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BRIEF COMMUNICATION

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Contrary to popular belief, chinchillas do not have a pure rod retina

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Abstract

Purpose: To establish whether there is cone contribution to retinal function and structure in chinchillas (*Chinchilla lanigera*), in view of the prevailing notion that this species possesses a pure rod retina.

Methods: Photopic electroretinography (ERG) responses to high-intensity flashes (10 and 25 cd*s/m^2) were recorded unilaterally in six pigmented chinchillas following 10 minutes of light adaptation (30 cd/m^2). Retinas of two animals were studied histologically, and immunohistochemistry (IHC) was conducted to detect the presence of short and medium/long wavelength cone photoreceptors.

Results: ERG recordings revealed photopic responses, albeit of low amplitudes. Histopathology demonstrated presumptive cone inner segments in the photoreceptor layer. Presence of cone photoreceptors was confirmed by IHC. Cone density was higher in the central retina, and red/green cones outnumbered blue cones.

Conclusions: Our results provide convincing evidence for the presence of functioning cone photoreceptors in the chinchilla retina, disproving the established belief that the species has a pure rod retina.

KEYWORDS

blue cones, diurnal, electroretinography, nocturnal, red/green cones

1 | INTRODUCTION

Even though it is becoming an increasingly popular pet animal, not much has been published regarding chinchilla ophthalmology, and specifically studies on retinal function and structure in this species are lacking. The fundus of the chinchilla is pigmented with prominent nerve fibers, and variable myelination and cupping of the optic disk; though the retina is described as anangiotic, a network of blood vessels is reported on the surface of the optic disk.[1,2] The retina was described as a pure rod retina in a pioneering study by Detwiler[3] and later Duke-Elder stated that "it is presumed, without clear histological proof" that chinchillas have a pure rod retina.[4] This assumption became an axiom that made its way into modern day literature, with current veterinary ophthalmology texts describing the chinchilla as a pure rod species, without providing any reference to support these statements.[1,5]

However, pure rod retinas have been demonstrated only in few species. These include some deep-sea fish that inhabit a scotopic environment, and some truly nocturnal species, such as the nocturnal gecko. As the retinas of many other nocturnal species (such as mice) have cones, and because in fact chinchillas are also adapted to a diurnal lifestyle,[6] we decided to determine whether indeed the chinchilla (*Chinchilla lanigera*) has a pure rod retina using electroretinographic (ERG), histopathologic, and immunohistochemical (IHC) methods.

Anna Boykova, Maya Ross and Alexey Obolensky contributed equally to this study.

2 | MATERIALS AND METHODS

Experimental protocols were approved by the Institutional Animal Care and Use Committee, Hebrew University of Jerusalem, and were conducted according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

2.1 | Electroretinography

Photopic ERG responses were recorded from the right eyes of six pigmented chinchillas (*Chinchilla lanigera*), 8-24 months of age, weighing 400-700 g. The animals were premedicated with an intramuscular (IM) injection of atropine 0.1 mg/kg 30-60 minutes before the recording (Atropine sulfate 0.1%, S.A.L.F Spa, Cenate Sotto, Italy). Animals were then anesthetized with an IM injection of ketamine (5 mg/kg) and medetomidine (0.05 mg/kg) (Ketamine 10%, Clorketam, Vetoquinol SA, Lure, France; medetomidine 0.1%, Domitor, Orion Corporation, Espoo, Finland). Pupils were dilated with two drops of tropicamide 0.5% (Mydramide, Fischer Pharmaceutical Laboratories, Tel-Aviv, Israel) applied 30-60 minutes before the recording.

Recordings were conducted using a Jet contact lens electrode (ERG-Jet, Fabrinal SA, La Chaux-de-Fonds, Switzerland). To improve conduction, the recorded eye was kept moist with a drop of 1.4% hydroxymethylcellulose (Celluspan, Fischer Pharmaceutical Laboratories, Tel-Aviv, Israel). Subcutaneous needles were used as reference and ground electrodes, and placed 3 mm lateral to the recorded eye and on the back, respectively. Impedance was checked and maintained at <5 KΩ. All recordings were conducted using a Handheld Multi-species Electroretinography system (HMsERG 2000, Ocuscience, Henderson, NV, USA) with a bandpass of 0.3–300 Hz. Flash stimuli and background adapting light were delivered using a handheld mini Ganzfeld placed about 3 cm from the recorded eye.

