Colour perception in a dichromat

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Summary

Most mammals have dichromatic colour vision based on two different types of cones: a short-wavelength-sensitive cone and a long-wavelength-sensitive cone. Comparing the signal from two cone types gives rise to a one-dimensional chromatic space when brightness is excluded. The so-called 'neutral point' refers to the wavelength that the animal cannot distinguish from achromatic light such as white or grey because it stimulates both cone types equally. The question is: how do dichromats perceive their chromatic space? Do they experience a continuous scale of colours or does the neutral point divide their chromatic space into two colour categories, i.e. into colours of either short or long wavelengths?

We trained horses to different colour combinations in a two-choice behavioural experiment and tested their responses to the training and test colours. The horses chose colours according to their similarity/relationship to rewarded and unrewarded training colours. There was no evidence for a categorical boundary at the neutral point or elsewhere.

This study suggests that dichromats perceive their chromatic space as a continuous scale of colours, treating the colour at the neutral point as any other colour they can distinguish.

Key words: colour vision, dichromat, horse, mammal, chromatic space.
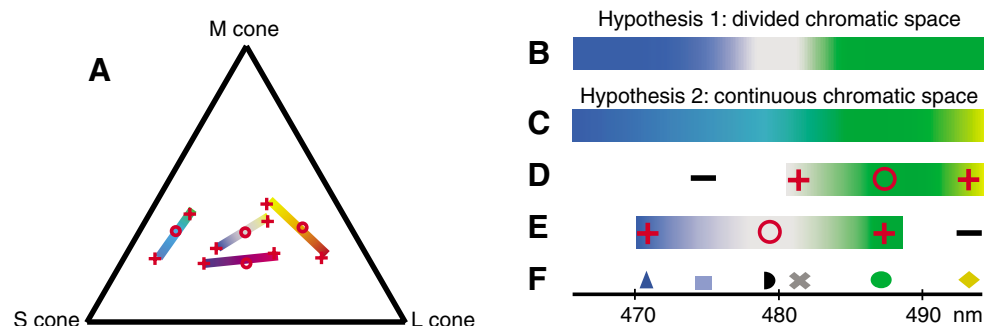
Introduction

Mammals have a nocturnal ancestry, when they probably depended on senses other than vision. Their nocturnality is today the most accepted hypothesis why most non-primate mammals have been left with only two of the four ancient vertebrate cone pigments and a high rod-to-cone ratio in their retina (Jacobs and Rowe, 2004; Walls, 1942). Because of the lack of strong diurnality, it was previously suggested that mammalian colour vision should be quite uncommon (Walls, 1942), but it is now known that dichromatic colour vision is widespread among mammals (Jacobs, 1993; Kelber et al., 2003).

Even though many behavioural studies have confirmed colour vision, it is difficult to establish how dichromats perceive colours in their colour space. The comparison of the short-wavelength-sensitive (S) and the long-wavelength-sensitive (L) cone gives rise to a two-dimensional colour space and to a one-dimensional chromatic space when brightness is excluded. In this one-dimensional space, colours that only stimulate the L cone type will be presented at one end and colours that mainly give rise to a response of the S cone type at the other end. In between, there will be a so-called 'neutral point' where both cone types are stimulated in the same ratio as (human-defined) white light would do. The wavelength that corresponds to the neutral point cannot be distinguished from achromatic colours, such as white and grey, which are defined, according to Wyszecki and Stiles (Wyszecki and Stiles, 1982), as '*perceived colour devoid of hue*'.

In a trichromatic colour space, grey shades are interpreted as qualitatively different from chromatic colours, such as green and red, and it is beautifully seen in Jones and colleagues' study on domestic chicks (Jones et al., 2001) that this is valid for animals other than humans. Chicks are tetrachromats, but by excluding UV light from the experimental situation the chicks were left with trichromatic colour vision, where all isoluminant colours can be visualized in a triangle with a 'grey point' in the centre. The trichromatic chicks in Jones and colleagues' experiment were trained to two similar colours as positive stimuli. When tested with a colour in between both, the chicks preferred the intermediate colour (Fig. 1A). For example, chicks trained to blue and red preferred the intermediate purple colour, indicating generalization. However, chicks trained to yellow and blue, which lie on opposite sides of the grey point, refused to choose grey, suggesting that they do not perceive grey as an intermediate colour between blue and yellow. Thus, they do not generalize over the grey point but treat colours on opposite sides as belonging to different categories.

