Journal of Clinical & Experimental
Ophthalmology

Azmanis et al., J Clin Exp Ophthalmol 2015, 6:3
DOI: 10.4172/2155-9570.1000431

Research Article Open Access

The Additional Diagnostic Value of Optical Coherence Tomography (OCT) and Its Application Procedure in A Wide Variety of Avian Species

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Received date: Mar 23, 2015, **Accepted date:** Jun 05, 2015, **Published date:** Jun 10, 2015

Abstract

Objective: The current study introduced OCT as a novel tool in clinical veterinary ophthalmology in a much wider variety of free-living avian species than hitherto studied.

Methods: OCT was tested and performed in 39 free-living birds (21 species of 12 families) and compared to direct ophthalmoscopy. Birds were examined with combinations of different restraints (manual restraint or fixation on a holding device) and different anaesthesia regimes (none, sedation, general anaesthesia). Inter- and intra-species specific variations of the general procedure, the restraint methods and the clinical findings were evaluated.

Results: OCT was possible in all examined avian species (from 40 g up to 7720 g) and superseded direct ophthalmoscopy in quality and quantity of ophthalmological findings. All restraint methods enabled OCT examination, however combination of general anaesthesia and a holding device provided the most rapid and subjectively the least stressful examination technique. Stability, stress reduction, head angle and distance from the OCT device were important factors influencing volume scanning quality. Sixteen, out of 39 birds, presented ocular abnormalities detected by OCT (compared to only five birds when using direct ophthalmoscopy). OCT with included fundus images offered an objective assessment of retinal changes. Retinal abnormalities included changes of fundus pigmentation, drusenoid changes and severe retinal and choroidal degenerations. Species-specific variations of retinal layer dimensions and of foveal structures were evident.

Conclusion: OCT is a promising, non-invasive method, which significantly compliments standard techniques. OCT is applicable to a wide variety of avian species; it provides high quality cross-sectional images of the retina, enabling accurate and improved diagnosis and prognosis of therapies. An assessment of the visual capabilities of traumatised birds is a major factor for their rehabilitation and survival in the wild. Finally, this method is an excellent tool in interdisciplinary retinal research, providing novel insights into the diversity of very specialized structural adaptations of avian retina.

Keywords: Avian ophthalmology; Direct ophthalmoscopy; Optical coherence tomography; Raptor; Retina

Introduction

Integrity of the visual system is of great importance for free-living birds, particularly raptors [1,2] and their successful rehabilitation and release back into the wild. Ophthalmology of wildlife birds is therefore important and research has been carried out in a different species in several large cohort studies [3,4]. A recent study investigated several free-living raptors by applying an extensive protocol and supplying overview data [5]. Additionally, similar clinical data is present for a small cohort of captive bald eagles [6] and a colony of screech owls [7]. However, optical coherence tomography (OCT) was not applied in these studies. The current investigation aims to address this gap by examining wildlife birds with this novel tool enabling in-depth retinal imaging.

In comparison to other eye diseases, retinal disorders are often under-diagnosed due to the small size of the avian eye and the difficulty in fully accessing the posterior segment of the eye and retinal layers in particular, e.g. due to species dependent globe size and shape (i.e. flat or tubular globes). Although variations of normal avian eye structures have been described [8-11], detailed descriptions of the *in vivo* retina and inter- and intra-species specific variations are missing. Additionally, there are only restricted descriptions of retinal pathologies in living birds [12,13]. Precise assessment of retinal disorders has thus far been limited to histopathologic evaluation of post-mortem tissue [14-20].

Frequently occurring retinal abnormalities described in different avian orders (e.g. Galliformes, Strigiformes and Falconiformes) have been classified as posttraumatic [21-23]), developmental or hereditary [14,15,17,19], idiopathic [16], or as manifestations of systemic infectious diseases [20,21].

In daily general veterinary practice, retinal diseases are usually detected with direct or indirect ophthalmoscopy. Advanced modalities such as ocular ultrasound or electroretinography [7,5] are infrequently applied and do not provide retinal imaging. In human ophthalmology, optical coherence tomography is a standard procedure to detect and monitor retinopathies. OCT is a non-invasive measurement technique bridging the imaging resolution gap between ultrasonography and confocal microscopy, providing non-contact, high-resolution, cross-sectional images of biological tissues [24-27]. To date, only few studies present normal and pathological retinal structures in living birds using OCT [12,13] and mostly in chicken [28,29]. Recently, Lan and colleagues applied OCT to laboratory animals (chicken), measuring choroidal thickness in order to study a physiological question [30] and McKibbin and co-workers presented a high-quality application of OCT to an animal model (also laboratory animals (chicken)) investigating retinal degeneration [31]. All these studies have used different examination protocols, different OCT devices and moreover, only few individuals or avian species were included. The current study introduced OCT as a novel tool in clinical veterinary ophthalmology on a large free-living avian population. Furthermore, the present investigation assessed the application and efficacy of OCT in avian species addressing its limitations and its diagnostic value based on a large cohort in order to address implications of body weight and eye size.