Light adaptation was achieved by background luminance of 30 cd/m^2 for 10 minutes prior to, and during, the recording session. Flash intensities were 10 and 25 cd*s/m^2. These high intensities, presented after 10 minutes of light adaptation, result in a pure cone response, as rods have saturated. Responses to 32 flashes were averaged in each step, with inter-stimulus interval of 0.5 seconds, and inter-step intervals of 0.5 seconds.

2.2 | Histology

The eyes of two animals, 1 year of age, were enucleated and fixed in 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 hours. After dehydration and embedding in paraffin (Paraplast Plus; Leica Biosystems Nussloch GmbH, Nußloch, Germany), 5 μm thick retinal sections were cut in the nasotemporal plane through the optic nerve and the center of the cornea. Sections were then stained with hematoxylin and eosin.

2.3 | Immunohistochemistry

Deparaffinized sections were incubated in a decloaking chamber (Biocare Medical, Pacheco, CA, USA) with 10 mmol/L 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 the following cone markers: anti-red/green opsin (rabbit polyclonal, 1:100; Chemicon International, Inc., Billerica, MA, USA), anti-blue opsin (goat polyclonal, 1:75; Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), or fluorescein peanut agglutinin (PNA, 1:100, Vector Laboratories, Burlingame, CA, USA).[7] After washing, proper secondary antibodies were applied for 1 hour: DyLight 488 donkey anti-rabbit IgG (1:250) and rhodamine Red-X-conjugated donkey anti-goat IgG (1:250; both from Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI)-containing mounting medium (Vector Laboratories, Burlingame, CA, USA). To determine the specificity of the antigen-antibody reaction, corresponding negative controls were performed with secondary antibody alone.

A semi-quantitative evaluation of the number of photoreceptors stained for red/green and blue opsins was conducted in a representative slide. Red/green and blue stained photoreceptors were counted in the nasal, central, and temporal retina. The nasal and temporal counts were performed 4-5 mm from the optic nerve head, in the respective directions; the central counts were performed 1 mm from the optic nerve head in both nasal and temporal directions. In each region, stained cells were counted in three randomly selected fields of equal size based on magnification (20X) and averaged, counts are presented as mean number of cells in a 450 μm segment. One way ANOVA was used to calculate the least square means differences between the different cell counts in the different areas of the retina. A *P*-value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | Electroretinography

The averaged responses recorded from all 6 animals are shown in Figure 1. Mean (± standard deviation) photopic

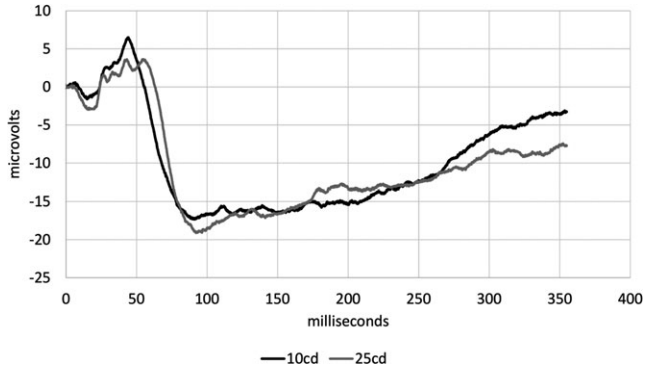

FIGURE 1 Photopic ERG traces from light-adapted retinas, in response to 10 and 25 cd*s/m^2 flash stimuli (black and gray lines, respectively). Each trace is an average of the signals recorded from all 6 animals at the given flash intensity. The actual a- and b-wave measurements were performed separately for each signal, and therefore the mean values cited in the text differ from that of the averaged trace presented in the figure. cd = cd*s/m^2

b-wave amplitudes were 10.2 (±2.9) and 8.7 (±3.1) μV for the 10 and 25 cd*s/m^2 white flash intensities, respectively. The corresponding b-wave mean implicit times were 43.6 (± 5.0) and 49.0 (± 9.6) milliseconds.

3.2 | Histology and IHC

Figure 2 shows an H&E stained cross-section of a chinchilla retina. Even though the chinchilla is regarded as a nocturnal species, consistent with other rodent species we did not see a tapetum. Presumptive cone inner segments were seen in the photoreceptor layer. IHC with the cone membrane marker PNA demonstrated a strong positive signal specific to cone outer segments, inner segments, and cone pedicles (Figure 3). However, only few labeled cones were found in each section, indicating that chinchillas have a rod-dominant retina. To determine whether the chinchilla is monochromatic or dichromatic, markers for red/green and blue cone opsins were used. Positive labeling for both opsins was found in the outer segments of cones (Figure 4). The majority of cones were found to contain red/green opsin (Figure 4B,D, solid arrows), while cones expressing blue opsin were rare (Figure 4C,D, dashed arrows). Increased cone density was found in the central retina compared with the peripheral retina (Table 1, Figure 5).