Still, reverting to the one-dimensional chromatic space of dichromats, it is not clear what role the neutral point plays in the perception of colours. According to one hypothesis (Hypothesis 1; Fig. 1B), the neutral point should divide the chromatic space into two colour categories (Vienot et al., 1995) or, as Jacobs and Deegan write in their paper (Jacobs and Deegan, 1994), '*For dichromatic observers, the neutral point*

Fig. 1. The chromatic space of a trichromat and a dichromat. (A) Chicks were left with a trichromatic vision by excluding UV light from the illumination. They were trained to two positive stimuli (red '+') and they generalized onto a novel intermediate test colour (red circle) in all cases but one. When tested with blue and yellow, which lie on opposite sides of the grey point, the chicks did not generalize onto the intermediate test colour, grey. They treated the grey point as achromatic. Redrawn from (Jones et al., 2001). (B) In dichromats, one hypothesis suggests that the neutral point divides the chromatic space into two colour categories (Jacobs and Deegan, 1994; Vienot et al., 1995). (C) A second hypothesis (Hemmi, 1999) proposes that dichromats perceive a continuous scale of colours. We trained horses in two experiments. (D) As a control, two horses were trained to two positive colours (red '+') with colour loci situated on the same side of the neutral point and tested with a novel intermediate green colour (red circle). The black '−' is the negative colour. (E) To test the first hypothesis – whether the neutral point operates as a categorical boundary in the dichromatic colour space – three horses were trained to two positive colours with colour loci situated on different sides of the neutral point and tested with a novel intermediate grey stimulus corresponding to the neutral point. (F) All colours are visualized and named as they appear to humans and with respect to the corresponding wavelength. The symbols shown in F represent all used colours and their corresponding wavelength, i.e. the wavelength of a monochromatic colour that has the same hue. All stimuli are marked with the same symbols in Fig. 2 (see Materials and methods).

effectively splits the spectrum such that subjects can make colour discriminations between lights whose spectral energy distribution fall principally to either side of the neutral point'. If this hypothesis were true, dichromats would perceive grey at the neutral point and recognize grey as different from chromatic colour, just as the chicks did.

A second hypothesis of colour perception in dichromats, suggested by Hemmi (Hemmi, 1999), assumes that their one-dimensional chromatic space contains a continuous scale of hues where the colour at the neutral point is not qualitatively different from other perceived colours (Hypothesis 2; Fig. 1C). According to this hypothesis, the neutral point does not split the chromatic space into two categories. If this is true, dichromats may be able to generalize between colours on opposite sides of the neutral point.

To investigate whether dichromats perceive their neutral point as qualitatively different from chromatic colours, we performed two experiments on horses (Experiments I and II) similar to those performed on the chicks (Jones et al., 2001). As a control we performed Experiment I (Fig. 1D) with both positive colours located on the same side of the neutral point. In Experiment II (Fig. 1E) we tested Hypothesis 1 (Fig. 1B), and two positive colours were therefore situated on either side of the neutral point. In both experiments intermediate colours were presented in tests to reveal whether the horses generalized between the positive stimuli. If the horses generalized and treated the intermediate colours similarly in both experiments, it would suggest that there is no categorical boundary at the neutral point.

Also, if Hypothesis 2 is accurate, the continuum of colours should allow dichromats to learn colours in a relative manner, which has been indicated already in Hemmi's study on the dichromatic tammar wallaby (Hemmi, 1999). Hemmi trained two wallabies to distinguish between two monochromatic lights and he clearly showed that they have colour vision. He then continued to train both wallabies to choose the colour of longer wavelength. For example, when 500 nm and 480 nm were

presented, 500 nm was treated as the positive stimulus. In combination with 520 nm, it was treated as the negative stimulus. The wallabies obviously learned this relative task and, in Experiment III, we tested whether horses are able to do the same.