Materials and Methods

Animals

The study was approved by Research and Ethics Committee of the local authorities (Faculty of Veterinary Medicine, University of Leipzig and Landesdirektion Leipzig) and was performed in accordance to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Birds were admitted to the Clinic for Birds and Reptiles, University of Leipzig, by zoo affiliated falconers or members of the public. All birds were medically assessed for suitability of complex OCT examinations and sedation procedures by an experienced veterinarian. Thirty-nine free-living birds (some of them were temporarily affiliated with a zoo), belonging to 21 species and weighing from 40 g up to 7720 g were included in this study. More than half of the examined birds (n=27) were diurnal or nocturnal raptors, which belonged to three different families (Table 1).

Procedures

After recording the history, each bird received a full body general examination as well as advanced examinations if indicated (i.e. parasitology, bacteriology, diagnostic imaging). Direct ophthalmoscopy was carried out in both eyes with the use of a simple monocular ophthalmoscope (Heine Beta 200, HEINE Optotechnik, Germany) by the same examiner. Afterwards the OCT examination was carried out with a spectral domain OCT system (Heidelberg SPECTRALIS® SD-OCT, Heidelberg Engineering GmbH, Germany) by the same handlers and examiner. Complete and comparable analysis of findings was exclusively based on the data of the right eye (OD) of all birds examined because in a few cases the full OCT examination protocol of the left eye was shortened to minimise overall examination time for the animal. Release back into the wild or euthanasia was decided based upon standard criteria (i.e. general condition, severity of injury, reconstitution and visual integrity).

OCT device and OCT settings

OCT scan setting options include star-shaped line scans, line scans and volume scans. Volume scans offer additional information and provides better resolution over a larger area. Furthermore, the integrated confocal scanning laser ophthalmoscopy enabled infrared reference fundus images of each region examined. In the present study the following scan settings were used for analysis: for every bird volume scanning with OCT was carried out with a field size of 15° (temporal-nasal) × 5° (superior-inferior), images were acquired in High Speed mode, therefore every B-scan contained 384 A-scans, corresponding to a distance between the single A-scans of 0.039°. 131 B-scans were acquired within the specified field, resulting in a distance between individual B-scans of 0.038°. Further details (e.g. full details on resolution, scanning procedure, OCT device) have been published elsewhere [13]. Such a volume scan takes approximately 1 minute under optimal conditions (i.e. without interruptions due to large eye movements or body movements).

Animal handling during OCT examination

OCT examination was conducted with dimmed room lighting to establish a calm environment for the birds. To ensure the desired stability, different forms of restraint (holding method and with or without chemical measures) in various combinations were applied. These combinations are listed in Table 2. The restraining method was chosen without pre-selection criteria on the basis of potential affinity to stress and according to the bird´s personality, body size and general condition. For OCT examination, birds were manually held or secured with the aid of a stabilizing-plate (Figure 1) and chemical restraint consisted of sedation (intranasal midazolam) or general anaesthesia with isoflurane (details of dose and application see Table 2).

Regardless of the restraining method applied, birds were always held in an upright position to facilitate normal respiration (Figure 1). Saline solution was used to safeguard corneal moisture and integrity. In general, an experienced avian veterinarian is needed in order to identify and act in anaesthetic emergencies.

Assessment of ophthalmological findings

Findings on ophthalmoscopy were compared to the information provided by the OCT examination, this was assessed in three steps: (i) simple numeric comparison of pathological findings revealed by monocular direct ophthalmoscopy (compared to published funduscopic images; [22]) and OCT; (ii) retinal alterations and pathological changes detected by OCT were categorised based on human OCT terminology and the consequences for visual impairment were estimated on the basis of experiences with human retinopathies; (iii) visual behaviour assessment by two experienced veterinarians (e.g. night vision, binocular focus, orientation) of the birds was carried out twice daily using practical subjective techniques (e.g. discrete observation, behaviour with absence or presence of a human, flight capacity, capture/obstacle avoidance, food intake and progressive weight gain).

