Ultraviolet colour perception in European starlings and Japanese quail

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Summary

Whereas humans have three types of cone photoreceptor, birds have four types of single cones and, unlike humans, are sensitive to ultraviolet light (UV, 320–400 nm). Most birds are thought to have either a violet-sensitive single cone that has some sensitivity to UV wavelengths (for example, many non-passerine species) or a single cone that has maximum sensitivity to UV (for example, oscine passerine species). UV sensitivity is possible because, unlike humans, avian ocular media do not absorb UV light before it reaches the retina. The different single cone types and their sensitivity to UV light give birds the potential to discriminate reflectance spectra that look identical to humans. It is clear that birds use UV signals for a number of visual tasks, but there are few studies that directly demonstrate a role for UV in the detection of chromaticity differences (i.e. colour vision) as opposed to achromatic brightness. If the output of the violet/UV cone is used in achromatic visual tasks, objects reflecting more UV will appear brighter to the bird. If, however, the output is used in a chromatic mechanism, birds will be able to discriminate spectral stimuli according to the amount of reflected light in the UV part of the spectrum relative to longer wavelengths. We have developed a UV 'colour blindness' test, which we have given to a passerine (European starling) and a non-passerine (Japanese quail) species. Both species learnt to discriminate between a longwave control of orange *vs* red stimuli and UV *vs* 'non-UV' stimuli, which were designed to be impossible to differentiate by achromatic mechanisms. We therefore conclude that the output of the violet/UV cone is involved in a chromatic colour vision system in these two species.

Key words: ultraviolet, UV, colour perception, European starling, Japanese quail, bird, vision, avian.

Introduction

Chromatic vs achromatic vision

Colour vision enables objects, or regions of objects, to be identified from differences in the intensity and spectral composition of light reflected from those objects. 'Achromatic' mechanisms are colour blind and are involved in the perception of brightness; they are reliant on either the response of a single cone type or the additive responses from several cone types. In contrast, 'chromatic' mechanisms are responsible for perception of chromaticity (colour) differences; they permit the discrimination of stimuli by their spectral composition and regardless of their relative intensity; they give an animal colour vision. Colour vision is achieved by comparing the output of two or more receptor types that differ in spectral sensitivity (Wyszecki and Stiles, 1982; Osorio et al., 1999). For humans, hue and saturation are the chromatic aspects of colour. Hence, the human perceptual sensations of brightness, saturation and hue are not inherent properties of a stimulus but are an interaction between the properties of light reflected from that stimulus and the properties of the visual system viewing that stimulus (Lythgoe, 1979; Jacobs, 1983; Endler, 1990).

Human vs avian colour vision

Humans have three different types of single cone photoreceptor. Each contains a different photopigment that is either short (SWS), medium (MWS) or long wavelength sensitive (LWS). For humans possessing normal colour vision, three primary colours are required to match any colour and, thus, humans are said to be trichromatic (Wyszecki and Stiles, 1982). In contrast, most birds studied have four spectral types of single cone (Bowmaker et al., 1997; Cuthill et al., 2000; Hart, 2001), so it is possible that they require four primary colours and hence are tetrachromatic (Burkhardt, 1989; Palacios et al., 1990; Palacios and Varela, 1992; Bennett et al., 1994; Vorobyev et al., 1998; Osorio et al., 1999; Cuthill et al., 2000). Like humans, birds have SWS, MWS and LWS single cones, although the cone photopigments of birds and primates are not homologous (Yokoyama and Yokoyama, 1996; Wilkie et al., 1998). Birds also have either a cone with peak sensitivity in violet wavelengths and considerable sensitivity in the near-ultraviolet (UVA, 320–400nm) region (VS cone) or a cone with maximal sensitivity in the UVA region (UVS cone) (Bowmaker et al., 1997). Possession of a

VS cone appears to be typical of non-passerine birds such as ducks and poultry (Wortel et al., 1987; Hart et al., 1999; Prescott and Wathes, 1999), whereas possession of a UVS cone appears to be typical of songbirds (oscine passerines; Hart et al., 1998; Bowmaker et al., 1997; Hart, 2001) and parrots (Bowmaker et al., 1997). Birds also have a substantial number of double cones across their retina. The function of these cells is unclear, although they are not thought to contribute to colour vision (Maier and Bowmaker, 1993; Vorobyev et al., 1998; Cuthill et al., 2000).