Materials and methods
Animals

We used the horse (Equus caballas L.) as a model species since it is easy to train and since horse colour vision has been convincingly confirmed by many studies (Grzimek, 1952; Macuda and Timney, 1999; Pick et al., 1994; Smith and Goldman, 1999). The neutral point has been determined to correspond to 480 nm for the horse (Geisbauer et al., 2004). Three large gelding ponies (Piccolo –99, Winsor –97 and Zimon –84), three half-blood horses, of which two were mares and one was gelding (Luna –99, Ametist –95 and Flash –98), and one fjord horse mare (Bianca –89) were used in the experiments. All experiments have been approved by the Swedish animal welfare agency (M148-05).

Stimuli

Stimuli were of size 15×21 cm and printed on white Munken Pure Copy paper (Arctic Paper Munkedals AB, Munkedal, Sweden) by an Epson Stylus Photo printer 2100. During experiments the stimuli papers were kept in transparent plastic cases, which did not influence their reflectance of light but made them resistant to dirt and moisture.

The reflectance of all stimuli $S(\lambda)$ was measured using an S2000 Spectrometer (Ocean Optics, Dunedin, FL, USA), and the standard daylight spectra (D65) (Wyszecki and Stiles, 1982) was used as light spectrum $I(\lambda)$. The spectral sensitivity of photoreceptor I, $R_i(\lambda)$, was calculated from the horse peak sensitivities (428 and 539 nm) (Carroll et al., 2001) with the Stavenga–Smits–Hoenders rhodopsin template (Stavenga et al., 1993). The relative number of quanta absorbed by the horse's cone types (Q_i) was calculated using
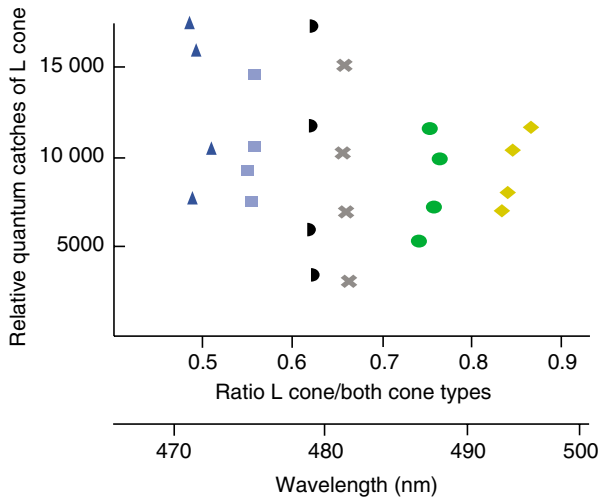
Fig. 2. Stimulus colours. All colours were measured with a photospectrometer and brightness is visualized as the quantum catch for S and L cones, calculated according to Eqn 1. The ratio between the absorption of L cones and the sum of both cone types determines the location of each colour on the *x*-axis. Below the chart, the corresponding wavelength is visualized.

Eqn 1. No significant amount of UV light was reflected from the stimuli:

$$Q_i = \int S(\lambda)\, I(\lambda)\, R_i(\lambda)\, d\lambda \,. \tag{1}$$

Both the quantum catch of the L cone type and the summed quantum catches of both cone types were taken into consideration when matching the intensities of the colour stimuli. Four versions of different brightness of each of the six colours (Fig. 2) were used in a pseudo-random order to minimize the possibility for the horses to use brightness as a cue. In addition, all results from the training combinations were examined and no correlation between correct choices and brightness was found. All horses showed a choice frequency well above 75% both when the positive stimulus was dimmer and brighter than the negative stimulus.