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Ordo	Familia	Species	No.	Age class[b]	pathological changes[b]
I[c]	Accipiters (*Accipitridae*)	Black kite (*Milvus migrans*)	1	adult	n.c.d.[a]
		Red kite (*Milvus milvus*)	2	all adult	n.c.d., drusenoid & pigment changes
		Goshhawk (*Accipiter gentilis*)	1	juvenil	n.c.d.
		Sparrowhawk (*Accipiter nisus*)	1	adult	n.c.d.
		White-tailed Sea Eagle (*Haliaaetus albicilla*)	1	adult	n.c.d.
		Common Buzzard (*Buteo buteo*)	5	all adult	n.c.d. (3), retinal degeneration (1), retinal detachment (1)
	Falcons (*Falconidae*)	Kestrel (*Falco tinninculus*)	6	juvenile (5) adult (1)	n.c.d. (4), pigment changes (1), drusenoid change (1)
		Peregrine falcon (*Falco peregrinus*)	3	all juvenile	n.c.d. (1), pigment changes (1), drusenoid changes (1)
II	Ducks (*Anatidae*)	Mute swan (*Cycgnus olor*)	1	adult	drusenoid change
III	Corvids (*Corvidae*)	Hooded crow (*Corvus corone cornix*)	1	juvenile	pigment changes
		Carrion Crow (*Corvus corone corone*)	4	juvenile (3), adult (1)	n.c.d. (3), retinal degeneration (1)
	Thruses (*Turdidae*)	Common Blackbird (*Turdus merula*)	1	juvenile	n.c.d.
IV	Plovers (*Charadriidae*)	Northern Lapwing (*Vanellus vanellus*)	1	adult	n.c.d.
V	Pigeons (*Columbidae*)	Pigeon (*Columba livia domestica*)	1	adult	n.c.d.
VI	Typical Owls (*Strigidae*)	Eagle Owl (*Bubo bubo omissus*)	2	all adult	n.c.d. (2)
		Tawny Owl (*Strix aluco*)	1	adult	drusenoid changes
		Long eared Owl (*Asio otus*)	3	all adult	drusenoid changes (1), RPE-detachment, drusenoid changes (1), pigment & drusenoid changes (1)
	Barn Owl (*Tytonidae*)	Barn Owl (*Tyto alba*)	1	adult	retinal & choroidal changes
VII	Swifts (*Apodidae*)	Commmon Swift (*Apus apus*)	1	adult	mild RPE-detachment
VIII	Hoopoe (*Upupidae*)	Hoopoe (*Upupa epops*)	1	adult	n.c.d.
IX	Woodpeckers (*Picidae*)	Great Spotted Woodpecker (*Dendrocopos major*)	1	juvenile	n.c.d.

[a] n.c.d. no changes detected
Ordo/Order: I – Birds of Prey (Falconiformes); II – Waterfowl (Anseriformes); III – Passerine (Passeriformes); IV – Plover like Waders (Charadriiformes); V – Plover and pigeons (Columbiformes); VI – Owls (Strigiformes); VII – Swifts (Apodiformes); VIII - Raven-like birds (Coraciformes); IX – Woodpeckers & allies (Piciformes)
[b] number in brackets represents the number of birds affected
[c] According to a publication by Hackett et al. (2008) falcons belong to a separate order besides birds of prey. In the current publication the conventional classification was employed, listing them as different families.

Table 1: Avian species examined and their OCT abnormalities.

Results

The most common reason for admission to the clinic were trauma (n=23, 59%) followed by orphanage or post-fledging emaciation (n=11, 28%) and systemic disease (n=5, 13%). From the 39 birds, 14 birds (36%) were released back to nature, 14 were sent to rehabilitation facilities for long-term care and eleven passed away (28%, three birds died naturally and eight birds were euthanized due to their poor general health resulting from their injury). Overall, 16 birds (41%) of the 39 were diagnosed with an ocular abnormality. 13 (81%) of these were admitted due to trauma and three were orphans. Of the birds with ocular abnormalities, seven (44%) were released, seven were sent

Fixation and anesthesia	Description	Human resources	No of Birds
Manual restraint of unsedated bird	The bird´s head held gently between thumb and index finger of one hand, while the extremities held among the fingers of the other hand. Eyelid kept opened with a moistened cotton-tip applicator. A soft towel/tissue was rolled to the birds, to prevent plumage damage.	1) OCT operator 2) Bird handler 3) Person holding the eyelid open	14
Non-sedated bird on a fixation plate	Stabilization plate: a light, wooden frame (length: 38 cm, width: 25 cm) with three horizontal movable wooden bars to support the bird´s dorsum. The head/neck held by an assistant to correct positioning. The body was strapped on with elastic bands. Legs stretched on two soft cushioned modified wooden processes of the frame (Figure 1). Eyelid kept opened with a moistened cotton-tip applicator.	1) OCT operator 2) Bird handler/Person holding the fixation plate upright 3) Person holding the eyelid open	1
Manual restraint of sedated bird	Manual restraint as in the first method described. Eyelid kept opened with a moistened cotton-tip applicator. Sedation: Intranasal midazolam (2-6 mg/kg, Midazolam-ratiopharm® 5 mg/1ml, Ratiopharm GmbH, Germany) reversed by intranasal flumazenil (0.05 mg/kg, Anexate, vial 0.5 mg/ml, Roche Ltd, UK) or combination of intranasal midazolam and intramuscular butorphanol (1 mg/kg, Toburgesic, 10 mg/ml, Fort Dogde, USA) reversed by intranasal flumazenil (0.05 mg/kg, Anexate, vial 0.5 mg/ml, Roche Ltd, UK).	1) OCT operator 2) Bird handler 3) Sedation monitoring and holding the eyelid open	5
Sedated bird on a fixation plate	Sedation as in the third method described. Stabilization plate as in the second method described.	1) OCT operator 2) Bird handler/Person holding the fixation plate upright, sedation monitoring and holding the eyelid open	5
Manual restraint of anesthetised bird	Manual restraint as in the first method described Anesthesia: Facemask and standard isoflurane (induction 5%, maintenance 1.5-2.5%, Forane USP, Baxter Healthcare Corporation, USA) and oxygen (induction 2 l/kg, maintenance 0.8-1 l/kg flow) concentrations. Intubation and basic anesthetic monitoring by vet (heart rate, respiratory rate, body temperature, ventilation).	1) OCT operator 2) Bird handler 3) Anaesthesia and intubation monitoring and holding the eyelid open	6
Anesthetised bird on a fixation plate	Anesthesia as in the fifth method described. Fixation plate as in the second method described.	1) OCT operator 2) Bird handler/Person holding the fixation plate upright and holding the eyelid open 3) Anaesthesia and intubation monitoring	8

