Early vertebrate colour vision

SIR — A feature of retinae in lizards, turtles and birds is the intercalation of a coloured oil droplet between the inner and outer segments of cone photoreceptor cells[1,2]. The colour in the droplets absorbs particular wavelengths of light, thereby narrowing the spectral sensitivity of the four opsins (visual-pigment proteins) that are located in the outer segments. The result is a tetrachromatic visual system with a broad sensitivity that extends from near-ultraviolet (~350 nm) to infrared (~750 nm) (birds[3,4], turtles[5,6]). The Australian lungfish, *Neoceratodus forsteri*, belongs to the order *Dipnoi* which diverged from the main vertebrate stock in the early Devonian. *N. forsteri* fossils have

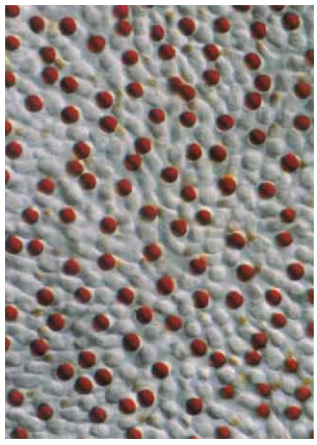

FIG. 1 Photomicrograph showing the array of coloured oil droplets in the cone photoreceptors of an unstained *Neoceratodus* retina. The small colourless droplets are not evident in this plane of focus. Lungfish were deeply anaesthetised, their eyes quickly removed, and the retinae immersed for 24 hours in 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The retinae were then flatmounted with the photoreceptor layer uppermost, and coverslipped in 50% glycerol in 0.1 M phosphate buffer. Magnification, × 150.

been dated at well over 100 million years, making this the oldest extant vertebrate species[7]. Here I show that *N. forsteri* has coloured droplets in its cone photoreceptors, suggesting that the common ancestors of lungfish and land vertebrates also had coloured oil droplets and, perhaps, tetrachromatic vision.

In the eight *Neoceratodus* retinae that I have studied, cones are distributed at densities of 2,500–4,600 mm^{-2} and they compose 50–56% of the photoreceptor population, the remainder being rods: 65–75% of cones possess red oil droplets, 5–10% have small colourless droplets, and the rest (15–25%) have a golden granular pigment at the base of the outer segment, instead of an oil droplet (Fig. 1). In some *Neoceratodus* the variety of oil droplets increases towards the retinal edge to include orange. The density of red droplets is always reduced in regions where orange droplets are present, but there is no reduction in the density of small colourless droplets or cones containing golden granular pigment.

The cone oil droplets in *Neoceratodus* have a larger diameter (6–15 μm) than those in turtles (3–10 μm)[8,9] or birds (1–6 μm)[9,10]. The droplet colours observed in *Neoceratodus*, as well as colourless droplets and cones with a yellow granular pigment in the ellipsoid, are found in birds[3,4,9,10] and turtles[5,6,8,9], but not all colours are present in every species.

Walls[1] proposed that coloured oil droplets appeared very early in vertebrate evolution, and that when species adopted a nocturnal habit, colour was lost from their droplets, or the droplets were eliminated. Once lost, the colours could not be regained, even if the descendants of a nocturnal animal colonized a diurnal niche, as has occurred among some mammals and frogs. This proposal was not widely accepted because of the restriction of coloured oil droplets to a small part of the vertebrate series. The restricted distribution could be explained more simply if coloured oil droplets originated about 250 million years ago in the common ancestors of turtles, birds and reptiles (Fig. 2). The discovery of coloured oil droplets in the lungfish provides much stronger support for the notion that coloured oil droplets were present in the lobe-finned ancestors of land vertebrates (*Sarcopterygii*) more than 400 million years ago (Fig. 2).

The number of cone opsins possessed by *Neoceratodus* is unknown. However, in all vertebrates that have been studied, colour vision is achieved through the use of two or more different photopigments, rather than by coloured oil droplets in combination with a single opsin[2,4]. The fact that coloured oil droplets are restricted to those vertebrates which have four or more cone opsins[4] implies that the colour in the droplets only conveys an

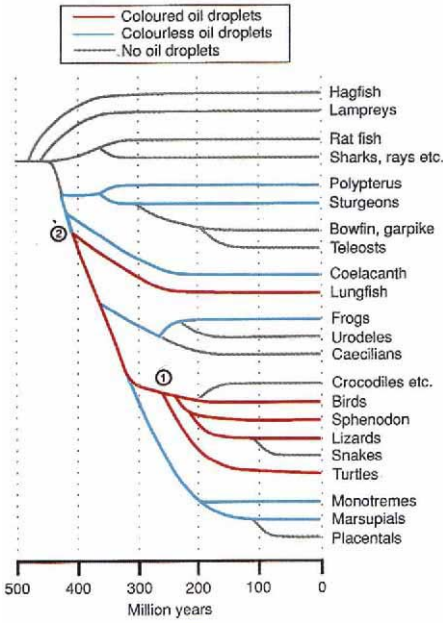

FIG. 2 Approximate origins of the main extant vertebrate taxa, based on data in refs 1,2,11. Taxons lacking oil droplets in their cone photoreceptors are indicated in grey, those with colourless droplets in blue, and those with coloured droplets in red. Until now, the distribution of coloured droplets was consistent with an origin about 250 million years ago. The finding of coloured droplets in lungfish supports a common origin 400 million years ago.

advantage when many opsins are present. This perspective favours the idea that *Neoceratodus* has four cone opsins, and raises the possibility that the ancestral *Sarcopterygii* had tetrachromatic vision.

