

Descriptive Histological Study and Detection of Serotonin (5-HT)-Associated Autofluorescence in the Neuro-Olfactory Epithelium of the Vampire Bat *Desmodus rotundus*

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Abstract

This study characterized the histological organization of the neuro-olfactory epithelium of the common vampire bat, *Desmodus rotundus*, using hematoxylin and eosin (H&E), Gomori's trichrome, and Holmes' silver impregnation. In addition, an induced autofluorescence technique was employed to investigate the distribution of serotonin (5-hydroxytryptamine, 5-HT)-associated fluorescence within the neuro-olfactory epithelium. Histological analysis demonstrated that the neuro-olfactory epithelium is in the dorsocaudal region of the nasal cavity and consists of a pseudostratified sensory epithelium composed of sustentacular cells, basal cells, and bipolar olfactory receptor neurons. Holmes' staining clearly identified unmyelinated nerve fibers associated with the olfactory nerve. Autofluorescence imaging obtained using two fluorescence filter sets revealed a yellow-to-orange colocalization signal indicative of serotonin (5-HT)-associated fluorescence within the neuro-olfactory epithelium and associated olfactory glands. These findings provide a detailed histological description of the neuro-olfactory epithelium of *Desmodus rotundus* and support the usefulness of induced autofluorescence as a complementary histological approach for detecting serotonin-associated fluorescence in formalin-fixed, paraffin-embedded tissues.

Keywords

Common Vampire Bat, *Desmodus rotundus*, Neuro-Olfactory Epithelium, Serotonin (5-HT), Autofluorescence, Histology

1. Introduction

The common vampire bat (*Desmodus rotundus*) is widely recognized in epidemiology because it serves as the principal reservoir and transmitter of rabies virus (RABV) to domestic animals, wildlife, and humans [1]. In addition to rabies, bats have been identified as reservoirs for multiple viruses with zoonotic potential, including Marburg virus, Ebola virus, coronaviruses (SARS-CoV-1 and 2) that cause severe acute respiratory syndrome, Middle East respiratory syndrome (MERS) virus, and henipaviruses such as Hendra and Nipah [2]. Despite its importance as a reservoir of zoonotic pathogens, *Desmodus rotundus* also plays essential ecological roles. As an obligate hematophagous species, it contributes to ecosystem dynamics by interacting with both wild and domestic vertebrate populations. Furthermore, bats are widely recognized as valuable bioindicators of environmental health because they accumulate contaminants, including pesticides and heavy metals, within their tissues. Consequently, analyses of organs such as the liver, kidneys, carcass, and guano provide useful information for monitoring environmental pollution and ecosystem quality [3] [4].

Vampire bats naturally inhabit caves, hillsides, crevices in rocky or mountainous areas, and ravines [5] [6]. They obtain their food by consuming the blood of both domestic and wild animals, including reptiles, birds, and mammals, which may partly explain their wide distribution and abundance [6]. They also remain near bodies of water such as ponds, rivers, and streams [7] [8]. The neuro-olfactory epithelium and the morphology of its primary sensory neurons were first described in the second half of the 19th century by the German anatomist and histologist Max Schultze [9]. This epithelium is described at the nasal cavity of vertebrates and is composed primarily of three cell types: olfactory receptor cells (bipolar neurons), supporting cells, and basal cells with regenerative capacity [9]. Cilia emerge from the apical border of the bipolar neurons, with a morphology that varies by species; 20th-century electron microscopy studies by Menco, as well as by Lidow and Menco, showed that amphibians possess approximately six very long cilia (up to 200 µm), whereas mammals have approximately 17 smaller stereocilia (15 to 50 µm) [10] [11].

In the bat species examined, the neuro-olfactory epithelium is in the dorsocaudal region of the nasal cavity, as reported for most mammals [12]. This specialized sensory tissue detects odorant molecules and initiates olfactory signal transduction. This function is particularly important for bats during environmental exploration, prey localization, and social communication. Histologically, the neuro-olfactory epithelium is pseudostratified and composed of sustentacular cells, bipolar olfactory receptor neurons, and basal cells, each contributing to the maintenance and function of the olfactory mucosa [13] [14]. Sustentacular cells extend throughout the thickness of the epithelium and exhibit an elongated morphology, with apical microvilli and cilia that help maintain the epithelial microenvironment. In addition to providing structural support, these cells participate in ionic homeostasis, metabolic regulation, detoxification, and the maintenance of the epithelial

barrier. Basal cells, located adjacent to the basal lamina, function as progenitor cells responsible for the continuous renewal of olfactory receptor neurons throughout the animal's life [13] [14].

