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The thickness of the ventral midline of the spinal cord in human embryos during the fifth week

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The thickness of the ventral midline of the spinal cord was determined in 9 human embryos aged five weeks (developmental stages 13–15). This part of the spinal cord consists of floor plate, mantle and marginal layers. The floor plate ependymal cells form pseudostratified columnar epithelium. The thickness of the investigated structure varied from 20 to 50 micrometers at different levels of the spinal cord. (Folia Morphol 2008; 67: 205–208)

Key words: human neuroembryology, spinal cord, ventral midline cells, floor plate

INTRODUCTION

Differentiation of neuronal types and the establishment of appropriate connections between neurons and their targets are critical phases of the early development of the nervous system, and these events form the basis for the proper operation of the mature nervous system. Cells at the midline of the neural tube control axon projections and influence motor neurons and several types of interneuron differentiation [2, 12, 16].

The formation of precise neuronal circuits depends upon the ability of the growth cone, which guides axons, to navigate over a long distance. The guidance cues exist in either diffusible or cell surface-associated forms [7, 21]. Growth cone-associated cell surface receptors interpret these signals as positive/attractive (netrin) or negative/repulsive (slit and semaphorin proteins) forces [15, 24].

The midline of the developing central nervous system represents an important choice point for pathfinding axons [8]. Recent experiments show that axial mesoderm can stimulate formation of specific ventral cell types in the spinal cord, including floor plate and motor neurons. Generation of these cell types is dependent on the secreted signalling molecule Sonic hedgehog (Shh), which is produced by axial mesodermal midline cells of the notochord. Shh signalling from the notochord induces development of the ventral midline cells and the floor plate which also expresses Shh together with other genes [4].

The ependymal lining floor plate has been investigated in ultrastructural and cytochemical studies on the human spinal cord by Gamble [3], Malinsky and Brichova [13] and Tanaka et al. [20]. In the present study we investigated the thickness and histological structure of the ventral midline of the spinal cord in human embryos aged five weeks.

Although morphological criteria do not permit delimination of the ventral midline cells and the floor plate in the transverse plane, it is possible to measure the thickness of the ventral midline cells of the spinal cord in the sagittal plane.

MATERIAL AND METHODS

A study was made on human embryos aged five weeks (developmental stages 13, 14 and 15, Table 1). The embryos were from the Collection of the Department of Anatomy of the University of Medical Sciences in Poznań. All embryos were embedded *in toto* in paraplast and serially sectioned in the

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Table 1. Crown-rump length, developmental stage, and age in days of the embryos examined

Catalogue number	C-R length [mm]	Developmental stage	Age in postovulatory days	Plane of section
B 171	4	13	32	Horizontal
B 194	6	13	32	Horizontal
B 218	4.5	13	32	Horizontal
A 19	7	14	32	Horizontal
B 207	6	14	32	Horizontal
P 41	5	14	33	Horizontal
B 115	8	15	33	Horizontal
PJK 20	7	15	33	Horizontal
PJK 21	10	15	37	Horizontal

horizontal plane. Measurements of the ventral midline of the spinal cord were made on sections from various levels of the spinal cord with the aid of a Leica Olympus Microscope using program AxioVision LE with the Canon Module. The thickness of the ventral midline was determined together with the floor plate.

The structure of the ventral midline of the spinal cord was also investigated in histological sections.

RESULTS

In embryos at stage 13 the mantle layer of the ventral midline of the spinal cord consists of 4 rows of cells with oval nuclei. The ependyma at this part is formed of columnar cells with basally located nuclei. The marginal layer is clearly visible (Fig. 1). The thickness of the ventral midline in the embryos studied varied from 20 to 40 μm at different levels of the spinal cord and was greater in the cervical and upper thoracic segments than in the lumbar segment of the spinal cord (Table 2).

In embryos at stage 14 the mantle layer of the ventral midline is thicker than that of embryos in stage 13 and consists of 5 or 6 rows of cells (Fig. 2). The ependymal layer consists of pseudostratified columnar epithelium. In the mantle layer the crossing commissural axons are visible (Fig. 3). The thickness of this ventral midline cells varies from 30 to 50 μm (Table 2).

In embryos at stage 14 the central canal of the spinal cord diminishes and the anterior horns are well developed. The structure and thickness of the ventral midline of the spinal cord are similar to that of embryos at stage 14 (Fig. 4).

DISCUSSION

The ventral midline cells of the central nervous system are important in the neural tube, playing a role in the differentiation part of motor neurons and the establishment of commissural projections [2, 14]. The differentiation of neurons in the ventral neural tube is dependent on inductive signals from the notochord and floor plate, which is formed of specialized glia in a narrow strip at the ventral midline [1, 14, 17, 18, 22].

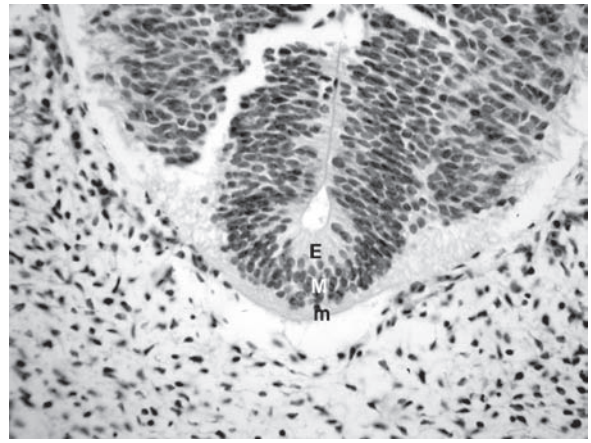

Figure 1. Horizontal section of the spinal cord of an embryo at stage 13. Staining with toluidine blue, × 400; E — ependymal of the floor plate, M — mantle layer, m — marginal layer.

