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Differentiation of the nuclear groups in the posterior horn of the human embryonic spinal cord

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The formation of nuclear groups in the posterior horns of the human embryonic spinal cord was traced in serial sections of embryos of developmental stages 13 to 23 (32 to 56 postovulatory days). The following observations, new for the human, are presented: 1. The differentiation of the neural tube into 3 zones (germinal, mantle and marginal) is detected in the middle of the 5th week. 2. The primordia of the posterior horns are marked at stage 14 (33 days). 3. In the middle of the 7th week the nucleus proprius and substantia gelatinosa are discerned. 4. Differentiation of the nuclei within the posterior horns proceeds in the ventrodorsal and rostrocaudal gradients. (Folia Morphol 2011; 70, 4: 245–251)

Key words: human neuroembryology, spinal cord, posterior horns

INTRODUCTION

The vertebrate spinal cord is responsible for the control and coordination of motor output and the relay of cutaneous, visceral, and proprioceptive sensory information to higher brain centres. Distinct neuronal subtypes are topologically positioned in the spinal cord, and this stereotypic organisation of cells reflects the function, and to some extent the developmental origin, of individual neurons [1]. The neurons that process and relay sensory input reside predominantly in the dorsal half of the spinal cord (Rexed laminae I to VI), whereas the circuits that participate in motor output are concentrated ventrally.

Ample and diverse evidence indicates that activity-dependent plasticity occurs in the spinal cord during development, with skill acquisition and maintenance later in life and in response to trauma and disease. Normal descending influence from the brain guides development of spinal cord plasticity that contributes to skill acquisition and maintenance of skilled behaviours [24].

The spinal cord plasticity depends on the pattern of afferent, efferent, and interneuronal activity. A certain level of appropriately timed sensory input appears to be essential [19].

Morphological differentiation of distinct clusters of neurons in the posterior horn of the spinal cord is important in the segregation of afferent inputs into laminar-specific projections, which is a key event in the development of the appropriate connections in the spinal cord. Specific genes, diffusible factors, and surface and matrix molecules have been implicated in this process [16, 17].

The functioning of the nervous system depends on its precise and complex spatial organisation. Creating this organisation during development involves solving two problems: first, arranging the cells in the correct locations, and second, establishing the correct pattern of neuronal connections [3].

Gross features and histological structure of the developing human spinal cord have been described by Hughes [4], O'Rahilly & Muller [13, 14], Węcle-

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Table 1. Crown-rump length (CRL) in mm, developmental stage, age in postovulatory days, and plane of section of investigated embryos

Catalogue no. of embryo	CRL [mm]	Stage	Age (days)	Plane of section
B-218	4.0	13	32	Horizontal
B-202	4.0	13	32	Horizontal
B-209	5.5	13	32	Horizontal
B-207	6.0	14	33	Horizontal
A-17	7.0	14	33	Horizontal
B-175	9.0	15	36	Horizontal
PJK-20	9.0	15	36	Horizontal
A-16	9.0	15	36	Horizontal
PJK-8	10.0	16	39	Horizontal
B-216	11.0	16	39	Horizontal
B-70	12.0	17	41	Horizontal
B-68	14.0	17	41	Horizontal
B-65	16.0	18	44	Horizontal
B-66	16.5	19	46	Horizontal
Bł-5	17.0	19	46	Horizontal
B-99	19.0	20	49	Horizontal
Bł-1	20.5	20	49	Horizontal
B-126	22.0	21	51	Horizontal
WR-II	25.0	22	53	Horizontal
A-71	29.0	23	56	Horizontal
B-184	30.0	23	56	Horizontal

wicz-Kruczyńska & Woźniak [23, 25], and Malinsky & Malinska [7, 8]. Ultrastructural studies of the human spinal cord have been undertaken by several investigators (27 and vast literature therein).

From these investigations it is clear that the first synaptic contacts are axodendritic and the mature synapses are preceded by surface specialisations of processes, which show electron dense patches that may represent protosynapses [20, 26].

Despite many studies on the development of the human spinal cord little is known about the differentiation of the various nuclear groups in the posterior horns during the embryonic period.

The aim of the present study is to trace the formation of the grey substance of the posterior horns in staged human embryos.