Table 2: Fixation methods and anesthesia used for OCT examination.

to rehabilitation facilities, and two (12%) were euthanized (none of those due to their ocular abnormalities).

Animal handling during OCT examination

Both, manual and stabilizing-plate supported (Figure 1) restraint permitted OCT examination. The anaesthetic protocol (A) and restraint method (B) was decided based upon if a bird was prone to stress or not and secondly, upon the size of the bird. (A): It was therefore preferred to examine species prone to stress (e.g. sparrow hawk, lapwing, hoopoe, woodpecker, swift) under general anaesthesia, to reduce potential agitation. Manual restraint under sedation was practical in larger, potentially dangerous species (e.g. sea eagle, eagle owl). (B): In general, manual restraint of a non-sedated bird could be applied to small (e.g. blackbird, woodpecker, and some baby raptors) and mid-sized birds (e.g. pigeon, red kite; body weight ≤ 1.000 g) or larger but docile species (e.g. waterfowl, non-raptor species). The fixation plate, due to its size, was applicable only in mid-sized species (e.g. barn owl, long-eared owl, crows, peregrine falcon, sparrow hawk) but impractical for smaller or larger species. General anaesthesia offered the shortest OCT examination times (approximately 10-15min) and optimal stability for volume scanning, sedation offered some relaxation and anxiolysis (averaged OCT examination times of 15-20 min) and therefore presented with improved stability plus reduced examination time compared to sole manual restraint (averaged OCT examination times of 25-30 min; for a survey of the procedures see Table 2).

Instability during the measurement could be caused by sources of excitation for the bird, for example by potential hand pressure during manual fixation or external stimuli. Such as the frequency of the galvano scanner voltage necessary to produce the infrared overview image alongside the OCT scan caused an inherent high-pitched noise during OCT measurement. Furthermore, the bird could possibly be affected visually by the fixation guiding light or the scanning laser during the measurement itself. Human resources for examination were not significantly different between the various combinations (i.e. 2-3 persons; Table 2). In most cases three examiners were needed: one examiner carried out the OCT measurements, one examiner attended to holding the bird and the third person monitored anaesthesia or sedation and/or kept the eyelid open. The restraint combination where only two examiners were able to carry out the desired measurements was the fixation of a sedated bird on the stabilizing device. Here, additionally to the examiner taking the OCT measurements, one examiner held the bird´s head with one hand and kept the eyelid open (with a moistened cotton-tip applicator) with his other hand (Figure 1).

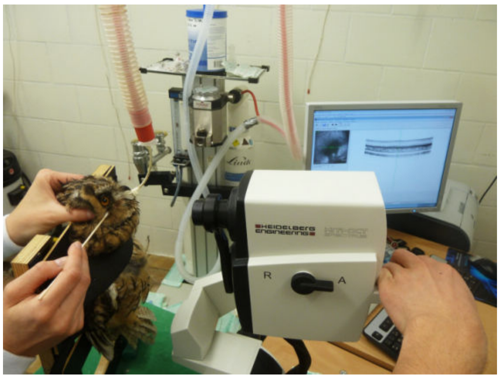

Figure 1: Photograph of the OCT device, fixation plate and anesthesia circuit used in this study. In this picture a long-eared owl (*Asio otus*) is under examination. The bird was anesthetized, and only one person was holding the plate and the animal's head, and kept the eyelid open. The original distance of the OCT lens during the volume scan was smaller.

The same examiner was able to simultaneously monitor sedation and anaesthesia. No major anaesthetic complication or anaesthetic-related death occurred during the study.

Ophthalmological evaluation and diagnostic value of OCT

The OCT examination was applicable in all cases and provided high quality images for central and most peripheral retinal areas, the pecten, the central and temporal foveae or the area centralis. Interspecies variations were evident in foveal structures (Figure 2), foveal dimensions, foveal locations or by the existence of an area centralis (e.g. pigeon; Figure 2). Retinal layer dimensions detected by OCT displayed minor variations between different species. An overview of the ophthalmological findings of each bird species is presented in Table 1. A quantitative comparison of both ophthalmological methods to detect pathological findings is given in Table 3.

Ophthalmoscopic method	No. of birds examined	No. of birds with presumed visual integrity	No. of birds with pathological changes[a]
Direct ophthalmoscopy	39	34	5
Infrared fundus image and OCT volume scan	39	23	16

[a] the classification of pathological changes indicates any alteration from the normal retinal structure (e.g pigment or drusenoid changes, retinal degeneration or detachment, RPE-detachment or choroidal changes).