The six colours (two blue colours, two grey colours, green and yellow) are named as they appear to humans, and the wavelengths they correspond to for the horses are given in Figs 1, 2.

Apparatus

The dual-choice discrimination apparatus, similar to those used on horses by Geisbauer et al. (Geisbauer et al., 2004) and Macuda and Timney (Macuda and Timney, 1999), was 166 cm wide and 160 cm high. One door, 30×30 cm in size, was placed on each side of a divider that was sticking out 40 cm, forcing the horse to make its choice of the two doors (Fig. 3). The apparatus was painted in light grey.

Experimental procedure

The training and testing took place in the mornings in an outdoor paddock during June to August. Ropes on both sides of the apparatus enclosed the experimental area. The horses were first led forward to entirely opened doors and fed carrots to

Fig. 3. Experimental apparatus. The horses were released at a distance of 3 m from the two stimuli placed on the doors of the experimental apparatus. A wooden divider forced them to choose a door at a distance of at least 40 cm from the stimuli.

make them accustomed to the stimulus apparatus. Then they were taught to open the doors by themselves while an experimenter was still holding them by the halter. It took approximately one week before we could release them 3 m from the apparatus, letting them choose between the unlocked doors, which both showed positive stimuli. Depending on the performance of the horses, it took an additional 2–4 days before we introduced them to their training sessions with both negative and positive stimuli.

For the training sessions we trained one horse at a time and presented each horse with 20 training combinations per day, each consisting of one positive stimulus and one negative stimulus. All horses were trained in a dual-choice situation. During the training sessions the door with the positive stimulus was unlocked, allowing the horse to reach carrot pieces as a reward. The door with the negative stimulus was locked but there were carrots behind it to avoid differences in odour cues. For all experiments, the position of the positive stimulus was altered in a pseudo-random manner, but it was shown equally often on the left and the right side.

In total, seven horses were trained in three different experiments. Five horses were trained to two positive colours (Experiments I and II). They received the same amount of training with both positive stimuli, each in combination with the same negative stimulus. Two horses were trained to one positive colour and one negative colour (Experiment III). After they had reached the learning criterion of 75% correct choices over three subsequent days, test stimuli were presented to the horses. The number of test presentations differed depending on the horse's performance. The horse had to chose correctly in 3–6 training presentations in sequence before tests were performed. This procedure made it impossible for the horse to learn when a test would appear. During tests, both doors were locked and no reward could be obtained. Directly after a test we continued the training. Binominal tests (Rohlf and Sokal, 1995) for each individual

animal were used to evaluate the statistical significance of the results.

Results

Experiment I – positive stimuli on the same side of the neutral point

In Experiment I, two colours, looking grey and yellow to humans and corresponding to wavelengths located on the same side of the neutral point (red '+'; Fig. 1D), were positive stimuli and combined with a negative blue stimulus (black '–'; Fig. 1D) located on the other side of the neutral point. Both horses trained in this experiment reached the learning criterion even though they reached a somewhat lower choice frequency for the positive stimulus that was more similar to the negative stimulus (Fig. 4A,B). After having reached the learning criterion, both horses were presented with tests.

Both horses chose the intermediate test colour (red circle,

Fig. 4C,D), green, as if it was a positive stimulus when combined with either of the positive colours. Thus, they generalized between two positive stimuli both corresponding to wavelengths longer than the neutral point. In Fig. 4C, the horses showed a significant preference for the colour corresponding to the longest wavelength, which was the colour that differed most from the negative blue stimulus. When tested with both positive stimuli they also preferred the colour corresponding to the longer wavelength, in this case yellow (Fig. 4E).

