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Spatial summation improves bird color vision in low light intensities

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Abstract

Color guides many important behaviors in birds. Previously we have shown that the intensity threshold for color discrimination in the chicken depends on the color contrast between stimuli and their brightness. The birds could discriminate larger color contrasts and brighter colors in lower light intensities. We suggested that chickens use spatial summation of cone signals, to maintain color vision in low light levels. Here we tested this hypothesis by determining the intensity thresholds of color discrimination using similar stimuli, patterns of grey tiles of varying intensity interspersed with color tiles, adjusted for this specific aim. Chickens could discriminate stimuli with a larger single color tile, or with a larger proportion of small color tiles, in lower light intensities. This is in agreement with the hypothesis that spatial summation improves color discrimination in low light levels. There was no difference in the intensity threshold for discrimination of stimuli with a single 6 x 6 mm color tile, stimuli with 30% colored tiles and stimuli in which color filled the whole pattern. This gives a first indication to the degree of spatial summation that can be performed. We compare this level of spatial summation to predictions from model calculations.

Keywords: spatial summation; vision; color vision; birds; dark noise; intensity threshold; visual modelling

1 Introduction

Color vision guides important behaviors of birds, such as finding food and choosing between mating partners (Bennett and Cuthill, 1994; Bennett et al., 1997; Church et al., 1998; Hunt et al., 2001; Maddocks et al., 2001). Bird color vision is mediated by four types of single cone photoreceptors sensitive to red light (long wavelengths, L), green light (medium wavelengths, M), blue light (short wavelengths, S) and violet or ultraviolet light (very short wavelengths, VS/UVS) (Hart, 2001; Osorio et al., 1999; Vorobyev et al., 1998). Bird cones are equipped with colored oil droplets that act as long pass filters and narrow cone spectral sensitivities. This is thought to improve color discrimination and color constancy (Barlow, 1982; Govardovskii, 1983; Vorobyev, 2003; Vorobyev et al., 1998) at the cost of the absolute sensitivity of color vision (Toomey et al., 2016; Vorobyev, 2003; Wilby et al., 2015).

We assume that color discrimination thresholds, including intensity thresholds, are set by noise (Lind and Kelber, 2009a; Vorobyev and Osorio, 1998; Vorobyev et al., 2001). Over a wide range of light intensities, Weber's law holds, so that sensitivity changes proportionally to light intensity (Lind et al., 2013), and a constant Weber fraction (ω) describes the signal-to-noise ratio that sets discrimination thresholds (Brown, 1951; Lind et al., 2013; Olsson et al., 2015; Yebra et al., 2001). At lower light intensities, the signal-to-noise ratio decreases as photon-shot noise and dark noise become more important (Osorio et al., 2004).

Photon-shot noise is caused by the stochastic nature of photon arrival that follows Poisson statistics. For a photon sample size N, the uncertainty, or photon-shot noise, is

$\sqrt{N}$, and the signal-to-noise ratio is $N/\sqrt{N}$, which is expressed as the de Vries-Rose law (De Vries, 1943; Rose, 1942; Rose, 1948). The absolute threshold of vision is set by dark noise, caused by spontaneous activation of the transduction cascade, indistinguishable from real photon absorption (Barlow, 1956; Rieke and Baylor, 1998; Rieke and Baylor, 2000). When the quantum catch of a photoreceptor is smaller than the standard deviation of the dark noise events, the light signal cannot be reliably detected.

In general, color vision is assumed to be restricted to higher light intensities than achromatic vision, because it requires comparison of the signals from two or more visual channels instead of summation, thus reducing the signal-to-noise ratio. Mathematical models predict that the higher the dimensionality of an animal's color vision the worse their color vision should be in low light (Vorobyev, 1997). Tetrachromatic birds, with light absorbing oil droplets, could therefore be at a disadvantage with regards to low light color vision compared to tri- and dichromatic mammals. The intensity threshold for color discrimination has been tested only in four bird species, and all of them loose color vision at higher light intensities than humans, by a factor of 2-10 (Gomez et al., 2014; Lind and Kelber, 2009b; Olsson et al., 2015; Kelber et al. 2002).

It has been proposed that visual systems can use spatial and temporal summation, integrating the signals from several photoreceptors over time and space, to increase the photon sample (N) and reduce the effect of photon-shot noise (Barlow, 1958), at the cost of spatial and temporal resolution. This phenomenon is well documented in achromatic pathways e.g. (Donner, 1987; Stöckl et al., 2016), but has only been suggested for chromatic vision (Kelber et al., 2002; Roth and Kelber, 2004).

In a previous experiment, we found that the intensity threshold for color discrimination of chickens depends on the chromatic contrast between the stimuli and on stimulus brightness (Olsson et al., 2015). We hypothesized that the chickens used spatial summation to maintain color discrimination in low light intensities. In this study we test this hypothesis, for the first time, by determining the intensity threshold for color discrimination in chickens, using stimuli which differ in the number and size of colored tiles.

2. Materials and methods

We determined the intensity threshold of color discrimination in chickens, by training them to a two-choice color discrimination task in successively lower light intensities. The stimuli were paper food containers, printed with color and grey tile patterns, similar to those that have been used with chickens before (Olsson et al., 2015; Olsson et al., 2016; Osorio et al., 1999). We used four types of stimulus patterns, in which either 100% of the tiles, 10% of the tiles, one single large tile or one single small tile of the stimulus were colored, see Fig. 1 for examples. Stimuli that contained more or larger color tiles, should be discriminable at lower light intensities if spatial summation was

important for color discrimination. We used a rewarded orange color (O+) and an unrewarded yellow color (Y-), and we repeated some tests with a rewarded green color (G+) and an unrewarded blue color (B-).

2.1 Animals

24 Lohman White chickens (Gimranäs AB, Herrljunga, Sweden) were obtained as eggs and hatched in a commercial incubator (Covatutto 24, Högberga AB, Matfors, Sweden) at the animal housing facility of Lund University. Both male and female chickens were used in the study. They were housed in 1x1 m boxes in groups of six to eight individuals. All experiments were carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and ethical approval was obtained from a local ethical committee (permit nr. M6-12, Swedish Board of Agriculture). Water was available *ad libitum* but during experimental days, access to food, commercial chick crumbs (Fågel Start, Svenska Foder AB, Staffanstorp), was restricted to training session and after the last training session of the day. On days with no training or testing, food was available *ad libitum*.