Table 3: Quantitative overview of the pathological changes of the right eye (OD) in free-living birds with two different ophthalmological examination methods.

Comparably, the findings detected by OCT outnumbered the observations by monocular ophthalmoscopy. The most common pathological findings by direct ophthalmoscopy were (i) retinal degeneration, (ii) retinal detachment, (iii) pigmentation changes of the fundus and (iv) bleeding. OCT imaging and infrared funduscopy provided additionally the possibility to discriminate: (i) retinal alterations in different retinal layers (e.g. the photoreceptor segments or inner retinal layers), (ii) drusenoid changes, (iii) grade of retinal neurodegeneration as well as (iv) detection of intra-retinal and/or intra-choroidal schisis (Figures 3 and 4). Descriptions of structural, morphological and biochemical changes were part of a process termed retinal neurodegeneration as a collective term. A drusen-like appearance was termed "drusenoid" throughout.

In general, retinal discoloration, and hyper- or hypo-pigmented changes respectively hyper- or hypo-reflective changes of the fundus were detected with both techniques employed and in five cases the fundus pigmentation was dramatically altered and inhomogeneous. OCT volume scanning enabled the accurate differentiation of funduscopic alterations and aided correct identification of severity. An example of this is presented in Figure 4. Here, funduscopy indicated two areas of different altered pigmentation near the pecten and the OCT scans revealed an unexpected different extent of neurodegeneration in the retinal tissue.

Pigmented round dots in the fundus were observed in a kestrel pulli (35 days old), a juvenile peregrine falcon, a juvenile red kite and a hooded crow. These pigmentary changes were observed on ophthalmoscopy and on the infrared reference fundus images of each region examined. However, after analysing the OCT images it was apparent that these areas possessed complete retinal integrity and it was therefore suspected that these pigmentary alterations present variations of the norm and are not related to disease processes. In favour of this, these four birds appeared to have clinically normal vision. This was concluded by assessing their flight behaviour in a large aviary, their visual perception of external visual stimuli (obstacles, food and handler), their weight stability and their interactions with conspecifics (i.e. crow, kestrel and peregrine falcon).

OCT enabled detailed imaging of the vitreous body. Vitreous body retraction and its irregular border shape were observed in many young and adults birds with and without retinal pathologies. Furthermore, OCT enabled determination of punctate drusenoid changes (Figures 3A and 3B) of the fundus and their localisation within the retinal layers and/or the subretinal space.

The classification of the ocular changes presented in this study, as well as the assessment of their impact on visual acuity/behaviour, is summarized in Table 4. All birds had the respective predicted food intake and weight gain during their treatment period. In 38 birds, visual behaviour (moving around, avoidance of a person or a hand, grasping of food items in raptors) was as expected for a free-ranging bird. In one case (carrion crow) the bird was unwilling to move or fly, even if coaxed, and was easily caught if approached from the affected side). This might be associated with the severity of his trauma (hit by stone) and/or potential concussion or general weakness. Nevertheless, the bird could feed itself.

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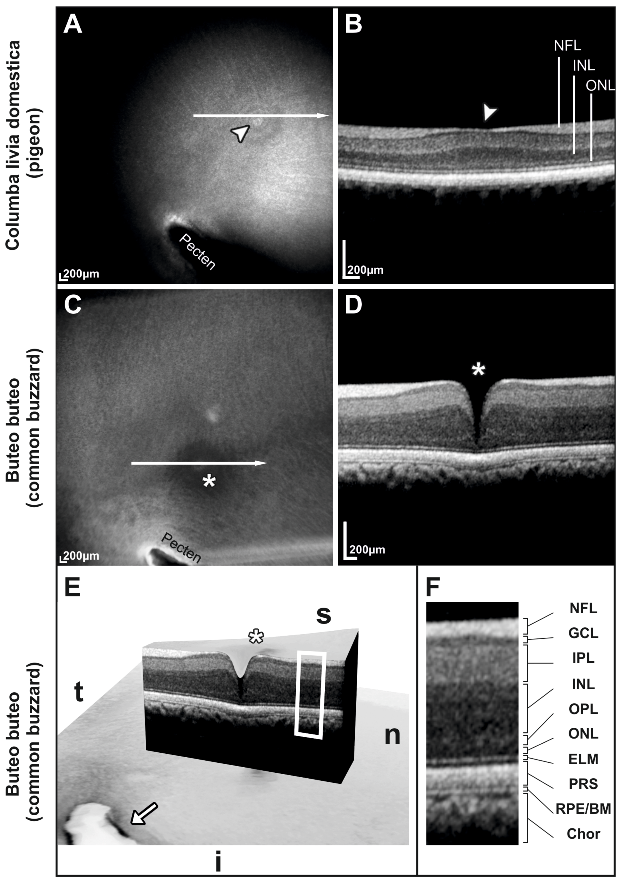

