FORMATION OF ACCESSORY OLFACTORY BULB IN INDIGENOUS RABBITS  
(*Oryctolagus Cuniculus*)

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ABSTRACT

Present study was carried out on 30 rabbit fetuses and 25 pups. The results showed that, the accessory olfactory bulb at 18-20 days old fetuses were not identified. At 28 days fetus the glomeruli of glomerular layer were first appeared and were smaller in their diameters than those of the main accessory bulb. At 30 days old fetus all the six layers of the accessory olfactory bulb were present. The study revealed that, Histologically, the accessory olfactory bulb in rabbits was developed before their birth. The width of accessory olfactory bulb layers was increased with age.

INTRODUCTION

The accessory olfactory bulb (AOB) is the first neural integrative center for the vomeronasal system originated from the rostral telencephalon (1). It is small laminar structure located dorso-posterior to the main olfactory bulb, the cell population homogenous to that of olfactory bulb. It is consisted of six layers (vomeronasal nerve layer, glomerular layer, external plexiform layer, mitral cells layer, internal plexiform layer, and granule cells layer) , (2)(3). The AOB is believed to be absent in animals without vomeronasal organ (VNO) like bird, fish and aquatic mammals. The animals with well-developed VNO are large in size and very well developed (4) (5)(6). The AOB process stimulation gets by the sensory neurons of the VNO like pheromone and large molecule non-volatile odor (7) (8). The stimulants transduced by the axons of mitral cells and through the olfactory tract to the amygdala and then into the hypothalamus (9)(10). The accessory olfactory system (VNO and AOB) regulates the sexo-social behavior of animals and plays a great role in feed strategy and maternal relation of animals,(11) (12).

The main objective of this study was to give the histological changes of the accessory olfactory bulb during its development.
MATERIALS AND METHODS

Thirteen rabbit fetuses at different ages (18, 20, 25, 26, 28, and 30) days of gestation period and 25 pups including 1, 7, 14, 21, and 30 days old were used in this study. The fetuses were obtained from pregnant by caesarian section; their ages were determined by counting the gestation periods and by measuring the crown-rump length according to (13). Experimental animals were sacrificed by anesthesia (xylazine 5mg/BW and Ketamine 15 mg/BW). Animals’ heads were removed and both the soft tissues and the skull bones were removed and the brains were exposed to the fixative solution (15% formalin solution with 2 mg ammonium bromide /100ml). After well fixation the olfactory bulbs were processed with paraffin method according to (14) The tissue sections were cut at 7 µm and stained with Harris Hematoxylin and eosin stain, and cresyl violet stain(15). The microscopic measurements were done by using Oculometer

RESULTS AND DISCUSSION

At 18-20 days old fetus

The olfactory bulb was appeared as cellular mass and there was no marked line of demarcation to determine the future main olfactory bulb and accessory olfactory bulb, except that the presence of the vomeronasal nerve fibers which were projected to enter from the dorsal aspect of the cellular mass, Fig.(1). The axons of developed sensory neurons of the medial wall of the vomeronasal organ were projected and token a pathway through the mesenchymal connective tissue to reach the telencephalon (16). The growth and migration of nerve fibers were influenced by many factors that produced by the mesenchymal tissue and the telencephalon (17)(18).

At 25-26 days old fetus

The accessory olfactory bulb was consisted of two cellular populations. The outer layer was consisted of large neurons, mitral cells, and inner granule cells layer which formed from aggregations of small round granule cells, Fig.(2)(3). These findings were in the agreement with the results of (19) in pig fetuses. The principal neurons (mitral cells) and interneurons of the olfactory bulb (granule cells were generated at the early stages of the prenatal development of the accessory olfactory bulb (20) (21).
At 28 days old fetus

At this age few individual glomeruli were begun to form. The glomerular layer was not clearly distinguished, Fig.(4). The glomeruli of the accessory olfactory bulb were developed in the early age compared with glomeruli of the main olfactory bulb, this fact improved what reported by (22) in rat pups in which the glomeruli of main olfactory bulb were appeared after birth, and also in agreement with result of (23) (19) in pig fetus. The glomeruli were spherical structures formed by the synapses of vomeronasal organ nerve fibers and the dendrites of mitral cells and periglomerular cells (2)(24)(25).

