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Original article

Histogenesis of Olfactory Bulbs in Human Foetus

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ABSTRACT

Histogenesis of olfactory bulb was studied in 62 human foetuses. The olfactory bulbs were removed en block with the surrounding bone and fixed in 10 percent formal saline. Serial sections of paraffin embedded tissue were stained with H/E, cresyl violet and Glees Marsland's modification of Bielschowsky's staining method for neurites. Klüver and Barrera's method was used for myelin and nissl granules. Sections of adult olfactory bulb stained with H/E and Klüver and Barrera's method were used as control. Microscopic study revealed extension of lateral ventricular cavity inside thebulbs even at 15th week of development. A laminar organization was detected inside the bulbs which gradually became more distinct reaching its peak at 26 weeks of development. Thereafter, the laminar organization began to disappear and at 34 weeks laminar organization was difficult to demonstrate. Mitral cells were the largest neurons which could be detected from the earliest age groups along with the nerve fibre layer. At 24 weeks mitral cells together with tufted cells formed a distinct lamina 3 – 4 cells thick. Glomeruli were seen from 20 weeks onwards but their number and size declined after 30 weeks. The internal and external plexiform layers were well identified upto 28th week after which they gradually became obscured. Granule cells were found confined in the centre of the bulb. Myelinated nerve fibre were detected in the adult olfactory bulbs but not in any of the foetal specimen. It was concluded that myelination in the olfactory bulbs begins only after birth.

1. Introduction

Every normal human being has a pair of olfactory bulbs, each of which is situated at the anterior end of the olfactory sulcus, apposed to the orbital surface of the frontal lobe. Olfactory receptors present in the nose send axons which pass through the cribriform plate of ethmoid bone and course into the olfactory bulb. The olfactory bulb has a laminar organization and consists of both gray matter and white matter [1] arranged in six distinct concentric layers. From superficial to deep these layers are olfactory nerve, glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer and granule cell layer [2].

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Although histology of the olfactory bulb is well documented, literature on its histogenesis appears to be surprisingly scanty. Moreover several authors have presented data suggesting that human neonates are able to identify the smell of their mothers but this ability seems to be lost as one grows up. The present work aims to study the histogenesis of the olfactory bulbs in the developing human foetus to broaden our understanding of this fascinating region of the human brain .

2. Materials and Methods

Sixty two normal fresh foetuses of different gestational ages ranging from 15 weeks to 38 weeks, products of terminated pregnancy under Medical Termination of Pregnancy (MTP) Act of India 1971 and still births collected from the Department of Obstetrics and Gynaecology, Regional Institute of Medical Sciences, Imphal were utilized for the study. The age of the foetuses were calculated from obstetrical history, crown rump length (CRL) and gross features. Statistically the specimens were categorized into four groups.

Group I	:	15 to 20 weeks
Group II	:	21 to 26 weeks
Group III	:	27 to 32 weeks
Group IV	:	33 to 38 weeks

The olfactory bulbs were dissected out en block with the surrounding bone and underlying cribriform plate of ethmoid and were fixed in 10%. Formal saline for one month. After proper fixation, the olfactory bulbs were freed from adjoining bone by fine dissection using the dissecting microscope and were then subjected to the standard histological procedures of dehydration, clearing and paraffin embedding. 5 to 10 mm thick paraffin sections were stained with H/E, Cresyl Fast Violet, Glees and Marsland's modification of Bielschowsky's staining method for neurites and Klüver and Barrera's method for myelin & nissl granules. Slides were studied under the light microscope and photographed. Sections of adult olfactory bulb stained with H & E and Klüver and Barrera's method were used as control.

3. Results

Group I (15 to 20 weeks)

In the earliest fetuses of 15 weeks age a slit like extension of the ventricular cavity was seen at the centre of the olfactory bulb. The cavity was surrounded by nerve cells arranged in three ill defined zones – an inner darkly stained zone, an outer pale stained zone and an intermediate zone (Photograph No.1). The three layers resemble and correspond to the neuroepithelial, mantle and marginal layers of the developing neural tube. By 18 weeks the ventricular cavity disappeared in all but one specimen. The nerve fibre layer was evident with numerous fibroblasts. Developing mitral cells, triangular in shape with round central nucleus were seen at a localized area beneath the nerve fibre layer (Photograph No.2). At 20 weeks glomeruli were seen as discrete round eosinophilic masses beneath the nerve fibre layer. The mitral cells appeared conspicuously larger than the surrounding cells.