The presence of multiple photoreceptor types raises the question of how the outputs of the receptors are neurally coded. If there are n cone types, it is plausible that the animal has n-dimensional colour vision. However, multiple pigments may instead only broaden the range of wavelengths to which the animal is sensitive and may not be involved in wavelength discrimination (D'Eath, 1998). Behavioural tests can distinguish between these alternatives and establish the dimensionality of an animal's colour vision (Jacobs, 1981; Goldsmith, 1990; Thompson et al., 1992; Varela et al., 1993).

Birds have been shown to use UV cues in tasks such as foraging and mate choice (e.g. Viitala et al., 1995; Bennett et al., 1996; Andersson and Amundsen, 1997; Bennett et al., 1997; Church et al., 1998; Johnson et al., 1998; Koivula and Viitala, 1999; Sheldon et al., 1999; Maddocks et al., 2002; Siitari et al., 2002; Siitari and Viitala, 2002). However, the way in which the output of the VS/UVS cone of birds is neurally coded is not clear. If the VS/UVS cone output is purely used in achromatic mechanisms, then surfaces that reflect more UV will appear brighter to the bird. Alternatively, if the output of the VS/UVS cone is utilised in chromatic vision, then birds could detect chromaticity differences between surfaces according to their UV reflectivity relative to longer wavelengths.

Three previous studies suggest that birds perceive UV as a chromatic rather than an achromatic signal, but none is conclusive. The first study found that songbirds could learn to discriminate between UV-reflective and non-UV-reflective paint marks under natural light (Derim-Oglu and Maximov, 1994). Discrimination performance was unaffected by small changes in intensity of the stimuli, so it was concluded that the birds could perceive UV chromaticity differences. However, UV reflectance in the stimuli was created by mixing powdered chalk, which is highly UV reflective, with white paint. This technique conceivably alters the surface properties of the paint as well as its UV reflectance. As no controls were employed to ensure that the birds really were using a UV cue, we cannot be sure whether the birds had learnt a UV chromaticity or a texture discrimination.

The second piece of evidence comes from a series of mate-choice studies (Bennett et al., 1996; Pearn et al., 2001; Maddocks et al., 2002) in which female birds preferred to associate with males viewed through UV-transmitting rather than UV-blocking filters but did not show a preference for males viewed under different levels of illumination. Although this provides strong evidence that the birds were seeing and responding to UV wavelengths, and not simply changes in intensity, it does not test directly how UV cues are neurally processed.

The third study (Osorio et al., 1999) provides compelling evidence that the four single cone outputs of birds are compared for use in a colour vision system. Pairs of domestic chicks learnt to discriminate between stimuli that were either UV rich or UV poor under lighting conditions that excluded the use of the LWS or MWS cones. However, although the stimuli were random in intensity, there were no controls to check that the birds were really using UV as the discriminatory cue for short wavelength discriminations. Also, we cannot be certain that double cones were not involved in the discrimination, as Osorio et al. (1999) acknowledge. Furthermore, the lighting conditions used in this experiment are unlikely to be found in nature, and it is possible that a different pattern of results would occur under lighting conditions more representative of natural light environments. Indeed, Neumeyer and Arnold (1989) found that goldfish only compare cone outputs under certain conditions. Goldfish shift from being tetrachromatic at high light intensities to being trichromatic at lower light intensities by dropping the LWS cone signal. Thus, even if we assume that the chicks really were making a UV chromaticity discrimination under the restricted lighting conditions of the Osorio et al. (1999) experiment, we still cannot be sure how birds would normally see UV under full-spectrum light. Natural light, which varies greatly in spectral irradiance, does contain UV wavelengths, but the spectrum is dominated by longer wavelengths, particularly under overcast conditions (Dixon, 1978; Lythgoe, 1979; Endler, 1990). So, it is possible that under natural full-spectrum lighting, the output of the UV cone is added to the output of the SWS cone to increase the intensity detection at the short wavelength end of the spectrum. What is clear, however, is that currently we do not know how the signal from the VS/UVS cone is wired up.