Figure 2: Images of OCT and fundus examinations of healthy retinas from two different bird species. **A**: Image of infrared funduscopy of a pigeon (Columba livia domestica) enables a general overview of the location of the Pecten oculi and the area centralis (arrowheads in A and B). The arrow in A indicates position and orientation of the 2D OCT scan shown in B. **B**: The OCT image reveals detailed morphological information: the inner and outer nuclear layer (INL and ONL) in the central area increases in thickness, whereas the thickness of the nerve fibre layer (NFL) decreases to a minimum. **C**: Infrared image of the fundus oculi of a common buzzard (Buteo buteo). The asterisks in C-E indicate the location of the central fovea. **D**: 2D OCT scan of the deep convexiclivate fovea centralis of the common buzzard located at the position shown by the arrow in C. **E**: Series of OCT-scans can be transformed into a 3D image of the examined retinal area (same bird as in C and D; s superior, i inferior, n nasal, t temporal). The arrow indicates the Pecten oculi. F Precise retinal layer structure labelling of the same bird: NFL: Nerve Fiber Layer; GCL: Ganglion Cell Layer; IPL: Inner Plexiform Layer; INL: Inner Nuclear Layer; OPL: Outer Plexiform Layer; ONL: Outer Nuclear Llayer; ELM: External Limiting Membrane; PRS: Photoreceptor Segment; RPE/BM: Retinal Pigment Epithelium/Bruch's Membrane complex; Chor: Choroid.

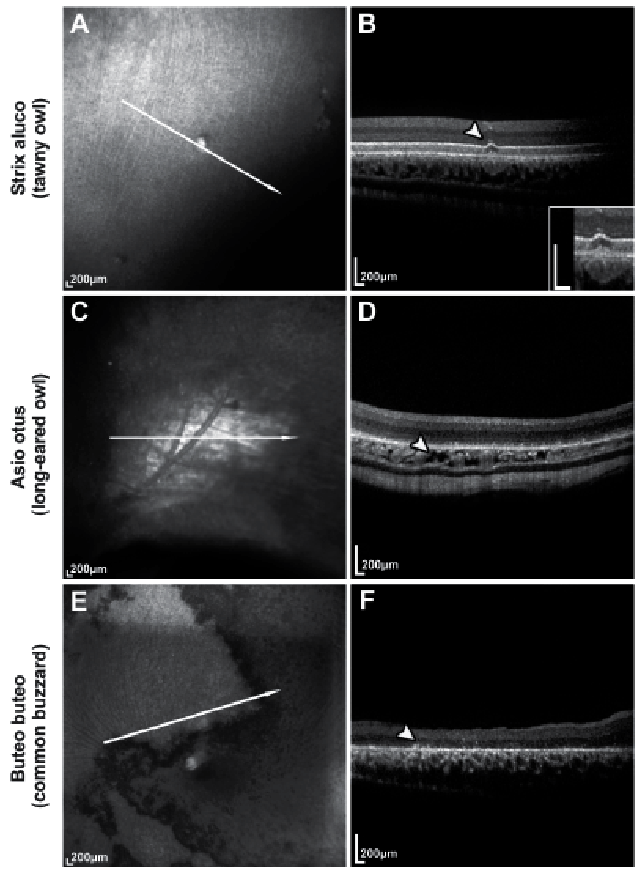

Figure 3: Images of infrared funduscopy (left column: A, C, E) and of 2D OCT scans (right column: B, D, F) show various morphological and pathological alterations in retinal and sub-retinal regions in eyes from three different bird species. **A** and **B** Bright spots in the fundus image of a tawny owl (Strix aluco) appear as sub-retinal drusenoid changes (arrowhead in B) in 2D OCT image (magnified in the inset). **C** and **D** Infrared fundus image and OCT of a long-eared owl (Asio otus) revealed pigmentation abnormalities (visibility of choroidal blood vessels). The arrowhead in D indicates the corresponding area in the 2D OCT image: related to the depigmented fundus area (C), one might assume washed-out and less distinguishable photoreceptor cell layers and this therefore might represent some evidence for retinal degeneration. **E** and **F** The infrared fundus image of a common buzzard (Buteo buteo) shows an extensive pathological altered fundus. 2D OCT reveals distinct atrophic neurodegeneration of the retina and loss and thinning of the retinal layers in the altered fundus areas (arrow head). White arrows in A, C, D indicate the orientation of the corresponding OCT-scan in the right column.