Figure.1. showing Human Foetus: 15 weeks, C.R. Length: 83 mm.VC : Ventricular Cavity, I: Inner Zone, II: Intermediate Zone, III: Outer Zone.(Cresyl Fast Violet Stain, 10x)

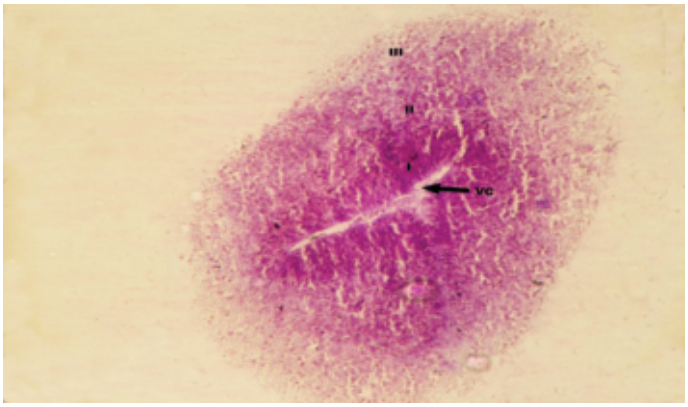
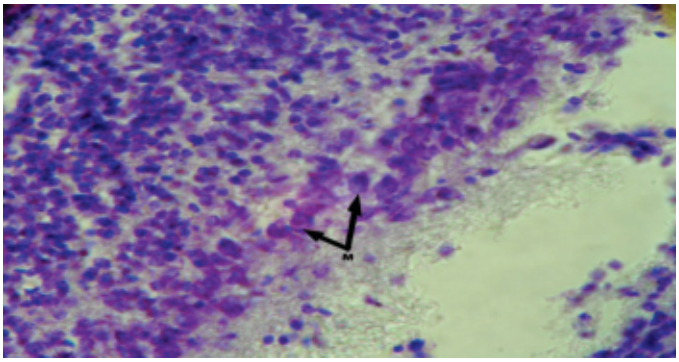


Figure.2. showing Human Foetus: 18 weeks, C.R. Length: 120mm.M : Mitral Cells (Cresyl Fast Violet Stain, 40x)



Group II (21 to 26 weeks)

The outer nerve fibre layer and glomerular layer appeared well developed by 22nd week. The external plexiform layer was evident and contained Scattered cells of various shape and size. The mitral cell layer beneath it formed a distinct lamina in cresyl violet stained sections. The internal plexiform layer was identified in isolated areas and was of variable thickness. The centre of the bulb contained a dense population of undifferentiated cells.

At 24 weeks all the six layers of the bulb were clearly identified (Photograph No.3). The mitral cells assumed their characteristic appearance and appeared 3 – 4 times larger than the surrounding cells (Photograph No.4). They formed a distinct lamina 3 -4 cells thick. The tufted cells were identified as multipolar neurons of slightly shorter variety intermingled between large mitral cells. The internal and external plexiform layers were prominent (Photograph No.5).

Figure.3. showing Human Foetus: 24 weeks, C.R. Length: 195mm.NFL : Nerve Fibre Layer, GL : Glomerular Layer.EPL : External Plexiform Layer, ML : Mitral Cell Layer,IPL : Internal Plexiform Layer, GCL : Granule Cell Layer.(Haematoxylin & Eosin Stain, 10x)

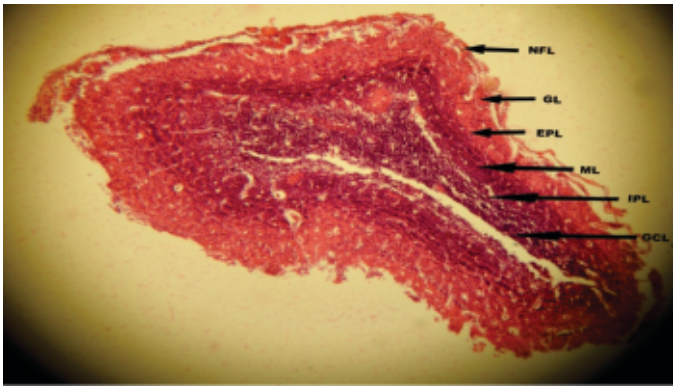


Figure.4. showing Human Foetus: 24 weeks, C.R. Length:195mm. M : Mitral Cells (Cresyl Fast Violet Stain, 100x)