To determine whether birds use their VS/UVS cone signal in a chromatic mechanism, it is necessary to show behaviourally that they can distinguish the UV part of the spectrum from other parts of the spectrum without using intensity cues. It is not known exactly how the visual system of birds produces the perception of brightness. However, it is possible to create stimuli in which intensity is a totally unreliable cue and in which chromatic signals are the only reliable predictive discriminatory cue (as in the study by Osorio et al., 1999). The ability to see UV chromatic signals can therefore be ascertained by giving the bird a discrimination task in which it learns to discriminate between patterns of random intensity that either do or do not contain UV reflectances. We used such an associative learning technique to test the ability of European starlings (*Sturnus vulgaris*) and Japanese quail (*Coturnix coturnix japonica*) to make both short wavelength (UV *vs* 'non-UV') and long wavelength (orange *vs* red) discriminations under full-spectrum lighting. The long-wavelength task was included as a positive control, so that any failure on the UV discrimination could not be attributed to some non-specific failure to learn the task. We chose to use

starlings and quail because they form models of the two main classes of avian colour vision systems; starlings have a UVS cone typical of oscine passerine species and quail have a VS cone typical of non-passerine species.

From an animal welfare perspective, if birds can see UV, the limited emission of UV from artificial lights (Lewis and Morris, 1998) may be detrimental to captive birds, as it may limit the functional capacity of their vision. There has already been some research in this area (e.g. Moinard and Sherwin, 1999; Prescott and Wathes, 1999; Sherwin, 1999; Sherwin and Devereux, 1999; Jones et al., 2001; Moinard et al., 2001; Maddocks et al., 2001). However, the previous visual experience of an animal may affect its ability to perceive UV, as many aspects of visual development rely on the animal being exposed to a normal mixture of wavelengths during development. Although Rudolph and Honig (1972) found that monochromatic rearing conditions did not affect the acquisition of spectral discrimination in chicks, it is plausible that absence of UV wavelength stimulation during rearing may lead to selective UV photoreceptor damage and subsequent perceptual impairment. It is therefore possible that supplementary UV may only benefit birds that have been reared under UV-containing light. Consequently, we also compared the perceptual abilities of quail that had been reared under UV-containing light (UV+) with quail that had been reared under lighting that was deficient in UV (UV–).

Materials and methods
Animals

Twelve female quail *Coturnix coturnix japonica* (Linnaeus) were kept in groups of six, each in a floor pen that measured 2.6 m×1.8 m. Six individuals had been raised in UV+ conditions, while six individuals had been raised in UV– conditions. The UV+ conditions consisted of full-spectrum fluorescent lamps [Durotest Truelite, for irradiance spectra, see Hunt et al., 2001, running on high-frequency ballasts (>30 kHz; Cooper Lighting and Security Ltd, Doncaster, UK)]. These tubes are designed to mimic natural sunlight in their approximate balance of UV and longer human-visible wavelengths (Bennett et al., 1996). The UV– lighting conditions were created by covering these lamps with a UV-blocking filter (Lee 226 UV– filter, Lee filters, Andover, UK; see Fig. 1 for transmission spectra). Two of the quail reared in UV+ conditions and two of the quail reared in UV– conditions were tested when they were between four and eight months of age. Four European starlings *Sturnus vulgaris* (Linnaeus) were wild-caught as juveniles under an English Nature licence (#20000069) in Somerset, UK and were maintained under UV+ conditions in the laboratory. The starlings were tested in our experiments when they were between six and eight months of age.

Stimuli

Perceptual ability was tested by giving the birds a discrimination task in which they were allowed to move freely around a foraging arena. In this arena, there were always eight stimuli that overlay separate food wells (1.5 cm×1.0 cm diameter × depth). In every trial, four stimuli of one colour were rewarded, and four stimuli of another were not rewarded with food. If birds can perceive and remember the difference between the two sorts of stimuli, then they should learn to ignore the unrewarded stimuli.

The stimuli were 2.5 cm×2.5 cm patterns consisting of a tiling of 121 grey squares of varying intensity (see Fig. 2 for examples). Birds are able to resolve at least four cycles per degree (Schmid and Wildsoet, 1997), so the discrete squares should have been perceptible to the animals. Each pattern was attached to the upper surface of a 37 g metal weight of the same size as the pattern (Fig. 2) to increase the energetic cost of moving the stimuli and thereby promote learning. On their lower surfaces, each weight was completely coated with matt black paint. The sides and bottoms of all the weights were laminated with Sellotape to prevent chipping of the paint, which may have provided the birds with alternative cues with which to solve the task during training. Birds were trained on three different visual discriminations, which generated the three different experiments described below. In each experiment, there were 12 pairs of training patterns.