According to O'Rahilly and Müller [16], the floor plate is formed by the ventromedial cells of the epinotochordal part of the neural plate or tube and is induced by the notochord. It differs regionally. Cells in the midbrain can induce the production of dopaminergic neurons, whereas those of the rhombencephalon develop into the septum medullae.

According to many authors, beginning with the descriptions of His [5], the floor plate is made up of columnar ependymal cells that span the width of the neural tube at its ventral midline. The anterior boundary of the floor plate has been placed at the hindbrain-midbrain junction [9, 10] but there are several

Table 2. Thickness of the ventral midline of the spinal cord in the embryos examined

Catalogue number of embryo	Developmental stage	Level of section	Thickness in micrometers
B 171	13	Upper thoracic	40
		Lumbar	30
B 194	13	Cervical	30
		Lower thoracic	20
B 218	13	Upper thoracic	20
		Lower thoracic	20
		Lumbar	20
P 41	14	Lower cervical	40
		Upper thoracic	40
		Lower thoracic	30
A 19	14	Upper thoracic	30
		Lower thoracic	40
B 207	14	Cervical	50
		Upper thoracic	50
		Lumbar	40
B 115	15	Cervical	40
		Upper thoracic	40
		Lower thoracic	40
PJK 21	15	Cervical	30
		Upper thoracic	30
		Lower thoracic	30
PJK 20	15	Cervical	50
		Upper thoracic	40
		Lower thoracic	40

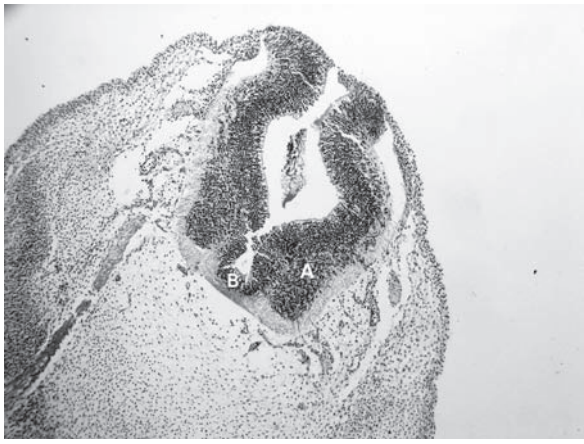

Figure 2. Horizontal section of the spinal cord in an embryo at stage 14. Bodian's protargol, × 100; A — anterior horn, B — ventral midline cells.

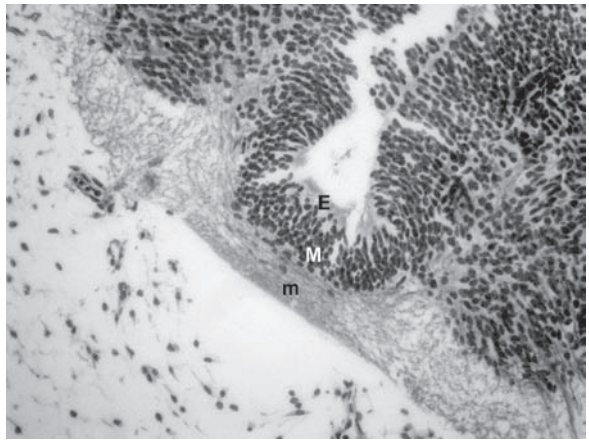

Figure 3. Horizontal section through the ventral part of the spinal cord in an embryo at stage 14. Bodian's protargol, × 400; E — ependyma of the floor plate, M — mantle layer, m — marginal layer with commissural fibres.

reasons for believing that this plate extends through the midbrain into the posterior diencephalon and ends near the mammillary region [2, 11, 17, 18].

In the present study it was shown that in the early embryonic human spinal cord the ventral midline of the spinal cord consists of ependymal lining cells, mantle, and marginal layers. The thickness of the part of the spinal cord under investigation slightly increases at stages 14 and 15, and during these stages commissural axons are visible crossing the marginal layer.

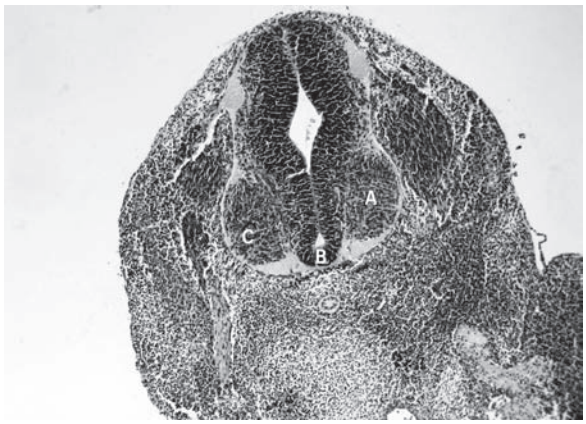

Figure 4. Horizontal section of the spinal cord in an embryo at stage 15. Haemotoxylin and eosin, ×100; A — anterior horn, B — ventral midline cells, C — spinal ganglion.

Sturrock [19] in mouse, Yoshioka and Tanaka in rats [23] and Gamble [3] and Malinsky and Brichova [13] in humans found that the floor plate ependymal cells differentiate during an earlier stage of embryonic development and are closely associated with the growth of decussating nerve fibres in the marginal layer, as was observed in our study. Midline-associated glial cells appear to be a source of contact-dependent or diffusible guidance cues for commissural axons [6].

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