracic nucleus was determined.

The method of determining the density of neurons and glial cells of the posterior horns of the human spinal cord in the prenatal period of ontogenesis was that five sections with an area of 0.01 mm2 were applied to each of five histological sections, after which cells were counted from the obtained data of 25 sections of the corresponding layer and determined the cell density. The glial index (GI) was calculated as the ratio of the number of gliocytes to the number of neurons, that is, the number of gliocytes per neuron. For clarity of comparison, the density of neurons was taken as one.

Statistical processing of the obtained morphometric parameters was carried out using the standard software package "Statistica 6.1" of StatSoft (owned by SRC National Pirogov Memorial Medical University, Vinnytsya, license № BXXR901E246022FA) using parametric and non-parametric criteria for evaluating the obtained results. Differences between samples were determined using the Mann-Whitney U-test and the Student's t-test, and average values for each characteristic and their standard deviations were determined.

## Results

We found that the proliferative activity of neural stem cells in the dorsal part of the neuroepithelium of 8-9 weeks fetuses is relatively greatest in the cervical segments (Ki-67 expression was observed in 10 % of cells) ($p<0.01$), in the thoracic, lumbar and sacral segments the same proliferative activity, which was 9 % ($p<0.05$) (Fig. 1).

Vimentin expression remains relatively strong in the

neuroepithelium, within the most posterior horns, and within the posterior cords. Fibers of radial glia have a radial direction and in a fan-like manner penetrate the posterior horns and end in the posterior cords. Short fibers of radial glia are located in the posterior horns dorso-medially. It should be noted that in this age period, CDX-2 is also weakly expressed in radial glia fibers in the segments of the spinal cord. S-100 expression was absent in radial glia cells and neurons of the posterior horns. Synaptophysin expression was moderate within the posterior horns and synaptophysin expression was absent within the neuroepithelial layer. Thus, the process of establishing synaptic connections of neurons of the posterior horns continues.

The average value of the density of neurons and neuroblasts in the cervical segments of fetuses of 8-9 weeks was equal to 25.26±2.04 unit/0.01 mm$^2$ ($p<0.01$), in the thoracic segments - 19.32±1.81 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments was 24.17±2.05 unit/0.01 mm$^2$ ($p<0.01$) and in the sacral segments - 17.88±1.64 unit/0.01 mm$^2$ ($p<0.01$). The density of glial cells of the posterior horns in the cervical segments was 42.80±2.19 unit/0.01 mm$^2$ ($p<0.05$), in the thoracic segments was equal 30.93±1.43 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments - 38.66±1.50 unit/0.01 mm$^2$ ($p<0.05$) and in the sacral segments - 26.51±1.44 unit/0.01 mm$^2$ ($p<0.05$). Thus, the GI was 1.7, in the thoracic segments - 1.6, in the lumbar segments it was 1.6 and in the sacral segments - 1.5.

In weeks 9-10, the proliferative activity of NSCs of the dorsal neuroepithelium is relatively greatest in the cervical segments - 9 % of cells ($p<0.01$), in the thoracic segments - 8 % ($p<0.05$), in the lumbar - 9 % ($p<0.05$) and in sacrum - 8 % ($p<0.05$). Only the proliferation of glial cells was noted within the posterior horns. Vimentin expression remained relatively strong in the neuroepithelium, within the most posterior horns and within the posterior cords. In this age period, CDX-2 expression was absent in segments throughout the spinal cord.

The average value of the density of neurons and neuroblasts in the cervical segments of fetuses of 9-10 weeks was equal to 23.48±1.41 unit/0.01 mm$^2$ ($p<0.05$), in the thoracic segments - 21.55±1.30 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments - 23.73±1.56 unit/0.01 mm2 ($p<0.01$) and in the sacral segments - 19.20±1.68 unit/0.01 mm$^2$ ($p<0.05$). Density of glial cells: in cervical segments - 42.12±2.39 unit/0.01 mm$^2$ ($p<0.05$), in the thoracic segments - 34.40±1.76 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments - 41.84±2.04 unit/0.01 mm$^2$ ($p<0.05$), and in the sacral segments - 28.81±1.46 unit/0.01 mm$^2$ ($p<0.05$). The GI is 1.8 in the cervical segments, 1.6 in the thoracic segments, 1.7 in the lumbar segments, and 1.5 in the sacral segments.

We found that the proliferative activity of NSCs in the dorsal neuroepithelium of 11-12-weeks fetuses is relatively greatest in the cervical and lumbar segments, the expression of Ki-67 was observed in 7 % of cells (6-7 cells reacted) ($p<0.05$), in the thoracic and sacral segments - 6 % (5-6 cells reacted) ($p<0.05$). Moderate expression of vimentin was noted within the area of the posterior horns in all segments of the spinal cord. Within the neuron complexes, radial glia fibers form mesh structures (Fig. 2).

