Histological Analysis of Sensory Nerve of the Ethmoid Bone of the Dog.
犬の篋骨に分布する知覚神経終末について.
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Since the classic and academic studies conducted in Germany (CSOKOR 1906) little knowledge has been published on comparative histology of domestic animals. In the special area of sensory innervation, the histology of the human nasal cavity as well is not fully understood. It is the intention of this study to create further interest in fundamental histology of the nasal cavity, especially the ethmoid bone of domestic animals. Thus, we have devoted ourselves to a consideration of morphological questions. We have attempted to analyze the extent of distribution of sensory nerve terminals within the ethmoid bone in one dog.

In addition, particular attention is concentrated upon morphological appearances of olfactory cells, previously overlooked by other researchers.

Portions of this study were presented at the 46th Meeting of the Japanese Society of Veterinary Science in 1958.

I. Material and Methods.

Working material. The ethmoid bone of the dog was fixed and kept in 10% neutral formalin for six years.

Procedure for decalcification. The ethmoid bone of the dog was decalcified with 5% nitric acid solution in 10% neutral formalin. Damage to the surface of mucous membrane was carefully avoided and particular attention given not to damage or otherwise injure the cilia.

Staining technic. Celloidin was used for embedding. Sections were cut 32 microns in thickness, transversely and sagittally.

KAWATA’s silver impregnation method (1943) and hematoxylin and eosin stains were applied. Celloidin-paraffin double embedding method was used for ultra-thin sectioning.

1. Selection of appropriate spot for ultra-thin sectioning: The silver impregnation technique itself has not been sufficiently explored because of its complicated and troublesome methodology. The study of sensory innervation has an additional restriction, that is, achievement of thin sectioning, since the silver impregnation requires frozen sectioning or celloidin method.

It is almost impossible to demonstrate the fine structure of the olfactory epi-
thelium and the incomprehensible combination of olfactory cells and supporting cells. It was desirable to improve thin sectioning procedure for the silver staining in order to solve a century old enigma.

2. Silver impregnation of the celloidin block: The most important area is detected by preliminary screening staining. The selected spot is minced into smaller block, approximately 2 mm by 3 mm and 2 mm in thickness, and KAWATA’s silver impregnation applied.

3. Paraffin embedding of the stained celloidin block: The minced small block impregnated with silver nitrate, has to be further embedded by paraffin method.

4. Ultra-thin sectioning and mounting: 200—600 mμ thin sections were cut with SPENCER rotary microtome. Transfer the floating sections on clean glass slide with delicate spatula-like needle, and warm the slide in order to smooth out the creases of the sections. Deparaffinization and dehydration are carried out simultaneously with xylene, applied by medicine dropper. Xylene should be absorbed as soon as soon as possible. Repeat three times. In this way the sections on the slide become transparent and ready for permanent mounting with balsam.

II. Results.

The ethmoid bone was divided and examined in two main portions, according to its anatomical structure, that is, the region of the ethmoturbinate and the region of the cribriform plate.

A. The Region of the Ethmo-Turbinate Bone.

The dog is singular animal that has extraordinary massive ethmo-turbinates macroscopically. They occupy the greater portion of nasal cavity (Fig. 1). Micro-

![Diagram](image_url)

Fig. 1. Diagram visualizes the location of materials dedicated to this study. The section is cut about one centimeter to the right of the median plane. DT dorsal turbinate, VT ventral turbinate, LC lamina cribriformis, HP hard palate, T tongue.

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which measures approximately from 80 \( \mu \) to 120 \( \mu \); the latter has comparatively thin or flat ordinary ciliated epithelium and its *lamina propria* too is shallow, where poor extension and distribution of nerve fibers and blood vessels will be recognized (Fig. 2).

![Image](image_url)

Fig. 2. Sagittal section of the part II (cf. Fig. 1). KAWATA's silver impregnation. *FB* thin paper-like ethmoid bone in massa lateralis, *NB* massive nerve bundle, *OE* olfactory epithelium measured 110 \( \mu \), *CE* thin ciliated epithelium (cf. the corner) interspersing no olfactory cell at all. Explanation in the text. Corner: high magnification (\( \times 1000 \)) of ciliated cells, 600 \( \mu \) sectioning. \( \times 100 \).

a) **Epithelium.**

The olfactory cells are evenly distributed between the sustentacular cells. However, the frequency of distribution is extraordinarily arbitrary, according to the site of the region.

The distribution of olfactory cells is poor in the part I, moderate in the part II and rich in the part III (Fig. 1). In fact it is difficult to detect the supporting cells around the *lamina cribiformis*. The shape and the length of olfactory cells are somewhat arbitrary, according to those three parts. At the apex, that is, the area of the part I the olfactory cells are small and less numerous.

