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The retina of the collared peccary (*Pecari tajacu*): structure and function

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Abstract

Objective To study retinal morphology and function in the collared peccary, an ungulate species distantly related to the domestic pig.

Animal studies Twenty captive peccaries anesthetized for routine health examinations.

Procedures No abnormalities were noted on a complete ophthalmic examination. Fundi were examined ophthalmoscopically and photographed. The eyes of an individual that died of unrelated, nonocular reasons were studied histologically and by immunohistochemistry. Scotopic, mixed rod–cone, and photopic electroretinography (ERG) responses were recorded using the 'QuickRetCheck' (n = 6) and 'Dog diagnostic' (n = 5) protocols of the Handheld Multispecies ERG (HMsERG).

Results The fundus of the peccary is atapetal, with varying amounts of pigmentation seen ophthalmoscopically, and histologically in the retinal pigment epithelium (RPE) and choroid. The retina is holangiotic with dichotomously branching vessels. These cross, and apparently loop on, the optic disk surface, but no venous circle was seen. Immunohistochemistry suggests a high concentration of cone photoreceptors with red/green cones being more abundant than blue cones. Rod ERG responses were very low with no evident dark adaptation. Mixed rod–cone and cone ERG response amplitudes were low compared to those of domestic pigs, but quite similar to those of minipigs.

Conclusions To the best of our knowledge, this study describes the collared peccary's retinal features for the first time. A comparison of our findings with data from other ungulate species shows some similarities between the peccary and pig retinas. Further studies are warranted to determine whether the peccary can be used alongside the pig as an animal model in retinal studies.

Key Words: animal model, cones, electroretinography, ERG, pig, ungulate

INTRODUCTION

The collared peccary (*Pecari tajacu*) is an ungulate species that inhabits the Amazon region and most of South and Central America, extending into the southern United States. It is the most widespread species of the Neotropical family Tayassuidae that together with the Old World family Suidae (which includes the domestic pig, *Sus scrofa*) forms the suborder Suiformes.[1–5] The peccary is classified as a stable species that easily adapts and reproduces in captivity and can therefore be found in many zoos.[1,6] In addition, the peccary is considered a dietary protein source for local communities in Latin America.[6]

McCowan *et al.*[7] reported on macroscopic and histologic findings in a peccary diagnosed with metastatic ocular transitional cell carcinoma. However, to the best of our knowledge, there are no published reports on the normal eye, or retinal function, of this species. Therefore, when a group of captive animals was anesthetized for a routine health examination and individual tagging, we decided to use the opportunity to study the retina of the collared peccary using indirect ophthalmoscopy, fundus photography, histology and immunohistochemistry (IHC) of an animal that later died of unrelated causes, and full-field electroretinography (ERG) recordings.

MATERIALS AND METHODS

Animals

The entire peccary population of the Tisch Family Zoological Gardens in Jerusalem, Israel, consisting of nine

males and 11 females (Table 1), was studied in the winter of 2013–2014, with ERG recordings performed at that time and again in February 2016. Animals live in an open, 200 m^2 yard that includes a house for shelter. The animals are fed vegetables, fruits, and herbivore pellets and have free access to water. Experimental protocols were conducted in accordance with the 'Statement on the Use of Animals in Research' of the Association for Research in Vision and Ophthalmology.

Anesthesia and ophthalmic examination

For purposes of this study, individual animals were herded into a squeeze cage where they were anesthetized with an intramuscular injection (5 mg/kg based on estimated body weight) of tiletamine HCl and zolazepam HCl (Zoletil 100, Virbac, Czech Republic). About 7 min after injection, the animal was removed from the cage and underwent a complete physical examination, including accurate weighing. Ears were notched, and a microchip was implanted subcutaneously for purposes of individual identification, and blood samples were collected for a complete blood count and serum biochemistry for routine health screening.

Animals underwent a full ophthalmic examination by a board-certified ophthalmologist or a veterinary ophthalmology resident, including slit-lamp biomicroscopy (SL-15, Kowa Co. Ltd., Tokyo, Japan) and applanation tonometry (Tono-Pen XL; Bio-Rad Inc., Santa Ana, CA, USA). Pupils were dilated (0.5% tropicamide, Mydramide, Fischer Pharmaceutical Laboratories, Tel Aviv, Israel) for indirect ophthalmoscopy (Omega 500, Heine, Herrsching, Germany) using a 20 D handheld lens and for fundus photography (SmartScope, Optomed, Oulu, Finland).