MATERIAL AND METHODS

The study was performed on 21 human embryos at developmental stages 13–23 (32 to 56 days) from the Collection of the Department of Anatomy at Poznań University of Medical Sciences (Table 1). The investigated embryos were embedded in toto in paraffin or paraplast, and serial sections were made in horizontal plane. The age of the embryos was estimated according to 23 developmental stages and was expressed in post-ovulatory days. Sections, 5 or 10 μm thick, were stained with haematoxylin and eosin or cresyl violet or impregnated with Bodian's protargol.

Microscopic pictures of the serially sectioned embryos were taken using a Canon S40 coupled with a computer and adjusted by using AxioVision LE Rel. 4.8 Digital Image Processing Software from Carl Zeiss MicroImaging GmbH.

RESULTS

In embryos at stage 13 the neural tube is closed and extends throughout the whole length of the embryo. It is flattened laterally and its wall begins

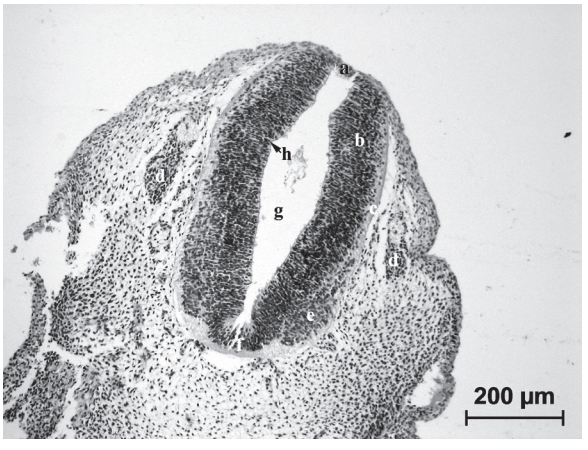


Figure 1. Horizontal section of embryo at stage 13. Cresyl violet; a — roof plate; b — germinal layer; c — marginal layer; d — spinal ganglion; e — mantle layer; f — floor plate; g — central canal; h — sulcus limitans.

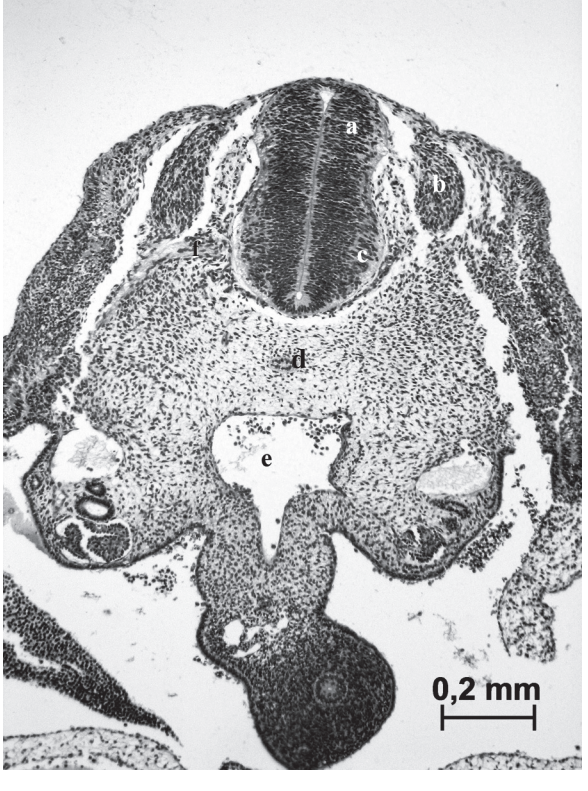


Figure 2. Horizontal section of embryo at stage 13. Cresyl violet; a — germinal layer; b — spinal ganglion; c — mantle layer; d — notochord; e — aorta; f — anterior root.

to differentiate into three zones, i.e. the germinal (matrix), mantle, and marginal. The sulcus limitans divides the neural tube into two parts: the alar lamina dorsally and the basal lamina ventrally. The mantle layer is thickest within the basal lamina and it forms the future ventral horn of the grey matter (Fig. 1).