Experiment 1

In experiment 1, the birds were trained to discriminate orange from red. All squares in the patterns were set within a grey grid, the intensity of which did not vary. Within each pattern, there were 35 randomly placed squares that were either all red or all orange. Patterns were printed onto paper using a colour inkjet printer (Epson Stylus Photo, 1440 d.p.i.). The patterns were overlain by a 3 mm thick UV-blocking Perspex filter to prevent the birds learning any discrimination based upon either UV reflection or UV-induced fluorescence in longer wavelengths (see Fig. 1 for transmission spectrum,
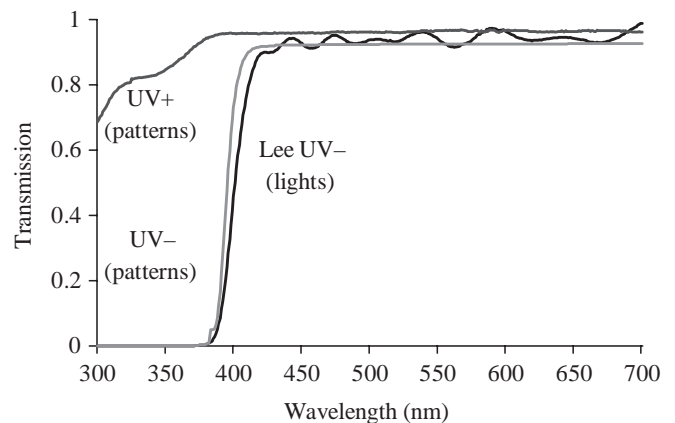

Fig. 1. Transmission spectra of the three filter types used in the three experiments. Lee UV– (lights) refers to the flexible Lee 226 UV-blocking filter used to remove UV wavelengths from the ambient light by covering the light sources; this occurred in probe trial 2 of experiments 1, 2 and 3. UV+ (patterns) and UV– (patterns) refer to the UV-transmitting and UV-blocking solid Perspex filters used to cover the training stimuli, respectively.
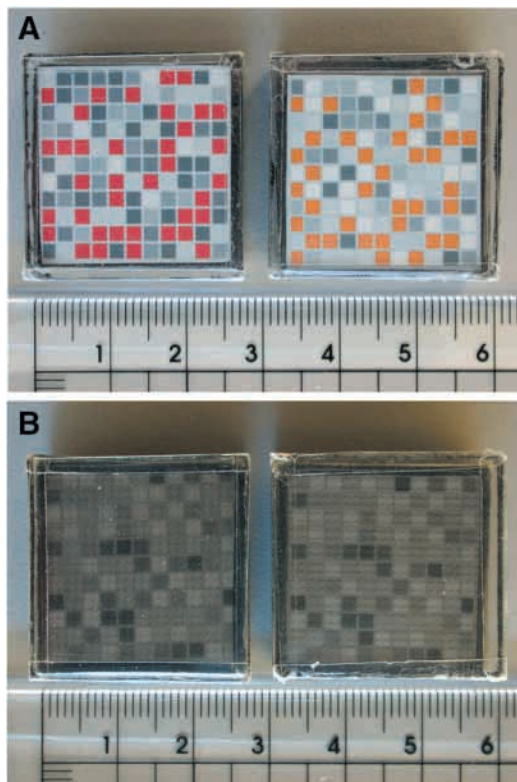
Fig. 2. Scale photographs of example stimuli. (A) orange *vs* red stimuli. (B) UV+ *vs* UV– stimuli.

Fig. 3. Reflectance spectra of the red, orange UV+ and UV– background colours on the stimuli, as measured through the appropriate overlying filter. All stimuli were covered by a UV-blocking filter, except the UV+ stimuli, which were covered by a UV-transmitting filter (see Fig. 1 for transmission spectra of the individual filters). Spectra are means of 10 measurements from different random locations on each background colour.

Fig. 2 for photographs of stimuli and Fig. 3 for the reflectance of colours within them).