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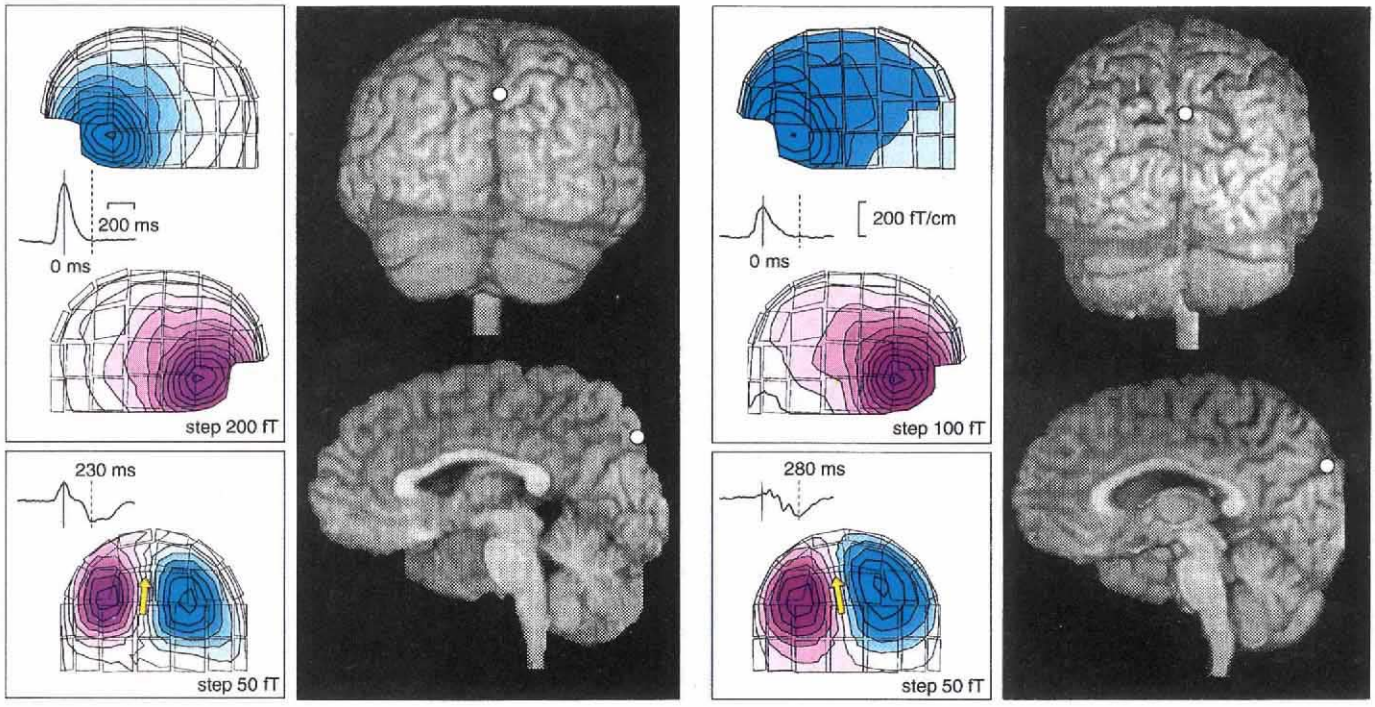
1. Walls, G. L. *The Vertebrate Eye and its Adaptive Radiation* (Bloomford Hills, Michigan, 1942).
2. Crescitelli, F. in *Photochemistry of Vision* (ed. Dartnall, H. J. A.) 245–363 (Springer, Berlin, 1972).
3. Partridge, J. C. *J. comp. Physiol.* **A165**, 415–426 (1989).
4. Bowmaker, J. K. in *The Perception of Colour; Vision and Visual Dysfunction Vol. 6* (ed. Gouras, P.) 108–127 (Macmillan, Basingstoke, 1991).
5. Lipetz, L. E. in *The Visual System* (eds Fein, A. & Levine, J. S.) 107–132 (Liss, New York, 1985).
6. Ohtsuka, T. *J. comp. Neurol.* **237**, 145–154 (1985).
7. Kemp, A. in *Vertebrate Palaeontology of Australasia* (eds Vickers-Rich, P. *et al.*) 465–496 (Pioneer Design Studio, Melbourne, 1991).
8. Kolb, H. & Jones, J. *J. comp. Neurol.* **209**, 331–338 (1982).
9. Kawata, A. *et al. Photochem. Photobiol.* **56**, 1157–1166 (1992).
10. Cserháti, P., Szél, Á. & Röhlich, P. *Invest. ophthal. Vis. Sci.* **30**, 74–81 (1989).
11. Marshall, C. & Schultze, H.-P. *J. molec. Evol.* **35**, 93–101 (1992).

