Lamination of Spinal Cord Gray Matter in Goat Foetii

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ABSTRACT

Architectural organization of neurons in the laminae of spinal cord gray matter in goat was studied using 52 specimens of various ages. Tubular primordium, neural tube, started to differentiate into gray and white matter of the spinal cord from 48 days. Gray matter presented its horns, and the laminae started to appear by this age. Even though all the ten laminae could not be differentiated at this stage, the onset of lamination was indicated by the formation of nuclear aggregations in lamina IX of ventral horn. These aggregations became better defined towards the end of second month at the enlargements. By beginning of third month, the lamination was clear at lamina II (substantia gelatinosa), VII (intermedio-lateral nucleus) and IX (nuclear aggregations in ventral horn). Towards the end of third month, other laminae started to differentiate and became better differentiated by fourth month. Gray matter presented all the ten laminae towards the end of gestation.

Key words: Goat foetus, Gray matter, Nuclear architecture, Spinal cord

The general microscopic structure of the spinal cord in animals has been described earlier (Jenkins, 1978; Clark, 1984). But as the data on the developmental aspect of nuclear architecture in goats are very limited, a study was undertaken to describe the normal structure and development of the same in the spinal cord gray matter during different stages of prenatal growth in goat.

MATERIALS AND METHODS

The study was conducted using 52 goat foetii of different ages. The age was calculated using the formula derived by Singh et al. (1979) for goat foetii:

$$W^{1/3} = 0.096 (t - 30),$$

where, $W =$ Body weight in g and $t =$ Age in days

Foetii were divided into five age groups, fixed in 10 per cent neutral buffered formalin for 48 h. The embryos of < 2 months age were fixed as such. From 3rd month onwards, spinal cord within vertebral column was fixed after cutting into region-wise pieces for the foetii of 4-5 months, the spinal cord was exposed by laminectomy, dissected out, cut into individual segments, fixed and processed for light microscopy. Serial sections of 5 µ were stained by Ehrlich’s haematoxylin and eosin, Holzer’s method for glial fibres, Sevier-Munger method for neural tissues, Van Gieson’s method for collagen fibres and Holmes silver nitrate method for axis cylinders and myelin sheaths. The cytological techniques like aldehyde-thionin-PAS method and phosphotungstic acid haematoxylin (PTAH) method for central nervous system and oil red ‘o’ in propylene glycol method for lipids (Luna, 1968) were also employed. The micrometrical data were recorded using an ocular micrometer.

RESULTS AND DISCUSSION

In the present study, the primordium had the shape of a neural tube up to the middle of second month (Fig. 1). By 48 days, in foetii of CRL 40 mm, the outer white matter and the inner gray matter with dorsal and ventral horns became distinguishable. The gray matter extended the length of the cord as a mass of nerve cells and fibres with neuroglial elements and blood vessels. Cells of gray matter were arranged in distinct groups-nuclei or cell columns. Neurons started to form nuclear aggregations from middle of second month onwards. Rexed (1952) described architectural organization of neurons in cat spinal cord and believed that a similar lamination or zoning of gray matter existed in all higher mammals. In the present study, the lamination of gray matter into its nuclei started from second month onwards and was complete only during the fourth and fifth month. All ten laminae could not be differentiated by second month but in ventral horn beginning of formation of nuclear aggregations

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in lamina IX were seen (Fig. 2). By third month, lamination was clear at lamina II (substantia gelatinosa), VII (intermedio-lateral nucleus) and IX (nuclear aggregations in ventral horn). Towards end of this month, other laminae started to differentiate and became better differentiated by fourth month. Gray matter presented ten laminae towards end of gestation. Cell size and thickness of different laminae varied between regions of cord. Dorsal horn was attained between fourth and fifth month of gestation, when, it presented a marginal zone, the substantia gelatinosa and body of dorsal horn, with nucleus proprius and Clarke’s column.

Lamina-I appeared by third month covering the surface of dorsal column as a cap. Cells were arranged tangential to the surface of dorsal horn and extended throughout the length of the cord. By fourth month, this lamina became measurable as a layer (Fig. 3). By fifth month, the cells were better differentiated (Fig. 4) and the lumbar enlargement presented more number of cells.

Lamina-II corresponded to substantia gelatinosa and extended entire length of the cord, forming a cap over dorsal horn and was located beneath marginal zone (Fig. 4). This layer was organized and presented prominent fibres and convolutions towards end of third month. It presented different shapes and two types of cells, which were tightly packed up to fourth month. Later, the layer was penetrated by fibre bundles and the cells became loosely packed. The size was more at enlargements. It was regarded as proper sensory nucleus of cord, especially of cutaneous sensibility. Waldeyer’s nucleus was always related both in size and disposition to substantia gelatinosa. Cells of substantia gelatinosa and those in Waldeyer’s nucleus sent their axons into fasciculus proprius that surrounded dorsal horn. They conduct thermal (pressor and depressor) and painful sensibilities (Papez, 1967).