2.2 Experimental arena and illumination

The experiments were carried out in a wooden arena (0.7 x 0.4 m) painted matte grey and illuminated by fluorescent tubes (Biolux L18W/965, Osram, München, Germany). We measured the spectral radiance of the illumination (Fig. S1 in supplementary information) as reflected from a white standard placed on the floor of the experimental arena using a spectroradiometer (RSP900-R; International Light, Peabody, MA, USA). The intensity of the illumination was reduced with neutral density filters and a potentiometer, which controlled the light intensity of the fluorescent tubes. We measured the luminance of white paper placed on the floor of the experimental arena using a photometer (Hagner ERP-105 Luminance meter, with an SD17 detector. Hagner AB. Solna, Sweden). We used luminances of 350 cd m^{-2}, 15 cd m^{-2}, 1.5 cd m^{-2}, 0.6 cd m^{-2}, 0.3 cd m^{-2}, 0.1 cd m^{-2} and 0.05 cd m^{-2} (see Fig. S1 in supplementary information).

2.3 Stimuli

Color stimuli similar to those used in previous studies (Olsson et al., 2015; Olsson et al., 2016; Osorio et al., 1999) were created in Adobe Illustrator CS5 (Adobe Systems Inc., San Jose, CA, USA) and printed on copy paper (Canon, Tokyo, Japan). A stimulus consisted of a printed pattern of tiles, forming a rectangle measuring 30 mm x 36 mm and folded into a cone-shaped food container. A given pattern contained only one of the colors (O+/Y-/G+/B-), besides grey tiles. We created four types of stimulus patterns. Two pattern types consisted of 270 tiles measuring 2x2mm each, with 100% or 10% (Fig. 1A and C) colored tiles respectively. A third pattern type consisted of 120 tiles, each measuring 3x3 mm, with only 1 color tile (Fig. 1B), and the fourth pattern type consisted of 30 tiles, each measuring 6x6 mm, again with only one colored tile (Fig. 1B). In the patterns with multiple color tiles, a random amount of black ink, random K

value in CMYK color coding, was added to adjust the intensity of each colored tile within a contrast range (the contrast between the highest and lowest intensity version of the colour) of 0.15 for O+ and Y- and 0.08 for G+ and B-. In patterns with a single color tile, no black ink was added to the color tile. The remaining tiles in each pattern were assigned a random grey intensity, and the achromatic contrast, between the highest and lowest intensity grey tile was 0.3. The intensity range of colored tiles was within the intensity range of the grey tiles. The achromatic contrast between the stimulus pairs (O+ vs Y- and G+ vs B-) was lower than 0.1, the achromatic contrast threshold of chickens (Jones and Osorio, 2004).

2.4 Training and testing

We performed experiments with two pairs of stimulus colors, training some chickens to discriminate a positive (rewarded) orange (O+) from a negative (unrewarded) yellow (Y-) color, and others to discriminate a positive green (G+) from a negative blue (B-) color. The color difference between the colors were 2.6 and 3.3 JND for the color pairs G+-B- and O+-Y- respectively. Each chicken had two training or testing sessions per day. Training started on the third day post-hatch. During the first day of training, groups of 4 to 6 chickens were placed in the experimental arena where they had access to two or three positive stimuli, orange (O+) or green (G+) food containers filled with food crumbs. The chickens learned to peck at the stimuli to spill out and eat the food. On the second day of training, the chickens were trained in pairs with only one positive stimulus, which was continuously refilled for ca. 5 minutes per session. On the third day, two chickens were initially placed behind a separating cardboard wall, and could access one positive stimulus filled with food after removal of the wall. This procedure was repeated on the fourth day, but with individual chickens, while a companion chicken was placed in an adjacent cage maintaining audio and visual contact to the experimental bird. On the fifth day of training, the negative stimuli, empty yellow (Y-) or blue (B-) food containers, were introduced. From this day onwards, each session consisted of 20 such trials. Tests started after chickens reached a learning criterion of 75% correct choices in two consecutive training sessions.

The first test was performed in the training illumination, and every consecutive day we reduced the intensity of the illumination, allowing two sessions of 20 trials for each chicken in each illumination, until the chicken's choice performance reached chance level. For comparison we also present the intensity thresholds for the same colors obtained in a previous study, in which 30% of the tiles in each pattern were colored (the colors O+ and Y- were named O+ and O4 and G+ and B- were named G+ and G4, in (Olsson et al., 2015)). The radiometer used to measure the intensity here was different from the one used in the previous study, there was a difference in measured intensity by a factor of two, which was corroborated by other instruments. We accordingly multiplied the thresholds from the previous study by two, to allow for comparison.

2.5 Visual modelling

Color differences, ΔS, were calculated using the receptor noise limited (RNL) model (Vorobyev and Osorio, 1998) as

$$\Delta S^2 = \frac{\begin{matrix}(\omega_1\omega_2)^2(\Delta f_4-\Delta f_3)^2+(\omega_1\omega_3)^2(\Delta f_4-\Delta f_2)^2 \\ +(\omega_1\omega_4)^2(\Delta f_3-\Delta f_2)^2+(\omega_2\omega_3)^2(\Delta f_4-\Delta f_1)^2 \\ +(\omega_2\omega_4)^2(\Delta f_3-\Delta f_1)^2+(\omega_3\omega_4)^2(\Delta f_2-\Delta f_1)^2\end{matrix}}{(\omega_1\omega_2\omega_3)^2+(\omega_1\omega_2\omega_4)^2+(\omega_1\omega_3\omega_4)^2+(\omega_2\omega_3\omega_4)^2} \quad \text{(Eq. 1)},$$

where Δf_i is the signal, or Weber contrast, within a photoreceptor mechanism calculated as

$$\Delta f_i = \ln\left(\frac{Q_i\ stimulus\ 1}{Q_i\ stimulus\ 2}\right) \quad \text{(Eq. 2)}.$$

where Q_i is the relative quantum catch of single cone of type i, which is calculated as

$$Q_i = \int_{300}^{700} R_i(\lambda)S(\lambda)I(\lambda)d\lambda \quad \text{(Eq. 3)},$$

where R_i is the spectral sensitivity of receptor type i, S is the reflectance spectrum of the stimulus and I is the radiance of the illumination.