Table 1. Signalment of 20 peccaries studied

No.	Sex	Weight (kg)	Estimated age (years)*	Additional examination
1	M	15.9	1.0	ERG 'QuickRetCheck'
2	M	15.4	3.0	ERG 'QuickRetCheck'
3	M	19.1	2.0	ERG 'QuickRetCheck'
4	F	15.9	2.0	ERG 'QuickRetCheck'
5	F	16.4	4.0	ERG 'QuickRetCheck'
6	M	15.1	1.0	ERG 'QuickRetCheck'
7	M	28.6	10.0	ERG 'Dog diagnostic'
8	M	25.7	7.0	ERG 'Dog diagnostic'
9	F	18.9	5.0	ERG 'Dog diagnostic'
10	M	5.3	1.0	
11	F	21.0	3.0	
12	F	11.6	0.5	
13	F	16.7	7.5	
14	F	23.2	10.0	ERG 'Dog diagnostic'
15	M	8.4	0.5	Histology & IHC†
16	F	6.8	0.5	
17	F	14.8	4.0	ERG 'Dog diagnostic'
18	M	21.5	7.5	
19	F	20.2	11.0	
20	F	8.0	1.0	

F: female; M: male; ERG: electroretinography.
*Estimated age at initial ophthalmic examination and recording of the 'QuickRetCheck' protocol; the 'Dog diagnostic' protocol was recorded 21 months later.
†Performed 15 months after initial examination.

Histology

A juvenile male (Table 1) died of renal disease about 15 months after the study. Both globes were rapidly enucleated, and the remnants of periocular skeletal muscle, fat, and connective tissue were gently trimmed to expose the sclera and visualize the posterior ciliary artery. The globe was submerged in a 100 mL Davidson's solution (Glacial acetic acid, 10 mL; 95% ethyl alcohol, 30 mL; 10% neutral buffered formalin, 20 mL; distilled water, 30 mL) for 48 h. The eye then was placed in 70% ethanol solution for 24 h, transferred to 90% ethanol solution for 24 more hours, and finally transferred to 100% ethanol solution for 24 h.

The fixed globe was then cut open by an incision starting adjacent to the optic nerve and ending with the cornea, and perpendicular to the posterior ciliary artery. The trimmed specimen was fit into a cassette, paraffin embedded, and submitted for standard histologic preparation. Sections 4 μm thick, containing the retina and optic nerve, were cut and stained with hematoxylin and eosin (H&E).

Immunohistochemistry

Sections of the eye from the same animal were deparaffinized and incubated in a decloaking chamber (Biocare Medical, CA, USA) with 10 mM citrate buffer (pH 6.0) at 125 °C, blocked with phosphate-buffered saline solution containing 1% (W/V) bovine serum albumin, 0.1% (W/V) Triton X-100, and 10% (W/V) normal donkey serum, and subsequently incubated overnight at a temperature of 4 °C with anti-red/green opsin antibody (rabbit polyclonal, 1:100; Chemicon International), anti-blue opsin (goat polyclonal, 1:100; Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), and anti-rhodopsin (mouse monoclonal, 1:120; Thermo Fisher Scientific, Fremont, CA, USA). After washing, the following secondary antibodies were applied for 1 hour: DyLight 488 donkey anti-rabbit IgG (1:250), rhodamine red-X-conjugated donkey anti-goat IgG (1:250), and DyLight 649 donkey anti-mouse IgG (1:250; all from Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI)-containing mounting medium (Burlingame, CA, USA).

Electroretinography (ERG)

To avoid the confounding effect of previous light exposure,[8] recordings preceded other diagnostic procedures. Recordings were conducted in a darkened indoor facility, and the recorded animal was covered with a black drape for dark adaptation. The animal was positioned in left lateral recumbency, while eyelids of the right eye were retracted with an eyelid retractor. As the globe was

centrally positioned throughout anesthesia, a stay suture for globe centralization was unnecessary. Pupil was dilated (0.5% tropicamide, Mydramide, Fischer Pharmaceutical Laboratories, Tel Aviv, Israel), and a drop of 1.4% hydroxymethylcellulose (Celluspan, Fischer Pharmaceutical Labs, Tel Aviv, Israel) applied to improve conduction. Signals were recorded using a Jet contact lens electrode (ERG-Jet, Fabrinal SA, La Chaux-de-Fonds, Switzerland). Subcutaneous needles (CareFusion, San Diego, CA, USA) served as reference and ground electrodes and were placed 5 mm away from the ipsilateral lateral canthus and at the superior aspect of the pinna of the ear, respectively (Fig. 1). Impedance was kept under 5 KΩ. All recordings were conducted using a Handheld Multispecies ERG system (HMsERG, OcuScience, Henderson, NV, USA) with a bandpass of 0.3–300 Hz and with the same anesthetic protocol.

In 2014, rod and mixed rod–cone responses were recorded in six randomly selected animals (Table 1) using the HMsERG's preprogrammed 'QuickRetCheck' protocol. This protocol allows a very rapid evaluation of retinal function with a total running time of 18 s, excluding dark adaptation.[9] Following 10 min of dark adaptation, four flashes at 0.01 cd·s/m^2, presented at 0.5 Hz, were averaged to generate the single scotopic flash response. This was followed by two single flashes at 3 cd·s/m^2 and 10 cd·s/m^2 to generate the standard- and high-intensity mixed rod–cone responses, respectively.

