The colourful world of the mantis shrimp

The colour-vision system of these crustaceans includes four types of UV photoreceptor.

Humans cannot see ultraviolet light, but many arthropods and vertebrates can because they have a single photoreceptor with a peak sensitivity to light at wavelengths of around 350 nanometres (ref. 1). Here we use electrophysiological methods to investigate the vision of the mantis shrimp, *Neogonodactylus oerstedii*. We find that this marine crustacean has at least four types of photoreceptor for ultraviolet light that are located in cells of the eye known as R8 cells. These photoreceptors are maximally sensitive to light of wavelengths 315, 330, 340 and 380 nm. Together with previous evidence[2], this finding indicates that the remarkable colour-vision system in these stomatopod crustaceans may be unique, as befits their habitat of kaleidoscopically colourful tropical coral reefs.

Many stomatopods inhabit the top few metres of water, which are bathed in light of ultraviolet-A wavelengths[3]. In this spectrally varied environment, they have evolved extremely complex retinae within their apposition compound eyes[2,4–6]. The top four rows of the midband (Fig. 1a) contain eight spectral sensitivities from wavelengths of 400 to 700 nm, each based on a different visual pigment[2]. Oversampling within the spectrum is avoided by elegant filtering mechanisms that narrow these sensitivities[2,6].

The R8 cells of stomatopods, like the cells R1 to R7 (see Fig. 1), have an extreme proliferation of spectral sensitivities. Three of the four ultraviolet spectral sensitivities are found in the midband, and one is in the periphery. Two of these sensitivities are relatively broad and two are very narrow, but they are all too narrow to be the result of visual-pigment absorption alone[7]. Filtering mechanisms like those in the rest of the eye are almost certainly the reason for this spectral tuning[2,6], with the filter being situated in the dioptric apparatus or within the microvilli of the R8 photoreceptors[8,9]. Midband row 2 or row 3 R8 spectral sensitivities have not yet been characterized, as they are hard to find or record from.

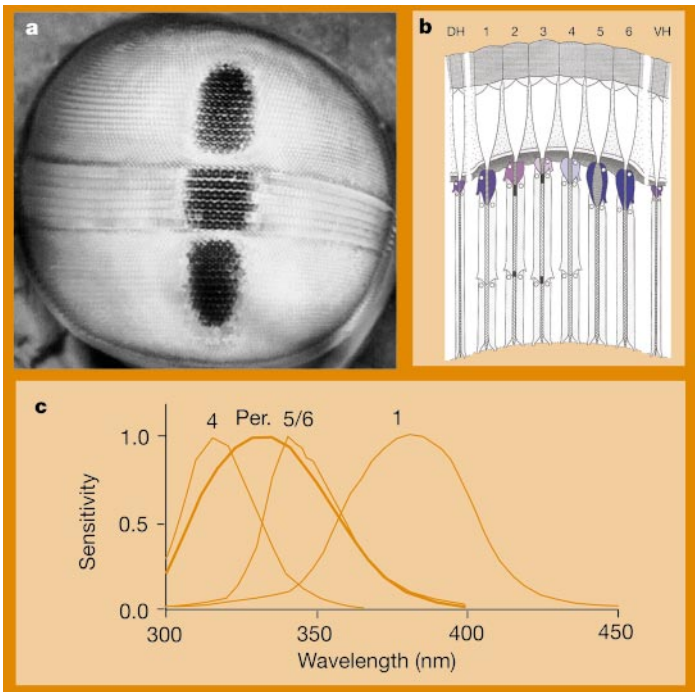

Figure 1 R8 photoreceptors in the midband of the stomatopod eye have multiple UV sensitivities **a,** Stomatopod eye showing the clearly defined midband, rows 1 to 6 (dorsal to ventral)[4,5]. The top four rows are concerned with colour information, and the remaining two are specialized for polarization[2,5]. **b,** Diagram of the photoreceptors in the six midband rows and the peripheral retinae. R8 cells are coloured. Chromatic channels from 400 to 700 nm are contained in a population of cells called R1 to R7 (ref. 4). VH and DH are representatives of the dorsal and ventral hemisphere regions in the peripheral retina. **c,** Ultraviolet spectral sensitivities of R8 cells. Per, peripheral retina (DH or VH).

