Corneal innervation in mesocephalic and brachycephalic dogs and cats: assessment using *in vivo* confocal microscopy

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Abstract

**Objective** To determine the density of the canine and feline corneal neural network in healthy dogs and cats using *in vivo* confocal microscopy (IVCM).

**Animals examined** A total of 16 adult dogs (9 Mesocephalic breeds, 7 Brachycephalic breeds) and 15 cats (9 Domestic Short-haired cats (DSH), 6 Persian cats) underwent IVCM.

**Procedure** Animals were examined with a confocal corneal microscope (HRTII/RCM; Heidelberg Retina Tomograph II/Rostock Cornea Module®, Heidelberg Engineering, Dossenheim, Germany). The investigations focused on the distribution of the corneal nerves and quantification of central subepithelial and subbasal nerve plexus.

**Results** The corneal stromal nerve trunks, subepithelial and subbasal nerve plexus were observed. The nerve fiber density (NFD) quantified in nerve fiber length in mesocephalic dogs were $12.39 \pm 5.25$ mm/mm$^2$ in the subepithelial nerve plexus and $14.87 \pm 3.08$ mm/mm$^2$ in the subbasal nerve plexus. The NFD of the subepithelial nerve plexus in DSH cats was $15.49 \pm 2.7$ and $18.4 \pm 3.84$ mm/mm$^2$ in the subbasal nerve plexus. The subbasal NFD of DSH cats was significantly higher than in mesocephalic dogs ($P = 0.037$). The subepithelial NFD in brachycephalic dogs, and Persian cats were $10.34 \pm 4.71$ and $9.50 \pm 2.3$ mm/mm$^2$, respectively. The subbasal NFD measured $11.80 \pm 3.73$ mm/mm$^2$ in brachycephalic dogs, and $12.28 \pm 4.3$ mm/mm$^2$ NFD in Persian cats, respectively. The subepithelial and subbasal NFD in Persian cats were significantly lower than in DSH cats ($P = 0.028$, respectively, $P = 0.031$), in contrast to brachycephalic vs. mesocephalic dogs.

**Conclusion** The noninvasive IVCM accurately detects corneal innervation and provides a reliable quantification of central corneal nerves.

**Key Words:** cat, corneal innervation, corneal nerve density, corneal nerve quantification, dog, *in vivo* confocal microscopy

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**INTRODUCTION**

Corneal innervation is important for the maintenance of corneal structure and function and protects against potential trauma.\(^1\) Denervation or decreased corneal sensitivity leads to increased epithelial cell permeability, decreased cell migration and cell mitosis, and is a risk factor for recurrent erosions, impaired wound healing, and infections.\(^2\)\(^-\)\(^6\) Yet, many aspects of corneal innervation in veterinary as well as in human ophthalmology remain incompletely understood. Corneal nerves are routinely injured by modern refractive surgery and by corneal disease. More attention has been focused on this subject in human ophthalmology in the last decade.\(^2\)\(^,\)\(^7\)\(^,\)\(^8\) Corneal anatomy has been studied mainly using *ex vivo* techniques.\(^9\)\(^-\)\(^11\)

*In vivo* confocal microscopy (IVCM) is a repeatable, rapid, noninvasive *in vivo* clinical examination technique capable of imaging corneal nerve anatomy, damage and repair.\(^12\)\(^-\)\(^14\)

In a previous pilot study, IVCM was adapted to veterinary use. In that study, real-time morphological images of the different corneal layers and nerve distribution in normal canine, feline and avian corneas were obtained and served as reference images.\(^15\)

To the best of our knowledge, this is the first corneal nerve investigation in veterinary medicine using IVCM, an established technique applied to several human investigations.\(^12\)\(^-\)\(^14\)

It was conducted to determine corneal nerve density in mesocephalic and brachycephalic dogs and cats.
MATERIALS AND METHODS