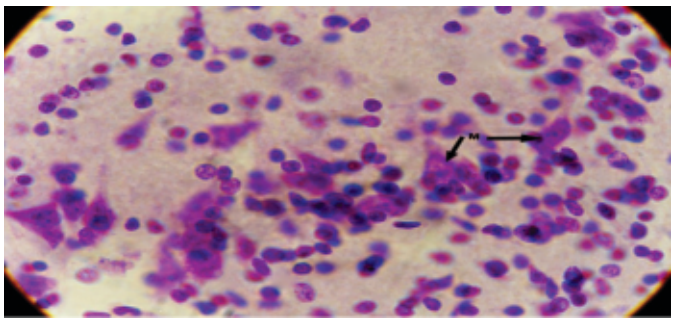
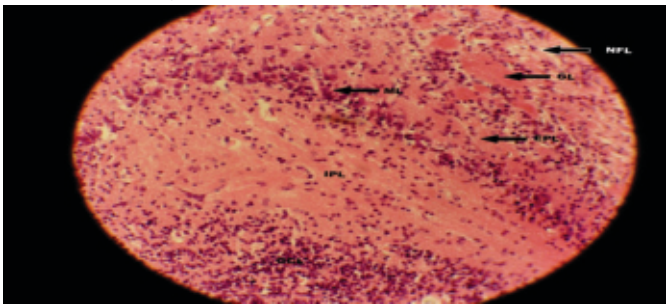


Figure.5. showing Human Foetus: 26 weeks, C.R. Length: 225mm.NFL : Nerve Fibre Layer, GL : Glomerular Layer.EPL : External Plexiform Layer, ML : Mitral Cell Layer,IPL : Internal Plexiform Layer, GCL : Granule Cell Layer.(Haematoxylin & Eosin Stain, 40x)

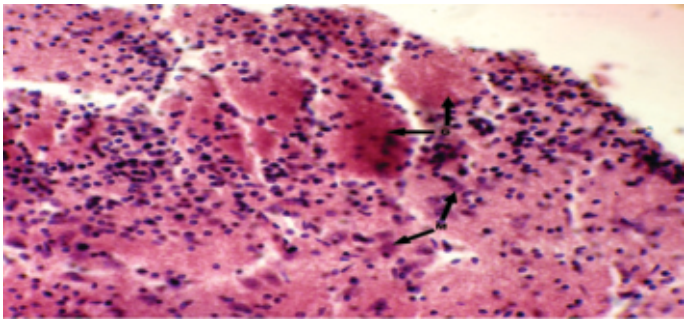


Group III (27 to 32 weeks)

At 28 weeks the laminated structure of the bulb though visible became slightly obscured. The nerve fibre and glomerular layers were prominent. The mitral cells no longer formed a distinct layer but appeared scattered over a wide zone (Photograph No.6). Hence the external and internal plexiform layers were not clearly demarcated from each other. Centre of the bulb was occupied by small granule cells.

The laminated appearance was indistinct in specimens of 30 week age. Mitral and tufted cells were seen scattered over a wide zone. External and Internal plexiform layers were ill defined. By the end of 32 weeks only nerve fibre and glomerular layers appeared as distinct entities.

Figure.6. showing Human Foetus: 28 weeks, C.R. Length: 260mm.G : Glomeruli, M : Mitral cells.(Haematoxylin & Eosin Stain, 40x)



Group IV (33 – 38 weeks)

At 34 weeks individual glomeruli were very much reduced in diameter (Photograph No.7). Mitral cells were reduced in number and appeared irregularly scattered. The internal and external plexiform layers were completely obscured. At 36 weeks the cellular architecture of the olfactory bulb remained almost the same as the preceding age group. The neuronal population at the centre of the bulb was markedly reduced.

At 38 weeks the overall cell population was greatly diminished. Nerve fibre layer was seen on one side of the section. Glomeruli were few, arranged into a small cluster. Granule cells were mostly undifferentiated and greatly reduced in number. The plexiform layers could not be identified. Mitral cells were large and well developed but they were few in number and no longer formed a distinct lamina.

There was no evidence of myelination in any of the foetuses, although myelin was detected in sections of adult olfactory bulb, used as control (Photograph No.8).