Experiment 2

In experiment 2, birds were trained to discriminate between UV-reflecting and non-UV-reflecting patterns. In this experiment, we made both intensity and chromatic cues available within the UV waveband to check that the birds could perceive UV wavelengths. Different UV colours cannot be printed from a standard inkjet printer, so the UV appearance of the patterns was manipulated using filters. Grey waterproof insulating tape (Elephant tape, Sellotape GB Ltd, Dunstable, UK), which is maximally reflective in the UV range, was used to make a UV-reflective surface. Tilings of grey squares of random intensity, similar to those used for experiment 1, were printed onto UV-transmitting acetate and stuck down by their edges over this UV-reflecting surface. Reflectance spectra (300–700 nm) taken with a Zeiss MCS 501 spectrophotometer (Carl Zeiss Ltd, Oberkochen, Germany) showed that increasing the density of grey squares printed onto acetate effectively reduced the reflected light intensity of all wavelengths, including UV wavelengths. To manipulate UV reflectance, these tilings of grey squares were subsequently overlain by UV-transmitting or UV-blocking Perspex (transmission spectra are shown in Fig. 1, a photograph of example stimuli is shown in Fig. 2 and the reflectance of the stimul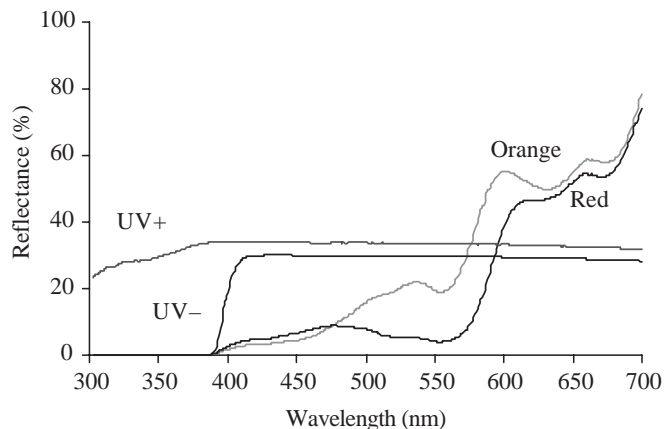i is shown in Fig. 3). This manipulated the chromaticity of all the squares within the pattern. To ensure that reliable intensity cues were available within the UV waveband, we left some of the squares in the overlying acetate pattern completely transparent and did not alter the absolute intensity of the pattern grid surrounding the squares.

We tested that there was no obvious visible difference between the UV and non-UV patterns barring UV cues by showing 24 naive human observers (12 males and 12 females, age range 18–24 years) the stimuli for both experiment 1 and experiment 2 outdoors under natural light. The patterns chosen were identical in pattern and orientation but not in chromaticity and were presented in a two-alternative choice design. Observers were asked to classify the pairs of patterns as being the 'same' or 'different' and were blind to both the aims of the study and the nature of the differences between patterns. Humans naturally classify red and orange patterns as looking different (mean percent choices correct ± S.E.M. 97.9±1.53%), but performance at discriminating UV from non-UV patterns was random (mean percent choices correct ± S.E.M. 50.0±4.14%). The highly significant difference (t=10.87, d.f.=23, P<0.001) in human performance on the two tasks confirmed that there were no obvious alternative cues for the birds to learn in the UV task except differences within the UV waveband.

Experiment 3

In experiment 3, only chromatic cues were available to solve the task. None of the squares within the pattern were left totally transparent, and the absolute intensity of the individual squares within the pattern was highly variable. The spatial layout of the pattern was always the same but there were 25 different levels of overall mean intensity. The intensity of our patterns was manipulated by increasing or decreasing the density of ink

printed on the overlying acetate as appropriate. Measurements with a Zeiss MCS 501 spectrophotometer confirmed that increasing ink density effectively decreased reflected light intensity in a linear manner and that our manipulation of intensity was effective. We also attempted to manipulate the perceived brightness of the squares within the patterns in an additional way by varying the intensity of the grid around the squares. For humans, a dark grid makes the squares within it appear less saturated than does a light grid. This visual effect has never been demonstrated to apply in birds; however, even if birds do not experience such induction effects, it was vital to ensure that intensity of every aspect of the pattern was randomised. Consequently, no two patterns had the same grid intensity, and grid intensity was randomised across all the patterns.

Procedure

A bird was placed in a foraging arena, consisting of a cage containing a 60 cm×90 cm white Conti board on which there were eight equidistant food wells. Because both quail and starlings are gregarious animals, a companion animal was placed behind a wire partition on either side of the arena, so that the test bird was never socially isolated at any time. The test birds did not have access to food during the trials, apart from the food they obtained via choosing stimuli. The apparatus was evenly illuminated by four wall-mounted Durotest Truelite fluorescent lamps running on high-frequency (>30 kHz) ballasts. During training, the ambient light was always UV+.

