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Behavioural evidence of dichromacy in a species of South American marsupial

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Colour vision in marsupials is a controversial issue, especially among the genus *Didelphis* (Didelphidae, Didelphimorphia). While behavioural tests have diagnosed these animals as trichromats and electrophysiological studies have diagnosed them as monochromats, recent molecular genetics studies provide evidence for dichromatic colour vision, having found two classes of cone opsins in a species of this genus. This study examines the colour perception of a male and female white-eared opossum, *Didelphis albiventris*, through a series of tasks involving a behavioural paradigm of discrimination learning. Both opossums succeeded in discriminating pairs of stimuli consisting of Munsell colour cards presented in random brightness values that are assumed to be easily discriminated by dichromats and trichromats (e.g. blues versus oranges). However, both subjects failed to discriminate between colours that are expected to be easily discriminated only by trichromats (e.g. greens versus oranges). The opossums were also unsuccessful in distinguishing a colour against itself (e.g. oranges versus oranges), demonstrating that discrimination was based only on visual cues. These results are consistent with recent predictions based on molecular genetics suggesting that the genus *Didelphis* is routinely a dichromat.

Five classes of photopigments are present in vertebrates; one rod class and four cone classes. However, most mammals have lost two types of cone photopigments and are classified as dichromats (Ahnelt & Kolb 2000; Hunt et al. 2009; Jacobs 2009). Excluding cave-living and fossorial animals, which have regressed vision relative to most mammals, the exceptions to dichromatism among mammals are concentrated in the order Primates. In this order a homogenous trichromatism (found in males and females) has evolved in all catarrhine primates (Old World monkeys, apes and humans) (Surridge et al. 2003) and in the howler monkey (*Alouatta*) (Jacobs et al. 1996; Araújo et al. 2008). Also, platyrrhine monkeys have X-chromosome opsin-gene polymorphism, resulting in more varied colour vision arrangements (Rowe & Jacobs 2004; Jacobs 2007). Finally, there is monochromatism found in the owl monkey (*Aotus*) (Jacobs 1993a) and in the prosimian bushbaby *Galago garnetti* (Wikler & Racic 1990). Further exceptions to dichromatism in mammals also include the monochromatic vision in three nocturnal carnivores (*Procyon cancrivorus*, *Procyon lotor*, *Potos flavus*) (Jacobs & Deegan 1992; Peichl & Pohl 2000), several rodents (Jacobs 2009) and some pinniped and cetacean species (Peichl et al. 2001).

However, recent studies with Australian marsupials suggest that trichromacy is not unique to primates. Using microspectrophotometry (Arrese et al. 2002) and microspectrophotometry and immunohistochemistry (Arrese et al. 2005), Arrese and collaborators identified three classes of cone photopigments in four species of Australian marsupials that are representative of the two major taxonomic divisions, the diprotodonts and the polyprotodonts (Springer & Murphy 2007). Behavioural tests were also performed for the fat-tailed dunnart, *Sminthopsis crassicaudata* (Arrese et al. 2006), a species previously diagnosed as trichromats by microspectrophotometry. This study showed that *S. crassicaudata* also behaved like trichromats. However, the determination of amino acid sequences of cone visual pigments in *S. crassicaudata* and in the stripe-faced dunnart, *Sminthopsis macroura*, indicated that while both species have long-wavelength-sensitive (LWS) pigments with a predicted maximum absorption (λ_{max}) of 530 nm and ultraviolet-wavelength-sensitive (UVS) pigments with a predicted λ_{max} of 360 nm (Strachan et al. 2004), there was no evidence of a third cone photopigment in either species. Furthermore, a recent in vitro expression study (Cowing et al. 2008) has expanded these results. Cowing and colleagues showed that although no cone opsin sequences for the middle wavelength cones could be extended, two genes for rod opsins (RH1) were found, which raises the possibility of RH1 being expressed in cones, leading to trichromacy.

Colour vision in marsupials remains to be satisfactorily resolved, not only among the Australian clades but particularly among the order Didelphimorphia, the American marsupials (see Table 1). Jacobs (1993b) and Jacobs & Williams (2010), performing electroretinographic measurements in the Virginia opossum, *Didelphis virginiana*, obtained responses for only one cone photopigment with a maximum sensitivity of about 560 nm. Previous colour discrimination behavioural tests were also conducted for *D. virginiana* by

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Table 1
Summary of colour vision studies in marsupials