The bipolar olfactory receptor neurons represent the primary sensory cells of the neuro-olfactory epithelium. Their dendrites extend toward the epithelial surface, where odorant molecules dissolved in the mucus interact with membrane receptors located on the olfactory cilia. This interaction initiates electrical impulses that are transmitted through unmyelinated axons, which converge into olfactory nerve fascicles. These fascicles pass through the foramina of the cribriform plate of the ethmoid bone and terminate within the glomerular layer of the olfactory bulb, where they establish synaptic connections with mitral cells, thereby transmitting olfactory information to the central nervous system [15].

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter widely distributed throughout the central and peripheral nervous systems. Beyond its well-established role in mood regulation, serotonin modulates sensory processing, neuronal excitability, and synaptic transmission. Within the olfactory system, serotonergic signaling modulates odor perception by regulating the activity of olfactory receptor neurons and associated neural circuits [16]-[20]. This study aimed to characterize the histological organization of the neuro-olfactory epithelium of the common vampire bat (*Desmodus rotundus*) using conventional histological techniques and to investigate the presence and localization of serotonin (5-HT) through an induced autofluorescence approach.

2. Materials and Methods

2.1. Collection and Fixation of Biological Samples

Two female adult specimens of the common vampire bat (*Desmodus rotundus*) were captured in Atotonilco el Grande, Hidalgo, Mexico, using mist nets, following the protocol described by Bracamonte [21]. The average weight of the specimens was 32.8 g, and their average wingspan was 39.5 cm. The criteria for identification and determination of the adult stage were based on the descriptions provided by Romero-Almaraz *et al.* [22]. Prior to tissue collection, the animals were sedated with ketamine (5 mg/kg, IM) and medetomidine hydrochloride (0.05 mg/kg, IM), followed by euthanasia through intrahepatic administration of sodium pentobarbital (0.1%), in accordance with the Mexican Official Standard NOM-033-SAG/ZOO-2014 for the humane euthanasia of domestic and wild animals [23].

Following euthanasia, the heads of the two selected specimens were fixed in a 3.5% aqueous formaldehyde solution, phosphate-buffered for 24 h [24].

Additionally, two tissue controls for the detection of serotonin-associated autofluorescence were obtained from the specimens studied and processed in the same manner: positive control (small intestine section) and a negative control (rectus femoris muscle section).

2.2. Tissue Processing and Paraffin Embedding

After fixation, tissue samples were processed using a Microm TP-1020 automated tissue processor. Samples were rinsed in distilled water, dehydrated through a graded ethanol series (80%, 96%, and 100%), cleared in xylene, and infiltrated with histological-grade paraffin. The processed tissues were embedded in paraffin blocks and semi-serial longitudinal sections (6 μm thick) were prepared using a Leica RM2125 RT rotary microtome. The sections were cut from the median plane of each half to the lateral edge (left or right) of the nasal cavity. This yielded 20 sections per specimen (10 from each half-head). For reference, the total length of the nasal cavity -measured from the nasal vestibule to the cribriform plate of the ethmoid bone- is 0.54 cm; the estimated width, accounting for the division of each half by the vomer bone, is 0.56 cm (0.28 cm for each half, left and right). It should be noted that the species is brachycephalic. Sections were floated on a tissue flotation bath (Premiere®) and mounted onto glass microscope slides [24].

2.3. Histological Staining

Tissue sections were stained with hematoxylin and eosin (H&E), Gomori's trichrome, and Holmes' silver impregnation methods. H&E staining was used to evaluate the general histological organization of the neuro-olfactory epithelium. In contrast, Gomori's trichrome facilitated the differentiation of connective tissue and associated glandular structures. Holmes' silver impregnation specifically highlighted the unmyelinated nerve fibers associated with the olfactory epithelium [24] [25]. After staining, sections were permanently mounted on glass coverslips using synthetic resin.

2.4. Induced Autofluorescence

Serotonin (5-hydroxytryptamine, 5-HT) was detected using the induced autofluorescence method described by Kaneko *et al.* [26]. This technique is based on the intrinsic fluorescence of monoamines after formaldehyde fixation, enabling visualization of serotonin-containing structures under fluorescence microscopy. Paraffin sections were deparaffinized in xylene, rehydrated through a descending ethanol series (100%, 95%, and 70%), rinsed in distilled water, and air-dried. The slides were examined using an epifluorescence microscope equipped with blue (460 - 495 nm) and green (530 - 550 nm) fluorescence filter sets. Under these conditions, the characteristic fluorescence of 5-HT-associated cells is emitted in the visible spectrum, appearing green through the blue filter and red through the green filter.