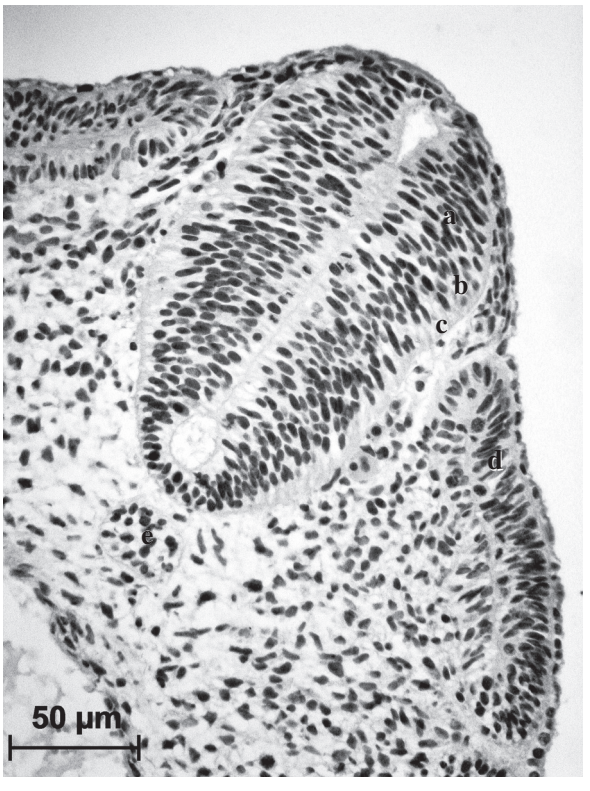


Figure 3. Horizontal section of lumbar part of embryo at stage 13. Cresyl violet; a — germinal layer; b — mantle layer; c — marginal layer; d — somite; e — notochord.

In the alar lamina the mantle layer presents a narrow string of loosely arranged cells. In the cervical and thoracic parts of the neural tube the ventral roots leaving the mantle zone of the basal lamina are observed (Fig. 2). In the lumbar and upper sacral levels the neural tube is less differentiated and the three zones are not clearly demarcated (Fig. 3). The lowermost sacral and coccygeal parts of the neural tube develop by secondary neurulation in the caudal eminence. The lumen of the neural cord in the caudal eminence develops by cavitation (Fig. 4).

At stage 14 the mantle layer of the alar lamina becomes more evident forming the primordium of the dorsal horn. Primordia of the dorsal funiculi are also seen and the posterior roots enter the spinal cord (Fig. 5). Intermediate grey substance consists of a narrow cellular group at the periphery of the germinal layer opposite the sulcus limitans. Paravertebral ganglia and rami communicantes are also observed. The spinal grey substance is thickest within the basal lamina.

During stage 15 the mantle layer within the alar lamina grows in width and may be considered as the posterior horn. The surface of the posterior horn

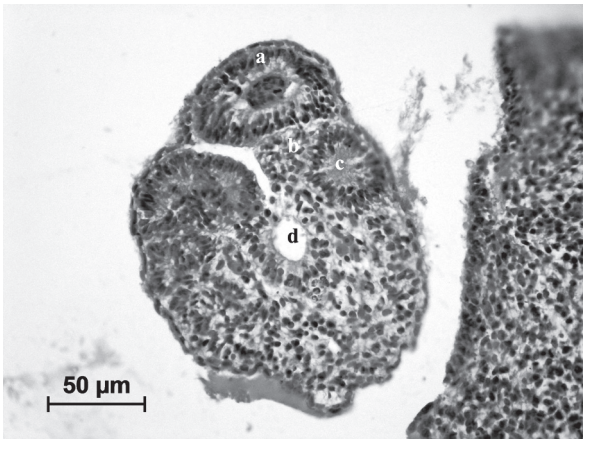


Figure 4. Horizontal section through the caudal eminence of embryo at stage 14. H+E; a — neural cord; b — notochord; c — somite; d — hindgut.