	Activity pattern	*N*	Method	Colour vision	Source
Didelphimorphia					
Didelphidae					
South American opossum, *Didelphis aurita*	Crepuscular	6	G	Dichromats	Hunt et al. 2009
Virginia opossum, *Didelphis virginiana*	Nocturnal	2	B	Trichromats	Friedman 1967
		NI	ERG	Monochromats	Jacobs 1993b
		NI	ERG	Monochromats	Jacobs & Williams 2010
Grey short-tailed opossum, *Monodelphis domestica*	Nocturnal	NI	G	Dichromats	Hunt et al. 2009
Mouse opossum, *Thylamys elegans*	Nocturnal	4/4	G/ERG	Dichromats	Palacios et al. 2010
Diprotodontia					
Tarsipedidae					
Honey possum, *Tarsipes rostratus*	Crepuscular	3	MSP	Trichromats	Arrese et al. 2002
		0	TI	Trichromats	Sumner et al. 2005
		3	G	Dichromats	Cowing et al. 2008
Macropodidae					
Tammar wallaby, *Macropus eugenii*	Crepuscular	2	B	Dichromats	Hemmi 1999
		1	G	Dichromats	Deeb et al. 2003
Quokka, *Setonix brachyurus*	Crepuscular	6	MSP/ICC	Trichromats	Arrese et al. 2005
Dasyuromorphia					
Dasyuridae					
Fat-tailed dunnart, *Sminthopsis crassicaudata*	Arrhythmic	3	MSP	Trichromats	Arrese et al. 2002
		1	G	Dichromats	Strachan et al. 2004
		2	B	Trichromats	Arrese et al. 2006
		3	G	Dichromats	Cowing et al. 2008
Stripe-faced dunnart, *Sminthopsis macroura*	Arrhythmic	1	G	Dichromats	Strachan et al. 2004
Peramelemorphia					
Peramelidae					
Quenda, *Isoodon obesulus*	Nocturnal	6	MSP/ICC	Trichromats	Arrese et al. 2005

Species are organized according to the marsupial evolutionary radiations, from the most primitive to the most derived group. Abbreviations: *N*: number of subjects; NI: not informed; B: behaviour; ERG: electroretinography; G: molecular genetics; ICC: immunocytochemistry; MSP: microspectrophotometry; TI: theoretical inference.

Friedman (1967), whose results indicated that subjects were able to discriminate among red, blue, green and yellow lights, which led Friedman to classify them as trichromats. However, the recent sequencing of the cone opsin of two nocturnal South American marsupials (*Didelphis aurita* and *Monodelphis domestica*) revealed only two classes of cone photopigments with peak sensitivities for ultraviolet and long wavelengths (Hunt et al. 2009).

A thorough survey of colour vision perception must also include behavioural tests with careful control for brightness cues (Jacobs 1999). The importance of behavioural analysis has been demonstrated in studies where the dimensionality of colour vision is assured in transgenic animals only after discriminative tests (Jacobs et al. 1999, 2007; Mancuso et al. 2009). Also, the trichromacy found in the marsupial *S. crassicaudata* (Arrese et al. 2006), which has only two cone opsins (Cowing et al. 2008), emphasizes the importance of behavioural approaches in colour vision investigation.

To provide a better understanding of colour vision in the genus *Didelphis*, we performed a series of experiments, based on a paradigm of discrimination learning, in the white-eared opossum, *Didelphis albiventris*. The white-eared opossum is a crepuscular South American marsupial, like the South American opossum, *Didelphis aurita* (Ahnelt et al. 1996), distributed from Colombia to Argentina (Talamoni & Dias 1999). *Didelphis albiventris* is a small mammal, its body mass ranges from 500 to 2750 g and its body length, from 305 to 890 mm. This is an omnivorous species and its diet consists of small mammals and birds, lizards, frogs, insects, eggs and fruits, beginning its foraging behaviour at twilight. The white-eared opossum is also a solitary animal that gathers only during breeding season (Rossi et al. 2006).

METHODS

Subjects

Two infant white-eared opossums, one male and one female, approximately 55–70 days old, were used in this experiment. The animals were housed individually in cages in the Laboratory of Neurosciences and Behaviour, University of Brasilia, and hand-fed a diet of fresh fruits and dry dog food after the experimental session.

Stimuli

The stimuli consisted of chromatic cards (12.5 × 7.5 cm) from the Munsell Color System (Nickerson 1940). In this system, every colour patch is specified by three attributes: hue, brightness and saturation. Hue is represented by a number and a letter, whereas brightness and saturation are expressed as fractions. For example, the notation 10YR 5/10 corresponds to a yellow-red (i.e. orange) colour card 10, with brightness 5 and saturation 10. Four categories of discrimination hues were used: yellow-red (10YR), blue (5B, 7.5B) and green-yellow (5GY). For each hue we used a set of four cards with different brightness values, paired and presented randomly, so we could assure that colour discrimination was based on the hue rather than on the brightness of the stimulus. The brightness values for each selected hue were as follows: 10YR *N*/10, *N* = 5–8; 5B *N*/6, *N* = 4–7; 7.5B *N*/4, *N* = 5–8; and 5GY *N*/10, *N* = 5–8. These stimuli were selected in a previous study (Gomes et al. 2002) involving colour-blind human subjects (protanopes and deuteranopes) and were also used in experiments with different Neotropical primates (e.g. Gomes et al. 2002; Pessoa et al. 2003, 2005a, b, c; Araújo et al. 2008; Prado et al. 2008). All Munsell cards were kept in acrylic covers to protect them from wear. When not being used during the experimental sessions, the cards were also protected from humidity and natural or artificial light. Stimuli that allowed the animals to gain a reward were designated positive discriminating stimuli (DS+), and stimuli that were nonrewarding were designated negative discriminating stimuli (DS−).

Apparatus

The test apparatus was placed on a table (0.58 × 1.88 × 0.77 m, length × width × height) and consisted of a plywood Y-shaped