The evenly spaced ultraviolet photoreceptors in the eye of *N. oerstedii* indicate that stomatopods have extended the high-frequency sampling system of the range 400 to 700 nm into the ultraviolet to form a single colour-vision system that is sensitive from 300 to 700 nm.

The narrowed sensitivities of all four cells can be modelled using existing visual-pigment nomograms[7] and a series of corneal ultraviolet cut-off filters[10] (Fig. 2). It is not possible to generate the spectral sensitivities observed by differential filtering of the same visual pigment. Therefore, in common with the longer-wavelength sensitivities in the R1 to R7 portion of the eye, ultraviolet spectral sensitivities of the R8 cells seem to be generated from a population of different visual pigments[10].

For colour vision, narrow-band photoreceptors have several advantages over broad-band systems, including fine colour discrimination[11] and improved colour constancy[11,12]. The main drawback is a drastic reduction in sensitivity[6], but many stomatopods are found in the brightly lit waters of coral reefs and are usually diurnally active, so highly sensitive vision is not required[7].

Stomatopods use colour in communication[13] and possess colour vision[14] but it has remained unknown whether these extend into the ultraviolet as they do in birds and

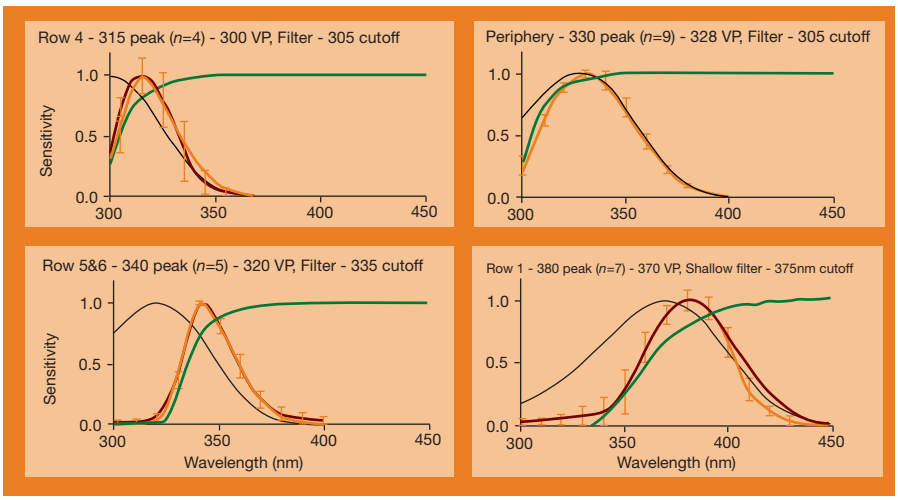

Figure 2 The multiple ultraviolet sensitivities are caused by several different rhodopsins that are heavily filtered. Electrophysiological spectral sensitivities (orange) are shown with standard errors for number of cells measured (*n*). A modelled spectral sensitivity (thick black line) has been fitted to these. Model components are possible visual-pigment absorbances calculated from ref. 7 (thin black lines) and putative filters that narrow the visual-pigment absorbance (green lines). Filter spectra are chosen from a series of known ultraviolet filters (J.M. and U. Siebeck, unpublished data) with 50% cut-off points in the range 300 to 400 nm. These intracellular recordings from stomatopod photoreceptors and our recordings from R1–7 cells in the range 400 to 700 nm (data not shown) broadly confirm previous microspectrophotometric results[2,6].

fish[1], although many coloured areas used by stomatopods in communication contain ultraviolet components[14]. Most of the colour information from natural objects can be decoded by just four types of photo-receptor in the 300–700 nm range[12,15], so there must be other reasons for the bizarre retinal design of stomatopods, which probably uses 12 channels for colour. There are two possible explanations that logically extend the sensitivity range of 400 to 700 nm.

The first possibility is that stomatopod eyes examine colour space from 300 to 700 nm in much the same way as the ear examines auditory space. The multiple, narrow-band spectral sampling channels, from 300 to 700 nm, may be analogous to the different auditory frequencies to which a cochlea is tuned along its length. This may be thought of as a kind of 'digital' colour vision.