The birds were trained to push weights off the food wells in the arena using behavioural shaping techniques. They were subsequently trained to discriminate the stimuli for experiments 1, 2 and 3. Prior to training, each bird was deprived of food for 1–2 h to ensure that they were motivated to forage. On each trial, food was placed in four of the eight food wells on the board. The location of the rewarded food wells and the selection of training stimuli presented on each trial was randomised. For each bird, a certain colour was always placed over the food, and the other colour was always unrewarded. For long-wavelength discrimination experiments, half the animals were rewarded for choosing red rather than orange and *vice versa*; for short-wavelength discrimination experiments, half the animals were rewarded for choosing UV over non UV patterns and *vice versa*. While the arena was being set up for each trial, an opaque screen was placed between the birds and the experimenters so that the birds could not see where the food was being placed or the behaviour of the experimenters while they set up the board.

In each trial, birds were considered to have made a choice when they uncovered the food well by pushing off the patterned weight. The order of food wells visited by the bird in each trial was recorded in real-time on a laptop computer using Etholog (E. B. Ottoni, Sao Paulo, Brazil). Trials lasted one minute for the quails and 30 s for the starlings, which move much faster.

Birds were given up to 40 trials per day, with trials presented in blocks of 10. Within each block, trials were separated by approximately four minutes. Birds were rested for an hour between each block of trials to ensure that they stayed motivated. Training continued until the birds were performing well above chance. The learning performance of quail was assessed by scoring the proportion of choices correct out of the total choices per trial. Starlings never stopped uncovering all the food wells, regardless of whether they contained food or not, so instead they were assessed by scoring the proportion of choices correct out of the first four food wells they chose to visit. When a bird was at least 80% correct, averaged over its previous 10 training trials, it was considered to have learnt the discrimination to criterion.

A series of probe trials was given to ensure that the animals had learnt the desired discriminatory cue. The bird had to be 100% correct over two consecutive training trials to receive a probe trial. In probe trial 1, the bird was given a test in which there was no food in any of the food wells and in which all the stimuli were similar to those used in training but had not previously been seen by the bird. Correct performance showed that the birds were not using olfaction or simply recognising individual characteristics of the training stimuli. Probe trial 2 also used novel stimuli, but UV wavelengths were removed from the ambient light by placing a Lee 226 UV-blocking filter (see Fig. 1 for transmission spectra) over the lights in the room. This ascertained what effect removal of UV wavelengths had on performance. Probe trials 1 and 2 were repeated twice for each bird, using novel stimuli each time, and were carried out for all of the experiments. The order of probe trials given was counterbalanced over time. Each bird was given regular training trials in between probe trials to ensure that its performance was still at criterion, as learning could potentially be extinguished by the presentation of unrewarded trials.

For experiment 3, the birds were given a third probe trial to ascertain whether they were using chromatic or achromatic cues to make the discrimination. Although absolute intensity within the grid arrangement on each tile had been randomised in the training stimuli, this did not ensure that the birds did not see UV patterns as being brighter. If birds are more sensitive to the UV waveband than to the rest of the spectrum, then it is plausible that the UV patterns still looked brighter on average. In probe trial 3, UV patterns were three times darker than the non-UV patterns. Nevertheless, intensity within any individual pattern was highly variable, as before. Consequently, if a bird trained to choose UV patterns uses achromatic mechanisms to solve the task, then it will use the algorithm 'always choose the brightest/lightest patterns'. In this case, a UV-trained bird should incorrectly select the much lighter non-UV patterns over the UV patterns. Conversely, if a bird trained to choose non-UV patterns uses achromatic mechanisms to solve the task, then it will use the algorithm 'always choose the darkest patterns'. In this case, the non-UV trained birds should incorrectly select the much darker UV patterns. Performance on the first presentation of probe trial 3 was therefore critical, as the birds could potentially learn the obvious intensity difference between the UV and non-UV patterns over

subsequent trials. Probe trial 3 was carried out three times per bird, interspersed by training trials and probe trials 1 and 2.