Spectral sensitivities, R, were derived by fitting a template (Govardovskii et al., 2000) to absorbance peak of chicken visual pigments and transmittance spectra of oil droplets (Bowmaker et al., 1997) and ocular media (Lind et al., 2014).

The noise within a receptor channel is expressed as a Weber fraction, ω_i, which is calculated as

$$\omega_i = \frac{\sigma_i}{\sqrt{\eta_i}} \quad \text{(Eq. 4)},$$

where σ_i is the standard deviation of the noise in receptor type i, and η_i is the relative abundance of receptor type i.

We used relative abundances of single cone types from the literature (Kram et al., 2010), resulting in η of 1:1.5:2.5:2 for the VS:S:M:L cone types. We assumed the same standard deviation of noise σ for all cone types such that the Weber fraction for the LWS channel was 0.06, based on the color discrimination thresholds measured in a previous study (Olsson et al., 2015).

We included photon shot noise in the calculation of color differences in low light by changing the calculation of the Weber fraction to

$$\omega_{i_{photon\ noise}} = \sqrt{{\omega_i}^2 + \frac{1}{N_i}} \quad \text{(Eq. 5)},$$

where the absolute quantum catch N_i of a cone of type i is calculated as

$$N_i = \left(\frac{\pi}{4}\right)^2 \left(\frac{d}{f}\right)^2 D^2\kappa\tau\Delta t \int_{300}^{700}\left(1 - e^{-kA(\lambda)l}\right)F(\lambda)S(\lambda)I(\lambda)\mathrm{d}\lambda \quad \text{(Eq.6)},$$

where d is the receptor diameter taken as the width of the ellipsoid, f is the focal length and D is the pupil diameter, κ is the electrical conversion coefficient, τ is the transmittance of the ocular media, Δt is the integration time obtained from flicker fusion frequency experiments (Lisney et al., 2011), k is the absorbance coefficient, A is the absorbance of the visual pigment filtered only by the ocular media, l is the length of the outer segment and S is stimulus reflectance, I is the radiance of the illumination. F is the fraction of light within the cross-sectional area of the inner segment that is focused into the outer segment by the oil droplet. All parameters can be found in table 1. F (Fig. S2) was calculated from an optical simulation of chicken single cone photoreceptors, which includes the optics of the ellipsoid, oil droplet and outer segment (see the supplementary material for more details). For comparison, the same method of calculating the absolute quantum catch as in a previous study (Olsson et al., 2015), is included in the supplementary material (Eq.S2).

Achromatic contrasts were calculated as the Michelson contrast, C, for the double cone as

$$C = \frac{Q_{DC_{stim1}} - Q_{DC_{stim2}}}{Q_{DC_{stim1}} + Q_{DC_{stim2}}} \quad \text{(Eq. 7).}$$

2.6 Modelling spatial summation

We estimated the level of spatial summation required to reach a modelled color difference of 1 JND at the behaviorally determined intensity threshold using the RNL model (Eq.1) with a Weber fraction that included the effect of photon shot noise (Eq.5). We assumed that the integrative field for color discrimination contained 1 VS, 1.5 S, 2.5 M and 2 L cones, on average. Two integrative fields for example, summed the photons from 2 VS, 3 S, 5 M, and 4 L cones. We modelled increasing levels of spatial summation, assuming that absolute quantum catches (Eq. 6 and Table S2) from each cone type are summed linearly and determined the number of photoreceptors that the model (Eqs. 1 and 5) required to sum signals from, to reach 1 JND at the intensity thresholds.

We calculated the retinal image size of color tiles, given a viewing distance of 30 cm (the distance between release point and stimuli) and 5 cm (the shortest observed choice distance). From these retinal image sizes and cone densities in the dorso-temporal retina of chickens (Kram et al., 2010) we estimated the number of cones that viewed a single color tile of a stimulus. Finally, we compared the modelled numbers of cones with the number of cones in the retinal image of single tiles.

2.7 Analysis

We derived intensity thresholds by fitting a logistic psychometric function to the choice data of each experimental group of chickens and individual chickens using the Matlab toolbox Palamedes (Prins and Kingdom, 2009):

$$\psi(x) = \gamma + (1 - \gamma + \lambda)\frac{1}{1+e^{\frac{a-x}{b}}} \quad \text{(Eq. 9)},$$

where ψ is the frequency of correct choices at stimulus value x, γ is the lower asymptote, set to 0.5, and λ is the lapse rate, set to 0.2. *a* and *b* are free parameters estimated from the distribution of the data using a least square approach. We used a frequency of 0.65 of correct choices as threshold, based on the binomial test ($p<0.05$ probability of correct choice by chance 0.5, n=40). The thresholds in Fig. 1 are fitted based on the data from the group, but individual thresholds were also derived and are available in the supplementary data (Fig S3-5). We compared intensity thresholds between experiments (Fig. 2) using the individual intensity thresholds (Fig S3-5), with the Kruskal-Wallis test in Matlab R2015b.

3 Results

3.1 Intensity thresholds of color discrimination in behavioral tests

We performed one experiment with both color pairs, O+ versus Y- and G+ versus B-. In this experiment we used full color stimuli and patterns with 10% colored tiles. We determined the illumination intensity, in which chickens chose the positive color significantly more often than the negative color. With both color pairs, the intensity thresholds (fitted threshold±S.E) for the full color stimulus (Fig. 1A and C; 0.08±0.01 cd m^{-2} for orange and 0.20±0.10 cd m^{-2} for green) were lower (Fig 2; $p<0.05$, Kruskal-Wallis) than the intensity thresholds for the stimuli with 10% colored tiles (Fig. 1A and C; 0.90±0.10 cd m^{-2} for orange and 1.55±0.35 cd m^{-2} for green).