Visual stability during eyeblinks

SIR — During each eyeblink we lose sight of the visual world for more than a tenth of a second without usually perceiving the discontinuity. A suppression of visual sensitivity during blinks[1,2] explains why darkening is not seen but it is not sufficient to account for the continuity of visual perception. Here we report on activation of the human parietal lobe after voluntary blinks. The posterior parietal cortex — an essential part of the spatial working memory system[3] — may have a role in maintaining a stable image despite the interruption of visual input during blinks.

We studied five adult subjects (ages 27–34 yr; 3 females) with a helmet-shaped 122-channel SQUID (superconducting quantum interference device) magnetometer (Neuromag-122™). The subjects



Field maps and MRI surface renderings for two subjects. The magnetic field patterns are shown during the maximum blink signal (upper boxes) and during the peak of the posterior response 230 and 280 ms later (lower boxes). The helmet-shaped sensor array is viewed from three angles (left, right and back). The patterns are based on simultaneous recordings with 122 sensors; each of the sensor units (squares in the figure) houses two orthogonal planar gradiometers. Red, magnetic flux out of the head; blue, flux into the head; the separation between isocontours ('step') is indicated in each box. Examples are shown of averaged magnetic signals from field extrema. The arrows show the sites and orientations of the equivalent current dipoles for the posterior signal patterns; dipole strengths are 23 and 26 nA m^{-1} for subjects 1 and 2, respectively. The white dots superimposed on the three-dimensional MRI surface rendering of the same subjects' brains (viewed from the back and from the mesial surface of the right hemisphere) illustrate the equivalent current dipoles for the late responses. The current flow is perpendicular to the course of the parieto-occipital sulcus.

fixated on a cross or a small picture and blinked voluntarily once every 3–5 s. Altogether 3×5 min of data were gathered, with two 1-min pauses in-between. The magnetic signals were averaged off-line using the onset slope of the vertical electro-oculogram as the trigger.

Strong magnetic signals were seen close to both orbits during the blinks (see figure): the magnetic flux emerged from the scalp over the right side and entered the head on the left side. More than 200 ms later (220–285 ms in the five subjects), when blink signals were no longer observed, new responses emerged over the upper posterior part of the head. Similar late responses have been observed previously in scalp EEG records[4]. The posterior field patterns were satisfactorily accounted for by current dipoles, which corresponded in strength to sources of strong evoked responses[5].

Superposition of the source locations on the individual three-dimensional magnetic resonance imaging (MRI) reconstructions (made for two subjects; see figure) implied activity close to the parieto-occipital sulcus, probably in the posterior association cortex (area 7). Conventional early visual evoked responses occurred at a much lower site. No corresponding magnetic signals were detected when blinking occurred in complete darkness (studied in one subject), indicating that an interruption of a visual stimulus is required for the appearance of this response. The relevant activity was obviously in the posterior parietal cortex, which receives afferent input from the striate cortex through a dorsal visual stream, a pathway considered to be involved in spatial rather than object vision[6,7]. No corresponding activation of the ventral stream was observed.

The posterior parietal cortex is reciprocally connected with prefrontal cortical areas[8] which seem to underlie spatial working memory[3]. It has been suggested that the monkey posterior parietal cortex updates continuously information about the nature and structure of visual objects in the ego-centred space[9,10]. The present results are consistent with similar pathways and mechanisms in man, inferring that the posterior parietal lobe is kept informed also about eyeblinks. Most blinks occur unnoticed by the subject, and blink-related information thus seems to be processed unconsciously.

We hypothetize that the observed blink-related responses in the human posterior parietal cortex are related to spatial working memory, necessary for maintaining a continuous image of the environment despite the 0.1-s loss of visual input during each blink. Such continuity is essential for stable visual perceptions. The latency of the parietal activation suggests that the eyeblink is reacted to after its occurrence, not in advance in connection with the motor command.

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1. Volkmann, F., Riggs, L. & Moore, R. *Science* **207**, 900–902 (1980).
2. Ridder III, W. H. & Tomlinson, A. *Vis. Res.* **33**, 1795–1802 (1993).
3. Wilson, F. A. W., O'Scalaidhe, S. P. & Goldman-Rakic, P. C. *Science* **260**, 1955–1958 (1993).
4. Berg, P. & Davies, M. *Electoenceph. Clin. Neurophysiol.* **69**, 1-5 (1988).
5. Hämäläinen, M. *et al. Rev. Mod. Phys.* **41**, 413 (1993).
6. Mishkin, M., Ungerleider, L. & Macko, K. *Trends Neurosci.* **6**, 414–417 (1983).
7. Horwitz, B. *et al. J. cog. Neurosci.* **4**, 311–322 (1992).
8. Cavada, C. & Goldman-Rakic, P. S. *J. comp. Neurol.* **287**, 422–425 (1989).
9. Goodale, M. A. & Milner, A. D. *Trends Neurosci.* **15**, 20–25 (1992).
10. Stein, J. F. *Behav. Brain Sci.* **15**, 691–700 (1992).