As the 'QuickRetCheck' recordings yielded mostly immeasurable scotopic responses (see below), 2 years later, it was decided to repeat the recordings in five other animals (Table 1) using the HMsERG's preprogrammed 'Dog diagnostic' protocol[10] that allows a more comprehensive evaluation of both dark- and light-adapted signals. Under scotopic conditions, responses to 10 flashes, presented at 0.5 Hz at a light intensity of 0.01 cd·s/m^2, were recorded and averaged to generate a single scotopic flash response. This was repeated every 4 min for a total of five scotopic flash responses recorded over 20 min to generate a dark adaptation curve. Next, mixed rod–cone function was recorded; responses to four flashes presented at 0.1 and 0.05 Hz at two light intensities (3 and 10 cd·s/m^2) were recorded and averaged. Animals were then light-adapted for 10 min (30 cd/m^2), and cone function was recorded. Responses to 32 flashes, presented at 1 Hz (3 cd·s/m^2), were recorded and averaged to generate the single photopic flash response. This was followed by a cone flicker response (128 flashes presented at 30 Hz).

A- and b-wave amplitudes (μV) of the scotopic, photopic, standard-, and high-intensity mixed rod–cone responses were measured from baseline to the first trough and from that trough to the next positive peak, respectively. Implicit times (IT, msec), which are the respective time intervals between the stimulus onsets to the trough or to the positive peak, were measured to examine the response kinetics. Cone flicker amplitude was measured from the trough to the positive peak of the following flicker wave.

RESULTS

The animals included in the study are listed in Table 1. Mean ± SE weight was 16.4 ± 1.4 kg, and mean estimated age was 3.3 ± 0.6 years. No abnormalities were observed in any animal during the physical examination or in the blood samples. The ophthalmic examination of all eyes was unremarkable.

The peccary fundus is atapetal, with regions of varying pigmentation (Fig. 2). All fundi examined were pigmented, and no albinotic or subalbinotic fundi were documented; similarly, all animals had pigmented brown irises (not shown) and a dark hair coat (Fig. 1). The optic disk is round and dark. The retinal blood supply is holangiotic, with dichotomously branching vessels seen throughout the fundus, without visible bulging into the vitreal cavity. No avascular zone, which in some species characterizes the area centralis, was seen. In each eye, about 10–15 (presumed) arteries and five-six larger vessels, presumably

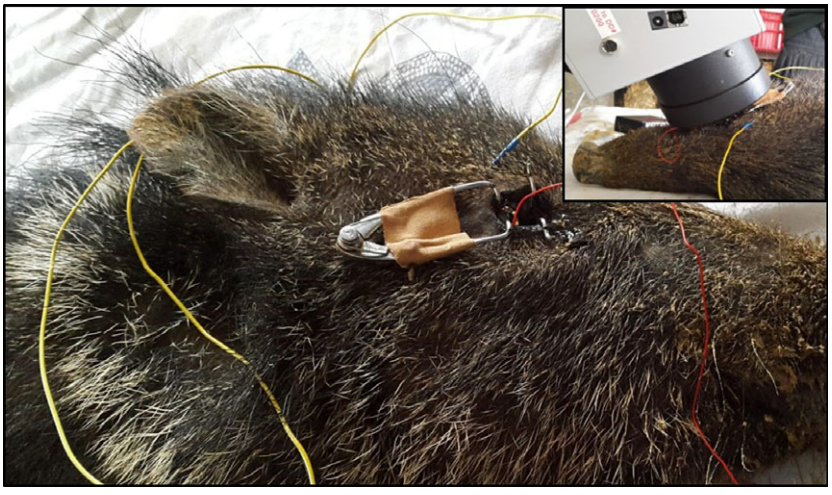

Figure 1. A peccary prepared for an ERG recording using a Handheld Multispecies ERG (HMsERG) system. Subcutaneous needles (yellow) were placed at the lateral canthus and the pinna of the ear and served as reference and ground electrodes, respectively. Lids were retracted, and signals were recorded with a Jet contact lens electrode (red). Distance between cornea and mini-Ganzfeld was approximately 1 cm.

veins, were seen encroaching onto the surface of the optic disk. No partial or complete venous circle was seen, but the larger veins formed vascular loops on the surface of the disk, sometimes extending into the peripapillary zone. Dark dots, similar to Stars of Winslow, were seen randomly dispersed throughout the fundus, but were more readily discerned in areas with less pigmentation. There were no noteworthy differences between two eyes of any individual, or between the eyes of individuals.

