HISTOGENESIS OF WHITE MATTER IN THE SPINAL CORD OF GOAT FOETUSES

S. Maya, J.J. Chungath, K.R. Harshan and N. Ashok

Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Mannuthy, Kerala – 680 651, India

ABSTRACT

Histogenesis of white matter of the spinal cord in goat was studied using 52 foetuses of various ages. By second month of gestation, the white matter became arranged in three funiculi. Thickness of white matter increased from fourth to fifth month and also from sacral region towards cervical region in the fifth month. The lateral funiculus exceeded all other funiculi in thickness. The fibre tracts like fasciculus gracilis, fasciculus cuneatus, dorsolateral fasciculus of Lissaeur, medial vestibulospinal tract and medial longitudinal fasciculus could be identified by middle of second month. Vascularity and cell density increased as the age advanced from middle of the second month onwards.

INTRODUCTION

The general structure of white matter in domestic animals has been described by many earlier workers. But a review of literature showed that the data on the developmental aspect of white matter in the spinal cord are very limited in goats. Therefore, this study was conducted to elucidate the various changes occurring in white matter at different stages of prenatal growth in this species.

MATERIALS AND METHODS

The study was conducted using 52 goat foetuses of different ages, which were freshly collected from slaughter houses and from cases of threatened accidental abortion and neonatal death. The age of the foetuses was calculated using the formula derived by Singh et al. (1979), for goat foetuses, \( W^{1/3} = 0.096 (t - 30) \), where, \( W = \text{Body weight in g and } t = \text{Age in days} \). The foetuses were divided into five age groups corresponding to five months of gestation. Upto two months of gestation, the embryos and foetuses, were fixed as such in 10 per cent neutral buffered formalin for 48 hours. In foetuses of three months of gestational age, the spinal cord within the vertebral column was fixed after cutting into region-wise pieces. In foetuses above three months of gestational age, the spinal cord was exposed by laminectomy, dissected out, cut into pieces of two to three segments each and fixed. The fixed specimens were washed, dehydrated and embedded in high melting paraffin (MP 58-60°C). Serial sections of 5μm thickness were made. Various techniques like Ehrlich’s haematoxylin and eosin (H & E) staining, Holzer’s method for glial fibres, Sevier-Munger method for neural tissues, Van Gieson’s method for collagen, Holmes silver nitrate method for axis cylinders and myelin sheaths, Aldehyde-Thionine-PAS method for central nervous system, phosphotungstic acid haematoxylin (PTAH) method for CNS tissue and oil red ‘O’ in propylene glycol method for lipids (Luna, 1968) were employed. The thickness of the marginal layer of the neural tube in the first month was recorded using an ocular micrometer by measuring the maximum distance between the outer margins of neural tube and mantle layer at dorsal, lateral and ventral aspects. The thickness of the different funiculi was measured as the maximum distance between the outer margins of spinal cord and gray matter at dorsal, lateral and ventral aspects. The data collected were analysed statistically following Snedecor and Cochran (1985).

RESULTS AND DISCUSSION

In the present study, the primordium of spinal cord had the shape of a closed neural tube by 24 days in the first month of gestation.

* Part of Ph.D. thesis submitted by first author to Kerala Agricultural University, Thrissur, Kerala - 680 656, India.

By 24 days, the 14 mm embryo presented three layers in the neural tube wall (Fig. 1), viz. an inner ependymal (germinal) layer with actively dividing neuroepithelial cells, a middle mantle layer consisting of developing neurons and glial cells and an outer marginal layer made up of fibres arising from the cells in the mantle layer. The marginal layer formed the outermost zone, generally lacked cells in this first month. But occasionally, spongioblasts were observed in this layer especially at floor plate and at the entry and exit (Fig. 2) of nerve roots, representing the cells, which accompany their processes. This agrees with the findings of Arey (1957) who also opined that the marginal layer was a fibrous mesh and lacked cells in early months of gestation by fourth week in man. In the present study, the marginal layer became thicker and was of loose consistency by 26 days in embryos with CRL 15 mm in the present study. So, the differentiation commenced earlier in goat than in the dog in which, the layer was differentiated only by 24 days, and became thicker by 28 days, as reported by Engel and Draper (1982). In goat foetuses, by the first month of gestation, the marginal layer was absent at the dorsal aspect of the neural tube. Towards the end of first month, by 27 days in 16 mm embryo, a fibrillar meshwork, the myelospongium was laid down by the better-developed cell processes. This concurs with the findings of Keith (1947) in man, who also found that many of these nerve fibres that grew into the marginal layer turned upward or downward in the cord or brain for long distances and synapsed with neurons elsewhere in the central nervous system later.