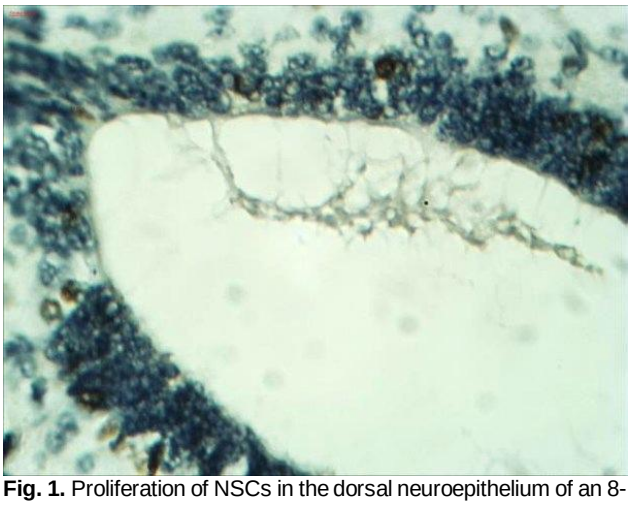

**Fig. 1.** Proliferation of NSCs in the dorsal neuroepithelium of an 8-9 weeks human fetus. Ki-67. x400.

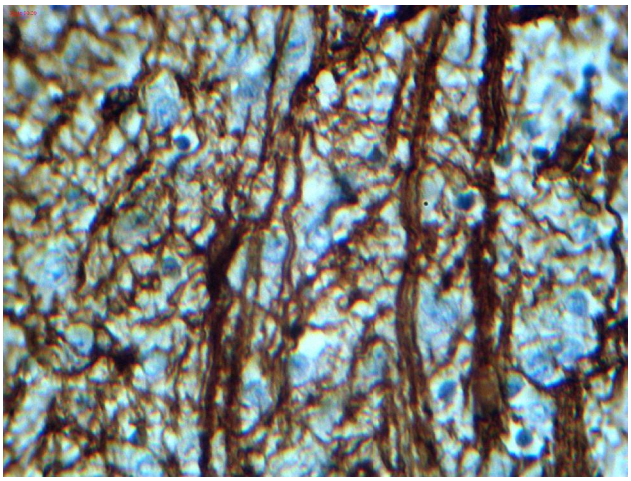

**Fig. 2.** The formation of reticular structures by radial glia fibers in the places of formation of neuron-glial complexes of the posterior horns of the spinal cord of fetuses of 11-12 weeks. Vimentin. x400.

Obviously, this phenomenon is related to the morphogenesis of the neuronal complexes themselves. Relatively strong expression of S-100 was observed in the posterior horns of the spinal cord. The expression of synaptophysin was relatively strong in the ventro-medial areas of the posterior horns, moderate in the thin bundle and other areas of the posterior horns, and weak in the wedge-shaped bundle. The expression of synaptophysin has not been established in the neuroepithelium itself.

The density index of neurons and neuroblasts in the posterior horns of fetuses of 11-12 weeks was obtained as follows: in the cervical segments - 22.33±1.39 unit/0.01 mm$^2$ ($p<0.01$), in the thoracic segments - 22.65±1.47 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments - 23.12±

1.51 unit/0.01 mm$^2$ (p<0.01) and in the sacral segments - 21.89±1.74 unit/0.01 mm$^2$ (p<0.05). Density of glial cells: in cervical segments 41.61±2.15 unit/0.01 mm$^2$ (p<0.05), in the thoracic segments - 38.40±1.83 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments - 41.64±1.97 unit/0.01 mm$^2$ (p<0.05) and in the sacral segments - 34.90±1.66 unit/0.01 mm$^2$ (p<0.05). Thus, GI in the cervical segments is 1.9, in the thoracic segments - 1.7, in the lumbar segments - 1.8, and in the sacral segments - 1.6.

In fetuses of 17-18 weeks, the proliferative activity of NSCs in the dorsal part of the neuroepithelium is relatively greatest in the cervical and lumbar segments, Ki-67 expression was observed in 5 % of cells (5-6 cells reacted) (p<0.05), in the thoracic and sacral segments - 4 % (4-5 cells reacted) (p<0.05). Long fibers of radial glia were observed only in the middle part of the posterior horns, and extended from the medial part of the base (starting from the dorsal neuroepithelium) to the dorso-lateral part. Relatively moderate expression of vimentin was noted in the posterior horns in all segments of the spinal cord. Relatively strong expression of S-100 in the posterior horns was noted at the base of the posterior horns and in the middle part. The expression of synaptophysin was relatively strong in the ventro-medial regions of the posterior horns, moderate in the thin bundle and the rest of the posterior horns.

The neuron density index in fetuses of 17-18 weeks in the cervical segments was equal 20.22±1.07 unit/0.01 mm$^2$ (p<0.05), in the thoracic segments - 20.43±1.14 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments - 22.06±1.48 unit/0.01 mm$^2$ (p<0.05) and in the sacral segments - 22.61±1.52 unit/0.01 mm$^2$ (p<0.05). Density of glial cells: in cervical segments 38.36±1.70 unit/0.01 mm$^2$ (p<0.05), in the thoracic segments - 36.72±1.83 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments - 40.39±1.75 unit/0.01 mm$^2$ (p<0.05) and in the sacral segments - 38.48±1.91 unit/0.01 mm$^2$ (p<0.01). Therefore, the GI was 1.9 in the cervical segments, 1.8 in the thoracic segments, 1.8 in the lumbar segments, and 1.7 in the sacral segments.