With the orange and yellow colors, we also tested patterns with one single small or large colored tile. The intensity thresholds for the large single tile stimuli (Fig 1B; 0.08±0.02 cd m^{-2}) were significantly lower than those for the small single tile color stimuli (Fig. 1B; 0.49±0.05 cd m^{-2}) (Fig. 2; $p<0.05$, Kruskal-Wallis) and for the pattern with 10% color tiles (Fig 2; $p<0.05$, Kruskal-Wallis). However, they were not lower than the thresholds for the full color pattern (Fig 2; $p>0.05$, Kruskal-Wallis).

3.2 Model estimations of absolute quantum catch and spatial summation required for successful colour discrimination

We used two different models to estimate the absolute quantum catch of individual photoreceptors. The first model included the optical effects of the ellipsoid, oil droplets and the outer segment, which have all been shown to be important in determining the amount of light available inside the outer segment (Wilby et al., 2015). The second model, which is simpler and assumes that the oil droplet only has a filtering effect, is used for comparison with previous data. The optical model resulted in lower quantum catches than the simple model (see Table 3, and compare Table 2 with Table S1 in supplementary material). Further analysis is performed using the data obtained from the optical model, which should be physiologically more relevant.

At the intensity thresholds for discriminating the patterns with more or larger color tiles, photon numbers are so low (Table 2 and 3) that photon-shot noise makes an important contribution to total noise levels. If photon-shot noise is included in the model and no spatial summation is assumed, our model suggests that the colors could not have been discriminated at their respective intensity thresholds (Table 4).

The level of spatial summation required for the model to predict 1 JND for the orange-yellow color difference at 0.08 cd m^{-2} was ca. 200 integrative fields, or 200:300:500:400 VS:S:MWS:LWS photoreceptors (Table 4). The level of spatial summation required to predict 1 JND for the green-blue color discrimination task was 124 integrative fields at 0.1 cd m^{-2} and 44 integrative fields at 0.3 cd m^{-2}. Colour discrimination will not gain from summing the signals from other than the cones that are actually viewing the color tiles. Therefore, we compared this theoretically predicted level of summation to the number of cones that absorb photons reflected from a single tile, when the chicken is looking at the patterns from 30 cm or 5 cm distance (Table 4). The level of spatial pooling predicted by the model is reasonably similar and does not surpass the number of cones in the retinal image of single tiles, seen from the distances from which the chickens made the discrimination.

4 Discussion

We tested the hypothesis that spatial summation of cone signals improves color discrimination in low light intensity in the chicken, by determining intensity thresholds of color discrimination using specifically designed stimuli. In line with this hypothesis, we found that the intensity thresholds were higher for stimuli with fewer or smaller color tiles and lower for stimuli with larger or more color tiles.

The intensity thresholds found with the full color stimuli and the large single color tile stimuli were not different to the intensity thresholds found earlier with stimuli containing 30% (6x2 mm) colored tiles (O+-O4/G+-G4, in (Olsson et al., 2015)) (0.067±0.01 and 0.40±0.39 cd m^{-2} respectively), (Fig 2; Kruskal-Wallis $p<0.05$). The similarity of the intensity thresholds measured in these experiments suggests that the results of the previous experiment (Olsson et al., 2015) were not limited by stimuli that had too few or too small colored tiles to estimate correct absolute intensity thresholds.

Spatial summation for achromatic visual tasks in low light is well known (Barlow, 1958; Donner, 1987; Stöckl et al., 2016). However, spatial summation for color vision in low light has been suggested for both invertebrates (Kelber et al., 2002) and vertebrates (Lind and Kelber, 2009b; Olsson et al., 2015; Roth and Kelber, 2004) but is not well studied. To maintain color information, signals from different spectral types of photoreceptors must remain separated. Can a visual system spatially sum cone signals over a larger area and still maintain separate spectral channels?

An optimal trade-off between spatial resolution and sensitivity requires a dynamic spatial summation mechanism recruiting more and more photoreceptors as the light intensity decreases (Barlow, 1958). The fact that we found a lower intensity threshold

for stimuli with larger or more color tiles suggests that a dynamic summation mechanism could be present, but is not proof for it. A dynamic spatial summation mechanism could be tested behaviorally by determining the acuity with isoluminant color gratings. The acuity for color gratings should decrease with lower light intensity if a dynamic summation mechanism is present. Unfortunately, there are no such studies published at the moment as far as we can find. There are two studies that have tested the contrast sensitivity function (CSF) to colour gratings in humans (Mullen 1985) and budgerigars (Lind and Kelber, 2011). In both cases the acuity to colour gratings are ca. half that which is found for achromatic gratings.

We found the same intensity threshold in tests with the large single color tile stimuli and the full color pattern stimuli suggesting that the level of summation may not increase beyond the angular size of the large single color tiles.

The intensity thresholds for the full color patterns and the large single color tile stimuli are similar to those found in our previous study (Olsson et al., 2015). In the previous study, the behaviorally determined intensity thresholds for stimuli with larger color differences suggested that the absolute color vision limit is presumably set by receptor dark noise (Olsson et al., 2015). Receptor dark noise may also set the intensity threshold for the stimuli with the larger and more abundant color tiles in this study, which would fit the observation of no decrease in intensity threshold despite a potential for higher levels of spatial summation between the full colour stimuli and the large single colour tile stimuli.

4.1 Modelling spatial summation

Using mathematical modelling allows us to speculate whether the predicted level of summed photoreceptor signals required to reduce the effect of photon shot noise is reasonable given the image of the color tiles of the stimuli on the retina and the density of cones on the retina. However, this modelling is sensitive to the parameters we have used and appropriate caution should be observed.

We used two models to estimate the absolute quantum catch of the photoreceptors, a simple model (SM), assuming that all photons that strike the ellipsoid of the photoreceptor cell are guided into the oil droplet, where they may be absorbed, and then into the outer segment and visual pigments. The second model includes the intracellular optics of the photoreceptor cells (OM) and is based on a previous study (Wilby et al. 2015). In the optical model light may be lost due to reflections at the oil droplet, caused by the high refractive index gradient between the ellipsoid and the oil droplet, and….