It was in the middle of the second month, by 48 days of gestation, in foetuses of CRL 40 mm, the outer white matter and inner gray matter became differentiated first (Fig. 3). The white matter became arranged in its three longitudinal columns, viz. dorsal, ventral and lateral funiculi of nerve fibres, on each side, extending the whole length of the cord. In the present study, in the middle of second month by 48 days, fibre tracts like fasciculus gracilis and fasciculus cuneatus could be identified in the dorsal funiculus at the medial and lateral aspects of the dorsal intermediate groove (Fig. 4). These tracts were seen in the dorsal funiculus between C1 and T13 segments. At the dorsolateral aspect of the dorsal funiculus, a dark stained area could be seen from middle of second month onwards, which formed the beginning of formation of dorsolateral fasciculus or fasciculus of Lissauer (Fig. 4).
third month, it was located at the entrance of dorsal root fibres. It became better developed by fourth month and later.

Ventral funiculus presented the medial longitudinal fasciculus and medial vestibulospinal tract by 48 days. Medial longitudinal fasciculus appeared as a fibre bundle located on either side of the ventrolateral aspect of central canal, on either side of the base of ventral median raphae (Fig. 3). It became well defined from the end of third month onwards. The medial vestibulospinal tract was seen at the base of ventral median fissure ventral to medial longitudinal fasciculus from middle of the second month onwards (Fig. 3), which also became well developed by the end of third month. By fourth month, in addition, the intersegmental tract, viz. the fasciculus proprius was seen as bundles of axons bordering the gray matter in all the funiculi. Considering the changes in the total length of gestation, this age of onset for the appearance of fibre tracts partially corresponded to that in man, as Keith (1947) reported that most of the ascending and descending tracts in the ventrolateral marginal zone were formed in the third month of gestation.

Except for these above mentioned tracts, other tracts were very difficult to be identified under light microscope. But at their respective area of location, clustering of fibres or glial cells could be seen. The definite levels of origin and termination were also very difficult to get identified. This agrees with the reports of King (1987) who observed that these tracts could not be distinguished unless they have been marked by special techniques. It is also reported that almost all tracts are mixed with other tracts in the spinal cord and are not separated into well defined bundles. The exceptions to this fact are the fasciculi gracilis and cuneatus, but in several domestic species for example, in sheep, even these tracts were blended with other tracts.

Blood vessels entered from the periphery through marginal layer to reach the mantle layer and ependymal layer by 26 days. By fourth month, blood vessels presented perivascular satellites with the astrocytic processes extending towards blood vessels (Fig. 5). These cells might contribute to the formation of blood-brain barrier. Eventhough the marginal layer presented very few cells in this first month, by 40 days of gestation in foetuses with 25 mm CRL, in addition to fibres, the white matter of the spinal
cord exhibited darkly stained spherical nuclei of oligodendrocytes and elongated nuclei of fibrous astrocytes. Later, the nerve fibres passing through the white matter presented blood vessels and fibrous astrocytes on their sides. The vascularity and the cell density in the white matter increased as age advanced towards the end of gestation, indicating the probable onset of myelination. Histochemical studies also revealed the presence of lipids in the white matter by third month of gestation. So, it is assumed that the myelination was initiated in the third month of gestation in the present study, so that the motor and sensory impulses through the cord became specific to make it ready for reflex activity. According to Sadler (2004) the myelination of nerve fibres in the spinal cord of man begins approximately by the fourth month of intrauterine life.

It appeared that the onset of myelination coincided with the beginning of a rapid increase in vascularity in white matter in the present study. This agreed with the observations of Sturrock (1982) in rabbit foetuses. But in contrary, in mouse he also observed that, the percentage vascularity in both gray and white matter remained fairly constant from onset of myelination by 18 days of gestation to the stage when myelination was fairly well established by 5 days postpartum.

The processes of multipolar neurons extending through the white matter could be well demonstrated towards the end of gestation as both cross section of bundles and longitudinal tracts. Oligodendrocytes formed myelin sheaths around axons towards the end of gestation by 142 days (Fig. 6). Rest of the oligodendrocytes without forming the myelin sheath were also seen associated with nerve tracts as interfascicular cells and free cells in the white matter. Even though a typical myelin sheath was observable only towards the end of gestation, clear and vacant spaces occurred around axons from fourth month itself by 102 days representing the sites of myelination (Fig. 5). Towards the end of gestation, the white matter became a mixture of myelinated and nonmyelinated axons, blood vessels and neuroglia. With silver stains, astrocytes were seen to possess numerous processes and microglia were seen as small elongate cells with polar processes. Rarely, nerve cell bodies were also seen in white matter. Collagen was absent in the spinal cord tissue except in the blood vessels, which agrees with the reports of Clark (1984) in mammalian spinal cord.

The thickness of the three funiculi at different stages of gestation are presented in Table 1. All the funiculi showed a progressive increase in the thickness during the gestation
Table 1. Thickness of funiculi of white matter in the precoccygeal spinal cord (Mean± S.E), μm.