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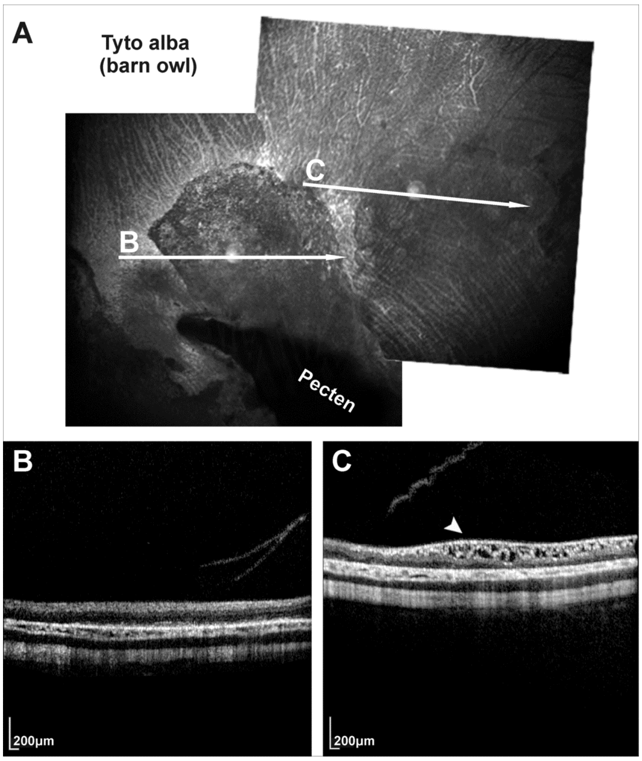

Figure 4: Arranged image of two adjacent infrared fundus pictures (A) of the right eye of a barn owl (Tyto alba) and the corresponding 2D OCT images (B and C). The white arrows indicate the orientation of the OCT scans. The infrared funduscopy reveals an extensive central area of considerably altered fundus pigmentation near the pecten (white arrow B in A) and a less conspicuous area of changed pigmentation beside it (white arrow C in A). Noticeable, the OCT scans indicate no such obvious pathological changes in the severe altered pigmentation area (B), whereas the less conspicuous area shows clearly disorganization with oedemantous gaps which can be characterized as intra-retinal degenerations (arrowhead in C).

Discussion

The current study focused on the assessment of the applicability, practicability, limitations and diagnostic value of the OCT in a wide variety of free-living birds. In this study different native European avian species were included to assess variability of body shape/weight, head/eye ratio, eye size, living environment and behaviour. And in fact, our study demonstrates the applicability and usefulness of OCT examinations in avian ophthalmology. However, all the above-mentioned species variations make it impossible to recommend a unique optimum procedure of OCT examination for all individual species.

OCT procedure, restraint and anaesthesia

In our study we tested two restraint methods in combination with two different general anaesthesia regimes (sedation vs. anaesthesia; see Table 2) to obtain high quality OCT images and to avoid excessive stress for the birds. One study applying OCT to avian species living in falconry captivity indicated that simple manual restraint of a non-sedated bird enabled qualitative OCT volume scanning [12]. In our experience, birds were agitated by the measurement with the OCT device in addition to the expected examination stress. Non-sedated or even sedated birds seemed to be stressed either from manual restraint, noise or the laser fixation light of the OCT device. This excitation of the birds produced instability, which may make it more difficult to obtain qualitative high resolution OCT volume scans and prolonged the overall duration of the procedure. Therefore, a bird-friendly holding device was designed and applied to advance stability and reduce human handling. However, the use of the fixation plate was limited by the species size and personality of the bird. In general manual restraint or restraint of non-sedated birds (specifically docile species) on a fixation device presented no important difference between these two techniques. On the other hand, the combination of fixation plate and sedation could possibly reduce the number of examiners to a minimum of two. In general, all applied restraint combinations needed three examiners (Table 2). Future research should focus on optimizing the OCT examination procedure for individual avian species and possibly, on an improvement of a holding device.

The current study indicated that faster volume scanning and best results were obtained in anesthetised birds. Isoflurane anesthesia, employed in this study, is a common procedure in avian routine, familiar to all avian practitioners. As alternative to intubation, air sac anaesthesia could be used, but was not employed in the current study due to its invasiveness possibly elongating time in captivity. Air sac anaesthesia has previously been found to provide more space to the head area since the anaesthetic circuit and intubation is on the body side [22]. Additionally, general anaesthesia supports mydriasis. Intracameral injection of vecuronium bromide, as proposed in another study [28], was avoided in our study.

The duration of an actual volume scan itself was similar to other studies [12,29], lasting only a few minutes. In general, the overall procedure duration (restraint/anaesthesia induction, best positioning etc.) as well as the OCT image quality is dependent on the experience of the OCT operator, the coordination of the involved staff and the familiarity with the species head and ocular anatomy.

Ophthalmological findings

The clinical results of monocular ophthalmoscopy of the current study are in concordance with previous studies regarding the prevalence of generalised trauma as well as that of ocular disorders in free-living raptors and birds in general [3-4,21-22,32-34]. Most of the ocular pathologies found were subsequent of blunt trauma and consistent with already described pathology [3-4,23,33]. The added diagnostic value of OCT, in contrast to the commonly used technique of direct ophthalmoscopy was evident from the presented study. OCT depicted precisely the retinal layers and posterior eye segment structures such as vitreous body, pecten, optic nerve, foveae and choroid, as indicated also in previous studies [12,28-29]. Clinically, the OCT provided more anatomical information on trauma localization and its extension within the retinal layers and the choroid, enabling a faster diagnosis, prognosis and decision-making for critical patients. In some difficult cases it contributed to a more accurate prognosis, as in the example of the barn owl (Figure 4). Additionally, the extraction of high quality 3D videos and infrared fundus images of the avian retina offered a complete retrospective evaluation, analysis and documentation.