In general, the number of photoreceptors required by the modelling to reach 1 JND at the specific intensity thresholds were well within the limits of the number of photoreceptors viewing the color tiles and therefore potentially available for summation (Table 4). We found that the level of spatial summation we needed to assume in order to consolidate mathematical modelling of colour discrimination and behavior, fitted

better assuming a physiologically more realistic optical model of quantum catch (Table 4).

4.2 Concluding remarks

Predation risks from visually guided predators are expected to increase with higher light intensities (Lima and O'Keefe, 2013). It may therefore be beneficial for prey animals, such as chickens, to remain active in low light environments. Color information, based on physical color contrasts, will remain available regardless of the light intensity. Therefore, maintaining color vision in lower light intensities, with strategies such as spatial summation, should enable these animals to successfully perform color-guided behaviors earlier in the day and later in the evening when light levels are low.

This is the first time that the hypothesis that spatial summation is important for color discrimination in low light has been tested. We show that spatial summation is important for determining the intensity thresholds for color vision. This suggests that it is difficult to estimate the intensity threshold of color vision for an animal based on morphological information alone, and that modelling color discrimination in dim light should be done with great care.

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Conflict of interest

All authors declare no conflict of interest.

Ethical statement

The behavioral experiments were approved by an ethical committee (permit nr. M6-12, Swedish board of agriculture).

Author contributions

PO initiated the study and developed the experiment together with AK. PO and DW performed the behavioral experiment and the mathematical modelling. All authors contributed to data analysis and interpretation. PO wrote the manuscript with input from DW and AK.

References

Barlow, H. B. (1956). Retinal noise and absolute threshold. *J. Opt. Soc. Am. A* **46**, 634–639.

Barlow, H. B. (1958). Temporal and spatial summation in human vision at different background intensities. *J. Physiol.* **141**, 337–350.

Barlow, H. B. (1982). What causes trichromacy? A theoretical analysis using comb-filtered spectra. *Vision Res.* **22**, 635–643.

Bennett, A. T. D. and Cuthill, I. C. (1994). Ultraviolet vision in birds: what is its function? *Vision Res.* **34**, 1471–1478.

Bennett, A. T. D., Cuthill, I. C., Partridge, J. C. and Lunau, K. (1997). Ultraviolet plumage colors predict mate preferences in starlings. *Proc. Nat. Acad. Sci.* **94**, 8618–8621.

Bowmaker, J. K. and Knowles, A. (1977). The visual pigments and oil droplets of the chicken retina. *Vision Res.* **17**, 755–764.

Bowmaker, J. K., Heath, L. A., Wilkie, S. E. and Hunt, D. M. (1997). Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Res.* **37**, 2183–2194.

Brown, W. (1951). The influence of luminance level on visual sensitivity to color differences. *J. Opt. Soc. Am. A* **41**, 684–688.

Church, S. C., Bennett, A. T. D., Cuthill, I. C. and Partridge, J. C. (1998). Ultraviolet cues affect the foraging behaviour of blue tits. *Proc. R. Soc. B* **265**, 1509–1514.

De Vries, H. L. (1943). The quantum character of light and its bearing upon threshold of vision, the differential sensitivity and visual acuity of the eye. *Physica* **10**, 553–564.

Donner, K. (1987). Adaptation-related changes in the spatial and temporal summation of frog retinal ganglion cells. *Acta Physiol. Scand.* **131**, 479–487.

Enoch, J. M. and Tobey, F. L., Jr (1978). Use of the waveguide parameter V to determine the difference in the index of refraction between the rat rod outer segment and the interstitial matrix. *J. Opt. Soc. Am. A* **68**, 1130–1134.

Gomez, D., Gregoire, A., Del Rey Granado, M., Bassoul, M., Degueldre, D., Perret, P. and Doutrelant, C. (2014). The intensity threshold of colour vision in a passerine bird, the blue tit (*Cyanistes caeruleus*). *J. Exp. Biol.* **217**, 3775–3778.

Govardovskii, V. I. (1983). On the role of oil drops in colour vision. *Vision Res.* **23**, 1739–1740.

Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K. (2000). In search of the visual pigment template. *Visual Neurosci.* **17**, 509–528.

Hart, N. S. (2001). The visual ecology of avian photoreceptors. *Progr. Retinal Eye Res.* **20**, 675–703.

Hunt, S., Cuthill, I. C., Bennett, A. T. D., Church, S. C. and Partridge, J. C. (2001). Is the ultraviolet waveband a special communication channel in avian mate choice? *J. Exp. Biol.* **204**, 2499–2507.

Johnsen, S. (2012). *Optics of life*. New Jersey: Princeton University Press.

Jones, C. D. and Osorio, D. C. (2004). Discrimination of oriented visual textures by poultry chicks. *Vision Res.* **44**, 83–89.

Kelber, A., Balkenius, A. and Warrant, E. J. (2002). Scotopic colour vision in nocturnal hawkmoths. *Nature* **419**, 922–925.

Kram, Y. A., Mantey, S. and Corbo, J. C. (2010). Avian cone photoreceptors tile the retina as five independent, self-organizing mosaics. *PLoS ONE* **5**, e8992.

Lima, S. L. and O'Keefe, J. M. (2013). Do predators influence the behaviour of bats? *Biol. Rev.* **88**, 626–644.

Lind, O. and Kelber, A. (2009a). Avian colour vision: Effects of variation in receptor sensitivity and noise data on model predictions as compared to behavioural results. *Vision Res.* **49**, 1939–1947.

Lind, O. and Kelber, A. (2009b). The intensity threshold of colour vision in two species of parrot. *J. Exp. Biol.* **212**, 3693–3699.

Lind, O., Chavez, J. and Kelber, A. (2013). The contribution of single and double cones to spectral sensitivity in budgerigars during changing light conditions. *J. Comp. Physiol. A* **200**, 197–207.