Histology

The optic nerve measured 1.4 mm in diameter as it exited through the sclera, but then it widened considerably, measuring 3.5 mm in diameter at its widest point, approximately 7 mm distal to the sclera (Fig. 3). Measurements were obtained postfixation. A gross comparison of the tissue before and after fixation suggests a 10–15% reduction in volume due to dehydration of the sample. This value is considered to be within the expected limits of volume reduction during modification of the tissue. The optic nerve fiber bundles exited the sclera through a single opening. The optic nerve in this area contained relatively thin strands of pigmented connective tissue, possibly scleral remnants. Similar to the related domestic pig and consistent with the funduscopic presentation, there was no visible tapetum lucidum, but there was a marked difference in pigment density in the retinal pigment epithelium (RPE). All of the examined RPE cells contained at least some pigment, but some (presumably in the ventral aspect of the globe) were pigment-rich while others (presumably in the dorsal aspect) were pigment-poor (Fig. 4). Regions of pigment-poor RPE were underlying relatively pigment-rich areas of the choroid. The rest of the retina contained no noteworthy features.

IHC

Retinal sections stained with anti-red/green and anti-blue opsin antibodies demonstrated protein expression mainly in the inner segments of cone photoreceptors, while staining with anti-rhodopsin antibody demonstrated protein expression in the outer segments of rod photoreceptors (Fig. 5). All analyzed sections revealed a high concentration of cone photoreceptors, with red/green cones being more abundant than blue cones.

ERG

Recordings using the 'QuickRetCheck' protocol yielded measurable single-flash scotopic responses in only two of six animals (animals #1 & #2); because of this small sample size, these were not averaged and are not presented. Furthermore, in animal #4, the mixed rod–cone responses were also discarded due to poor signal/noise ratio which made it impossible to analyze the signals. A- and b-wave parameters of the standard- and high-intensity mixed rod–cone responses recorded from the remaining five animals using this protocol are presented in Table 2.

As these recordings yielded mostly immeasurable scotopic responses, it was decided to repeat the recordings in five other animals using the 'Dog diagnostic' protocol. The five recorded animals had measurable scotopic, mixed rod–cone, and photopic responses. Averaged traces of the responses are presented in Fig. 6, and their a- and b-wave parameters are summarized in Table 2.

DISCUSSION

The aim of this study was to evaluate the function and structure of the peccary's retina, provide baseline retinal data for this species, and compare it to that of the

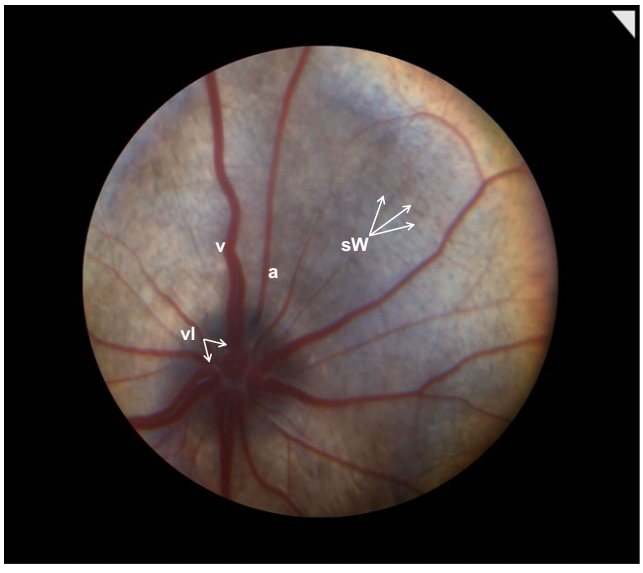

Figure 2. Representative fundus image of the right eye of animal #11. **v** – retinal vein, **a** – retinal artery, **vl** – venous loops, **sW** – Stars of Winslow (the black dots seen throughout the fundus).

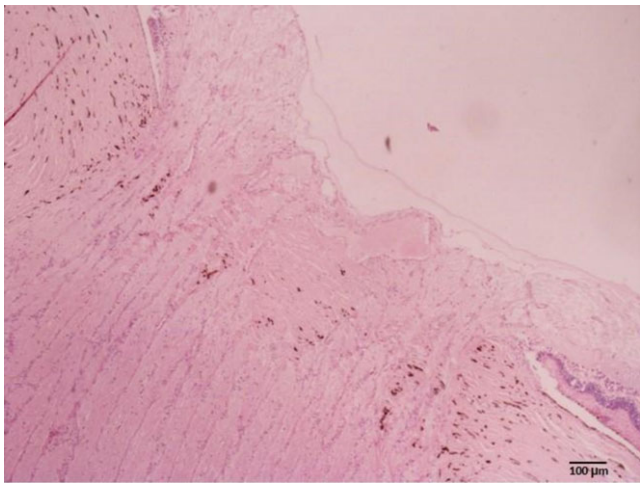

Figure 3. The proximal optic nerve of the peccary. There are thin strands of pigmented connective tissue, possibly scleral remnants, dispersed between the nerve bundles. One can appreciate the increased diameter of the optic nerve as it exits the globe. H&E, ×4.