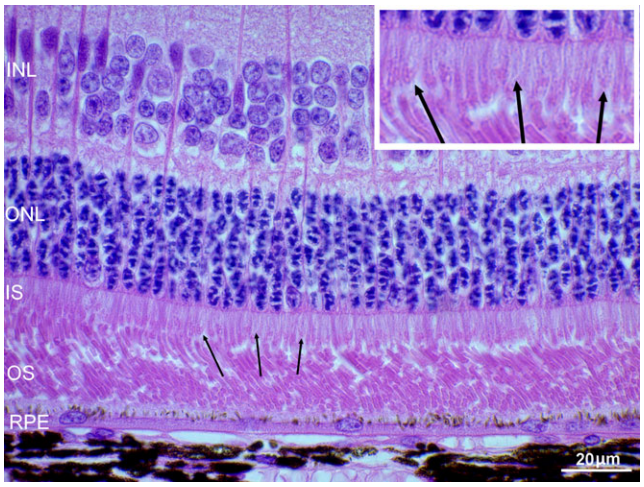

FIGURE 2 H&E staining of a cross-section of the central retina of a one-year-old chinchilla. The arrows point to presumptive cone inner segments in the photoreceptor layer An enlarged view is shown in the inset. Note also the distinct Müller cells spanning the entire width of the retina and contributing to the outer limiting membrane. INL, inner nuclear layer; IS, inner segments of photoreceptors in the photoreceptor layer; ONL, outer nuclear layer; OS, outer segments of photoreceptors in the photoreceptor layer; RPE, retinal pigment epithelium layer

4 | DISCUSSION

Few species have a pure rod retina. In fact, species such as seals and whales, that were once believed to have a pure rod retina,[4] were proved later to be cone monochromats (ie, having one population of cones in addition to rods). Similarly, tarsiers (nocturnal lemurs) that were once considered to have a pure rod retina [4] were later found to be cone dichromats. We show here that the retina of the chinchilla is also dichromatic, possessing both red/green and blue cones. As in most species, the former outnumber the latter. However, as we did not study the peak sensitivity (ie, the specific wavelength that produces maximal response) of the chinchilla's red/green cones, we cannot determine whether the species resembles other rodents,[8] where peak sensitivity is shifted toward the middle part of the visible spectrum, resulting in increased sensitivity to green/yellow hues at the expense of low sensitivity for red, or whether it resembles dogs, in which the reverse is true.[9] Furthermore, the extent to which the short wavelength (ie, blue) sensitive cones, which are scarce, affect vision cannot be determined. Of course, as we used only blue and red/green antibodies in our IHC assay, we cannot rule out the possibility that chinchillas are in fact trichromatic, though we are not aware of any trichromatic rodent species. As in most species, there was a higher cone density in the central chinchilla retina (Table 1, Figure 5). However, as we did not perform retinal flat mounts, we were unable to determine whether chinchillas have a region analogous to an area centralis, or dorso-ventral asymmetry in cone distribution as demonstrated in mice.[10]

While it is obvious that chinchillas have distinct light-adapted ERG responses (Figure 1), their b-wave amplitudes were very low compared to mice and rats recorded in our laboratory, namely ~10 μV in chinchilla vs ~120 μV in mice and rats.[11] As dark-adapted responses were similarly