Lind, O., Mitkus, M., Olsson, P. and Kelber, A. (2014). Ultraviolet vision in birds: the importance of transparent eye media. *Proc. R. Soc. B* **281**, 20132209.

Lisney, T. J., Ekesten, B., Tauson, R., Håstad, O. and Ödeen, A. (2012). Using electroretinograms to assess flicker fusion frequency in domestic hens Gallus gallus domesticus. *Vision Res.* **62**, 125–133.

Lisney, T. J., Rubene, D., Rózsa, J., Løvlie, H., Håstad, O. and Ödeen, A. (2011). Behavioural assessment of flicker fusion frequency in chicken Gallus gallus domesticus. *Vision Res.* **51**, 1324–1332.

Maddocks, S. A., Church, S. C. and Cuthill, I. C. (2001). The effects of the light environment on prey choice by zebra finches. *J. Exp. Biol.* **204**, 2509–2515.

Olsson, P., Lind, O. and Kelber, A. (2015). Bird colour vision: behavioural

thresholds reveal receptor noise. *J. Exp. Biol.* **218**, 184–193.

Olsson, P., Wilby, D. and Kelber, A. (2016). Quantitative studies of animal colour constancy: using the chicken as model. *Proc. R. Soc. B* **283**, 20160411.

Osorio, D. C., Smith, A. C., Vorobyev, M. and Buchanan Smith, H. M. (2004). Detection of Fruit and the Selection of Primate Visual Pigments for Color Vision. *Am. Nat.* **164**, 696–708.

Osorio, D. C., Vorobyev, M. and Jones, C. D. (1999). Colour vision of domestic chicks. *J. Exp. Biol.* **202**, 2951–2959.

Prins, N. and Kingdom, F. A. A. (2009). Palamedes: Toolbox for analysing psychophysical data. *www.palamedestoolbox.org*.

Rieke, F. and Baylor, D. A. (1998). Single-photon detection by rod cells of the retina. *Rev. Mod. Phys.* **70**, 1027–1036.

Rieke, F. and Baylor, D. A. (2000). Origin and functional impact of dark noise in retinal cones. *Neuron* **26**, 181–186.

Rose, A. (1942). The relative sensitivities of television pickup tubes, photographic film, and the human eye. *Proc. IRE* **30**, 293–300.

Rose, A. (1948). The sensitivity performance of the human eye on an absolute scale. *J. Opt. Soc. Am. A* **38**, 196–208.

Roth, L. S. V. and Kelber, A. (2004). Nocturnal colour vision in geckos. *Biol. Lett.* **271**, S485–S487.

Stöckl, A. L., O'Carroll, D. C. and Warrant, E. J. (2016). Neural summation in the hawkmoth visual system extends the limits of vision in dim light. *Curr. Biol.* **26**, 1–6.

Toomey, M. B., Lind, O., Frederiksen, R., Curley, R. W., Riedle, K. M., Wilby, D., Schwartz, S. J., Witt, C. C., Harrison, E. H., Roberts, N. W., et al. (2016). Complementary shifts in photoreceptor spectral tuning unlock the full adaptive potential of ultraviolet vision in birds. *eLife* **5**, e15675.

Vorobyev, M. (1997). Cost and benefits of increasing the dimensionality of colour vision systems. In *Biophysics of vision: Molecular and phototransductive events* (ed. Taddei-Ferreti, C., pp. 280–289. Singapore: World Scientific.

Vorobyev, M. (2003). Coloured oil droplets enhance colour discrimination. *Proc. R. Soc. B* **270**, 1255–1261.

Vorobyev, M. and Osorio, D. C. (1998). Receptor noise as a determinant of colour

thresholds. *Proc. R. Soc. B* **265**, 351–358.

Vorobyev, M., Brandt, R., Peitsch, D., Laughlin, S. B. and Menzel, R. (2001). Colour thresholds and receptor noise: behaviour and physiology compared. *Vision Res.* **41**, 639–653.

Vorobyev, M., Osorio, D. C., Bennett, A. T. D., Marshall, J. N. and Cuthill, I. C. (1998). Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A* **183**, 621–633.

Wilby, D., Toomey, M. B., Olsson, P., Frederiksen, R., Cornwall, M. C., Oulton, R., Kelber, A., Corbo, J. C. and Roberts, N. W. (2015). Optics of cone photoreceptors in the chicken (*Gallus gallus domesticus*). *J. Roy. Soc. Interf.* **12**, 20150591.

Yebra, A., Garcia, J. A., Nieves, J. L. and Romero, J. (2001). Chromatic discrimination in relation to luminance level. *Color Res. Appl.* **26**, 123–131.

Tables

Table 1. Parameters used to calculate absolute quantum catches and the enhancement factors.

Parameter (unit)	Value	Ref.
Cone outer segment length (μm)	30	(Olsson et al., 2015)
Cone outer segment width	1.73	(Wilby et al., 2015)
Cone outer segment refractive index	1.45	(Wilby et al., 2015)
Absorption coefficient	0.035	(Bowmaker and Knowles, 1977)
Ellipsoid diameter (μm)	3.1	(Olsson et al., 2015)
Ellipsoid length	3.5	(Wilby et al., 2015)
Ellipsoid refractive index	1.43	(Wilby et al., 2015)
Oil droplet refractive index	as in	(Wilby et al., 2015)
Surrounding medium refractive index	1.35	(Enoch and Tobey, 1978)
Focal length (μm)	8300	(Olsson et al., 2015)
Pupil size (max-min) (μm)	4900-3500	(Olsson et al., 2015)
F-number (min)	1.66	(Olsson et al., 2015)
Transmittance of ocular media τ (%)	80 (at max)	(Johnsen, 2012)
Quantum transduction efficiency κ (%)	50	(Johnsen, 2012)
Integration time (max-min) (ms)	50-12	(Lisney et al., 2011; Lisney et al., 2012)

Table 2. Quantum catches of single cones per integration time from the stimuli at the different intensities, including photoreceptor optics (OM). Bold letters signify the intensity thresholds.