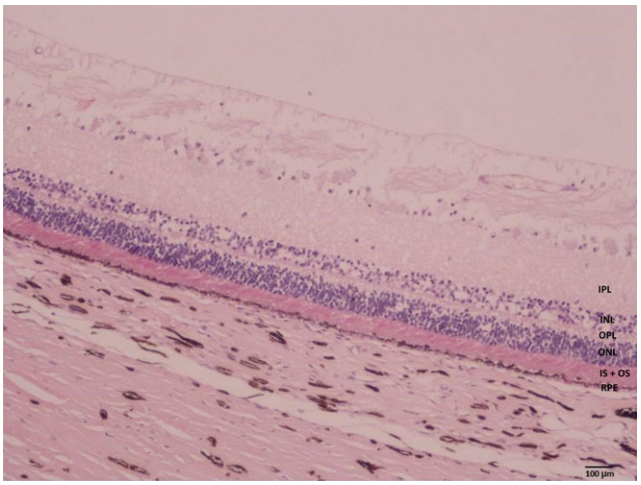

Figure 4. The retina of the peccary. A transitional zone between pigment-rich (on the left) and pigment-poor (on the right) retinal pigment epithelium can be seen. H&E ×4. INL, inner nuclear layer; IPL, inner plexiform layer; IS+OS, inner and outer segments of photoreceptors; ONL, outer nuclear layer; OPL, outer plexiform layer; RPE, retinal pigment epithelium.

domestic pig, a related ungulate that is a commonly used animal model and therefore has a well-studied retina.

The peccary's retinal structure was assessed by means of indirect ophthalmoscopy, fundus imaging, histology, and immunohistochemistry. Indirect ophthalmoscopy and fundus imaging of the peccary eye revealed an atapetal fundus, similar to that of the pig, a finding that is usually associated with a diurnal lifestyle.[11] All of the animals we examined had pigmented fundi; regions with varying degrees of RPE pigmentation were seen both histologically and ophthalmoscopically, and it seems that regions of pigment-poor RPE are surrounded by a relatively pigment-rich choroid. This choroidal pigmentation has been suggested as the cause of the gray reflectivity seen in the fundi of atapetal animals,[12] including our study animals (Fig. 2). In the domestic pig, in which many fundi are subalbinotic,[11,13] changes in choroidal melanocytes and melanosomes have been associated with aging and dietary concentration of trace elements.[14–16]

The retinal vasculature of the peccary is holangiotic with numerous arteries and veins encroaching onto the surface of the optic disk. The vasculature of the domestic pig's retina is quite similar, although the venous circle seen on the surface of the porcine disk[17,18] was not seen on the surface of the peccary optic disk. The looping of the larger veins on the surface of the peccary's optic disk is a feature that, to the best of our knowledge, has not been previously reported as a normal element of the fundus in other species. In humans, looping of retinal veins has been described as a rare manifestation of diabetic retinopathy[19] or a benign congenital anomaly[20] that presents in the retinal periphery and peripapillary regions, respectively, but not on the surface of the disk. Angiography is needed for a more detailed investigation of the peccary retinal vasculature, and to elucidate the apparent looping of some of the vessels on the surface of the optic disk and the suspected Stars of Winslow.[21]

IHC revealed a high concentration of cones in the peccary retina, with red/green cones being more abundant than blue cones. Similarly, the domestic pig retina has a high cone density, with blue cones making up an average of only 13.5% of the cone population.[22,23] Future studies using retinal flat mount staining may allow to calculate the densities of rods, the two cone populations, and retinal ganglion cells of the peccary retina and to determine whether the species has an area centralis.

Retinal function was assessed with two ERG protocols. The 'QuickRetCheck' allows a rapid evaluation of retinal function,[9] while the 'Dog diagnostic' protocol allows a comprehensive evaluation of rod and cone function using a series of dark-adapted tests for rod-driven and mixed rod–cone responses, and a set of light-adapted tests for assessing photopic responses.[10] The 'QuickRetCheck' yielded mostly immeasurable scotopic signals, possibly because the dark drape we used did not provide for sufficient darkness, or because 10 minutes are insufficient for dark adaptation in this species. Standard- and high-intensity mixed rod–cone parameters recorded with this protocol were quite similar to those measured later under different conditions, using the 'Dog diagnostic' protocol, providing validation of these results (Table 2).

All five animals had measurable ERG responses using the 'Dog diagnostic' protocol. Scotopic parameters were low, and there was virtually no increase in a- and b-wave amplitudes in the five consecutive scotopic responses, indicating lack of a dark adaptation process (Table 2). These results are incompatible with behavioral data collected by Galleti *et al.*[24] showing activity of the peccary mostly at night and in the early morning. A possible reason for this apparent contradiction could be a difference between the behaviors of the captive peccary we studied and that of the wild, free-ranging animals studied by Galleti *et al.* Another reason might be the limitations of 'field' ERG recording in the present study. As we were unable to move the animals to a dark room, a dark drape was used to minimize exposure to light and create a scotopic environment; however, it is possible we did not obtain complete darkness, and this might have affected the dark-adapted responses.