The study included one eye from each of 16 dogs and 15 cats. The mesocephalic dogs’ ages ranged from 0.4 to 10.1 years, with a mean age of 3.01 years. All but three dogs were purebred, represented by two Weimaraners and one Doberman Pinscher, Poodle, Cocker Spaniel and German shepherd, respectively. The group of brachycephalic dogs ranged from 0.5 to 4.9 years, with a mean age of 1.94 years and consisted of 4 Boxers, 2 Pekingese dogs and one English Bulldog. The Domestic Short-haired cats (DSH) cats’ ages ranged from 1.8 to 10.0 years, with a mean age of 5.3 years and the age of the 6 Persian cats ranged from 0.6 to 13.1 years with a mean age of 6.2 years.

Ophthalmic examination included Schirmer’s tear test (STT), slit-lamp biomicroscopy and applanation tonometry. All subjects received a complete physical examination and were found to be free of any significant ocular and systemic disease, and no diabetic patients were included in the study.

The patients were investigated under general anesthesia for reasons unrelated to this project.

The central area of each cornea was examined using a confocal corneal microscope (HRTII/RCM; Heidelberg Retina Tomograph II/Rostock Cornea Module®, Heidelberg Engineering, Dossenheim, Germany). The cornea module is a detachable lens objective system, used to convert the HRTII into a digital laser scanning microscope for investigating the anterior segment of the eye. All the elements of the device were fastened to a microscope floor stand (S3®, Carl Zeiss Meditec AG, Jena, Germany).

A swivelling holder for the HRTII/RCM was constructed and mounted on the arm of the microscope floor stand, as previously described.

The field-of-view was 384 × 384 μm with an optical lateral resolution of 2 μm. The focal plane could be moved through the entire cornea within a range of up to 1500 μm. Acquisition time was 0.024 s/image. Entire examination time per eye was < 5 min.

The focus level could be defined and changed in an axial direction by external manual or internal z-scan. Three images were evaluated from each patient (Fig. 1). The nerve fiber density (NFD) was measured using following parameter.

The total nerve fiber length is defined as sum of the length of all nerve fibers and branches per square millimeter of cornea tissue. The Scion image 4.02.b program package (Scion Corporation, Frederick, MA) was used for morphometric measurements of the corneal nerve fibers length. The summarized central nerve fibers in μm/147 456 μm² (size per image) were converted in mm/mm².

The subepithelial nerve plexus arise from the penetrating stromal nerves and is a dense, highly anastomotic plexus located in the superficial corneal stroma, immediately below the stromal–epithelial interface. Axons from the subepithelial plexus penetrate the epithelial basal lamina and give rise to the subbasal nerve plexus.

The subbasal nerve plexus in humans, cats, and dogs are morphologically similar and are arranged as a series of long horizontal fibers with short interconnecting branches in the subnuclear region of the basal epithelial cell layer.

Statistics
Detailed statistical analysis of the central nerve fiber lengths was performed by multivariate analysis of variance (ANOVA) and paired Student’s t-tests using spss 11.0.1 (SPSS Inc., 1991–2000 Lead Technologies, Chicago, IL). A P < 0.05 was considered to be statistically significant.

RESULTS

The superficial, wing, and basal cells of the corneal epithelium, the stroma with keratocytes, the Descemet’s membrane and adjacent corneal endothelial cells were observed in all patients. Similar to histological investigations, the corneal nerve architecture could be followed and demonstrated through all different layers. The corneal stromal nerve trunks, subepithelial and subbasal nerve plexus were observed in all patients, especially with regard to variations in different skull types (Fig. 2).

The mean NFD determined as mean nerve fiber length of the central subepithelial and central subbasal nerve plexus in mesocephalic, brachycephalic dogs, DSH cats and Persian cats was summarized in Table 1.

The mean NFD in mesocephalic dogs was 12.39 ± 5.25 mm/mm² in the subepithelial nerve plexus and 14.87 ± 3.08 mm/mm² in the subbasal nerve plexus. These values were not significantly different (P = 0.98, Student’s t-test). The mean NFD of the subepithelial nerve plexus in DSH cats was 15.49 ± 2.7 and 18.4 ± 3.84 mm/mm² in the subbasal nerve plexus. The results were not significantly different (P = 0.053, Student’s t-test).