At 30 days old fetus to 1 day old pup

The entire six layers are shown in Fig.(5). the outer the vomeronasal nerve fibers layer was formed from the nerve fibers. The width of the layer was $146.8\pm3.8 \mu m$. The width of the glomerular layer, which consisted of the many pinkish-spherical glomeruli, was $112.6\pm5 \mu m$. The mean diameter of the glomeruli was $112.6\pm5 \mu m$. The glomeruli were small and not completely surrounded by the periglomerular cells. Where the glomeruli of the main olfactory bulb were completely surrounded by the periglomerular cells, as well as described by (22) (26) (27). The periglomerular cells forming cellular zone which separate the glomerular layer form the external plexiform layer, Fig.(6). These findings were similar to the results of (19) in pigs and result of (4) that studied the histological structure of accessory bulb in bats. The external and internal plexiform layers were thin, measured $(21.8\pm0.6)\mu m (8.46\pm0.3)\mu m$ respectively. The internal plexiform layer was thinner. The expansion of the glomerular layer and the mitral cells layer caused these layers to be thin in width. These data revealed similar results of (4) in bats and (19) in pig fetuses. The mitral cells layer was consisted of many cell layers which extended between the external and internal plexiform layers. The mitral cells were appeared large pyramidal in shape with large round nucleus surrounded by thin cytoplasm, Fig.(6). The results were incompatible with the results of (28)and (4). The granule cells layer was the deepest and the widest layer which was formed by cellular populations of granule cells, Fig.(7) and the width of this layer was $448.4\pm1.8\mu m$. This result was in agreement with the result of (20) (29) (30) whom claimed that these cells continuously generated in pre and postnatal life which caused continuous increasing in the width of the layers which led to an increase in the accessory olfactory bulb volume. At these ages the accessory olfactory bulb was appeared well developed. The results suggest that, the
accessory olfactory bulb was functional before birth, and this conceded with result of (31) in sheep and (19) in pig, whereas in mice and rats the accessory olfactory bulb completed its developments at the first week after birth (17) (32) (31).

At 7-14 days old pup

This age showed no important histological changes, Fig.(8), except that an increasing in the width of the layer and the diameter of glomeruli as shown in tables (1) and (2). The accessory olfactory bulb was well developed. An increase in the width of the layers expressed the development and the function of the bulb which appeared complete at this age. This was in agreement with the results of (33) (34) (35).

At 21-30 days old pup

The ideal six layers of the accessory olfactory bulb were appeared clearly, Fig.(9). The diameter of the individual glomerulus was $41.4\pm 1.2\mu m$ it was smaller than the glomerulus diameter of the main olfactory bulb on 30 days old rabbit measured by (36). The width of the granule cells layer was $747.4\pm 2.24\mu m$. The continuous increase in the measurement with age was caused by the effect of exposure of growing pups to different environments cues (nonvolatile odor and pheromones) (37) (10). The newly generated granule cells regulate the social and sexual behavior of animals (38) (39).

Table (1) The width measurements of accessory olfactory bulb layers at different ages.

<table>
<thead>
<tr>
<th>Age /days</th>
<th>VNNL /µm</th>
<th>GL /µm</th>
<th>EPL /µm</th>
<th>MCL /µm</th>
<th>IPL /µm</th>
<th>GCL /µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 days</td>
<td>102±5</td>
<td>92.6±2.2</td>
<td>11.2±0.2</td>
<td>238±19.6</td>
<td>Nil</td>
<td>396±4</td>
</tr>
<tr>
<td>30 days</td>
<td>146.8±3.8</td>
<td>112.6±5</td>
<td>21.8±0.6</td>
<td>291±6.6</td>
<td>8.46±0.3</td>
<td>448.4±1.8</td>
</tr>
<tr>
<td>7-14 day</td>
<td>198.4±1.8</td>
<td>1394±0.8</td>
<td>25.4±0.4</td>
<td>299±0.8</td>
<td>10.6±0.4</td>
<td>566±3.7</td>
</tr>
<tr>
<td>(21-30)day</td>
<td>248±1.8</td>
<td>182.6±1.9</td>
<td>25.6±0.4</td>
<td>224±3.7</td>
<td>11.2±0.7</td>
<td>747.4±2.24</td>
</tr>
</tbody>
</table>