Lamina-III was represented by a less packed area with larger cells. It appeared by the end of third month. By the fifth month this zone entered into the substantia gelatinosa (Fig. 4). Lamina-III consisted of large projection neurons and small interneurons. By the end of the third month, lamina-IV appeared as an indistinct cell column and even by the fifth month, it was poorly defined and presented nucleus proprius (Figs. 2, 3). The cells had either polygonal or spindle-shape and gave origin for spinothalamic and spino-tectal tracts. Nucleus proprius corresponded in size to substantia gelatinosa. The cell column contained small to medium sized cells and was largest in lumbosacral region and smallest in thoracic region (Truey and Carpenter, 1969) as observed during the present study.

Lamina-V started to appear by the middle of the second month (Fig. 2) and presented reticular nucleus and reticular processes with small to medium-sized cells (Figs. 5, 6). Lamina had medial and lateral divisions from fourth month (Fig. 7) and extended entire length of spinal cord. Reticular formation was considered as seat of consciousness (Jenkins, 1978). Lamina-VI became identifiable as a broad layer with indistinguishable boundaries by fourth month, lateral to the central canal and consisted of a compact medial zone and a lateral less compact zone (Fig. 8). Many dorsal root group I muscle afferents terminated in medial zone but descending pathways project to cells in lateral zone. Some axons left gray matter to enter fasciculus proprius system and lateral white matter (Truey and Carpenter, 1969).

Lateral horn: Lamina-VII represented intermediate gray matter, with intermedio-lateral nucleus, intermedio-medial nucleus, cervical nucleus of Stilling and Clarke’s column. Lateral horn with intermedio-lateral nucleus (Figs. 2, 3) extended from C8 or T1 to anterior lumbar segments and was seen at sacral region. The neurons were not as differentiated as that of the ventral horn. The nucleus was better differentiated with spindle-shaped cells by the end of third month, was seen as a clear cell column in lateral horn and included two dispersed nuclear groups by fourth month, viz. the medio-posterior and intercornual columns.

Intermedio-medial nucleus (Fig. 3) appeared first by 81 days in thoracic and lumbar regions ventral to Clarke’s column. By 142 days, it became better developed but was smaller at L1 and T12. Nissl bodies were finer in intermedio-medial and intermedio-lateral nuclei. Dorsal dendrites of neurons of lateral horns in thoracic segments reached lamina II and ventral ones reached lateral group of motor nuclei. Dendrites directed medially run to medial area of intermediate gray while those directed laterally enter lateral funiculus to run a short distance. Dorsal dendrites of neurons of lateral horns in thoracic segments reached lamina II and ventral ones reached lateral group of motor nuclei (Jenkins, 1978).

Cervical nucleus of Stilling (Figs. 5, 8) was noticed by end of third month in cervical region at the base of dorsal column. At the same position in sacral region, sacral nucleus of Stilling was seen. It became continuous with
Figs. 1-3. 1. Cross section of neural tube at thoracic region (24 days). Note diamond-shaped lumen (1); sulcus limitans (2); ependymal layer (3); mantle layer (4); marginal layer (5); alar plate (6); basal plate (7); floor plate (8); dorsal funiculus (9); lateral funiculus (10); ventral funiculus (11) H. & E. × 100. 2. Cross section of thoracic region (48 days). Note ventral median fissure (1); ventral commissure (2); central canal (3); gray matter (4); white matter (5); lateral horn (6); reticular formation (7); narrow ventral horn (8); nucleus proprius (9); clark’s column (10); piamater extending into ventral median fissure (11); linea splendens (12); ganglion (13); medial longitudinal fasciculus (14); vestibulospinal tract (15) H. & E. × 100. 3. Cross section of T segment (81 days). Note apex of dorsal horn (1); head of dorsal horn (2); cervix of dorsal horn (3); lamina I (4); narrow ventral horn (5); dorsolateral fasciculus (6); intermediolateral nucleus (7); intermediomedial nucleus (8); clark’s column (9); nucleus proprius (10) H. & E. × 100.