2.5. Microscopy and Image Analysis

Histological sections were examined using an Olympus BX41 light microscope equipped with epifluorescence and an Evolution VF digital camera (Media Cybernetics, USA). Bright-field and fluorescence micrographs were captured in TIFF format using Image-Pro Plus software (Media Cybernetics).

Micrographs were acquired at a total magnification of 100X (10X objective and 10X eyepiece); the scale bar represents 100 μm . To ensure comparability between samples and minimize variations in image quality, all autofluorescence images were captured consecutively on the same day, with an interval of approximately 20 seconds between shots while switching filters (blue to green). During the capture session, acquisition parameters remained fixed and identical for all sections: an exposure time of 3 s per photograph and a handling time of 20 minutes per slide. The camera software gain was set to a fixed value of 1X.

Micrographs captured in the blue and green channels were processed using Fiji software to identify serotonin-associated fluorescence, which appeared as a yellow-to-orange colocalization signal [27]. First, the RGB images corresponding to each emission channel were imported (File > Open). Subsequently, the signals were merged using the Image Calculator tool (Process > Image Calculator) by selecting both images and applying the averaging operation (Operation: Average). Finally, contrast and brightness levels were adjusted uniformly across all images to optimize the visualization of the autofluorescence signal observed in 5-HT-associated cells.

2.6. Ethical Approval

All animal handling, capture, sedation, and euthanasia procedures were conducted in accordance with the Mexican Official Standard NOM-033-SAG/ZOO-2014. Field collection and experimental procedures were approved by the corresponding Institutional Animal Care and Use Committee (CICUAL-V-I/018/2023).

3. Results

Both specimens exhibited similar morphological characteristics and patterns of serotonin-associated autofluorescence.

Hematoxylin and eosin (H&E) staining demonstrated that the neuro-olfactory epithelium consisted of a pseudostratified sensory epithelium composed of sustentacular cells, bipolar olfactory receptor neurons, and basal cells (**Figure 1**). Sustentacular cells occupied the superficial region of the epithelium and exhibited elongated nuclei. In contrast, bipolar receptor neurons were characterized by centrally located nuclei containing finely dispersed chromatin. Basal cells were located adjacent to the basal lamina, separating the neuroepithelium from the underlying lamina propria. The lamina propria contained numerous olfactory glands, blood vessels, adipose tissue, and bundles of olfactory nerve fibers.

Holmes' silver impregnation method clearly demonstrated bundles of unmyelinated nerve fibers within the lamina propria, corresponding to the olfactory nerve fascicles. These nerve bundles were closely associated with numerous olfactory glands distributed throughout the connective tissue (**Figure 2**).

Gomori's trichrome staining provided clear differentiation of the connective tissue associated with the neuro-olfactory epithelium. The three principal cellular components of the sensory epithelium—sustentacular cells, bipolar receptor neu-

rons, and basal cells—were readily identified. The stain also highlighted the olfactory glands, olfactory nerve fascicles, and the relatively sparse connective tissue within the lamina propria and periosteum (**Figure 3**).