In weeks 20-21, we established that the proliferative activity of NSCs in the dorsal neuroepithelium of fetuses remains relatively greatest in the cervical and lumbar segments, where Ki-67 expression was observed in 5 % of cells (5-6 cells reacted) (p<0.01), in breast and sacral segments - 4 % (4-5 cells reacted) (p<0.05). The expression of vimentin in radial glia indicates the presence of short and long fibers of this formation. Moreover, the long fibers had an intermittent course in the middle part of the rear horns, and extended from the middle part of the base to the dorso-lateral part. Short fibers together with radial glia cell bodies form the neuroepithelium itself. In general, a relatively strong expression of vimentin was observed only in the neuroepithelium (radial glia cells), in the remaining areas of the posterior horns in all segments of the spinal cord, a relatively weak expression of vimentin was noted. Relatively strong expression of synaptophysin was noted in all areas of the posterior horns.

The density of neurons in the rear horns of fetuses of 20-21 weeks was as follows: in the cervical segments - 18.62±1.04 unit/0.01 mm$^2$ (p<0.01), in the thoracic segments - 20.21±1.15 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments - 20.67±1.22 unit/0.01 mm$^2$ (p<0.01), and in the sacral segments - 21.90±1.41 unit/0.01 mm$^2$ (p<0.05). Density of glial cells: in cervical segments 37.24±1.75 unit/0.01 mm$^2$ (p<0.05), in the thoracic segments 36.33±1.92 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments - 39.11±1.86 unit/0.01 mm$^2$ (p<0.01) and in the sacral segments - 39.44±1.79 unit/0.01 mm$^2$ (p<0.05). The glial index in the cervical segments was 2.0, in the thoracic segments - 1.8, in the lumbar segments - 1.9, and in the sacral segments - 1.8.

In fetuses of 25-26 weeks, the density of neurons of the posterior horns throughout the spinal cord in the cervical segments was equal to 17.80±0.91 unit/0.01 mm$^2$ (p<0.05), in the thoracic segments - 19.36±1.15 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments 19.92±1.15 unit/0.01 mm$^2$ (p<0.05) and in the sacral segments - 21.37±1.40 unit/0.01 mm$^2$ (p<0.05). Density of glial cells: in cervical segments 35.12±1.62 unit/0.01 mm$^2$ (p<0.05), in the thoracic segments - 36.13±1.93 unit/0.01 mm$^2$ (p<0.01), in the lumbar segments - 38.06±1.87 unit/0.01 mm$^2$ (p<0.05) and in the sacral segments - 39.11±1.74 unit/0.01 mm$^2$ (p<0.05). Thus, the GI was 2.0 in the cervical segments, 1.9 in the thoracic segments, 1.9 in the lumbar segments, and 1.8 in the sacral segments.

We established that the density of neurons in the posterior horns of the spinal cord of fetuses of 29-30 weeks in the cervical segments was 17.29±0.85 unit/0.01 mm$^2$ (p<0.01), in the thoracic segments - 18.46±1.11 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments - 18.54±1.02 unit/0.01 mm$^2$ (p<0.01) т and in the sacral segments 20.43±1.33 unit/0.01 mm$^2$ (p<0.05). Density of glial cells: in cervical segments - 34.30±1.65 unit/0.01 mm$^2$ (p<0.05), in the thoracic segments - 35.87±1.92 unit/0.01 mm$^2$ (p<0.05), in the lumbar segments - 36.77±1.61 unit/0.01 mm$^2$ (p<0.05) and in the sacral segments - 37.63±1.50 unit/0.01 mm$^2$ (p<0.05). GI in the cervical segments was 2.0, in the thoracic segments - 2.0, in the lumbar segments - 2.0, and in the sacral segments - 1.8.

It was established that the proliferative activity of NSCs in the dorsal neuroepithelium of fetuses at 34-35 weeks remains relatively greatest in the cervical and lumbar segments, expression of Ki-67 was observed in 5 % of cells (3-4 cells reacted) (p<0.01), in the thoracic and sacral segments - 4 % (2-3 cells reacted) (p<0.05). The expression of vimentin was determined to be relatively moderate in the remnants of radial glia near the dorsal neuroepithelium (area of the base of the posterior horns), and focal expression of vimentin was observed around the vessels within the boundaries of the posterior horns (Fig. 3).

The expression of S-100 was relatively strong in the base of the posterior horns and the middle part. It should

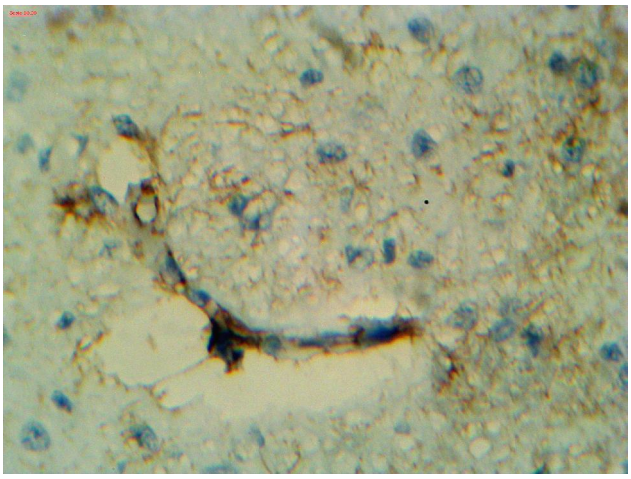

**Fig. 3.** Posterior horns of the spinal cord of a human fetus at 34-35 weeks. Focal expression of vimentin in remnants of radial glia near vessels. Vimentin. x400.