Both the scotopic and photopic ERG amplitudes measured in the present study are considerably lower than those reported in domestic pigs.[25–27] While we recorded scotopic b-wave amplitudes of 16.5 μV in response to a 0.01 cd·s/m^2 stimulus in peccaries, amplitudes of 396 μV were recorded in pigs in response to a similar stimulus.[25,26] Similarly, the dark-adapted, standard intensity mixed rod–cone responses in domestic pigs and peccaries are 400 and 124 μV, respectively, while the dark-adapted, high-intensity mixed rod–cone responses of the two species

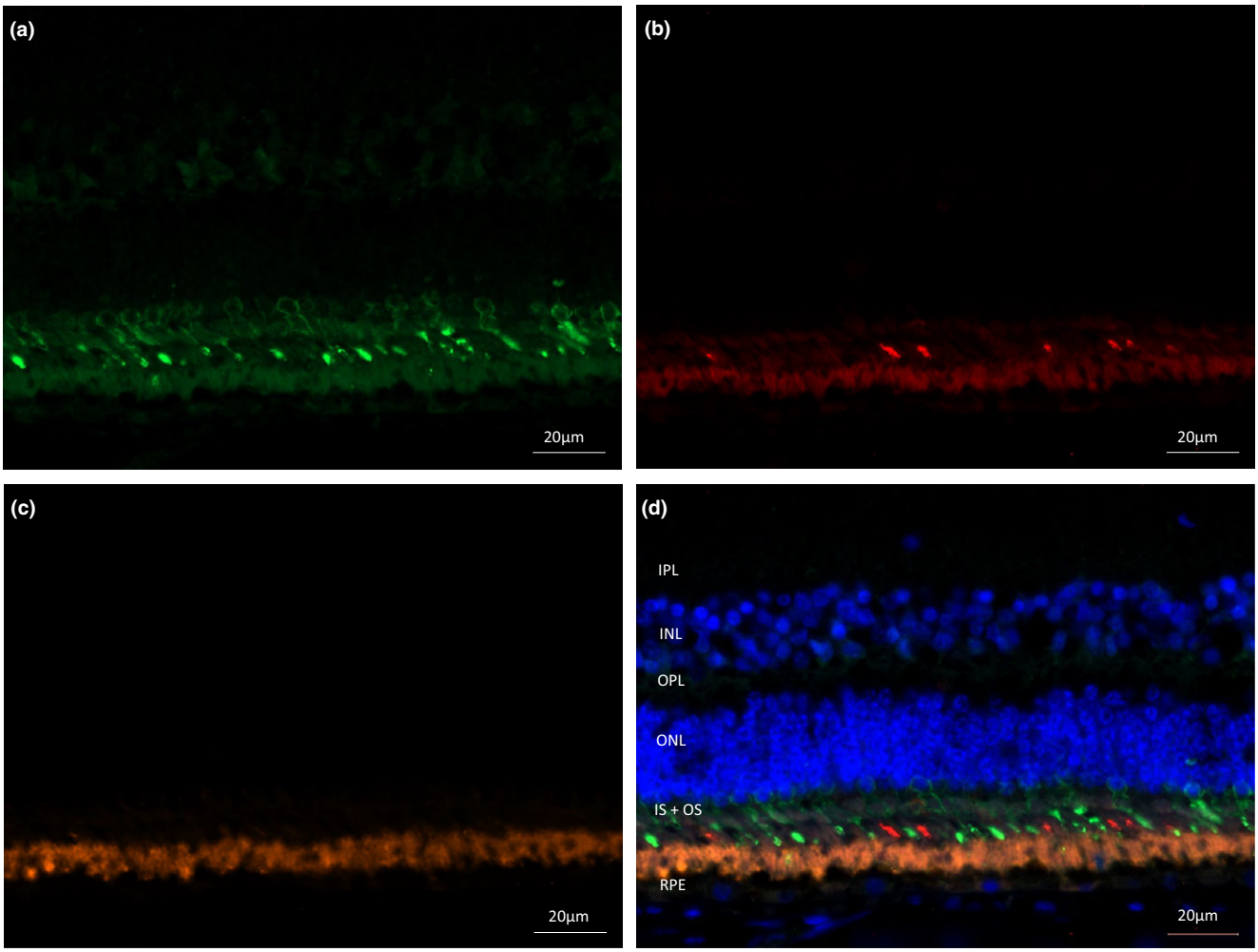

Figure 5. Immunohistochemistry of the peccary retina. (a) Anti-red/green opsin (green) and (b) anti-blue opsin (red) staining revealed a large number of red/green cones and a smaller number of blue cones. (c) Anti-rhodopsin (orange) staining reveals protein expression in the outer segment of rod photoreceptors. (d) Merged image of rod, blue cone, and red/green cone IHC staining. INL, inner nuclear layer; IPL, inner plexiform layer; IS+OS, inner and outer segments of photoreceptors; ONL, outer nuclear layer; OPL, outer plexiform layer; RPE, retinal pigment epithelium. Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (blue). Original magnification ×40. Scale bars = 20 μm.