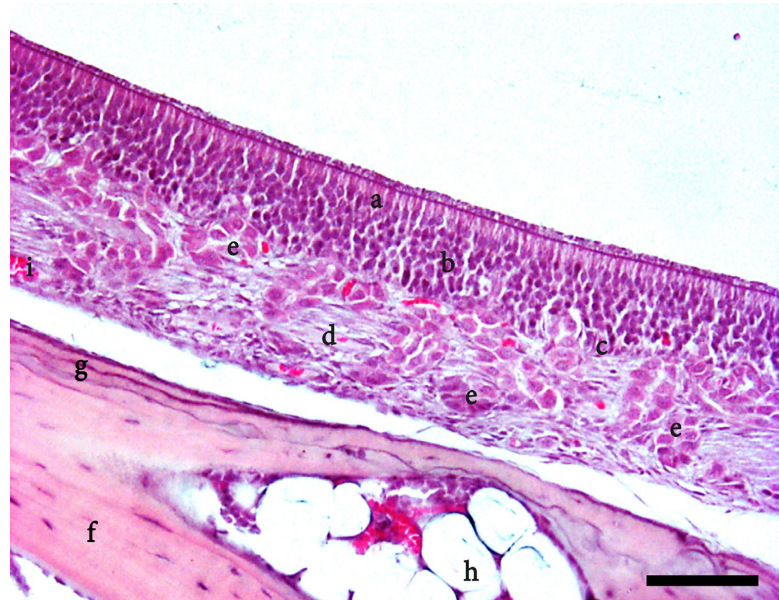


Figure 1. Histological organization of the neuro-olfactory epithelium of the common vampire bat (*Desmodus rotundus*). H&E staining. The pseudostratified sensory epithelium consisted of sustentacular cells (a), bipolar olfactory receptor neurons (b), basal cells (c), olfactory nerve fibers (d), olfactory glands (e), ethmoid bone (f), periosteum (g), adipose tissue (h), and blood vessels (i). Scale bar = 100 μm .

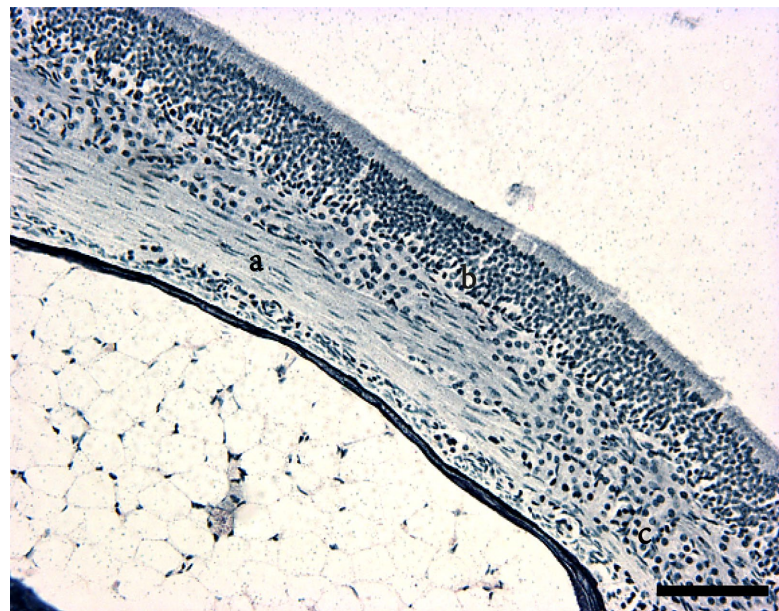


Figure 2. Neuro-olfactory epithelium. Hematophagous bat *Desmodus rotundus*. Holmes' silver impregnation demonstrating unmyelinated olfactory nerve fascicles (a), bipolar olfactory receptor neurons (b), and olfactory glands (c) within the lamina propria. Scale bar = 100 μm .

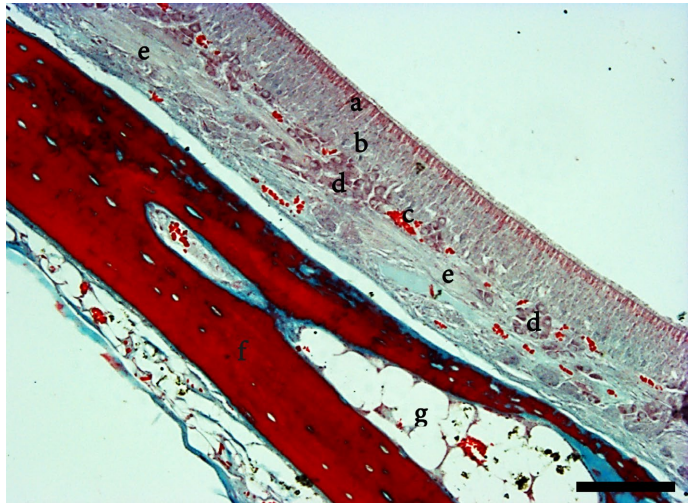


Figure 3. Neuro-olfactory epithelium. Hematophagous bat *Desmodus rotundus*. Gomori's trichrome staining of the neuro-olfactory epithelium showing sustentacular cells (a), bipolar receptor neurons (b), basal cells (c), olfactory glands (d), olfactory nerve fascicles (e), ethmoid bone (f), and adipose tissue (g).