In order to test whether the horses had simply learned to avoid the negative colour, one horse was presented with a novel blue colour (black circle). This blue colour corresponded to an even shorter wavelength than the negative training stimulus and was presented together with the test colour, green (Fig. 4F). Here, the horse confidently chose the green stimulus (red circle), again implying that it generalized between the positive training colours.
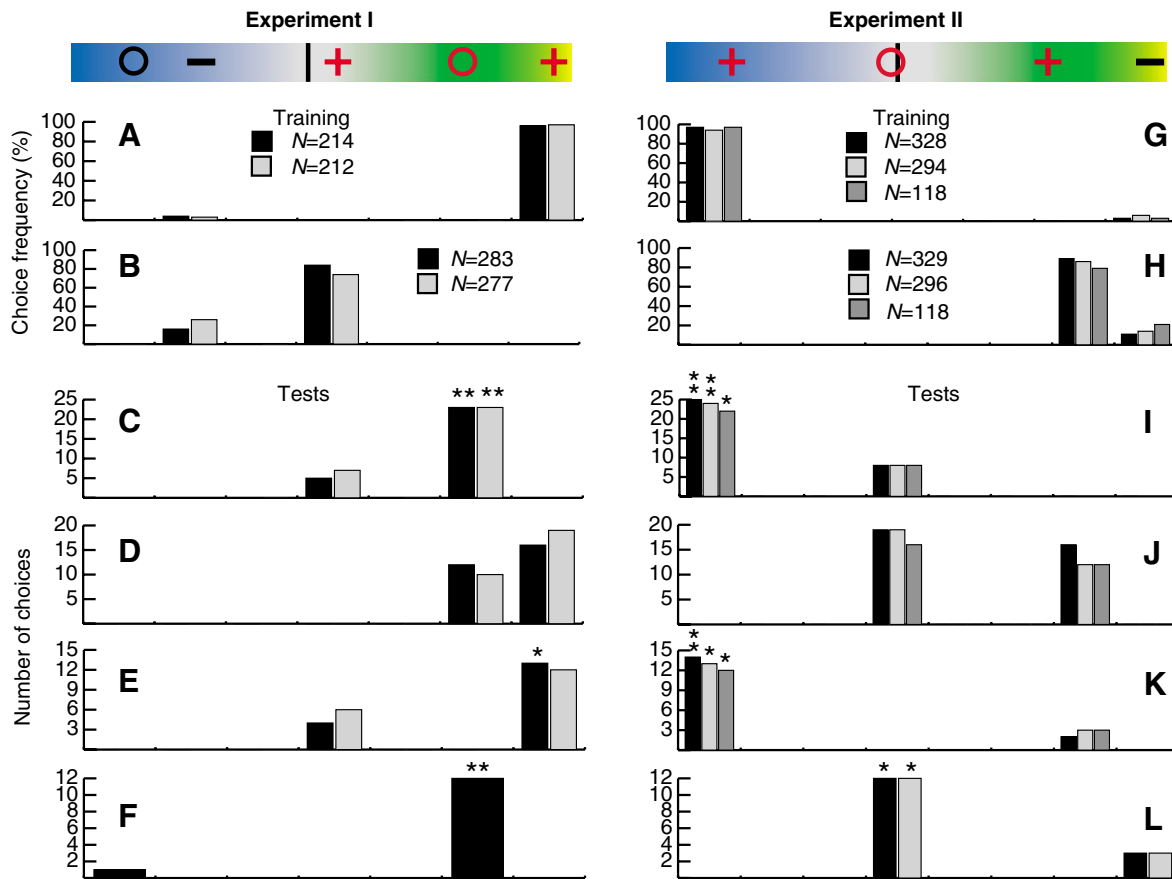

Fig. 4. Experiments I and II. The results bars are located corresponding to the chromatic space shown at the top, and the black vertical line corresponds to the neutral point in horses. Different shades of grey in the bars signify different horses (binominal tests; *P<0.05, **P<0.01). (A–F) In Experiment I, two horses were trained to positive grey and yellow stimuli (red '+'). Blue was the negative stimulus (black '–'). (A,B) Both horses reached high choice levels in both training combinations. In tests (C) and (D), they treated the novel green stimulus (red circle) as a positive colour and showed a significant preference for green when it corresponded to the longer wavelength of the stimuli presented. In test (E), the horses again showed preference for the stimulus corresponding to the longer wavelength. In a final test (F), one horse chose the novel green test colour as if it was a positive stimulus. (G–L) In Experiment II, blue and green were positive training stimuli (red '+') and yellow was the negative training stimulus (black '–'). (G,H) All three horses reached high choice frequencies for both training combinations. In tests (I,J) with one positive training stimulus and the novel intermediate stimulus, grey, corresponding to the neutral point, grey was treated as a positive stimulus in the experiment. In test (I), a significant preference for the colour corresponding to the shortest wavelength was seen. (K) When both positive colours were presented to the horses a strong preference for blue was again seen. (L) Two horses tested with the negative yellow stimulus and the novel colour grey significantly preferred grey.