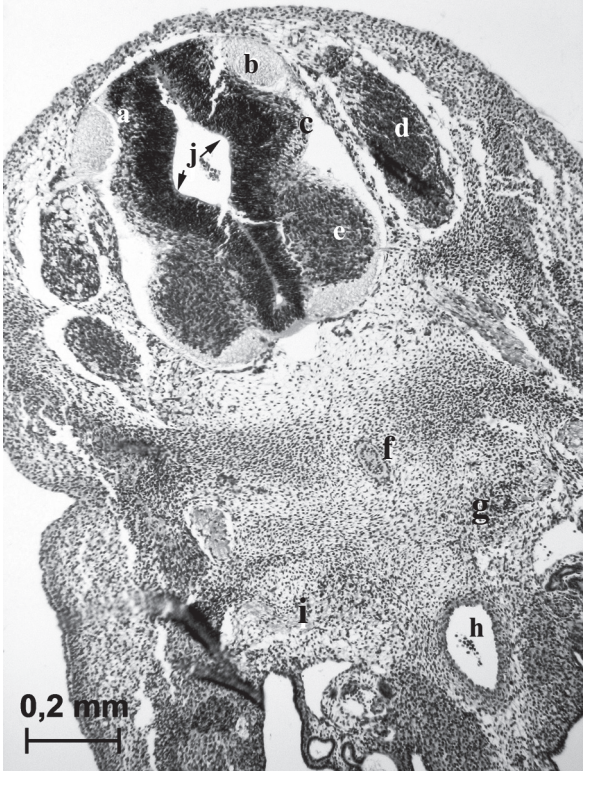
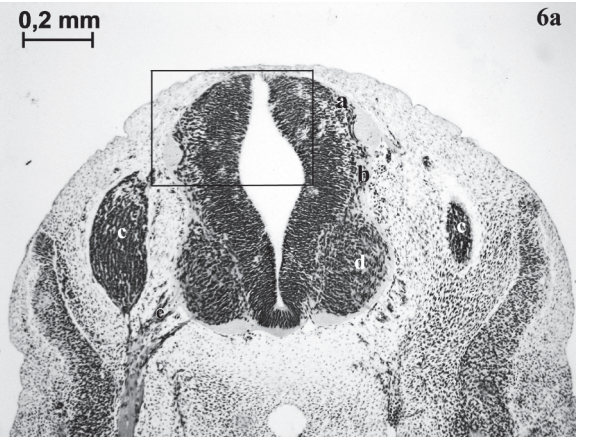


Figure 5. Horizontal section of embryo at stage 14. Cresyl violet; a — primordium of posterior horn; b — primordium of posterior funiculus; c — primordium of intermediate grey substance; d — spinal ganglion; e — anterior horn; f — notochord; g — sympathetic ganglion; h — aorta; i — rami communicantes; j — sulcus limitans.

consists of a narrow band of densely packed neurons which form a distinct cap-like cluster of cells (Fig. 6). The spinal ganglia are migrated ventrally and they are at the upper limit of the anterior horns.



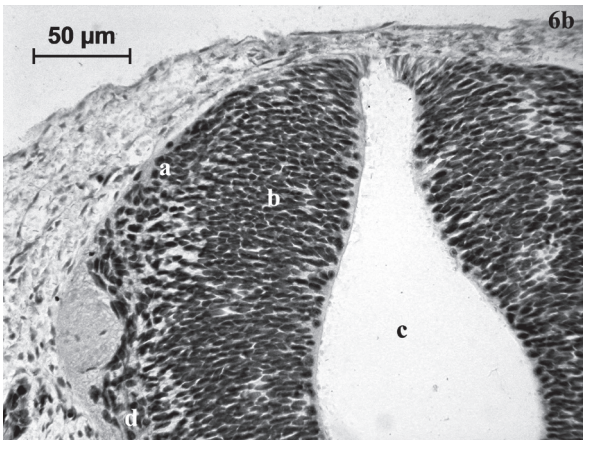


Figure 6. A. Horizontal section of embryo at stage 15. Impregnation with Bodian's protargol; a — posterior horn; b — intermediate gray substance; c — spinal ganglion; d — anterior horn, anterior roots; **B.** Horizontal section of alar lamina of spinal cord in embryo at stage 15. Higher magnification of framed area of Figure 6A. Impregnation with Bodian's protargol; a — posterior horn; b — germinal layer; c — central canal; d — intermediate grey substance.