Table 2. ERG parameters in peccaries

		a-wave		b-wave	
	Protocol	Amp (μV)	IT (msec)	Amp	IT
Standard-intensity mixed rod–cone response	QuickRetCheck*	40.8 ± 4.0	16.4 ± 1.8	109.8 ± 14.6	36.8 ± 2.1
High-intensity mixed rod–cone response	QuickRetCheck	46.6 ± 6.3	14.3 ± 1.9	146.8 ± 23.6	33.8 ± 1.6
Scotopic response 1	Dog diagnostic	4.3 ± 3.3	10.7 ± 4.7	10.2 ± 6.7	28.3 ± 4.4
Scotopic response 2	Dog diagnostic	4.7 ± 1.5	17.3 ± 3.9	9.5 ± 3.6	27.9 ± 4.2
Scotopic response 3	Dog diagnostic	3.51 ± 1.3	15.4 ± 7.0	10.6 ± 3.9	36.9 ± 4.1
Scotopic response 4	Dog diagnostic	6.0 ± 2.7	19.7 ± 6.3	11.5 ± 4.3	37.7 ± 7.4
Scotopic response 5	Dog diagnostic	7.3 ± 8.0	18.6 ± 2.0	16.5 ± 13.9	34.8 ± 10.8
Standard-intensity mixed rod–cone response	Dog diagnostic	51.7 ± 25.3	15.6 ± 2.2	124.1 ± 62.3	36.0 ± 2.5
High-intensity mixed rod–cone response	Dog diagnostic	65.3 ± 32.8	13.6 ± 2.2	154.5 ± 65.2	36.9 ± 3.3
Cone response	Dog diagnostic	18.9 ± 11.9	12.1 ± 1.0	96.9 ± 62.2	27.0 ± 1.3
30 Hz Flicker amplitude	Dog diagnostic	107.0 ± 67.9			

Mean ± SE a- and b-waves amplitudes and implicit times (IT) of rod, standard- and high-intensity mixed rod–cone, and cone responses recorded in five peccaries using the 'Dog diagnostic' protocol. Parameters of mixed rod-cone responses recoded and in six animals using the QuickRetCheck protocol are also shown.
*Parameters of the scotopic QuikcRetCheck recording are not presented, as they were mostly immeasurable.

are 796 and 154 μV, respectively.[26] Following light adaptation, the amplitude of the photopic b-wave was 96.9 μV in the peccary, while values of 519, 350, and 300 μV were reported in two studies in domestic pigs.[25–27] Finally, the 30 Hz flicker response amplitude in pigs is approximately four times higher than in peccaries (350 and 107 μV, respectively).[27]

One possible reason for the differences in the scotopic parameters of the two species might be the shorter dark adaptation period we used in the peccary recordings, which may not have been sufficient to allow significant rod adaptation and complete mydriasis. Dark adaptation in our study was limited to 20 min with periodic scotopic recordings every 4 min, a protocol that is traditionally used to generate a dark adaptation curve in dogs.[10] This is in contrast to the 30 min, uninterrupted dark adaptation period used in the pig studies, which would provide superior dark adaptation.[25,26] More importantly, our inability to achieve complete darkness in the zoo might also have affected the dark-adapted responses of the peccaries However, the differences in mixed rod–cone and cone function between the two species cannot be fully explained by the incomplete dark adaptation. Some of them may be due to differences in experimental design. For example, both Noel *et al.* and Fernandez de Castro *et al.* light-adapted their animals at an intensity of 20 cd/m^2,[25,27] while we used an intensity of 30 cd/m^2; thus, it is possible that their animals were not completely light-adapted, and the high amplitudes of their photopic responses may have been partially due to rod contributions not present in our recordings. Another possible explanation for our low amplitudes is incomplete pupil dilation, as we applied a single dose of 0.5% tropicamide and did not measure pupil diameter. Both Noel *et al.* and Fernandez de Castro *et al.* used a combination of 2.5% phenylephrine hydrochloride and 1% tropicamide to obtain dilation in pigs, while Luu *et al.* applied 1% tropicamide every 5 min.[25–27] It has been reported that, in mice, a combination of topical phenylephrine and tropicamide is required for maximal pupillary dilation and optimal ERG amplitudes.[28] Of course, it is also possible that the low amplitudes recorded in peccaries represent a genuine anatomical or physiological difference between this species and pigs.