Figs. 4-6. 4. Cross section of T segment (142 days). Note cell in lamina I (1); lamina II cells (2); fibres in lamina II (3); blood vessels (4); lamina III (5) H. & E. × 100. 5. Cross section of cervical region (81 days). Note central canal (1); nucleus proprius (2); cervical nucleus of stilling (3); cuneate nucleus of medulla (4); reticular formation (5); spinal accessory nucleus (6); dorsomedial nucleus (7); dorsolateral nucleus (8); ventromedial nucleus (9); ventrolateral nucleus (10) H. & E. × 100. 6. Cross section of L1 segment (81 days). Note nucleus in ventral horn (1); clark’s column (2); lamina I (3); lamina II (4); lamina III (5); reticular formation (6); lateral horn (7); nucleus proprius (8); dorsomedial nucleus (9); dorsolateral nucleus (10); ventromedial nucleus (11); ventrolateral nucleus (12); intermediomedial nucleus (13) H. & E. × 100.

Figs. 7-9. 7. Middle lateral part of spinal cord at T segment (124 days). Note lateral part of lamina IV showing cells (1); reticular formation (2); intermediolateral nucleus (3) H. & E. × 100. 8. Cross section of C segment showing gray matter ventral to substantia gelatinosa (124 days). Note cervical nucleus of stilling (1); medial zone of lamina VI (2); lateral zone of lamina VI (3) H. & E. × 100. 9. Cells of laminae VIII and IX at L4 segment (102 days) H. & E. × 400.

Fig. 10. Cross section of lumbar enlargement (58 days). Note dorsomedial nucleus (1); dorsolateral nucleus (2); ventromedial nucleus (3); ventrolateral nucleus (4); central nucleus (5); retrodorsolateral nucleus (6) H. & E. × 100.

de the lateral cuneate nucleus of medulla. Clarke’s column (Figs. 2, 3) became clear by 81 days at the anterior lumbar region. It occurred at the lateral aspect of the central canal from T1 level extending backwards. By fourth month, it had neurons with eccentrically placed nucleus. By fifth month, it was well developed from T9 to L2 level. Clarke’s column had intimate relation to fasciculus gracilis, which sent numerous collaterals into it. Cells of Clarke’s column gave origin to dorsal spinocerebellar tract of same side and also to crossed ventral spinocerebellar tract of other side. Proprioceptive impulses were sent by these tracts into cerebellar cortex (vermis). These tracts were also believed to conduct exclusively deep or muscular sensibility to cerebellar cortex (Papez, 1967).
Ventral horn: It contained lamina-VIII and IX. The shape and size of the horns varied from one region to the other so that the ventral horns were wider in the cervical and lumbar enlargements but were narrower in the anterior cervical and thoracic levels.

From fourth month onwards lamina-VIII appeared as a mixture of small and medium sized cells but was not differentiated from lamina-VII (Fig. 6). By fourth month, lamina-VIII contained small and medium sized cells and was not sharply differentiated from lamina-VII (Fig. 9). Lamina-VIII excluded motor neuron pools and consisted entirely of interneurons, the axons of which projected via propriospinal tracts to a number of different spinal levels, serving as receptor sites for fibres of suprasegmental origin, whose excitation patterns were then synaptically transferred to nearby motor neurons. Vestibulospinal, pontine reticulospinal and tectospinal tracts and medial longitudinal fasciculus end as synapse on lamina-VIII interneurons in order to affect, ultimately, final common pathway in man (Clark, 1984).

Lamina-IX was composed of alpha and gamma cells being larger at enlargements. By third month (Figs. 3, 5, 6), ventral horn presented multipolar neurons with large vesicular and eccentrically placed nucleus and eosinophilic cytoplasm (Fig. 9). Among these somatic motor neurons, larger alpha motor neurons innervated striated muscle fibres (extrafusals) and smaller gamma motor neurons innervated intrafusal fibres of muscle spindles (Clark, 1984). By middle of second month towards ventrolateral aspect of ventral horn, nuclear aggregations were formed (Fig. 2). Lateral and medial nuclear masses were seen. Former always had sharply limiting boundaries at enlargements whereas latter were less defined with diffused borders with lamina-VIII. Medial nuclear group consisted of dorsomedial and ventromedial nuclei, former was smaller and most distinct in cervical and lumbar enlargements and latter extended throughout whole cord. Medial group innervated short and long muscles attached to axial skeleton. Lateral nuclear group became enlarged with a number of subgroups in enlargements. This group innervated most distal portions of extremities (Truex and Carpenter, 1969).

Enlargements presented central and retrodorso-lateral nuclei also (Fig. 10). Central nucleus innervated muscles attached to shoulder and pelvic girdle.

Lamina-X surrounded central canal. By fifth month, neurons in lamina-X could be clearly identified without much regional variation in size. Functionally these cells were homologous with cells of reticular formation (Papez, 1967). In the present study, the development of nuclear aggregations occurred corresponding to progression of development of muscles and skeleton of foetus.

REFERENCES