During the 6th week (stages 16 and 17) and at the beginning of the 7th week (stage 18) slow growth of the posterior horn is observed. It consists of a narrow group of cells partly surrounded by the posterior funiculus (Fig. 7).

In the middle of the 7th week (stage 19) the posterior horn is distinctly wider as compared with the previous stage and is composed of highly concentrated groups of small neurons. In the cervical and thoracic parts of the spinal cord in the developing posterior horn the primordia of the substantia gelatinosa and the nucleus proprius are visible (Fig. 8). Some of the neurons invade the posterior funiculus. They may be considered as the primordium of the postero-marginal nucleus (substantia marginalis). In the lumbar region of the spinal cord the posterior horn neurons are narrower than in the

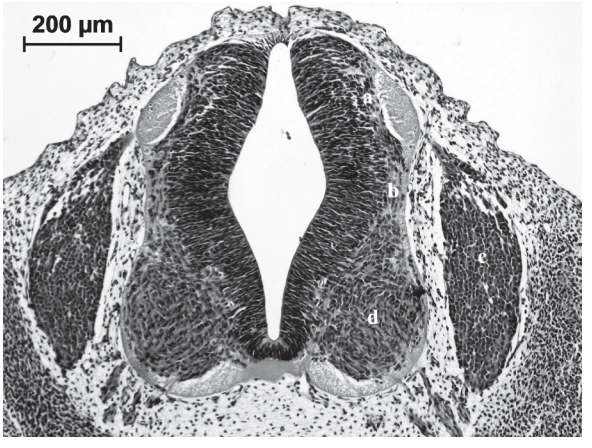


Figure 7. Horizontal section of embryo at stage 16. H+E; a — posterior horn; b — intermediate grey substance; c — spinal ganglion; d — anterior horn.

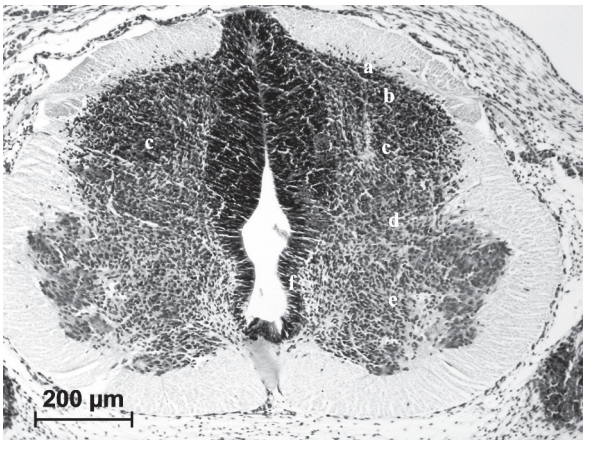


Figure 10. Horizontal section of embryo at stage 21. H+E; a — substantia marginalis; b — substantia gelatinosa; c — nucleus proprius; d — intermediate grey substance; e — anterior horn; f — germinal (ependymal) layer.

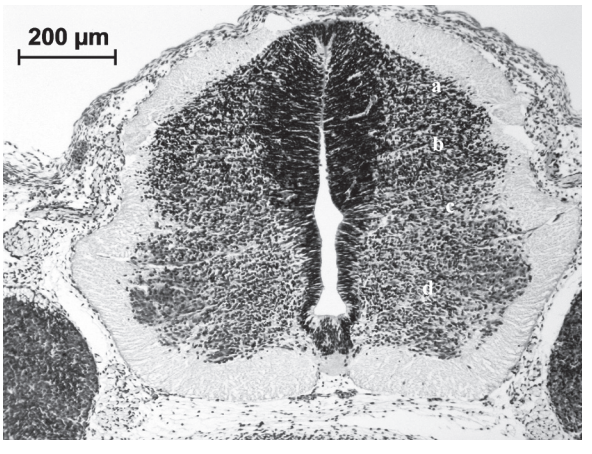


Figure 8. Horizontal section of embryo at stage 20. H+E; a — substantia gelatinosa; b — nucleus proprius; c — intermediate grey substance; d — anterior horn.