Analysis

For each experiment, the mean percentage of correct choices made was calculated for the last 10 training trials prior to starting probe trials and for the appropriate probe trials. For quail, the proportion of correct choices was calculated from the total number of choices they made in a trial. For starlings, the proportion correct out of their first four choices was calculated. We compared the performance of the birds during their last 10 training trials with their performance on each type of probe trial using one-sample t-tests.

Results

The two quail reared in UV+ conditions performed similarly to the two quail that had been reared in UV– conditions, so we have therefore pooled the data for all four quail. The results for experiments 1–3 for all four starlings and all four quail are shown in Tables 1–3, respectively.

Discussion

The main results to emerge from this study are that, under lighting conditions designed to mimic natural light conditions, both quail and starlings see UV wavelengths and use UV for colour vision. This is consistent with opponent processing of the output from the VS/UVS cone photoreceptor in at least one chromatic channel. All four Japanese quail and all four European starlings gradually learnt to discriminate between orange *vs* red stimuli and UV *vs* non-UV stimuli. Both species could perform these discriminations without reliable intensity cues and, during subsequent unrewarded probe trials,

Table 1. *Results of experiment 1 for quail and starlings*

Experiment 1	Mean % choices correct±S.E.M.	
	Quail	Starlings
Last 10 training trials	87.8±3.5	92.4±3.4
Probe 1 Novel stimuli, no food reward	96.4±3.6 t=2.52, d.f.=3, P=0.086	88.7±5.2 t=1.63, d.f.=3, P=0.2
Probe 2 Novel stimuli, UV– conditions	85.4±5.9 t=0.16, d.f.=3, P=0.886	80.7±3.9 t=3.01, d.f.=3, P=0.057

All the birds were over 80% correct during their last 10 training trials. In probe trial 1, the birds remained over 80% correct when novel stimuli were used without reward. In probe trial 2, when UV was removed from the ambient light (UV– conditions), performance remained high.

Statistics show one sample t-test on difference in performance between the last 10 training trials and probe trials.

Table 2. *Results of experiment 2 for quail and starlings*

Experiment 2	Mean % choices correct±S.E.M.	
	Quail	Starlings
Last 10 training trials	94.0±1.8	88.9±2.2
Probe 1 Novel stimuli, no food reward	94.4±3.2 t=0.09, d.f.=3, P=0.932	80.2±8.9 t=0.9, d.f.=3, P=0.435
Probe 2 Novel stimuli, UV– conditions	53.8±3.3 t=19.97, d.f.=3, P=0.000	50.0±11.6 t=4.38, d.f.=3, P=0.022

All birds were over 80% correct during their last 10 training trials. In both species, performance did not decline when novel stimuli were used without reward (probe trial 1), but performance dropped significantly and to random levels when UV was removed from the ambient light (probe trial 2).

Statistics show one sample t-test on difference in performance between the last 10 training trials and probe trials.

performed in a way that was consistent with the birds perceiving UV as a chromatic signal.

Tables 1, 2 and 3 show that the results for both species were very similar for experiments 1, 2 and 3. In all three experiments, both quail and starlings were over 80% correct during their last 10 training trials on each task. In probe trial 1, the birds always remained over 80% correct when novel

Table 3. *Results of experiment 3 for quail and starlings*

Experiment 3	Mean % choices correct±S.E.M.	
	Quail	Starlings
Last 10 training trials	86.0±2.5	83.8±2.4
Probe 1 Novel stimuli, no food reward	82.3±4.0 t=1.22, d.f.=3, P=0.309	87.5±5.4 t=0.73, d.f.=3, P=0.517
Probe 2 Novel stimuli, UV– conditions	40.7±5.2 t=10.85, d.f.=3, P=0.002	52.1±5.2 t=3.29, d.f.=3, P=0.046
Probe 3 Selects appropriate wavelength regardless of intensity	81.3±5.4 t=1.10, d.f.=3, P=0.350	87.5±2.4 t=1.00, d.f.=3, P=0.391

Both species are still over 80% correct during their last 10 training trials and when novel stimuli were used without reward (probe trial 1). Again, performance drops significantly and to random levels when UV is removed from the ambient light (probe trial 2). Finally, the performance of the birds on the task was resistant to large variations in intensity, with birds correctly selecting patterns of the appropriate wavelengths (probe trial 3).