Alternatively, stomatopods may divide the spectral world from 300 to 700 nm into six dichromatically examined windows (two in the ultraviolet, mediated by row 1–4 R8 cells), each of which is subject to very fine spectral discrimination[14].

One of the ultraviolet sensitivities is in row 5 of the midband, a region of the eye that is probably used in polarization vision[4,5]. Anatomical[4] and microspectrophotometry[6] comparisons show that the same sensitivity exists in row 6 and that this is theoretically optimal for polarized-light vision[16].

As ultraviolet-blind humans, we have created a barrier in the spectrum at 400 nm. For the colour or polarization vision of stomatopods, this barrier is meaningless.

Justin Marshall*, Johannes Oberwinkler†
**VTHRC, University of Queensland, Brisbane 4071, Australia*
†Department of Neurobiophysics, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands

1. Tovee, M. J. *Trends Ecol. Evol.* **10,** 455–460 (1995).
2. Cronin, T. W. & Marshall, N. J. *Nature* **339,** 137–140 (1989).
3. Smith, R. C. & Baker, K. S. *Appl. Optics* **20,** 177–184 (1981).
4. Marshall, N. J. *Nature* **333,** 557–560 (1988).
5. Marshall, N. J. *et al. Phil. Trans. R. Soc. Lond. B* **334,** 33–56 (1991).
6. Cronin, T. W. *et al. Vision Res.* **34,** 2639–2656 (1994).
7. Hart, N. S., Partridge, J. C. & Cuthill, I. C. *J. Exp. Biol.* **201,** 1433–1446 (1998).
8. Hardie, R. C. *Trends Neurosci.* **9,** 419–423 (1986).
9. Arikawa, K. *et al. Vision Res.* **39,** 1–8 (1999).
10. Cronin, T. W. & Marshall, N. J. *J. Comp. Physiol. A* **166,** 261–275 (1989).
11. Neumeyer, C. in *Vision and Visual Dysfunction: Evolution of the Eye and Visual System* Vol. 2 (eds Cronly-Dillon, J. R. & Gregory, R. L.) 284–305 (Macmillan, London, 1991).
12. Osorio, D., Marshall, N. J. & Cronin, T. W. *Vision Res.* **37,** 3299–3309 (1997).
13. Caldwell, R. L. & Dingle, H. *Sci. Am.* **234,** 80–89 (1976).
14. Marshall, N. J., Jones, J. P. & Cronin, T. W. *J. Comp. Physiol. A* **179,** 473–481 (1996).
15. Vorobyev, M., Osorio, D., Bennett, A. T. D. & Cuthill, I. C. *J. Comp. Physiol. A* **183,** 621–633 (1998).
16. Seliger, H. H., Lall, A. B. & Biggley, W. H. *J. Comp. Physiol. A* **175,** 475–486 (1994).

AIDS

Re-emergence of HIV after stopping therapy

A dormant reservoir of human immunodeficiency virus (HIV) is established early on during primary infection[1] which consists of latently infected, resting CD4$^+$ T cells carrying replication-competent HIV. This pool can persist even in individuals who are receiving highly active antiretroviral therapy (HAART)[2–4]. Here we show that this pool rapidly re-emerges within weeks of discontinuing HAART in two patients, and that this re-emergence is associated with the appearance of HIV in the plasma (viraemia) of these patients. Both had been aviraemic while receiving HAART and intermittent treatment with interleukin-2 (ref. 5), and repeated attempts to isolate replication-competent HIV in this population of cells during therapy had been unsuccessful. This finding raises the possibility that there may be other tissue reservoirs of HIV that contribute to early plasma viral rebound following discontinuation of HAART in infected patients.