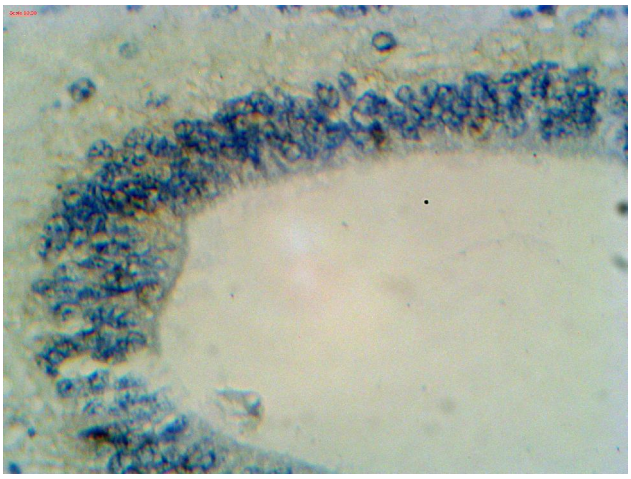

**Fig. 4.** Proliferation of NSCs in the dorsal neuroepithelium of a 39-40 weeks fetus. Ki-67. x400.

be noted that some neurons also expressed S-100. Relatively strong expression of synaptophysin was observed within the neck (proper nucleus) and head (gelatinous substance). Moderate expression of synaptophysin was at the apex of the posterior horns (posterior marginal nucleus).

The value of the density of neurons of the posterior horns in fetuses of 34-35 weeks in the cervical segments was 16.08±0.74 unit/0.01 mm$^2$ ($p<0.05$), in the thoracic segments - 17.25±1.05 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments 17.54±0.91 unit/0.01 mm$^2$ ($p<0.05$) and in the sacral segments - 19.00±1.26 unit/0.01 mm$^2$ ($p<0.05$). Density of glial cells: in cervical segments 33.34±1.55 unit/0.01 mm$^2$ ($p<0,05$), in the thoracic segments 35.48±1.72 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments - 35.66±1.49 unit/0.01 mm$^2$ ($p<0.05$) and in the sacral segments - 36.93±1.52 unit/0.01 mm$^2$ ($p<0.05$). Accordingly, the GI of the posterior horns in the cervical segments was 2.1, in the thoracic segments - 2.1, in the lumbar segments - 2.0, and in the sacral segments - 1.9.

The proliferative activity of NSCs of the dorsal neuroepithelium at 39-40 weeks remains relatively greatest in the cervical and lumbar segments, expression of Ki-67 was observed in 4 % of cells (2-3 cells reacted) ($p<0.05$), in the thoracic and sacral segments - 3 % (1-2 cells reacted) ($p<0.05$) (Fig. 4). The expression of vimentin in the posterior horns was relatively moderate in the remnants of radial glia around the neuroepithelium, and in the neuroepithelium itself, vimentin expression was absent and focal vimentin expression was observed around the vessels. The expression of S-100 protein in the posterior horns is relatively strong at the base of the posterior horns and in the middle part. It should be noted that some neurons also expressed S-100.

Expression of synaptophysin was noted in all areas of the posterior horns. Relatively strong expression of synaptophysin was observed within the neck (proper nucleus) and head (gelatinous substance). Moderate expression of synaptophysin was at the top of the posterior horns (posterior marginal nucleus).

The parameter of the density of neurons in the posterior horns during the spinal cord in fetuses of 39-40 weeks was as follows: in the cervical segments - 15.04±0.59 unit/0.01 mm$^2$ ($p<0.05$), in the thoracic segments - 16.44±0.83 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments - 16.11±0.81 unit/0.01 mm$^2$ ($p<0.05$) and in the sacral segments - 17.74±1.03 unit/0.01 mm$^2$ ($p<0.05$). Density of glial cells: in cervical segments 31.72±1.46 unit/0.01 mm$^2$ ($p<0.05$), in the thoracic segments - 34.42±1.57 unit/0.01 mm$^2$ ($p<0.05$), in the lumbar segments - 33.84±1.38 unit/0.01 mm$^2$ ($p<0.05$) and in the sacral segments - 35.40±1.40 unit/0.01 mm$^2$ ($p<0.05$). Therefore, the GI was 2.1 in the cervical segments, 2.1 in the thoracic segments, 2.1 in the lumbar segments, and 2.0 in the sacral segments.

Thus, in the course of the study, we determined the density of neurons and glial cells of the posterior horns of human fetuses, as well as the glial index and proliferative activity of NSCs of the dorsal neuroepithelium.

## Discussion

The parameter of the density of neurons and neuroblasts in the posterior horns during intrauterine development is quantitatively asynchronous. Thus, in the cervical and lumbar segments, this parameter increases to 8-9 weeks, in the thoracic segments - to 11-12 weeks, in the sacral segments - to 14-15 weeks. In the following, before birth, the density of neurons and neuroblasts in the posterior horns of all segments gradually becomes smaller.