Statistics show one sample t-test on difference in performance between the last 10 training trials and probe trials.

stimuli were used without rewards. This confirms that (i) on each test the birds had learnt something general about the patterns rather than individual characteristics of the training stimuli and (ii) that they were not using olfaction. When UV wavelengths were removed from the ambient light (probe trial 2), performance remained high in experiment 1, indicating that removal of UV wavelengths does not alarm the birds sufficiently to prevent them from making discriminations (see Table 1). However, in experiments 2 and 3, performance dropped to random in probe trial 2, confirming that UV was the cue that the birds had been using to make the discriminations (see Tables 2, 3). In experiment 3, in which only chromatic signals were available as reliable cues, the performance of the bird on the task was resistant to large variations in the overall intensity of novel patterns, with birds still correctly selecting patterns of the appropriate wavelengths (see probe trial 3, Table 3). This further suggests that the birds were not making the discrimination between stimuli using achromatic cues but were instead using chromaticity differences.

Despite much evidence that birds see and use UV for ecologically relevant tasks such as mate choice and foraging (reviewed in Cuthill et al., 2000), there has been relatively little investigation into the perceptual experience of UV. Previous studies have strongly suggested that birds can discriminate spectral stimuli, according to the signal of the UV cones relative to that in other single cone types (Derim-Oglu and Maximov, 1994; Bennett et al., 1996; Osorio et al., 1999), but all are open to alternative interpretations. The experiments we describe here form a more watertight case that exemplars of both poultry (Japanese quail) and passerines (European starlings) use UV signals in a chromatic mechanism and can do so under full-spectrum lighting.

The ability of these two species to make wavelength discriminations based upon the presence or absence of UV shows that the output of the VS/UVS cone is being compared with the output of one or more other cone types, not simply being added to it. Although this is not a demonstration of tetrachromacy, this experiment provides firm evidence that the VS/UVS cone is in some way opponently coded, and that birds have a dimension to their colour vision that humans do not. However, this experiment does not tell us with which other cone type, or types, the VS/UVS cone is being compared.

It is also still not known whether the VS/UVS cone contributes to the perception of brightness as well as the perception of chromaticity differences. It is well known that the SWS cone of humans contributes little, if at all, to achromatic mechanisms (Mollon, 1989), so it is plausible that the VS/UVS cone of birds may, likewise, not be involved in brightness perception. Current evidence suggests that this may be the case, as avian perception of longer wavelengths appears to be involved in the detection of both motion (Campenhausen and Kirschfeld, 1998) and visual texture (Osorio et al., 2001).

It has been suggested that supplemental UV lighting may benefit bird welfare (e.g. Sherwin, 1999; Sherwin and Devereux, 1999; Maddocks et al., 2001; Maddocks et al., 2002). However, if rearing birds without UV wavelengths selectively impairs their ability to perceive it, birds that have been reared in UV– conditions will not be able to benefit from supplemental UV. As quail reared in UV– conditions could perceive UV wavelengths, rearing quail without UV wavelengths does not seem to impair their ability to see and use UV cues. This is consistent with previous work on birds showing that rearing under restricted spectral distributions does not affect subsequent ability to make spectral discriminations (Rudolph and Honig, 1972). The two birds reared without UV appeared to learn the UV discrimination as easily as their counterparts reared in UV+ conditions. With such a small sample size, it is not possible to make a firm judgement as to whether rearing without UV wavelengths affects the rate at which birds respond to and learn about UV cues. However, it is possible that the visual rearing environment affects later learned colour preferences and colour learning (Miklósi et al., 2002). We could not test whether or not absence of UV during development prevents the UVS cone of passerines from developing normally, as the starlings were wild caught and had developed their visual systems under natural light. The VS cone of quail would have been stimulated by blue light during development even in the absence of UV and may be at lower risk of impairment through rearing in the absence of UV compared with the UVS cones of passerines. As it is currently thought that the provision of supplemental UV lighting may be beneficial to bird welfare, this topic seems worthy of further investigation.

In conclusion, both starlings and quail learnt colour differences in the UV waveband that are invisible to human observers, and the birds were clearly making choices based upon perceived wavelength differences in the stimuli. From these experiments, although we are now confident that the UV-sensitive cones of both passerines and poultry are involved in a colour opponency mechanism, we do not know with which cone types their output is compared nor the nature of the opponency. Further psychophysical and neurophysiological studies are needed to ascertain precisely how these particular photoreceptors work.

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