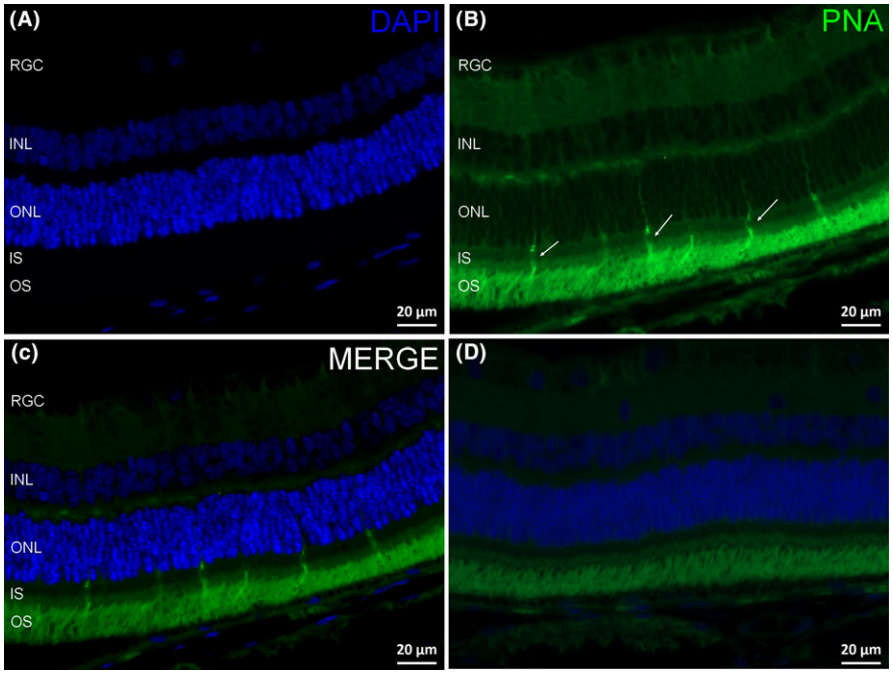

FIGURE 3 Immunohistochemistry with the cone photoreceptor marker PNA in the peripheral retina of a one-year-old chinchilla. A, DAPI labeling of nuclei. B, PNA labeling, cone inner segments in the photoreceptor layer are marked with arrows. C, merge of A +B. D, negative control without PNA. Note the intense auto-fluorescence of the outer segments of photoreceptors. INL, inner nuclear layer; IS, inner segments of photoreceptors in the photoreceptor layer; ONL, outer nuclear layer; OS, outer segments of photoreceptors in the photoreceptor layer; RGC, retinal ganglion cell layer

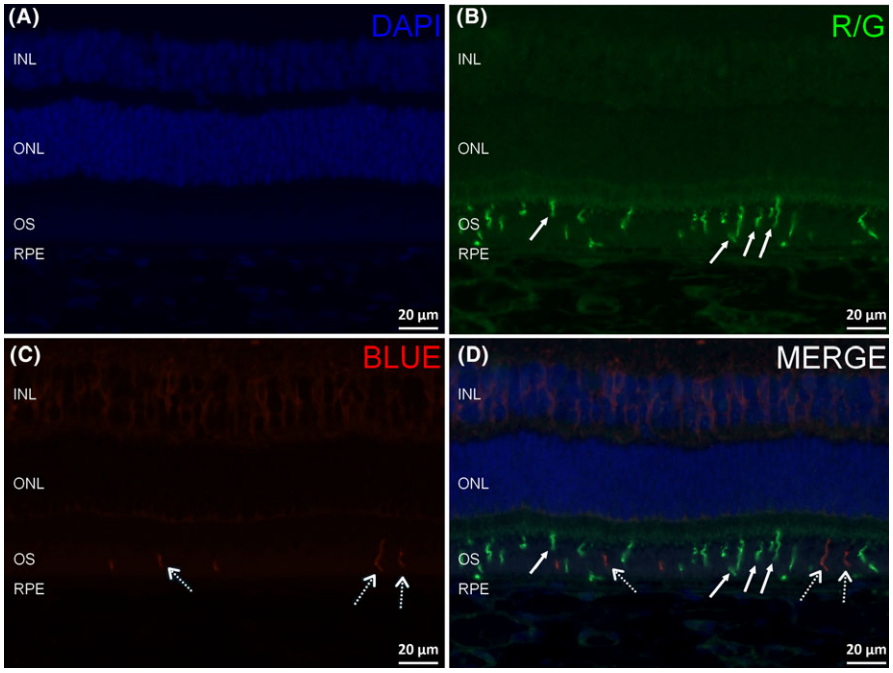

FIGURE 4 Immunohistochemistry for cone photoreceptor opsins in the central retina of a one-year-old chinchilla. A, DAPI labeling of nuclei. B, labeling of the red/green (mid wavelength) opsin in the outer segments of cone photoreceptors. C, labeling of the blue (short wavelength) opsin in the outer segments of cone photoreceptors. D, merge of A + B + C. Red/green opsins are marked with solid arrows, while blue opsins are marked with dashed arrows. INL, inner nuclear layer; OS, outer segments of photoreceptors in the photoreceptor layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium layer

attenuated (data not shown), this finding cannot be attributed to a specific cone feature. A trough following the positive b-wave peak (Figure 1) might contribute to the low amplitudes recorded. However, this is not an established parameter, and thus was not analyzed. On the other hand, the implicit times of the light-adapted b-waves were similar to mice and rats, at about 45 milliseconds. The lack of a measurable a-wave in light-adapted responses is normal in rodents, for example, see Mojumder et al (Figure 3, right panel) in mice,[12] or Sandalon et al (Figure 4A) in rats.[11]

Although chinchillas are naturally a nocturnal species, they easily adapt to a diurnal lifestyle.[6] This can be partly explained by the presence of cones as was shown here, but may also be due to the extremely narrow stenopaic (slit-like) aperture of the chinchilla pupil, which may be contrasted with the round pupil of nocturnal species such as mice and rats.[2,4]

In summary, we have shown the *Chinchilla lanigera* to possess a functional cone photoreceptor system and to be structurally a dichromatic mammal.