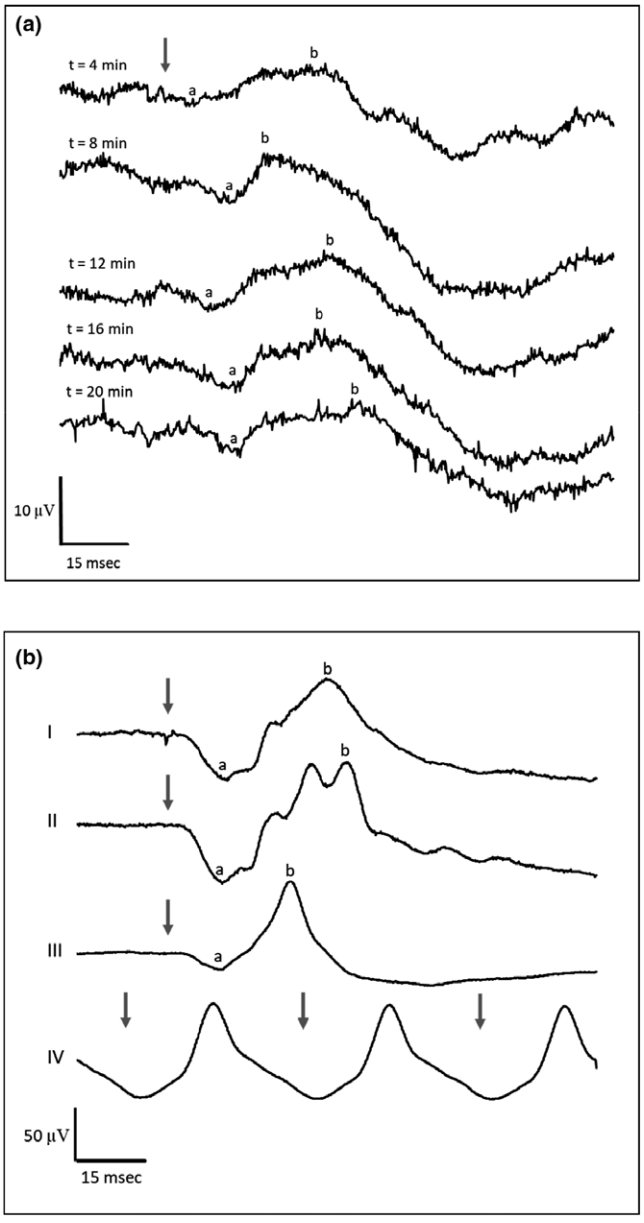

Figure 6. Averaged ERG traces of five peccaries recorded with the HMsERG Dog diagnostic protocol. (a) Five consecutive scotopic responses recorded at 4-min intervals (b) I – standard-intensity mixed rod–cone response; II – high-intensity mixed rod–cone response; III – single-flash photopic response; IV – 30 Hz flicker photopic response. Flash onset indicated by the arrows, and a- and b-wave peaks are marked.

Another marked difference between our study and those conducted in domestic pigs is anesthesia; the recordings in domestic pigs were conducted using a closely monitored inhalant anesthesia (with either halothane or isoflurane), while our recordings were conducted using a less stable, injectable anesthesia.[25,26] This might have contributed to the high degree of variation in our results, but cannot account for the marked differences in amplitudes.

Recently, Augsburger *et al.* conducted photopic and scotopic ERG recordings in 162 healthy minipigs using a protocol similar to the 'Dog diagnostic' protocol used in the present study.[29] In their work, photopic ERG was recorded first and revealed slightly lower a- and b-wave amplitudes (7.7 and 45.5 μV, respectively) than those we recorded in peccaries. Scotopic b-wave amplitudes after 20 min of dark adaptation in minipigs were approximately four times higher than those of the peccary (74.6 μV), but standard-intensity, mixed rod–cone a- and b-wave amplitudes (28.1 and 113.1 μV respectively) were very similar to our measured responses. The fact that only scotopic values were markedly different in the two studies supports the explanation that the dark adaptation conditions of our

study were insufficient to assess pure scotopic function, as indeed Augsburger *et al.* performed their recordings in an indoor research facility that allows complete dark adaptation conditions. Indeed, because Augsburger *et al.* used both a similar recording protocol and a similar injectable anesthetic, it may be more valid to compare our results to theirs, rather than to results in the domestic pig; this may account for the similarities in the photopic and mixed rod–cone responses of the peccaries and minipigs and supports the explanation that differences in anesthesia and recording protocols may account for the significant differences in the ERG parameters of pigs and peccaries.

Of course, comparisons of the peccary ERG responses to those of the domestic pig or minipigs are rather speculative as the species are rather distantly related, belonging to different families in the same suborder. Of the two species, the minipigs' retinal function seems to resemble that of the peccary more than that of the domestic pig. Nonetheless, it would seem that in ungulates, as in other species, longer dark adaptation periods result in higher amplitudes of rod and mixed rod–cone responses. Despite the aforementioned study limitations, our recordings reveal distinct rod, mixed rod–cone, and cone responses, which can serve as preliminary baseline values in the peccary.

A PubMed search of the words 'pig retina' reveals more than 1700 publications, as the species is widely used to study retinal diseases and physiology, as well as drug delivery. However, domestic pigs weigh 150–300 kg, and even so-called 'miniature' pigs, such as the Vietnamese potbelly pig, can grow up to 30–60 kg in weight,[30] and become expensive, difficult, and dangerous to handle. These limitations are significantly decreased in the much smaller peccary (Table 1). The apparent similarities between the pig and peccary retina suggest that the latter species can potentially be used alongside the pig as an animal model for retinal research.

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