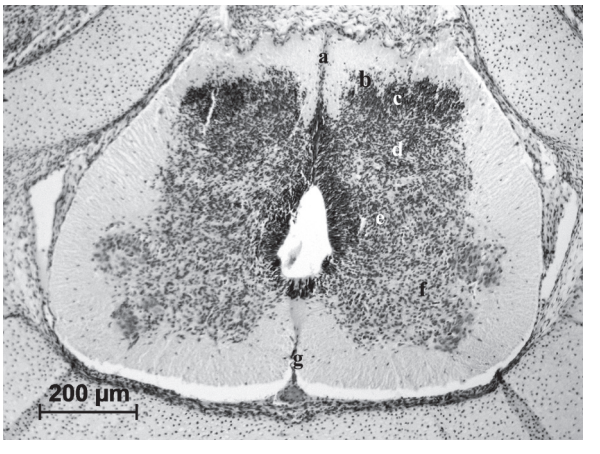


Figure 11. Horizontal section of lumbar part of embryo at stage 23. H+E; a — posterior median septum; b — substantia marginalis; c — substantia gelatinosa; d — nucleus proprius; e — nucleus dorsalis; f — anterior horn; g — anterior median fissure.

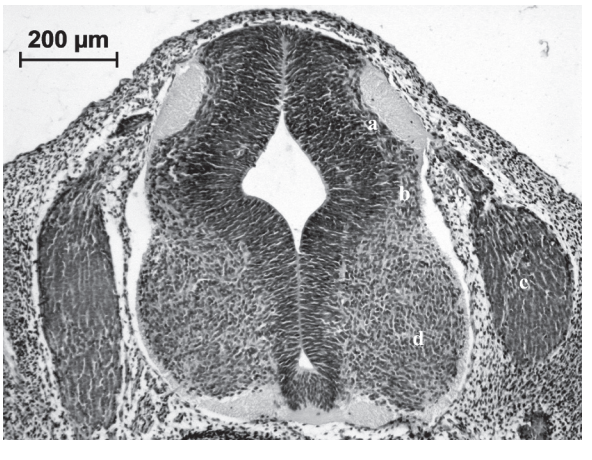


Figure 9. Horizontal section of lumbar part of embryo at stage 19. H+E; a — posterior horn; b — intermediate grey substance; c — spinal ganglion; d — anterior horn.

upper parts and resemble those of the cervical spinal cord in the 6th week of development (Fig. 9).

Beginning from stage 21 the posterior horn nuclei (i.e. the postero-marginal nucleus, the substantia gelatinosa, and the nucleus proprius) are well differentiated in the cervical and thoracic parts of the spinal cord. The width of the posterior horn is greater than the intermediate grey matter (Fig. 10). At the end of the embryonic period (stage 23) the postero-marginal nucleus, the substantia gelatinosa, and the nucleus proprius of the posterior horn are evident throughout the whole length of the spinal cord (Fig. 11). At this stage of development the medial part of the base of the posterior horn in the thoracic and upper lumbar parts of the spinal cord

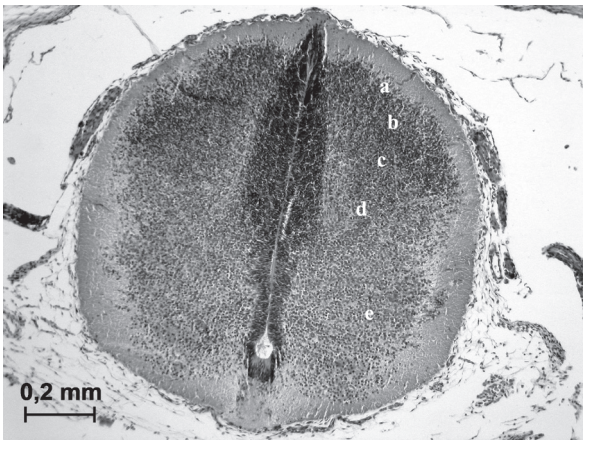


Figure 12. Horizontal section of embryo at stage 23. H+E; a — substantia marginalis; b — substantia gelatinosa; c — nucleus proprius; d — nucleus dorsalis; e — anterior horn.

is partly occupied by the dorsal nucleus (posterior thoracic nucleus) (Figs. 11, 12).