No. of birds with detected ophthalmological abnormalities[a,b]	**No. of birds with presumed mild or severe visual impairment [b]**		**No. of birds with presumed reduced visual acuity (evaluated by behavior)**
	mild (birds with pigment/drusenoid[c] changes mainly)	severe (birds with a considerable area of neurodegeneration[d])	
16	11	5	1
- red kite (1)	- red kite (1)	- common buzzard (2)	- carrion crow
- common buzzard (2)	- kestrel (2)	- carrion crow	
- kestrel (2)	- peregrine falcon (2)	- long-eared owl	
- peregrine falcon (2)	- mute swan	- barn owl	
- mute swan	- hooded crow		
- hooded crow	- tawny owl		
- carrion crow	- long-eared owl (2)		
- tawny owl	- common swift		
- long-eared owl (3)			
- barn owl			
- common swift			

a the term 'detected abnormalities' indicates any alteration from the normal retinal structure present (e.g. pigment or drusenoid changes, retinal degeneration, retinal detachment, RPE-detachment, or choroidal changes)

b number in brackets represents the number of birds

c a drusen-like appearance was termed 'drusenoid' as a collective term throughout

d structural, morphological and biochemical changes were a part of a process termed retinal neurodegeneration as a collective term

Table 4: Qualitative overview of the pathological changes in free-living birds (identified by OCT) and its presumed consequence for visual acuity.

OCT revealed key aspects of additional information to evaluate fundus pigment changes and to investigate drusenoid changes. Furthermore it gave a novel overview of the vitreous body in the birds examined, for example, vitreous retraction and irregular border shapes in birds seem to be a normal feature and are not generally associated with retinal pathologies as often observable in human eyes [35]. Furthermore, drusenoid changes can be compared with drusenoid alterations observed in older human patients [36-37]. To our knowledge, drusenoid retinal changes in bird eyes were not explicitly described in the literature and are not routinely diagnosed by direct or indirect ophthalmoscopy in living birds. Therefore, OCT examination offers a new possibility to correlate drusenoid changes with assumed aetiologies and influences such as light regimes, food, genetic factors and/or aging.

Nevertheless, the diagnostic findings of the OCT examination, as well as their impact on visual performance of the avian patient should in any case be evaluated further based on the bird's behaviour. For example, two owls in this study (i.e. barn owl and long eared owl) with unremarkable behaviour but with presumed visual impairment of various degrees (Table 4) might have balanced their visual disability with their advanced echolocation. Therefore, a final clinical decision (release, long care facility or euthanasia) should be still based upon overall visual acuity, behaviour and health of the patient additionally to ophthalmological findings or OCT.

Although the current price of an OCT device is a limiting factor for the integration of OCT in routine avian practice, it could be proposed as an advanced diagnostic tool for valuable breeding birds (in ex-situ conservation projects or falconry birds) or in cases of rare wildlife casualties. The OCT diagnostic add substantial possibilities to improve accuracy of diagnosis, therapy and prognosis in avian ophthalmology.

Conclusions

OCT is a valuable, applicable, non-invasive method and therefore a promising tool for the detection of retinal abnormalities in free-living avian species, which present significant variation in shape, size, behaviour and stress reaction. The OCT requires extensive financial and human resources. However, the diagnostic value clearly supersedes monocular ophthalmoscopy. Further research on restraint methods and interpretation of retinal abnormalities are needed to standardize and promote OCT application.

OCT will greatly advance the diagnostic and prognostic accuracy of the assessment of retinal alterations in traumatised birds. An accurate prognosis of the visual capabilities of free-living birds is a major factor for their rehabilitation and survival in the wild. Moreover, breeding programs of rare and endangered species will also greatly benefit from OCT investigations: many retinopathies and ocular diseases are inherited and their dissemination in the population can thus be prevented by OCT examination of the potential parents prior to breeding. Similar as in human medicine, OCT, may also be used to learn more about the time course and progression of retinal diseases in birds, as well as for an early diagnosis of systemic diseases which induce retinal symptoms. Finally, this method also is an excellent tool in interdisciplinary retinal research, as it provides novel insights into the diversity of very specialized structural adaptations of the avian retina.

Conflict of Interest Statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

The authors would like to thank Heidelberg Engineering GmbH, Heidelberg, Germany for the loan of the OCT device to make this study possible. Furthermore, the authors would like to acknowledge the support of the veterinarian staff of the Clinic for Birds and Reptiles, University of Leipzig and we wish to thank the anonymous members of the public for rescuing the avian patients.

The work presented in this publication was kindly supported by funding from the German Federal Ministry of Education and Research (BMBF, 1315883 to CK, NK, MF), from the Deutsche Forschungsgemeinschaft (GRK 1097/1 and 1097/2 to AR, BW; RE 849/17-1 to AR and FR 1825/1-1) and the Medical Faculty of the University of Leipzig (Project No. 990101-089, Admin. No. 78190941 to WV).

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