At the area of the part III the olfactory cells are thin and tall. The peripheral part of the cell body is thick and sometimes extends as a straight, cylindrical process from the nucleus to the surface. They are bending or spiral. In general, they have an enlarged or swollen tip, shaped like pineapple (Fig. 3). It is imposible to explain the fine structure of olfactory cells in detail from this figure (Fig. 3). Still we can declare with certainty that the head is enlarged and has cilia.

The proximal end rapidly tapers into a thin, smooth filament. It is an axon—a fiber of the olfactory nerve. It passes into the subjacent connective tissue and here, together with similar fibers, forms small nerve bundles (Fig. 4).
b) Lamina propria.

The subjacent connective tissue layer of the olfactory epithelium of ethmo-tur-
Histological Analysis of Sensory Nerve of the Ethmoid Bone of the Dog.

Bivariate is not remarkable. However, the lamina propria is enriched with many blood vessels and networks of capillaries. In its deeper layers it contains a plexus of large veins and dense networks of lymph capillaries. The olfactory glands of BOWMAN are packed tightly in this layer (Fig. 5). Among these olfactory gland and blood vessels, numerous kinds of nerve elements and an aggregate of nerve bundles are found. Sometimes the terminal portion of the nerve is club-like or as shown, a simple glomerular end apparatus. In other cases, it is a bifurcated, wavy terminal fiber, and extending like a loop or snake. Distributions of thick twig-like nerve bundles are noted. But their significance is not understood. (Fig. 6).

B. The Region of the Cribriform Plate.

The proximal end of the olfactory cells tapers into a thin, smooth filament — a fiber of the olfactory nerve — passes into the subjacent connective tissue layer of
the olfactory epithelium and here, together with similar fibers, forms small nerve bundles. The nerve bundles are constantly anastomosing and form huge massive trunks which are directed toward the lamina cribroformis (Fig. 5). The bundles of nerve fibers are non-myelinated. They are frequently provided with a sheath of SCHWANN. Myelinated nerve fibers are sometimes noted. The nerve fibers are enveloped or surrounded by a delicate connective tissue. The combinations of these anatomic structures advance to the openings of the cribiform plate of ethmoid bone (Fig. 7).

III. Discussion.

There are numerous references describing the histological aspects of the nasal cavity, especially of the sensory innervation of the nasal mucous membrane. We may summarize the classical studies by indicating that the mucous membrane of the Regio olfactoria consists of three kinds of cells: 1. sustentacular or supporting, 2. basal, and 3. olfactoria.

In a span of a hundred years many discussions have been carried out concerning the problem of the existence of special olfactory cells in the epithelium of the regio olfactoria. Recently SAITO (1947) studied the nasal cavity of the human being. He concluded that the epithelium of the regio olfactoria has no special olfactory cells, but branched or unbranched nerve fiber terminals which are richly interspersed within the epithelium. In contrast, study of the comparative animal histology of the nasal cavity is of interest. KAWATA and OKANO (1958) examined the septum nasi of the horse and dog, and demonstrated olfactory cells in limited areas of septum nasi of both animals.

IV. Summary.

We have been studying the comparative histology of the nasal cavity of domestic animals. In this particular paper the sensory innervation of the ethmoid bone of the dog has been emphasized.

The ethmoid bone is divided into two main parts, according to its anatomical structure, namely, the region of the ethmo-turbinate and cribiform plate. Subdivided the ethmo-turbinate into three portions, I, II, and III.

1. The region of the ethmo-turbinate.

Epithelium. The distribution of olfactory cells is poor in the part I, moderate in the part II, and rich in the part III. The shape and the length of olfactory cells differ according to the three parts outlined. The peripheral part of the cell body is thick, and forms a tuft at the enlarged apex. The proximal end rapidly tapers into a thin, smooth filament—a fiber of the olfactory nerve.

Lamina propria. The olfactory glands of BOWMAN are packed tightly in the subjacent connective tissue. Among the olfactory glands, the blood vessels, few kinds of nerve elements, and rich plexus of nerve bundles are existing thickly.

2. The region of the cribiform plate.

In this region comparatively thin nerve bundles are constantly anastomosing and form huge massive nerve trunks which are directed toward the lamina cribr-
Histological Analysis of Sensory Nerve of the Ethmoid Bone of the Dog. 615

iformis.

The nerve fibers are non-myelinated. They are frequently provided with a sheath of SCHWANN. Myelinated nerve fibers are sometimes detected. These combined heterogeneous nerve fibers pass through the openings of the cribriform plate of ethmoid bone.

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References.