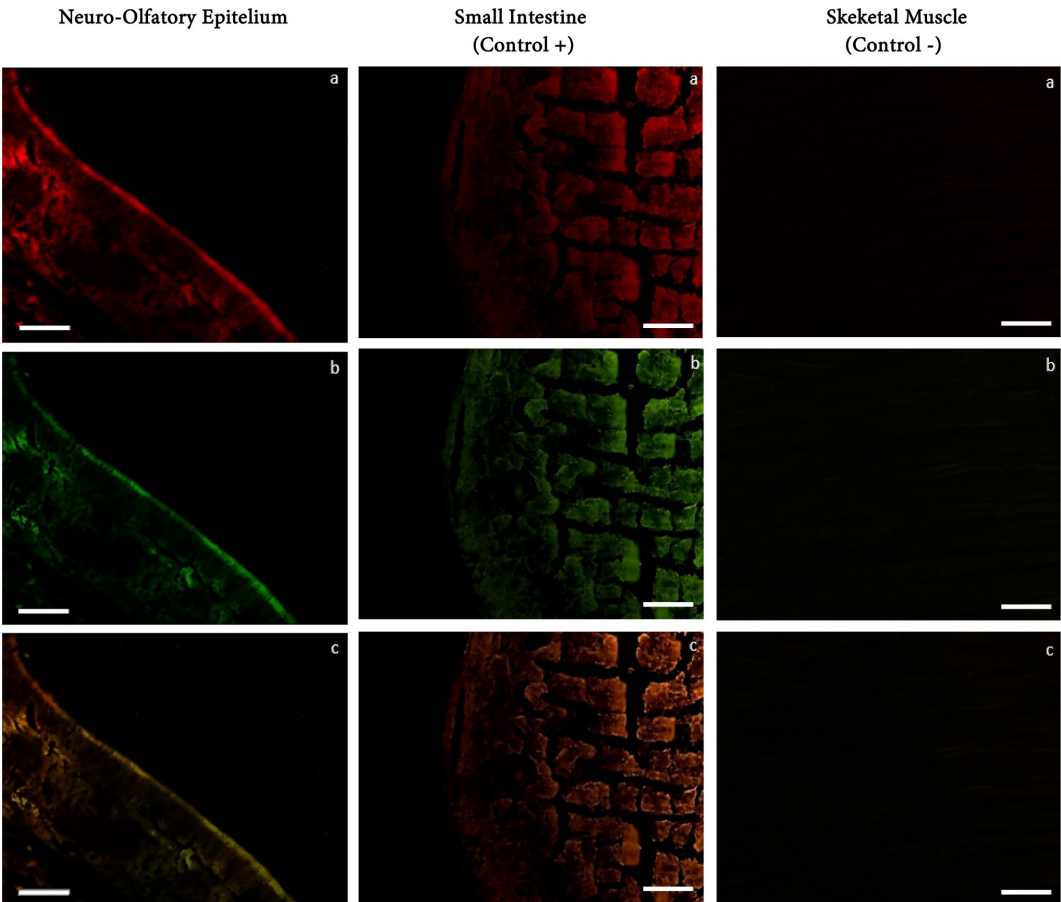


Figure 4. Detection of serotonin-associated autofluorescence in *Desmodus rotundus*. Fluorescence images were acquired using blue (460 - 495 nm) (a) and green (530 - 550 nm) (b) filter sets. Superimposition of both images generated a yellow-to-orange fluorescence signal (c), indicating serotonin-associated fluorescence. Scale bar = 100 μ m.

Autofluorescence was detected using blue (460 - 495 nm) and green (530 - 550 nm) fluorescence filter sets. Superimposition of the fluorescence images generated a yellow-to-orange signal within the neuro-olfactory epithelium, indicating the presence of serotonin-associated fluorescence (**Figure 4**). The fluorescence signal was predominantly localized to the apical region of the sensory epithelium, corresponding to the terminal portions of bipolar receptor neurons' dendritic processes. Additional fluorescence was observed within glandular cells of the lamina propria.

In the positive control, serotonin-associated fluorescence was observed in the intestinal epithelium associated with intestinal villus (**Figure 4**). In the case of negative control, there was no signal indicating serotonin-associated fluorescence to skeletal myocytes (**Figure 4**).

4. Discussion

The present study demonstrated that the neuro-olfactory epithelium of *Desmodus ro-tundus* exhibits the characteristic histological organization observed in most mammalian species, consisting of a pseudostratified sensory epithelium comprising sustentacular cells, bipolar olfactory receptor neurons, and basal cells. This structural organization is consistent with previous anatomical descriptions of the olfactory mucosa in bats and other mammals, indicating that the general organization of the olfactory neuroepithelium has been evolutionarily conserved despite the ecological specialization of hematophagous bats [12]-[15]. Furthermore, the neuro-olfactory epithelium was observed in the dorsocaudal region of the nasal cavity, a distribution that agrees with previous reports in chiropteran species, [12]. This anatomical arrangement, coupled with local serotonergic signaling mechanisms in mammalian olfactory tissues [28], has been associated with efficient odor detection and environmental perception. In addition, Holmes' silver impregnation clearly demonstrated the presence of unmyelinated olfactory nerve fascicles within the lamina propria, confirming the structural organization of the peripheral olfactory pathway observed in other mammalian species [15].