Experiment II – positive stimuli on both sides of the neutral point

Next, we investigated whether horses can generalize between two colours on opposite sides of the neutral point. In Experiment II, three horses were trained to discriminate blue and green as positive stimuli (red '+'; Fig. 1E), from a negative yellow stimulus (black '−'). All horses reached the learning criterion although they seemed to have more difficulty in distinguishing green and yellow than blue and yellow (Fig. 4G,H). They thereby showed the same pattern as the horses in Experiment I, reaching a somewhat lower choice frequency for more similar colours.

After having reached the learning criterion, they were presented with tests similar to those in Experiment I. The first test consisted of one positive training colour and a new test colour (red circle; Fig. 1E), grey, corresponding to their neutral point. All three horses chose this test colour, grey, as if it was a positive stimulus, clearly indicating generalization between two colours on opposite sides of the neutral point. In addition, they showed a preference for the colour most different from the negative yellow stimulus. When tested with blue and grey (Fig. 4I), all three horses strongly preferred the blue stimulus, i.e. the colour corresponding to the shortest wavelength. In tests with green and grey (Fig. 4J), the difference was not significant according to binominal tests.

When tested with both positive training stimuli (Fig. 4K), all three horses significantly preferred blue to green. These results again show that the horses choose in a relative manner, preferring the shortest wavelength. In a final test, two horses were given the choice between the test colour, grey, and the negative stimulus, yellow (Fig. 4L). Both horses preferred grey, again suggesting that they had learned to generalize between the positive colours, to the neutral point. The similarities with the results from Experiment I clearly show that the neutral point is not treated any differently from other colours that the dichromatic horse can perceive.

Experiment III – relative colour learning

In Experiment III, we trained and tested two horses with the goal to better understand whether they learn colour in a relative manner. Green was the positive stimulus (red '+') and grey the negative (black '−'). Both horses learned to discriminate these two training stimuli, which required that they chose the colour corresponding to the longer wavelength (Fig. 5A).

The first test included the positive colour, green, and a novel yellow stimulus to see whether the horses would prefer the colour corresponding to the longer wavelength, in this case yellow (Fig. 5B). However, the horses chose both colours equally often. From these results we conclude that they did not discriminate between yellow and green in this experiment. Therefore they might have interpreted both colours as positive stimuli.

In the second test, we presented the negative stimulus, grey, together with a novel blue colour. Here, both horses were very reluctant to choose. It often took a long time for them to choose but grey was most often preferred to blue (Fig. 5C). This was a critical test for the horses. Even though they had learned that grey was negative, in the test it was still the colour corresponding to the longer wavelength. The result from
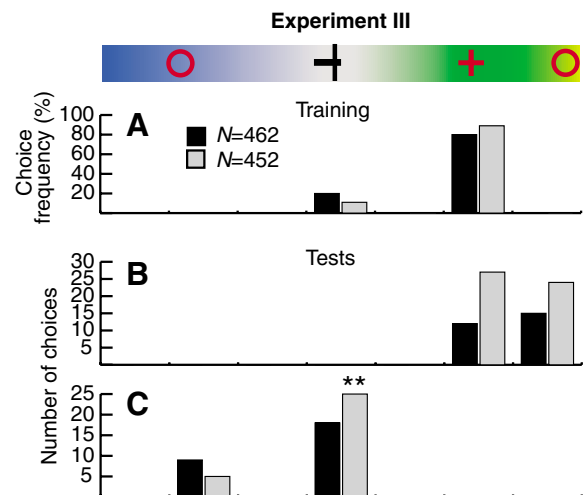