Despite the success of HAART in driving plasma viraemia to below the levels of detectability in many HIV-infected individuals[6,7], the persistence of a latent reservoir of HIV in resting CD4$^+$ T cells in HAART-treated individuals is considered to be a major impediment to the long-term control of HIV infection[8]. We have recently shown that intermittently administering interleukin-2 during continuous HAART reduces the size of this reservoir in resting CD4$^+$ T cells[5]. The only way to demonstrate that cellular reservoirs of HIV have been eradicated is by clinical trial in which therapy is discontinued in individuals who have become aviraemic with therapy, so we enrolled two patients from a previously reported cohort[5] in such a trial. This trial, involving 18 patients, will be the subject of a separate report[9].

Before HAART was discontinued in these two patients, we were unable to detect replication-competent HIV in the resting CD4$^+$ T cells of peripheral blood at two consecutive time points, despite culturing up to 330 million cells[5], and the virus could not be isolated from the lymph nodes of either patient[5]. After HAART was discontinued, we were able to detect plasma viraemia within three weeks in both patients.

We carried out quantitative co-culture assays[5,10] by using highly purified resting CD4$^+$ T cells at several time points. The pool of resting CD4$^+$ T cells carrying replication-competent HIV emerged shortly after plasma viraemia was detected (Fig. 1). This pool of cells increased in size by 3.8 log by week 4 and by 2.0 log by week 6 in patients 1 and 13 (numbered according to ref. 5), respectively, following the discontinuation of HAART. The integrated form of HIV DNA, which was not detected by Alu-LTR polymerase chain reaction (Table 1) in 10^6 resting CD4$^+$ T cells from either patient during HAART, was readily detectable during the reappearance of the virus in both patients (142 copies per million cells for patient 1 at week 6, and 420 copies for patient 13 at week 8).

Our results demonstrate the speed with which the latent HIV reservoir in the resting CD4$^+$ T-cell compartment emerges during the reappearance of plasma viraemia following discontinuation of HAART. This is as fast as the initial establishment of the reservoir in patients during primary infection[1]. Furthermore, given that replication-competent HIV was not detectable in resting CD4$^+$

Table 1 Quantitative analysis of integrated HIV-1 DNA in resting CD4$^+$ T cells

Patient	*Integrated HIV-1 proviral load (copies per 10^6 cells)*	
	Week 0	Week 6
1	< 0.5	142
	Week 0	Week 8
13	< 0.5	420

Genomic DNA from resting CD4$^+$ T cells was serially diluted and subjected to the polymerase chain reaction (PCR) in duplicate using nested 5′ primer from conserved Alu and 3′ primer from conserved HIV-1 long-terminal repeat (LTR) sequences as described[2]. A portion of the diluted first PCR product was further subjected to the second round of PCR using nested HIV-1 LTR-specific primers. The product of two rounds of PCR was detected by dot blotting using LTR-specific probe. Week 0 represents the time when patients discontinued HAART.

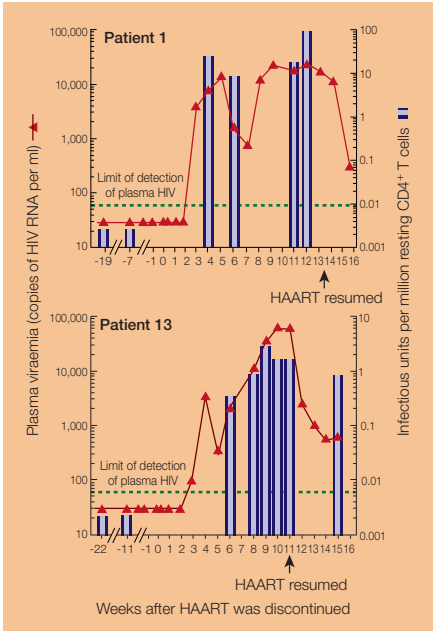

Figure 1 Rebound in plasma viraemia and re-emergence of the pool of latently infected, resting CD4$^+$ T cells in two patients after HAART was discontinued. Plasma HIV RNA was measured by using the bDNA assay (Chiron) with a detection limit of 50 copies per ml. Frequencies of resting CD4$^+$ T cells carrying replication-competent HIV were determined by quantitative co-culture assays as described[5,10]. Patients 1 and 13 received HAART for 33 and 30 months, respectively, before it was discontinued. Week 0 represents the time when patients discontinued HAART.