In our previous studies, it was established that the proliferative activity of NSCs of the dorsal epithelium and the maximum density of neurons of the posterior horns occurs in the embryonic period, up to 8-9 weeks [26]. Comparing such indicators with the results of this study, we can conclude that from the beginning of the fertile period, the above-mentioned parameters before birth have a

**Table 1.** Quantitative composition of neuron-glial cells of the spinal cord of an adult according to Bahney J. and von Bartheld C. S. (2018).

| Segments | Total number of cells, million | Neurons, % | Glia cells, % | Neuron-glial relation |
|---|---|---|---|---|
| Cervical | 535.5±98.9 | 13.5 | 86.5 | 1:7 |
| Thoracical | 670.3±131.9 | 14.0 | 86.0 | 1:5 |
| Lumbar | 457.9±97.7 | 12.6 | 87.4 | 1:7 |

tendency to gradually decrease.

We have established that the parameter of the density of gliocytes in the posterior horns during intrauterine development is also quantitatively asynchronous. An increase in the density of glial cells in the cervical segments was observed up to 8-9 weeks [26], in the thoracic segments - up to 11-12 weeks, in the lumbar segments - up to 9-10 weeks, and in the sacral segments - up to 20-21 weeks. Before birth, the density of glial cells in the posterior horns of all segments gradually becomes smaller.

Before the beginning of the fetus period (8-9 weeks), the cervical segments have the maximum GI value of the posterior horns (GI - 1.7), the thoracic and lumbar segments have the GI value - 1.6, and the sacral segments have the minimum GI value - 1.5 [26]. By 29-30 weeks, the GI index in the cervical, thoracic and lumbar segments is the same and is 2.0. At 34-35 weeks, the value of GI in the rear horns of the lumbar segments, in contrast to the cervical and thoracic segments, becomes somewhat smaller, but before birth, the GI indicator becomes the same again in the above-mentioned segments (GI - 2.1). It should be noted that the GI value in the posterior horns of the sacral segments remains smaller throughout the fetal period and at the time of birth its value was 2.0.

S.V. Ryhlik [22] found that with increasing human age, there is a progressive decrease in the distribution density, number and size of nerve cells, a decrease in the amount of Nissl's substance, dystrophy and degeneration of neurons, accumulation of lipofuscin in them, a decrease in protein-synthesizing function, an increase in the number of gliocytes, reduction of the capillary network, polymorphism of endothelial cells. In her own research, M. A. Berezhnaya [6] also came to the conclusion that with age in the V layer in the upper frontal gyri of the human brain, a decrease in the number of neurons and capillaries was observed, but the number of glial cells increased, which is characterized by pronounced dynamics of changes in the glial index. The most pronounced changes for all parameters were noted between the age groups of 35-50 years and 51-75 years. According to O. Ya. Zhurakivska [29], the numerical density of glial cells also decreases with age. It should be noted that similar studies were concerned with the study of neuron-glial relations in the formations of the central nervous system of adults. However, the scientific conclusions regarding the decrease with age in the density of neural cells and the increase in the glial index are also confirmed by our research.

After long contradictory statements regarding the number of neurons and glial cells in the brain of humans and primates, scientists have recently reached a consensus [27]. However, the number of cellular components in relation to another component of the central nervous system, i.e. the spinal cord, remains uncertain [3, 13]. Thus, Burish M. J. [8] proposed the highest limit of the neuron-glial ratio, which is 1:40. Analyzing similar works by other authors and taking into account the above results from available literature sources, similar ratios in animals are on average 1:2 or 1:3 [7]. In general, most scientists adhere to the opinion that in such structures of the central nervous system as the cerebral cortex, cerebellum, brain stem, etc., a more consistent and gradual change in the neuron-glial ratio occurs, including in humans [12]. Therefore, we believe that the obtained data of the neuron-glia ratio in the spinal cord Burish M. J. [8] do not look expected.

In our opinion, more accurate results regarding the number of neurons and glial cells, as well as establishing the neuron-glial ratio in the spinal cord of an adult person and animals, were obtained by Bahney J. and von Bartheld C. S. [3]. The authors studied the spinal cord of three monkeys and three adults using the method of isotropic fractionation; there were no CNS diseases. Scientists determined the number of neurons and glial cells, as well as the neuron-glia ratio separately in the cervical, thoracic, and lumbar segments (Table 1).

Therefore, the ratio of glial cells and neurons was determined in human brain tissue [14], and not so long ago in the entire human spinal cord [3]. However, there is almost no purposeful description of the ratios of different populations of glial cells and neurons in the posterior horn of the segments of the human spinal cord, except for isolated reports. Thus, in their research Ruiz-Sauri A. [21] studied the relationship of glial cells to neurons in the posterior horns of the spinal cord of an adult and only in the thoracic segments (T8-T11). The authors emphasized the importance of understanding the anatomical and histological structure of the posterior horn in the above segments. The results of Ruiz-Sauri A. [21] showed that the number of neuron bodies in the gray matter of the segments is much smaller than the number of glial cells. The neuron-glia ratio in the gray matter was 1:12.