From the performed study it is evident that the differentiation of the grey substance of the spinal cord proceeds in the ventrodorsal and rostrocaudal gradients.

DISCUSSION

The nervous systems of both vertebrates and invertebrates are derived from a single cell layer, the neuroepithelium, from which progenitor cells are specified and which then divide to produce a large number of specialised cell types that differ in their position, morphology, connectivity, ion channel composition, neurotransmitter repertoire, and other properties [6].

In different animals and in the human the spinal cord develops during the primary and secondary neurulation. Primary neurulation is the formation of the spinal cord to the lumbosacral level. It begins morphologically during stage 8 (23 days) and is completed during stage 12 (30 days) [10].

Secondary neurulation begins with the closure of the caudal neuropore (stage 12), which occurs in the human embryo when about 29 somites are present [13]. The site of closure corresponds to future somite 31, the level of the future second sacral vertebra. Secondary neurulation ends at stage 17 (40 days).

Primary and secondary neurulation is under the control of different genes [18].

In the present study it was shown that in early human embryos of stage 13 (32 days) the neural tube is divided into the alar and basal laminae by the sulcus limitans and is already differentiated into three zones. The alar laminae are united by a thin roof plate, and the basal laminae are joined by a thin floor plate.

The notochord induces the floor plate and, together with the floor plate, controls the pattern of cellular types that appear along the dorso-ventral axis of the neural tube [13]. The floor plate acts as an intrinsic organiser of axons by releasing chemoattractants [13, 20].

Our study indicated that in embryos aged 5 weeks (developmental stage 13 to 15) there is differentiation of neurons migrating from the germinal zone. This differentiation proceeds in the ventrodorsal and rostrocaudal gradients. Similar gradients of differentiation were observed by other investigators but in later periods of embryonic development [13, 22].

The formation of synapses in the human embryonic spinal cord is also in the ventrodorsal and rostrocaudal directions. The sequences of synaptogenesis in the simple cutaneous reflex pathway occur in a retrograde fashion with respect to the direction of flow of nerve impulses [11, 12, 27].

It was demonstrated that during the 5th week within the alar lamina which forms the posterior horn there is a narrow band of closely packed neurons which form a cap-like structure typical for the future substantia gelatinosa.

Ozaki and Snider [15] showed that differentiation of morphologically and biochemically distinct groups of neurons in the posterior horn of the developing mouse spinal cord is apparent well before penetration of the grey matter by sensory axons. The onset of collateral branching does not occur until well after differentiation of certain cell groups. They also demonstrated that different classes of sensory axons enter the developing spinal cord in sequence and project directly to their target laminae, avoiding inappropriate laminae en route. Neurotrophin-3 (NT-3) has been considered as a primary candidate to regulate collateral branching of spinal sensory axons [2, 17].

Marti et al. [9] showed that calcitonin gene-related peptide, galanin, somatostatin, neuropeptide Y, and its C-flanking peptide (CPON) were the first to appear localised to motoneurons (weeks 6–14 in humans). The number of immunoreactive motoneurons decreased toward birth. In the youngest human embryos at 6 weeks immunoreactivity was present throughout the spinal cord.

According to Marti et al. [9], the sequence of appearance of nuclear groups in the human spinal cord is as follows: (1) cells of the floor plate; (2) motor

neurons; (3) cells of the nucleus proprius; and 4) substantia gelatinosa.

Woźniak and Węclewicz [25] showed early formation of the nuclear groups in basal lamina of the spinal cord.

In the present study it was demonstrated that the substantia gelatinosa and the nucleus proprius are visible in embryos early during the 7th week.

Yip et al. [27] showed that cyclin-dependent kinase 5 (Cdk5), which is crucial for neuronal migration and survival in the brain, affects the migration of different populations of neurons in the developing spinal cord. In the absence of Cdk 5 the sympathetic, parasympathetic preganglionic neurons, as well as dorsally originating and ventrally originating dorsal horn neurons, failed to migrate to their final destinations.

Afferent fibres reach the dorsal horn well before their peripheral field expresses sensory peptides. This suggests that peripheral tissue does not direct the formation of central termination [5, 13].

The performed study gives evidence that the differentiation of nuclear clusters in the posterior horns takes place early in human intrauterine life.

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