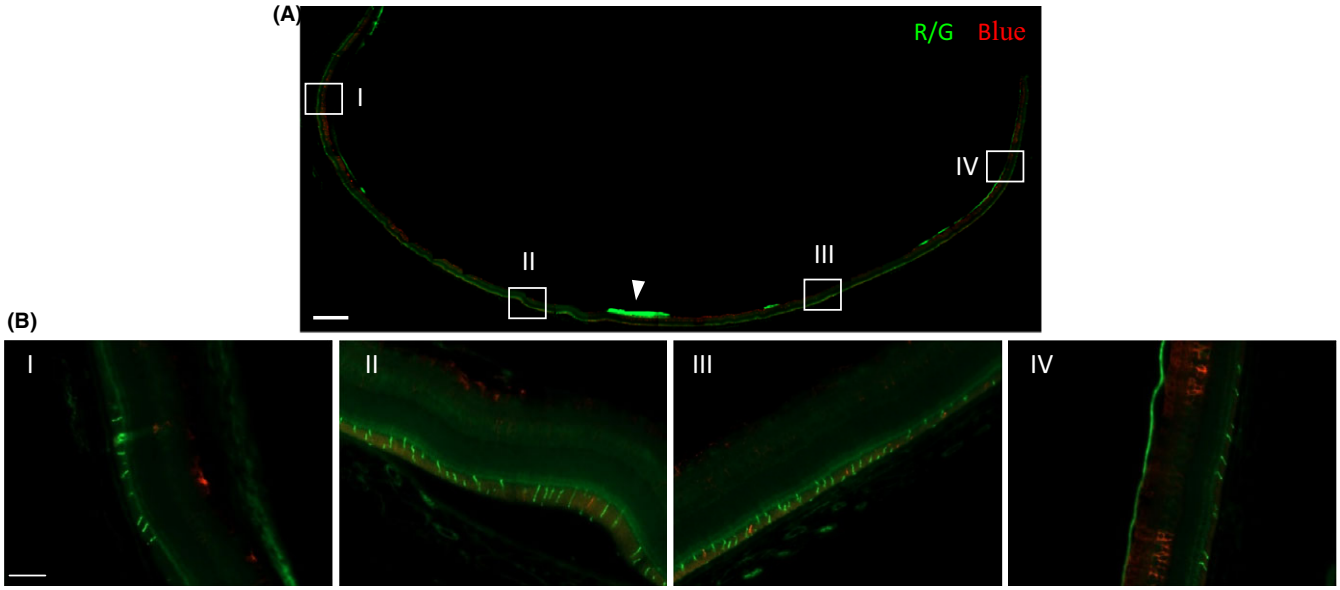

FIGURE 5 Immunohistochemical staining for red/green and blue cone opsins of a one-year-old chinchilla. A, Staining of the whole retina cut along the nasal-temporal plane White arrowhead marks the optic nerve head. Horizontal bar for panel A = 450 μm. B, 20 × magnification of four representative areas of the retina presented in panel A (I—nasal, II and III—central and IV—temporal). Horizontal bars in B = 50 μm. R/G, red/green (mid wavelength) opsins; Blue, blue (short wavelength) opsin

TABLE 1 Semi-quantitative evaluation of the number of cones in three regions of a single chinchilla retina

	Nasal	Central	Temporal
No. of cells stained for red/green opsin per 450 μm segment (mean ± SE)	9 ± 09^{bc}	40 ± 12^{a}	11 ± 35^{b}
No. of cells stained for blue opsin per 450 μm segment (mean ± SE)	1 ± 07^{c}	3 ± 15^{bc}	1 ± 09^{c}

Photoreceptors stained for red/green (mid wavelength) opsin and blue (short wavelength) opsin were counted in three regions of the retina; Nasal, Central, and Temporal.

In each region, cells were counted in three randomly selected areas of equal size based on magnification (20X) and averaged cell counts are presented as number of cells per 450 μm segment.

Values with different superscript letters are significantly different from each other ($P < .05$) as determined by ANOVA (ie, 'a' is significantly different from 'b', 'c' and 'bc'. 'b' is significantly different from 'a' and 'c' but not from 'bc'. 'c' is significantly different from 'a' and 'b' but not from 'bc').

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