Figure.7. showing Human Foetus: 34 weeks, C.R. Length: 345mm.G : Glomeruli (Haematoxylin & Eosin Stain, 10x)

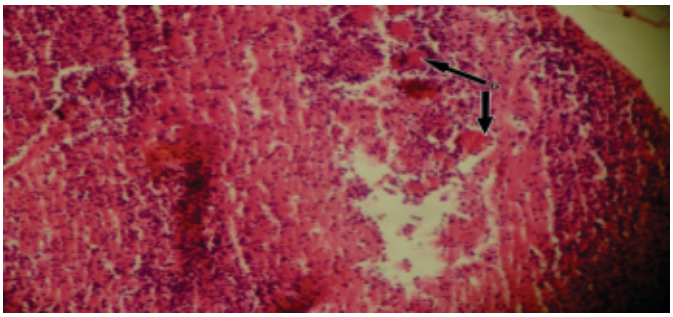
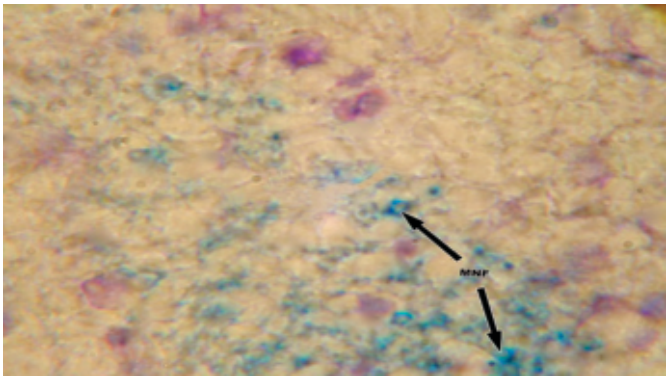


Figure.8. showing Adult Human.MNF : Myelinated Nerve Fibres.(Klüver & Barrera's Stain, 100x)



4. Discussion and Conclusion

Hamilton WJ and Mossman HW [3]and Romanes GJ [4]observed that the olfactory bulbs were formed as outgrowths from the ventral aspect of the cerebral hemisphere and at first contained an extension of the lateral ventricle which was later obliterated. The findings of the present study were in agreement with those of the above workers. Extension of ventricular cavity

was confirmed from microscopic study of serial sections at 15 weeks. However it is in contrast to the opinion expressed by Kiernan JA [5] that the lateral ventricle extends into the bulbs only during the embryonic stage. Mori K et al [6] opined that zonal organization is a characteristic feature of the mammalian olfactory system. Another view by Carpenter MB [2] mentions that the olfactory bulb had a laminar organization but it is difficult to demonstrate in humans. In partial agreement to the above findings the present study showed that the developing olfactory bulbs gradually acquired a laminar organization but the lamination was lost towards term. This is in accordance to Kiernan JA [5] who mentioned that the layers of the olfactory bulbs were irregular and indistinct in the adult humans although they were obvious in the foetal stage of development. Right from the 15th week an indistinct but obvious laminar organization was observed with the laminar arrangement reaching its zenith at 26 weeks of development. Thereafter the lamination began to disappear and at 34 weeks laminar organization was difficult to demonstrate.

Mitral cells were observed to be the largest and most prominent neurons in the olfactory bulb. This is in accordance to reports from Shepherd GM [7] and Bhatnagar KP et al [8]. These cells were present in the earliest specimen of the study and gradually increased in size and attained their characteristic shape by 24 weeks. However their number and orderly arrangement gradually diminished after 28th week. The glomerular layer was well developed at 22 weeks. After 28th week the glomerular layer thickness and the size of individual glomerulus showed a gradual decline. Both Copenhaver WM et al [1] and Kiernan JA [5] described an external plexiform layer between the glomeruli and mitral cells but stopped short of acknowledging the existence of an internal plexiform layer. In the present study both layers were detected with the external plexiform layer becoming apparent earlier (18 weeks) than the internal plexiform layer (22 weeks). Both layers were well identified upto 28 weeks after which they gradually became indistinct. Granule cells were confined to the centre of the bulb and their population showed a steady decline after 30 weeks. The olfactory bulbs in adults were found to be composed of both gray matter and white matter with myelinated fibres mostly confined to the centre of the bulb. This is in accordance to findings of Copenhaver WM et al [1] but in contrast to the opinion of Le Gros Clark WE [9] that the olfactory bulbs were composed only of gray matter. Myelin was however not detected in the foetal specimens implying that the process of myelination in the bulb begins only after birth.

All these findings apparently indicate that the structure of the human olfactory bulbs in foetal stage, although for few weeks, closely resemble the laminar structure of the olfactory bulbs of lower mammals e.g. mouse, dog etc. Thus the olfactory bulb may serve as a model in the central nervous system where ontogeny and phylogeny are strikingly related.

5. References

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