	Quantum catch							
	Rewarded				Unrewarded			
Illumination	**L**	**M**	**S**	**VS**	**L**	**M**	**S**	**VS**
Orange								
300 cd m^{-2}	86.8	37.8	53.8	75.3	77.8	42.1	54.1	67.7
15 cd m^{-2}	19.3	8.5	11.1	15.9	17.3	9.5	11.1	14.3
1.5 cd m^{-2}	3.44	1.73	1.74	3.33	3.11	1.94	1.77	2.98
0.6 cd m^{-2}	1.32	0.74	0.59	1.53	1.20	0.84	0.60	1.37
0.3 cd m^{-2}	0.69	0.39	0.30	0.75	0.62	0.44	0.30	0.66
0.15 cd m^{-2}	0.35	0.20	0.14	0.49	0.32	0.23	0.15	0.35
0.1 cd m^{-2}	0.23	0.13	0.10	0.26	0.21	0.15	0.10	0.23
0.08 cd m^{-2}	**0.20**	**0.11**	**0.08**	**0.22**	**0.18**	**0.13**	**0.08**	**0.19**
0.05 cd m^{-2}	0.10	0.06	0.04	0.12	0.10	0.07	0.04	0.10
Green	**L**	**M**	**S**	**VS**	**L**	**M**	**S**	**VS**
300 cd m^{-2}	25.5	29.6	62.43	81.7	24.1	28.0	69.1	100
15 cd m^{-2}	5.65	6.69	12.8	17.2	5.34	6.32	14.21	20.9
1.5 cd m^{-2}	1.04	1.39	2.02	3.61	0.98	1.32	2.22	4.44
0.6 cd m^{-2}	0.41	0.61	0.68	1.66	0.39	0.58	0.74	2.05
0.3 cd m^{-2}	**0.22**	**0.31**	**0.34**	**0.81**	**0.20**	**0.30**	**0.37**	**1.00**
0.15 cd m^{-2}	**0.11**	**0.17**	**0.17**	**0.43**	**0.11**	**0.16**	**0.18**	**0.53**
0.1 cd m^{-2}	**0.07**	**0.11**	**0.11**	**0.28**	**0.07**	**0.10**	**0.12**	**0.35**
0.05 cd m^{-2}	0.03	0.05	0.05	0.12	0.03	0..05	0.05	0.16

D = 0.35, 0.415, 0.47, 0.47, 0.47, 0.475, 0.48 and 0.5 cm at 300, 15, 1.5, 0.6, 0.3, 0.15,

0.1 and 0.05 cd m^{-2}. Δt = 12.5, 25 and 50, 50, 50, 50, 50 and 50 ms at 300, 15, 1.5, 0.6, 0.3, 0.15, 0.1 and 0.05 cd m^{-2}

Table 3. Comparison of the absolute quantum catch (photons per integration time) at the intensity thresholds using an optical model (OM) and a simple model (SM) of quantum catch.

	Quantum catch							
	Optical model (OM)				Simple model (SM)			
	Photoreceptor type							
Stimulus (intensity)	L	M	S	VS	L	M	S	VS
O+ (0.08 cd m^{-2})	**0.20**	**0.11**	**0.08**	**0.22**	**2.12**	**1.14**	**0.39**	**0.39**
G+ (0.15 cd m^{-2})	**0.11**	**0.17**	**0.17**	**0.43**	**1.21**	**1.93**	**0.82**	**0.77**

D=0.48 and 0.475 cm and Δt = 50 ms and 50 ms for 0.08 and 0.15 cd m^{-2} respectively

Table 4. The size of the stimulus image and the number of receptors available and required to overcome photon shot noise at the intensity threshold. At a photoreceptor density of 3618 VS cones mm^{-2}, there are ca. 1.5, 2.5 and 2 times as many S, M and L cones respectively, in a given retinal image size.

Tile size	Intensity threshold (cd m^{-2})	Image size (mm^2)		#VS photoreceptors required assuming OM (or SM)	# VS photoreceptors in image from 30 – 5 cm
		From 30 cm	From 5 cm		
			Orange		
2x2 mm tiles	1	0.003	0.11	>5<17 (1)	11 – 398
3x3 mm tiles	0.5	0.007	0.25	17-32 (1-2)	25 – 897
6x6 mm tiles	0.08	0.028	0.99	200 (20)	100 – 3600
			Green		
2x2 mm tiles	1.8	0.003	0.11	11 (1)	11 – 398