<table>
<thead>
<tr>
<th>Month</th>
<th>Thickness of funiculi</th>
<th>Regions</th>
<th>Cervical</th>
<th>Cervical enlargement</th>
<th>Thoracic</th>
<th>Lumbar</th>
<th>Lumbosacral enlargement</th>
<th>Sacral</th>
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<tbody>
<tr>
<td>I</td>
<td>Dorsal funiculus</td>
<td></td>
<td>40.0±3.2</td>
<td>54.4±9.8</td>
<td>39.2±2.4</td>
<td>45.0±6.7</td>
<td>55.0±11.4</td>
<td>22.5±2.2</td>
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<tr>
<td></td>
<td>Lateral funiculus</td>
<td></td>
<td>35.0±6.3</td>
<td>58.1±13.7</td>
<td>37.6±2.5</td>
<td>39.4±3.9</td>
<td>52.5±12.1</td>
<td>40.0±2.5</td>
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<tr>
<td></td>
<td>Ventral funiculus</td>
<td></td>
<td>45.0±9.5</td>
<td>63.8±7.4</td>
<td>53.3±6.7</td>
<td>46.9±5.3</td>
<td>62.5±8.2</td>
<td>40.0±6.6</td>
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<tr>
<td>II</td>
<td>Dorsal funiculus</td>
<td></td>
<td>60.0±5.4</td>
<td>83.1±0.5</td>
<td>103.5±3.1</td>
<td>70.5±4.1</td>
<td>56.2±0.1</td>
<td>37.7±0.1</td>
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<tr>
<td></td>
<td>Lateral funiculus</td>
<td></td>
<td>165.0±16.43</td>
<td>160.0±8.3</td>
<td>155.8±4.3</td>
<td>127.6±1.0</td>
<td>165.0±17.3</td>
<td>127.5±12.9</td>
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<td></td>
<td>Ventral funiculus</td>
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<td>255.5±5.4</td>
<td>230.0±6.8</td>
<td>202.6±4.9</td>
<td>195.0±2.2</td>
<td>232.5±12.9</td>
<td>147.5±7.2</td>
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<td>III</td>
<td>Dorsal funiculus</td>
<td></td>
<td>81.8±2.3</td>
<td>142.5±7.2</td>
<td>147.5±8.8</td>
<td>125.0±4.1</td>
<td>118.8±0.6</td>
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<td></td>
<td>Lateral funiculus</td>
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<td>215.5±4.2</td>
<td>179.1±2.3</td>
<td>214.3±15.7</td>
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<td>180.7±0.4</td>
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<td>Ventral funiculus</td>
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<td>277.5±9.8</td>
<td>274.1±10.6</td>
<td>224.6±1.6</td>
<td>277.6±17.0</td>
<td>297.0±8.9</td>
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<td>IV</td>
<td>Dorsal funiculus</td>
<td></td>
<td>84.3±2.9</td>
<td>232.6±26.1</td>
<td>177.6±4.9</td>
<td>126.0±2.3</td>
<td>132.5±18.7</td>
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<td>530.0±19.2</td>
<td>534.3±13.7</td>
<td>448.3±24.5</td>
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<td>645.0±16.4</td>
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<td>526.3±28.4</td>
<td>702.6±16.9</td>
<td>575.0±28.8</td>
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<tr>
<td>V</td>
<td>Dorsal funiculus</td>
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<td>747.0±25.7</td>
<td>428.7±50.6</td>
<td>356.3±22.4</td>
<td>401.3±13.5</td>
<td>341.2±32.4</td>
<td>75.0±21.6</td>
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<td></td>
<td>Lateral funiculus</td>
<td></td>
<td>986.2±41.9</td>
<td>1014.5±17.1</td>
<td>927.1±17.3</td>
<td>952.1±19.9</td>
<td>720.0±38.9</td>
<td>171.2±26.7</td>
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<tr>
<td></td>
<td>Ventral funiculus</td>
<td></td>
<td>1281.7±64.4</td>
<td>1289.2±29.2</td>
<td>1170.8±17.3</td>
<td>996.7±6.5</td>
<td>900.0±43.3</td>
<td>285.0±26.0</td>
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</table>

* Part of Ph.D. thesis submitted by first author to Kerala Agricultural University

period and a tremendous increase in the amount of white matter was noticed towards the latter half of gestation especially between fourth and fifth month of gestation. A remarkable increase was also noticed in the amount of white matter from the sacral region towards the cervical segments in the fifth month of gestation. This observation is found to agree with the observations of Jenkins (1978), who found that since all levels of the spinal cord were connected with the brain by long ascending and descending fibres, the white matter increased from lower to higher levels of the cord and therefore in mammals the cervical segments contained the largest number of fibres.

The lateral funiculus exceeded other funiculi in thickness in all regions at all ages from second to fifth month. In contrary to this, Nyberg (1966) reported that in adult animals, the ventral funiculus was larger than the lateral funiculus. This difference may be attributed to a change during the developing stages in the present study.
REFERENCES