• (n=5).
• The measurement represents: Mean±Standard Error (SE).
Table (2) The diameters of the accessory olfactory bulb glomeruli at different ages.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Diameters (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-days old fetus</td>
<td>10.6±0.8</td>
</tr>
<tr>
<td>30 days fetus-one day pup</td>
<td>112.6±5</td>
</tr>
<tr>
<td>(7-14) days old pup</td>
<td>29.2±1.1</td>
</tr>
<tr>
<td>(21-30) days old pup</td>
<td>41.4±1.2</td>
</tr>
</tbody>
</table>

- (n=5).
- The diameter represents: Mean±Standared Error (SE).

Figure-1 histological section of the olfactory bulb at (18-20) day’s old fetus shows:

A: Olfactory bulb mass. B: Vomeronasal nerve layer. H&E satin 100x.
Figure-2 histological section of the olfactory bulb at (25-26) days old fetus shows:
A: Outer mass.(Mitic cells) ,B: Inner mass.(Granule cells) ,(Arrows show large neurons) , H&E stain 100x.

Figure-3 histological section of the olfactory bulb at (25-26) day’s old fetus (arrows show Mitral cells population) Cresyl violets stain 400x.
Figure-4 histological section of the olfactory bulb at (28) days old fetus shows (Arrows show newly formed glomeruli). H&E stain 400x.

Figure-5 histological section of the olfactory bulb at 1 day old fetus shows:

A. Olfactory nerve layer., B.Glomerular layer. ,C.External plexiform layer., D.Mitral cells layer. E.Internal plexiform layer. ,F.Granule cells layer. ,H&E stain100x.
Figure-6 Histological section of the olfactory bulb at one day old pup shows:
- (Red arrow) Glomerulus.
- (White arrows) Periglomerular cells.
- (Yellow arrows) Mitral cells.
- (Black arrow) External plexiform layer). H&E stain x 400.

Figure-7 histological section of the accessory olfactory bulb at one day old pup shows granule cells layer and the arrows show granule cells).
H&E stain 400x.
Figure-8 histological section of the olfactory bulb at 7-14 days old fetus shows:

Figure-9 histological section of the main and accessory olfactory bulb at 30 days old pup shows: (Capital litters represented layers of accessory olfactory bulb, small litter represent layers of main olfactory bulb).

- (D, d). Mitral cells layer. (E, e). Internal plexiform layer.
- (F, f). Granule cells layer. (H&E stain 40x)
دراسة تطورية للبصلة الشمية الإضافية في الأرانب الهجينة
علي فارس رشك
فرع التشريح والأنسجة والاجنحة كليه الطب البيطري جامعة بغداد، العراق

الخلاصة

أجريت الدراسة على ثلاثون جنين وخمس وعشرون من جراء الأرانب. بينت النتائج النسجية أن البصلة الشمية الإضافية في الاجنزة بعمر 18-20 يوما كانت غير متغيرة. عند الاجنزة بعمر 28 يوما ظهرت الكبيبات في الطبقة الكبيرة للبصلة الشمية الإضافية وتم تمايز واضح لأول المطبقات متغايرة لكن أقطار الكبيبات في البصلة الشمية الإضافية كان أقل مما كانت عليه أقطار الكبيبات في البصلة الشمية الرئيسية. عند الاجنزة بعمر 30 يوما جميع طبقات البصلة الشمية الإضافية بدأ تتمايز. نسجيا استنتجت الدراسة أن البصلة الشمية الإضافية في الأرانب متطورة قبل الولادة. بينت الدراسة أن فترة ما بعد الولادة تأخذ طبقات البصلة الشمية الإضافية بالزيادة في سمكها.

REFERENCES