Further, the subepithelial NFD of mesocephalic dogs and DSH cats was not significantly different (P = 0.45, Student’s
t-test), however, the feline subbasal NFD was significantly greater than those in mesocephalic dogs (P = 0.037, Student’s t-test).

The subepithelial NFD in brachycephalic dogs and in Persian cats was 10.34 ± 4.71 and 9.50 ± 2.3 mm/mm², respectively. These values were not significantly different (P = 0.058, Student’s t-test). The subbasal nerve plexus in Persian cats and in brachycephalic dogs was 12.28 ± 4.3 and 11.80 ± 3.73 mm/mm² NFD, respectively (P = 0.45, Student’s t-test).

The subepithelial and subbasal NFD in Persian cats was significantly lower than in DSH cats (P = 0.028, respectively; P = 0.031, Student’s t-test), in contrast to brachycephalic vs. mesocephalic dogs (P = 0.98, Student’s t-test).

The basal lamina of the anterior epithelium and the epithelial nerve endings could not be demonstrated with this technique.

Table 1. Mean nerve fiber density (NFD) and standard deviation (SD) calculated in mm/mm² in mesocephalic and brachycephalic dogs and cats

<table>
<thead>
<tr>
<th>Animals</th>
<th>Mean NFD ± SD (mm/mm²)</th>
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<tr>
<td></td>
<td>subepithelial</td>
</tr>
<tr>
<td>Domestic Short-haired cats (n = 9)</td>
<td>15.49 ± 2.7*</td>
</tr>
<tr>
<td>Mesocephalic dogs (n = 9)</td>
<td>12.39 ± 5.25</td>
</tr>
<tr>
<td>Brachycephalic dogs (n = 7)</td>
<td>10.34 ± 4.71</td>
</tr>
<tr>
<td>Persian cats (n = 6)</td>
<td>9.50 ± 2.3*</td>
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</tbody>
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*Statistically significant differences (P = 0.028, Student’s t-test) between the mean subepithelial NFD of Domestic Short-haired cats and Persian cats.
†Statistically significant differences (P = 0.031, Student’s t-test) between the mean subbasal NFD of Domestic Short-haired cats and Persian cats.
‡Statistically significant differences (P = 0.037, Student’s t-test) between the mean subbasal NFD of Mesocephalic dogs and Domestic Short-haired cats.

DISCUSSION

Recently, confocal microscopy has been used for microstructural clinical investigations of the human cornea, primarily focusing on qualitative observations in response to local or systemic disease and after refractive surgery.16–23 Quantitative investigations of the corneal nerve supply were conducted in normal healthy corneas, in diabetic patients, in keratitis sicca patients with and without Sjögren’s syndrome and in corneal transplant patients.12,14,24–28

In veterinary ophthalmology, there has been very limited experience with this investigative method.15 The present study focused on quantitative analysis of the corneal innervation in dogs and cats using IVCM.

On the basis of several human studies dealing with nerve quantification and IVCM, the total nerve fiber length of each single nerve fiber seen, was added and the density was calculated in mm/mm². Therefore, the data of animals and humans should be comparable.12–14,24,25 Interestingly, the NFD of the central subbasal nerve plexus in healthy humans varied considerably and the data ranged between
The varying results may be explained by the use of different generations of diagnostic devices and different kind of devices like tandem scanning confocal microscopes, slit scanning confocal microscopes and the RCM/HRTII (a point-like detector) used in the present study, which provide a very high contrast even in the edges of each image. The more recent devices, slit scanning confocal microscopes with a higher lateral resolution and contrast, yield high detail resolution images even of the corneal nerve plexus. The definition of NFD used in several reports varies. Parameter used includes the sum of individual nerve fibers per frame or the total nerve fiber length in μm or mm per frame. Furthermore, several studies used different parameters to analyze the NFD of the subbasal nerve plexus, which could lead to false estimation; for example, Chang et al. reported 26.5 ± 7.5 (number/mm²) and Rosenberg et al. measured 4.9 ± 1.1 (number/frame).23,26 In the present report, the total nerve fiber length per square millimeter was utilized as adequate parameter, because of lack of consistency in randomized single evaluations to count single nerve fibers and its branches by two different observers.

