Double cones are used for colour discrimination in the reef fish, *Rhinecanthus aculeatus*

Vincenzo Pignatelli[1], Conor Champ[1],
Justin Marshall[1] and Misha Vorobyev[2,*]

[1]*Sensory Neurobiology Group, Queensland Brain Institute,
University of Queensland, Brisbane 4072, Australia*
[2]*Department of Optometry and Vision Science,
University of Auckland, Auckland 1142, New Zealand*
Author for correspondence (m.vorobyev@auckland.ac.nz).

Double cones (DCs) are the most common cone types in fish, reptiles and birds. It has been suggested that DCs are used for achromatic tasks such as luminance, motion and polarization vision. Here we show that a reef fish *Rhinecanthus aculeatus* can discriminate colours on the basis of the difference between the signals of individual members of DCs. This is the first direct evidence that individual members of DCs are used in colour vision as independent spectral channels.

1. INTRODUCTION

Double cones (DC) are two cone photoreceptor cells that are fused together and may be optically and/or electrically coupled (Marchiafava 1985; Smith *et al.* 1985). They are present in the eyes of most vertebrate animals, but are conspicuously absent from retina of placental mammals, elasmobranches and catfish (Walls 1942; Ali & Anctil 1976; Ebrey & Koutalos 2001). Although DC are the most common cone photoreceptors in fish, reptiles and birds, the function of DC is largely unknown. Here, we use a behavioural method to reveal the role of DC in colour vision in a reef fish *Rhinecanthus aculeatus* (Linnaeus 1758). *Rhinecanthus aculeatus* belongs to the family of triggerfish (Balistidae, Order Tetraodontiformes) and known as Blackbar triggerfish. Recent behavioural observations have demonstrated that this fish is capable of colour vision, but the role of cone types in colour vision has not been determined (Marshall *et al.* 2004). Blackbar triggerfish possesses one type of single cone (SC), with visual pigment peaking at 413 nm (S for short-wavelength), and a DC with different visual pigments in each member, one peaking at 480 nm (M for middle-wavelength) and the second peaking at 530 nm (L for long-wavelength) (Marshall *et al.* 2004).

For a number of animal groups including fish, it has been suggested that, while SCs certainly contribute to colour vision, DCs are likely to be involved in achromatic tasks, such as luminance, motion and polarization vision (Boehlert 1978; Lythgoe 1979; Cameron & Pugh 1991; McFarland 1991; Hawryshyn *et al.* 2003). Indeed, analysis of colour thresholds in

some birds suggests that DC do not participate in colour vision (Maier & Bowmaker 1993; Vorobyev & Osorio 1998; Goldsmith & Butler 2003), and it has been demonstrated that motion vision in goldfish and chickens is mediated by the long wavelength-sensitive visual pigment housed in DCs (Schaerer & Neumeyer 1996; Campenhausen & Kirschfeld 1998). Summation of the signals of individual members of DC would be beneficial for luminance vision, as this would broaden the spectral sensitivity and improve the ability of fish to detect targets contrasting to background in different parts of the spectrum (Lythgoe 1979; Lythgoe & Partridge 1989; Marshall & Vorobyev 2003; Marshall *et al.* 2003). Numerous gap junctions between members of fish DCs form an anatomical basis for such summation (Marchiafava 1985). Therefore, it has been hypothesized that the signals of the two members of DC are summed in the retina and the signals of separate members of DC are not conveyed to the brain (Marshall & Vorobyev 2003; Marshall *et al.* 2003). Hence, a fish with three types of visual pigments, one housed in single cones and two pigments housed in two members of DC may be predicted to be dichromatic (Lythgoe 1979; Marshall & Vorobyev 2003; Marshall *et al.* 2003). Several modelling papers on colour perception by reef fishes were based on the assumption that reef fishes are effectively dichromatic (Chiao *et al.* 2000; Marshall & Vorobyev 2003; Marshall *et al.* 2006).

A behavioural outcome of the DC summation hypothesis is that a fish should not be able to discriminate between colour stimuli that differ in signals from individual DC members, given that such stimuli provide similar summed DC signal and SC signals. Here we test this prediction by training a fish to discriminate stimuli with adjusted spectral properties.

2. MATERIAL AND METHODS

(a) *Stimuli and their spectral properties*

Coloured circular targets (5 cm in diameter) were printed on white paper using an Epson Stylus Photo 1290 colour printer and, in order to water-proof them, were laminated. Reflectance spectra of the laminated stimuli and the aquarium illumination were measured using an Ocean Optics USB 2000 spectrometer (figure 1b,c). The spectral properties of stimuli were adjusted by changing the red, green and blue (RGB) values of colours in Photoshop (Giurfa & Vorobyev 1997; Giurfa *et al.* 1997). Spectral sensitivities of S, M and L cones were modelled by combining the visual pigment absorption curve with the ocular media spectrum (Siebeck & Marshall 2001; Marshall *et al.* 2004; figure 1a). Cone signals, q_i, were calculated as normalized quantum catches:

$$q_i = k_i \int S(\lambda) I(\lambda) R_i(\lambda)\, \mathrm{d}\lambda, \qquad (2.1)$$

where i denotes the spectral type cone ($i = $ S, M, L), λ the wavelength, $R_i(\lambda)$ the spectral sensitivity of a cone i, $S(\lambda)$ the reflectance spectrum of a stimulus, $I(\lambda)$ the illumination spectrum, $k_i = 1/\int I(\lambda) R_i(\lambda) \mathrm{d}\lambda$ is the scaling factor whose value is chosen so that the quantum catch corresponding to an ideal reflector is equal to 1 (Kelber *et al.* 2003). Such scaling describes adaptation of cones to ambient illumination. Because members of DCs in *R. aculeatus* are of equal size (Marshall *et al.* 2004), the combined DC signal can be modelled as:

$$q_\mathrm{D} = \frac{q_\mathrm{L} + q_\mathrm{M}}{2}. \qquad (2.2)$$

Three spectral types of stimuli have been chosen for experiments, which we label according to their colour appearance to our eyes as 'magenta', 'purple' and 'blue'. The reflectance spectra of the stimuli are given in figure 1, and the quantum catches are given in table 1.

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