Fig. 5. Experiment III. The results bars are located under the corresponding colour in the chromatic space shown at the top (binominal test; **$P<0.01$). The black vertical line corresponds to the neutral point. Two horses were trained on positive green stimulus (red '+') and negative grey stimulus (black '−'). (A) Both horses reached high choice levels for the training combination. (B) In tests with the positive green stimulus and a novel yellow stimulus (red circle) no significant difference could be seen. (C) In tests with the negative grey stimulus and a novel blue stimulus (red ring) both horses preferred the negative grey stimulus corresponding to the longest wavelength in the combination.

Experiment III, besides confirming relative colour learning, again suggests that horses treat grey as any other colour in their colour space.

Discussion

All horses reached the learning criterion for their training combinations. However, the choice frequency depended on the similarity of the different stimuli. In Experiment I, both horses were slightly better at discriminating between yellow and blue than between grey and blue. This is most certainly due to the fact that the colours in the former combination are more different from each other than the colours in the latter combination. The same result was found in Experiment II, where the horses performed slightly better at distinguishing between blue and yellow than between green and yellow. However, horses that were not repeatedly trained but only occasionally tested with similar colours such as green and yellow chose them equally often. This happened in Experiments I and III (Fig. 4D, Fig. 5B), where no significant differences could be seen. This is in agreement with results obtained by Macuda that show rather poor wavelength discrimination in this particular spectral range (Macuda, 2000).

The results of Experiments I and II show that horses generalize to a novel test colour located between the two positive stimuli from the training procedure. In contrast to the domestic chicks tested by Jones et al. (Jones et al., 2001), the horses did not always prefer the intermediate colour when presented together with a positive stimulus. The choices made by the horses depended on which colour differed most from the negative stimulus. The results of all three experiments suggest

that the horses learn colours in a relative manner. However, relative colour learning does not exclude the recognition of specific colours, which is seen in Experiment III, where the horses only reluctantly chose the negative stimulus, grey, when it was presented with blue (green was the positive stimulus).

The most obvious difference between the horses and the chicks (Jones et al., 2001) is their behaviour in tests that involve the neutral point in horses or the grey point in chicks. Chicks did not generalize between colours on opposite sides of this point whereas the horses did in Experiment II. Our results from Experiments II and III suggest that the dichromats treat grey like any other colour that they can perceive in their chromatic space. Their neutral point does not split up their chromatic space into two colour categories, as suggested earlier (Jacobs and Deegan, 1994; Vienot et al., 1995). Instead, our results confirm Hemmi's findings on the tammar wallaby (Hemmi, 1999) that dichromatic mammals, such as the horse, perceive a continuous scale of colours. In addition, recent studies show that human dichromats use the term 'green' for colours at the neutral point (Wachtler et al., 2004), again indicating that the neutral point does not divide the chromatic space and that dichromats can perceive a chromatic colour at the neutral point. Our results, together with Hemmi's and Wachtler's studies, make it very likely that these conclusions apply to dichromats in general.

Grzimek previously performed a study where he showed that horses could distinguish grey from blue, green, yellow and red colours (Grzimek, 1952). However, in later studies (Macuda and Timney, 1999; Pick et al., 1994; Smith and Goldman, 1999) some horses seemed to have difficulties in discriminating green, and sometimes even yellow, from grey. Given that previous studies have tested different colours *versus* grey shades, it strongly depends on which green has been used, since different colours that human perceive as green can be located quite close or far away from the neutral point of the horse. This is probably one of the reasons for the differences in the results and makes it difficult to compare the studies. In general, if dichromats do not perceive grey as qualitatively different from red, blue, green or yellow, a better test of colour vision would be to test colours different from the neutral point instead of the classic grey *versus* colour tests.

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