The results of the canine and feline NFD range between these mean data of the human literature, even considering the fact that different diagnostic devices and different units were used, so a direct comparison could lead to misinterpretations. A recent study described and quantified changes in corneal nerve fiber bundles in patients with diabetic retinopathy and revealed a high NFD (number/mm²) in the normal patient group, that seem to correlates with the high corneal sensitivity in humans. Likewise, the functional anatomical corneal nerve density findings in cats could explain the higher corneal sensitivity in comparison to dogs.

Additional parameters for corneal nerve evaluations, such as the number of headings, the sum of nerve branches, and the grade of nerve tortuosity, were not included in this study due to high observer variability. Nerve fiber headings were described as micronodules and were characteristic of metabolically active transmitter-containing nerve fibers. Until now, 17 different neurotransmitters have been described, for example, substance P and Calcitonin-Gene-Related-Peptide (CGRP).

In our experience, only the measurements in the central cornea revealed high quality images that could be used to quantify the corneal nerve. Images made of the peripheral cornea produced neither reliable nor evaluable images and could be misinterpreted. The patients were under general anesthesia and the investigator could rotate the globe to the correct plane. Images with clearly distinguishable nerve fibers could only be obtained by slight contact with a gel bridge and without compression of the tissue. The slightest compression leads to tissue folds and artifacts. In our observations we could not obtain analyzable nerve fiber images of the superficial regions when there was the slightest compression, scars or edema. Our results of the cats’ NFD being greater than those of dogs corresponds with the results of esthesiometric investigations, where cats had significantly lower corneal touch thresholds than dogs. Our data support the conclusion that the value of the corneal touch thresholds is dependent on the corneal nerve density of the subbasal nerve plexus.

Focusing on different skull types, brachycephalic breeds show significantly less corneal sensitivity compared to mesocephalic or dolichocephalic breeds. This corresponds to our results, even though we examined a small number of animals. Despite the limitations of a small number of patients within the different groups, the significantly decreased NFD of the Persian cats obtained by IVCM corresponds to the esthesiometric results. The relationship between the anatomical features of the Persian cat cornea and their lower corneal nerve densities compared to DSH cats is not understood. In the human literature, brachycephaly belongs to a syndrome that includes further disabilities and retardations. Interestingly, there is a similar syndrome in brachycephalic dogs and humans (Treacher Collins syndrome; Dysostosis craniofacialis). It is possible that a functional connection exists.

However, the nerve fiber densities of brachycephalic dogs were not significantly different in comparison between the two nerve plexi and to mesocephalic dogs. This needs to be studied in future investigations with a larger number of patients. The data show a trend and we hypothesize that the difference of the corneal nerve density in brachycephalic dogs compared to mesocephalic dogs is not as great as the difference in mesocephalic vs. brachycephalic cats.

Additional studies of corneal nerve density according to skull type could be useful, as brachycephalic breeds are predisposed to spontaneous chronic corneal epithelial defect and corneal sequestra. Currently, the different etiologies of these corneal defects are incompletely understood. The influence and coherence of decreased corneal nerve density, corneal epithelial integrity and the increased risk in development of corneal defects needs to be investigated.

These baseline data are reference values that should be taken into account for further clinical pathological studies. This study provides the first reference values of corneal NFD in dogs and cats. IVCM can be a very useful tool in veterinary ophthalmology and future clinical investigations.

In conclusion, IVCM is a rapid, noninvasive, and repeatable technique to quantify the corneal innervation in the central region of healthy corneas and offers exciting insight into microstructural anatomic conditions.

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