Kaneko *et al.* [26] adapted the classical Falck-Hillarp fluorescence principle to formalin-fixed, paraffin-embedded tissues, demonstrating that serotonin-containing cells can be identified by induced autofluorescence. Their study showed a close correspondence between autofluorescence and serotonin immunohistochemistry, supporting the reliability of this approach for detecting serotonin in histological sections. In the present study, a similar fluorescence pattern was observed within the neuro-olfactory epithelium and associated olfactory glands, suggesting that induced autofluorescence is a useful complementary technique for the histological localization of serotonin in *Desmodus rotundus*.

Kaneko *et al.* [26] demonstrated that the autofluorescence pattern obtained with this technique closely corresponded to serotonin immunohistochemical labeling in enterochromaffin cells, supporting the reliability of induced autofluorescence as a complementary method for detecting serotonin in formalin-fixed,

paraffin-embedded tissues. In the present study, serotonin-associated autofluorescence was predominantly localized to the apical region of the neuro-olfactory epithelium, corresponding anatomically to the terminal dendritic processes of bipolar olfactory receptor neurons. This distribution is consistent with the proposed involvement of serotonergic signaling in modulating olfactory sensory processing, as described in previous studies [29]-[30]. Serotonin-associated autofluorescence was also detected in olfactory gland cells within the lamina propria. Although serotonin has been implicated in regulating secretory activity in several tissues [31], its specific role in the olfactory glands of *Desmodus rotundus* remain unknown and warrants further investigation.

Serotonin (5-hydroxytryptamine, 5-HT) is a well-established neuromodulator involved in regulating sensory processing in the olfactory system. Previous studies have demonstrated that serotonergic signaling modulates the activity of olfactory receptor neurons and contributes to odor information processing before transmission to higher olfactory centers [32]-[35]. In the present study, serotonin-associated autofluorescence was predominantly localized to the apical region of the neuro-olfactory epithelium, corresponding anatomically to the terminal dendritic processes of bipolar olfactory receptor neurons. This distribution is consistent with the proposed involvement of serotonergic signaling in olfactory sensory modulation described in previous experimental studies [33]-[35]. However, because receptor expression and neuronal function were not evaluated, the present findings should be interpreted as morphological evidence of serotonin-associated fluorescence rather than direct evidence of its physiological role. Serotonin-associated autofluorescence was also observed in olfactory gland cells within the lamina propria. Although serotonin has been implicated in the regulation of exocrine secretion in other tissues [31], its functional significance in the olfactory glands of *Desmodus rotundus* remains unknown and further investigation warrants.

Although detection using fluorescence filters (e.g., blue and green) allows for the visualization of fluorescent compounds associated with serotonin, confirming molecular colocalization and precisely identifying the source of the fluorescence require complementary methodologies—such as hyperspectral analysis, fluorescence spectroscopy, or validation via immunohistochemistry for 5-HT—as indicated by Kaneko *et al.* [26].

5. Conclusions

The present study provides a detailed histological characterization of the neuro-olfactory epithelium of *Desmodus rotundus*. The olfactory mucosa exhibited the typical mammalian organization, consisting of pseudostratified sensory epithelium comprising sustentacular cells, bipolar olfactory receptor neurons, and basal cells, with olfactory nerve fascicles and numerous olfactory glands within the lamina propria.

The induced autofluorescence technique successfully revealed serotonin-associated fluorescence within the neuro-olfactory epithelium and olfactory glands of

D. rotundus. These findings support the usefulness of induced autofluorescence as a complementary histological approach for detecting serotonin-associated signals in formalin-fixed, paraffin-embedded tissues.

Overall, this study expands the current knowledge of the sensory anatomy of *Desmodus rotundus* by providing the first histological description of serotonin-associated autofluorescence within its neuro-olfactory epithelium. These observations establish a morphological basis for future immunohistochemical, molecular, and functional studies aimed at confirming the presence and clarifying the role of serotonergic signaling in the olfactory system of hematophagous bats.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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