Therefore, the obtained results of our research coincide with the main provisions of the proposed concept of Herculano-Houzel S. [13], that as the brain matter increases, the following processes should occur: an obvious decrease in the number of neurons and their enlargement, an increase in the number of glial cells per neuron (since glial cells are engaged in providing the needs of neurons and do not actually increase in size), then the density of the distribution of neurons in the brain tissue should decrease. In addition, in our opinion, the growth of satellite glia indicates a high degree of functional activity of neurons, therefore the nature of structural and quantitative changes

of glia demonstrates high plasticity of nervous tissue.

The issue of discussion today is the study of the intensity of the proliferation processes of the dorsal neuroepithelium of the human spinal cord during the prenatal period [23] and their effect on the density of neural cells in the posterior horns [2, 14], as this is the basis for the formation of the NGC [5].

Again taking into account our previous works [26] and analyzing the results of this study, we concluded that the greatest proliferative activity of the dorsal neuroepithelium occurs during the embryonic period. Starting from the beginning of the fetal period and before birth, the proliferative activity of the dorsal neuroepithelium becomes less. After proliferation, NSCs of the dorsal neuroepithelium migrate into the mantle layer along radial glia fibers. The fibers of the radial glia of the posterior horns express vimentin relatively strongly and are characterized by a clear radial direction. With its short fibers, radial glia forms a "stripedness", which, in our opinion, enables NSCs to move not only radially, but also in other directions. T. J. Nowakowski and others [15] also describe the presence of intermittent fibers of radial glia already in the early stages of embryogenesis in brain formations. In addition, in this age period, radial glia fibers are part of the posterior roots of the spinal cord. This phenomenon can be explained by the fact that the neural cells of the dorsal neuroepithelium have the ability to migrate not only to the mantle layer, but also to the spinal nodes. Also, at 11-12 weeks. in the formations of the posterior horns, radial glia fibers form mesh structures, which is obviously connected with the beginning of the formation of neuron-glial complexes of the posterior horns. The final involution of radial glia in the posterior horns of the spinal cord of human fetuses begins at 34-35 weeks, i.e. these are the terms when neuron-glial complexes are already formed. The results we obtained correspond to the concept of Rakic P. [19] regarding the leading role of radial glia in the mechanisms of development of CNS structures, as well as in the formation of NGK. But, in general, we also support the opinion of Balazs A. [4] that the development of neurons of the posterior horn of the spinal cord is not completed before birth and in the prenatal period proceeds slowly and moderately. Although inhibitory and excitatory neurons appear in the early embryonic period.

## Conclusions

1. In the fetal period, greater proliferative activity of the dorsal neuroepithelium is determined at 8-9 weeks: in the cervical segments - 10 % ($p<0.05$), in the thoracic, lumbar and sacral segments - 9 % ($p<0.05$). By 39-40 weeks, this indicator gradually becomes smaller: in the cervical and lumbar segments, 4 % of cells (2-3 cells reacted) ($p<0.05$) and in the thoracic and sacral segments - 3 % (1-2 cells reacted) ($p<0.05$).

2. Throughout the fetal period, there is a tendency towards a gradual decrease in the density of neurons and gliocytes. The glial index, on the contrary, up to 39-40 weeks. increases and at the time of birth was equal to 2.1 in cervical, thoracic and lumbar, and 2.0 in sacral.

3. At 11-12 weeks, radial glia fibers form mesh structures within the neuronal complexes, which coincides with the beginning of the formation of neuron-glial complexes of the posterior horns. At 17-18 weeks, the fibers of radial glia keep the radial direction only in the middle part of the posterior horns. At 34-35 weeks, vimentin expression was determined to be relatively moderate in the remnants of radial glia near the dorsal neuroepithelium and focal expression of vimentin around vessels within the posterior horns. Expression of vimentin in the neuroepithelium of fetuses of 39-40 weeks was absent. In this age period, the neuroepithelium is structured from ependymocytes and radial glia cells are absent, as there is a relatively strong expression of S-100 in the neuroepithelium. Relatively strong expression of synaptophysin occurred in the posterior horns of 8-9 weeks fetuses. This age period is the beginning of the establishment of synaptic connections.

## References


[1] Arora, M. (2016). Fetal anatomy: morphometry of vertebral canal and spinal cord of cervical region in human fetuses. *Annals of International Medical and Dental Research,* 2(3), 128-131.

[2] Arulkandarajah, K. H., Osterstock, G., Lafont, A., Le Corronc, H., Escalas, N., Corsini, S. ... Mangin, J.-M. (2021). Neuroepithelial progenitors generate and propagate non-neuronal action potentials across the spinal cord. *Curr. Biol.,* 31(20), 4584-4595. doi: 10.1016/j.cub.2021.08.019

[3] Bahney, J., & von Bartheld, C. S. (2018). The cellular Composition and glia-neuron ratio in the spinal cord of a human and a non-human primate: comparison with other species and brain regions. *Anat. Rec.,* 301(4), 697-710. doi: 10.1002/ar.23728

[4] Balazs, A., Meszar, Z., Hegedüs, K., Kenyeres, A., Hegyi, Z., Docs, K., & Antal, M. (2017). Development of putative inhibitory neurons in the embryonic and postnatal mouse superficial spinal dorsal horn. *Brain Struct. Funct.,* 222(5), 2157-2171. doi: 10.1007/s00429-016-1331-9

[5] Bentivoglio, M., & Mazzarello, P. (1999). The history of radial glia. *Brain Res. Bull.,* 49(5), 305-315. doi: 10.1016/s0361-9230(99)00065-9

[6] Berezhnaya, M. A., Gargin, V. V., & Maslovskiy, S. Yu. (2013). Межполушарная ассиметрия нейроно-глиально-капиллярных взаимоотношений V слоя в верхних лобных извилинах головного мозга человека [Interhemispheric asymmetry of the neuron-glial-capillary interrelated layer V in the upper frontal gyri of the human brain]. *Вісник проблем біології і медицини - Bulettin of problems in biology and medicine,* 3(2), 274-277.