Figures

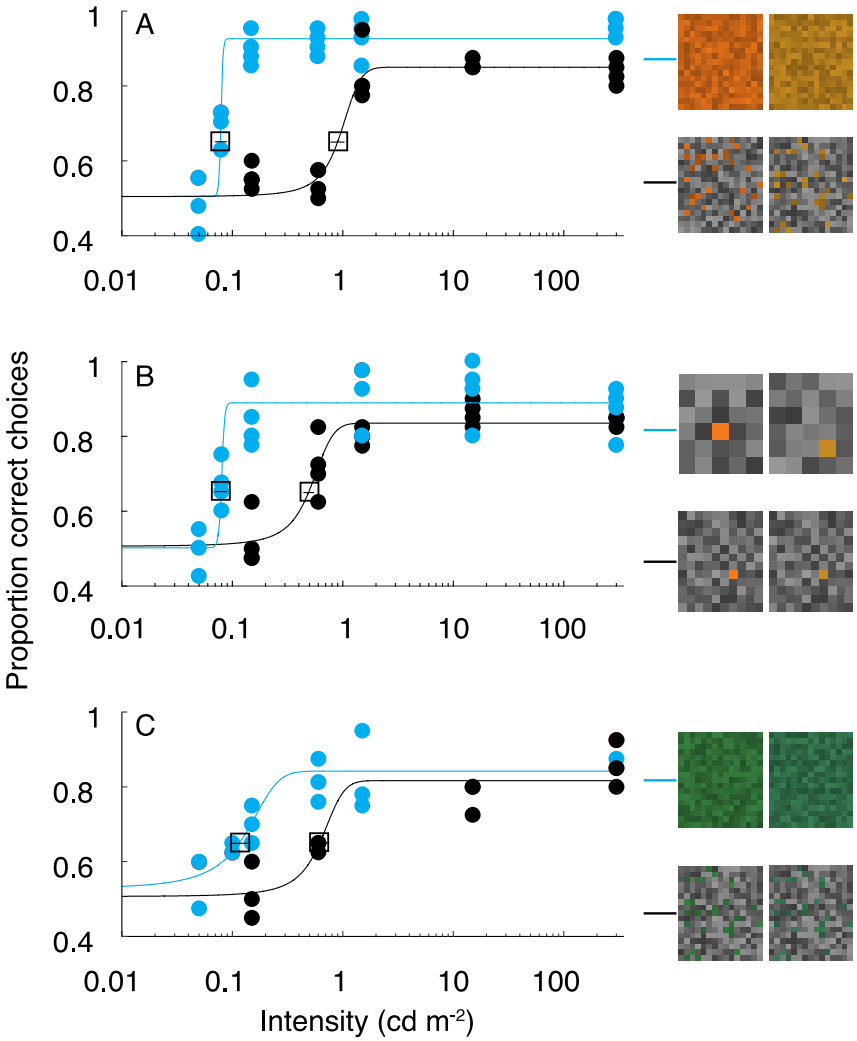

Fig. 1. Color discrimination performance at different light intensities. The proportion of correct choices as a function of light intensity. Each data point represents the choice frequency of one individual. A logistic psychometric function with a threshold estimate (*open box)* is fitted to the data of each group. The inserts and the colours of the data sets show the type of stimulus patterns that the chickens discriminated. (A) Intensity thresholds with full color stimuli and 10% color stimuli for the O+-Y- color discrimination task. (B) Intensity thresholds with large single color tile stimuli and small single color tile stimuli for the O+-Y- color discrimination task. (C) Intensity thresholds with full color stimuli and 10% color stimuli for the G+-B- color discrimination task.

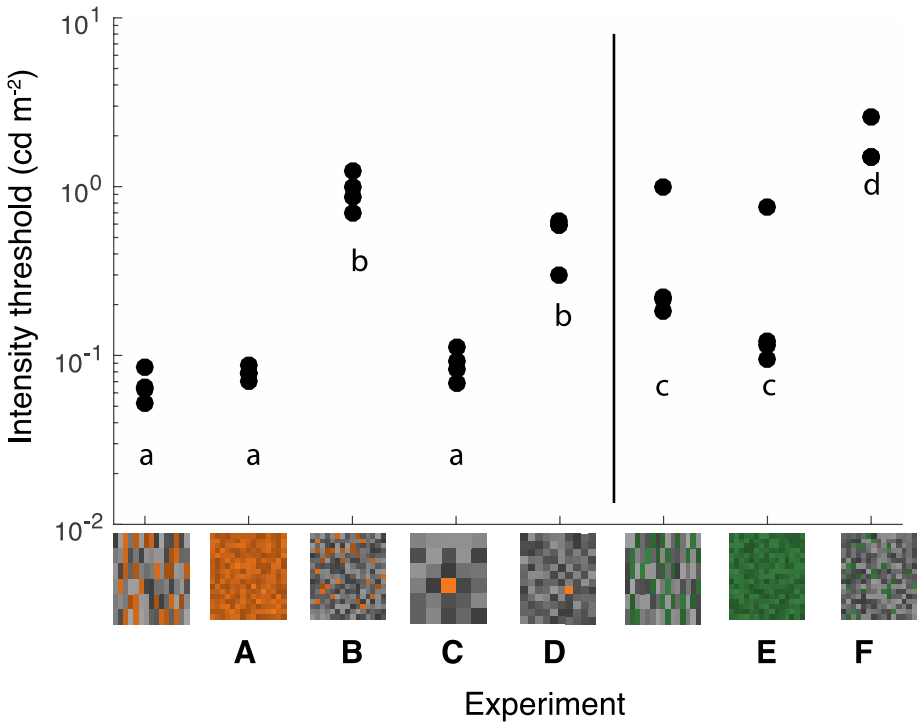

Fig. 2. Individual intensity thresholds of all experiments. The individual (n=4 in all cases, except (F) where n=3) intensity thresholds (see Figs. S3-5 in supplementary material) for each type of color pattern. The same subscript letter (e.g. (a) denotes thresholds that were not significantly different from each other ($p>0.05$ for a Kruskal-Wallis test). Different subscript letters denote groups with significantly different intensity thresholds ($p<0.05$ for a Kruskal-Wallis test). The results of orange-yellow and green-blue color discrimination tasks were not compared. The data points without a letter come from a previous study (Olsson et al., 2015).

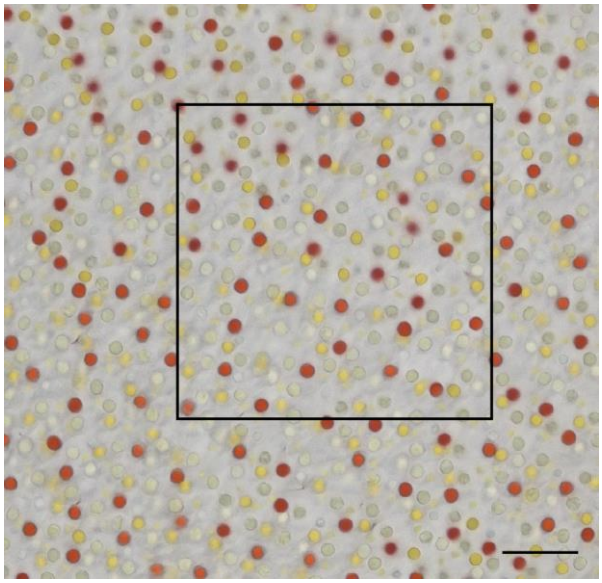

Figure 3. Cone mosaic and the retinal image of a single color tile. The box shows the image of a single 3x3 mm tile from 30 cm viewing distance superimposed on the cone mosaic of a chicken. The photoreceptors within the box, differentiated by the color of the oil droplets, can be assumed to be the maximum number of photoreceptors that the visual system can sum visual information from, for this specific stimulus and distance. Scale bar = 20μm.