[7] Bjugn, R. (1993). The use of the optical dissector to estimate the number of neurons, glial and endothelial cells in the spinal cord of the mouse - with a comparative note on the rat spinal cord. *Brain Res.*, 627(1), 25-33. doi: 10.1016/0006-8993(93)90744-8

[8] Burish, M. J., Peebles, J. K., Baldwin, M. K., Tavares, L., Kaas, J. H., & Herculano-Houzel, S. (2010). Cellular scaling rules for primate spinal cords. *Brain Behav. Evol.,* 76, 45-59. doi:

10.1159/000319019
[9] Cassaro, M., Rugge, M., Tieppo, C., & Giacomelli, L. (2007). Indefinite for non-invasive neoplasia lesions pn gastric intestinal metaplasia: the immunophenotype. *J. Clin. Pathol.,* 60, 615-621. doi: 10.1136/jcp.2006.040386
[10] Cedeno, D. L., Smith, W. J., Kelley, C. A., & Vallejo, R. (2020). Spinal cord stimulation using differential target multiplexed programming modulates neural cell-specific transcriptomes in an animal model of neuropathic pain. *Mol. Pain,* 16, 1744806920964360. doi: 10.1177/1744806920964360
[11] Costa, V., Lugert, S., & Jagasia, R. (2015). Role of adult hypocampal neurogenesis in cognition in physiology and disease: pharmacological targets and biomarkers. *Handbook of Experimental Pharmacology,* 228, 99-155. doi: 10.1007/978-3-319-16522-6_4
[12] Herculano-Houzel, S. (2014). The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia,* 62, 1377-1391. doi: 10.1002/glia.22683
[13] Herculano-Houzel, S. (2017). Numbers of neurons as biological correlates of cognitive capability. *Curr. Opin. Behav. Sci.,* 16, 1-7. doi: 10.1016/j.cobeha.2017.02.004
[14] Kuzmicz-Kowalska, K, & Kicheva, A. (2021). Regulation of size and scale in vertebrate spinal cord development. *Rev. Dev. Biol.,* 10(3), e383. doi: 10.1002/wdev.383
[15] Nowakowski, T. J., Pollen, A. A., Sandoval-Espinosa, C., & Kriegstein, A. R. (2016). Transformation of the radial glia scaffold demarcates two stages of human cerebral cortex development. *Neuron,* 91(6), 1219-1227. doi: 10.1016/j.neuron.2016.09.005
[16] Ma, J. J., Zhang, T. Y., Diao, X. T., Yao, L., Li, Y. X., Suo, Z. W. … Liu, Y. N. (2021). BDNF modulated KCC2 ubiquitylation in spinal cord dorsal horn of mice. *Eur. J. Pharmacol.,* (5), 906. doi: 10.1016/j.ejphar.2021.174205
[17] Peirs, C., Williams, S. G., Zhao, X., Arokiaraj, C. M., Ferreira, D. W., Noh, M. C. … Seal, R. P. (2021). Mechanical allodynia circuitry in the dorsal horn is defined by the nature of the injury. *Neuron,* 109(1), 73-90. doi: 10.1016/j.neuron.2020.10.027
[18] Popele, R., & Bosco, G. (2003). Sophisticated spinal contributions to motor control. Trends Neurosci., 26(5), 269-276. doi: 10.1016/S0166-2236(03)00073-0
[19] Rakic, P. (2003). Elusive radial glial cells: historical and evolutionary perspective. *Glia,* 43, 19-32. doi: 10.1002/glia.10244
[20] Rueger, M., & Schroeter, M. (2015) In vivo imaging of endogenous neural stem cells in the adult brain. *World Journal Stem Cells,* 7(1), 75-83. doi: 10.4252/wjsc.v7.i1.75
[21] Ruiz-Sauri, A., Orduna-Valls, J. M., Blasco-Serra, A., Tornero-Tornero, C., Cedeno, D. L., Bejarano-Quisoboni, D. … Vallejo, R. (2019). Glia to neuron ratio in the posterior aspect of the human spinal cord at thoracic segments relevant to spinal cord stimulation. *J. Anat.,* 235(5), 997-1006. doi: 10.1111/joa.13061
[22] Ryhlik, S. V. (2014). Морфологічні особливості вентролатеральної групи ядер таламуса проміжного мозку людини [Morphological features of the ventrolateral group of nuclei of the thalamus of the human midbrain]. *Вісник проблем біології і медицини - Bulettin of Problems in Biology and Medicine,* 4(1), 285-289.
[23] Skarlatou, S., Herent, C., Toscano, E., Mendes, C. S., Bouvier, J., & Zampieri, N. (2020). Afadin Signaling at the Spinal Neuroepithelium Regulates Central Canal Formation and Gait Selection. *Cell. Rep.*, 31(10), 107741. doi: 10.1016/j.celrep.2020.107741
[24] Smith, T. M., Lee, D., Bradley, K., & McMahon, S. B. (2020). Methodology for quantifying excitability of identified projection neurons in thedorsal horn of the spinal cord, specifically to study spinal cord stimulation paradigms. *J. Neurosc. Methods,* 330, 108479. doi: 10.1016/j.jneumeth.2019.108479
[25] Stepanov, A. S., Akulinin, V. A., Mysik, A. V., Stepanov, S. S., & Avdeev, D. B. (2017). Neuro-glio-vascular complexes of the brain after acute ischemia. *General Reanimatology,* 13(6), 6-17. doi: 10.15360/1813-9779-2017-6-6-17
[26] Vernygorodskyi, S. V., Shkolnikov, V. S., & Prykhodko, S. O. (2021). The morphology and neuronal-glial correlations of the spinal cord posterior horns of human embryos. *International Medical Journal,* 3(3), 93-100. doi: 10.5455/im.41865
[27] Von Bartheld, C. S., Bahney, J., & Herculano-Houzel, S. (2016). 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.,* 524, 3865-3895. doi: 10.1002/cne.24040
[28] Zhou, W., Xie, Z., Li, C., Xing, Z., Xie, S., Li, M., & Yao, J. (2021). Driving effect of BDNF in the spinal dorsal horn on neuropathic pain. *Neuroscience Letters,* 756, 135965. doi: 10.1016/j.neulet.2021.135965
[29] Zhurakivska, O. Ya. (2014). Становлення нейрон-гліо-капілярних співвідношень у вентромедіальному ядрі гіпоталамуса в постнатальному періоді онтогенезу [Formation of neuron-glio-capillary ratios in the ventromedial nucleus of the hypothalamus in the postnatal period of ontogenesis]. *Науковий вісник міжнародного гуманітарного університету - Scientific Bulletin of the International Humanitarian University,* (7), 15-18.

**НЕЙРОНО-ГЛІАЛЬНІ ВІДНОШЕННЯ ЗАДНІХ РОГІВ СПИННОГО МОЗКУ ПЛОДІВ ЛЮДИНИ**

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*Не дивлячись на відносно достатнє вивчення будови та функціонування нервової системи, зацікавленість до проблеми нейроно-гліальних відношень продовжує неухильно зростати, оскільки даний параметр віддзеркалює динаміку розвитку нервової тканини та може бути використаний для оцінювання рівня якості морфологічних змін. Мета дослідження: встановити морфогенез та нейроно-гліальні взаємовідносини задніх рогів спинного мозку людини у плодовому періоді онтогенезу. Дане дослідження виконано на препаратах 104 плодів людини від 8-9 тижнів до 39-40 тижнів при використанні анатомічних, гістологічних, імуногістохімічних та морфометричних методик. Статистичну обробку числових даних отриманих результатів проводили за допомогою ліцензійного програмного пакету "Statistica 6.1" фірми StatSoft із застосуванням параметричних і непараметричних методів. У процесі дослідження встановлено, що у плодовому періоді більша проліферативна активність дорзального нейроепітелію визначається у 8-9 тижнів: у шийних сегментах - 10 % ($p<0,05$), у грудних, поперекових та крижових - 9 % ($p<0,05$). До 39-40 тижнів даний показник поступово повільно стає меншим: у шийних і поперекових сегментах 4 % клітин (прореагувало 2-3 клітини) ($p<0,05$) та у грудних і крижових сегментах - 3 % (прореагувало 1-2 клітини) ($p<0,05$). Виявлено, що увесь плодовий період триває тенденція до поступового зменшення щільності нейронів та гліоцитів. Гліальний індекс, навпаки, до 39-40 тиж. збільшується, і на момент народження дорівнює*

*у шийних, грудних та поперекових сегментах - 2,1, а у крижових - 2,0. З'ясовано, що у 11-12 тижнів у межах нейронних комплексів волокна радіальної глії формують сітчасті структури, що співпадає з початком формоутворення нейроно-гліальних комплексів задніх рогів. У 17-18 тижнів волокна радіальної глії зберігають радіальний напрямок лише у середній частині задніх рогів. У 34-35 тижнів експресія віментину визначалась відносно посередньою у залишках радіальної глії біля дорзального нейроепітелію та вогнищева експресія віментину навколо судин у межах задніх рогів. Експресія віментину у нейроепітелії плодів 39-40 тижнів була відсутня. У даному віковому періоді нейроепітелій структурований з епендимоцитів та відсутні клітин радіальної глії, оскільки є відносно сильна експресія S-100 у нейроепітелії. Відносно сильна експресія синаптофізину відбувалась у задніх рогах у плодів 8-9 тижня. Даний віковий період є початком встановлення синаптичних зв'язків.*

**Ключові слова:** *плоди людини, спинний мозок, задні роги, нейроно-гліальні відношення, радіальна глія, нейральні стовбурові клітини.*