1 **Cone excitation ratios correlate with color discrimination**

2 **performance in the horse**

3

4 **Abstract**

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6 Six horses (*Equus caballus*) were trained to discriminate color from grays in a

7 counterbalanced sequence with lightness cues irrelevant. Subsequently, the pre-

8 trained colors were then presented in a different sequence. Two sets of novel colors

9 paired with novel grays were also tested. Performance was just as good in these

10 transfer tests. Once the horse had learned to select the chromatic from the achromatic

11 stimulus, regardless of the specific color, they were immediately able to apply this

12 rule to novel stimuli.

13 In terms of the underlying physiology, we showed for the first time that the spectral

14 sensitivity of horse cone photopigments, measured as cone excitation ratios, was

15 correlated with color discrimination performance, measured as accuracy, repeated

16 errors and latency of approach.

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19 **Keywords: Horse; Color vision; Cone excitation ratios; Discrimination learning.**

Introduction

21 An animal is said to possess color vision if it can discriminate between stimuli that

22 differ in their distributions of spectral energy, independently of any differences in

23 total energy (Jacobs, 1993). Although many animals in most phyla have been shown

24 to have some color vision (Kelber *et al.*, 2003), the extent of these abilities and how

25 they differ from one animal to the next requires further investigation. In particular,

26 normal human color vision provides no basis to predict what stimuli will be salient to

27 the horse. For example, one consequence of dichromacy is that within the spectrum a

28 wavelength will exist that is confusable with white, called the dichromatic neutral

29 point. This is generally located within the middle wavelengths, for example at around

30 505 nm (green) in the tree shrew (Jacobs and Neitz, 1986). This point has been

31 located at about 480 nm in the horse (Geisbauer *et al.*, 2004).

32 Research into the ability of the horse to see colors has resulted in conflicting findings,

33 both in studies adopting a behavioral approach (Grzimek, 1952; Pick *et al.*, 1994;

34 Macuda and Timney, 1999; Smith and Goldman, 1999) and in those investigating the

35 spectral sensitivity of the cone photopigments of the equine retina (Ver Hoeve *et al.*,

36 1999; Yokoyama and Radlwimmer, 1999; Macuda, 2000; Carroll *et al.*, 2001). There

37 is evidence that the horse, like other ungulates, possesses two types of cone

38 photopigment, one maximally sensitive to short wavelengths, the other maximally

39 sensitive to medium-long wavelengths. Values for the spectral peaks of these

40 photopigments have been estimated at 545 nm (Ver Hoeve *et al.*, 1999; Yokoyama

41 and Radlwimmer, 1999; Macuda, 2000) and 429 nm (Macuda, 2000). These values

42 vary slightly from those obtained by Carroll *et al.* (2001) (539 nm and 428 nm), but

43   both are consistent with the range found in other ungulates (Jacobs, 1993). The former

44   values were used in the current study.

45   Behavioral studies into the ability of animals to see colors have generally involved

46   training them to discriminate between chromatic and achromatic stimuli, where all

47   other cues (in particular differences in lightness/brightness, and olfactory or spatial

48   cues) have been made irrelevant. The length of time required for this training has

49   limited both the number of subjects used and the range of colors that have been tested

50   by this method. A maximum of four colors have so far been tested on the horse.

51   Although all previous studies have concluded that this species has the ability to

52   discriminate blue from gray, the results for red, green and yellow have varied.

53   Grzimek (1952) found that horses could discriminate yellow best, followed by green

54   and then blue, but had some difficulty with red. Horses tested by Pick *et al.* (1994)

55   could reliably discriminate blue and red, but not green, from gray. Similar results

56   were obtained by Macuda and Timney (1999) whose horses could discriminate red

57   and blue, but not green or yellow, from gray (though with the green stimulus used in

58   this study there was evidence that at least some of the chromatic information was

59   available).

60   A study into color vision in another ungulate, the fallow deer (*Dama dama)*,

61   demonstrated that this species could discriminate greens from grays (Birgersson *et al.*,

62   2001). Given the conformity that has been found in the peak sensitivities of the

63   photopigments of a number of other ungulates (Jacobs, 1993), it is likely that the

64   horse has the ability to discriminate at least some greens from gray.

65   One notable difference between the early study by Grzimek (1952) and the more

66   recent investigations (Pick *et al.*, 1994; Macuda and Timney, 1999; Smith and

67   Goldman, 1999) is the height at which the stimuli were presented. Successful

68   discrimination of greens was demonstrated when the stimuli were presented at ground

69   level both in horses (Grzimek, 1952) and deer (Birgersson *et al.,* 2001), but not when

70   they were presented to horses at nose height (Pick *et al.*, 1994; Macuda and Timney,

71   1999). Smith and Goldman presented their stimuli at a height of 1.22 meters from the

72   ground and found individual differences in the performance of the horses. We have

73   found that presenting stimuli at ground level enhanced the performance of horses in a

74   task of lightness discrimination (Hall *et al.*, 2003). Thus stimulus position is likely to

75   have been a contributing factor to the discrepant findings of the previous studies.

76   As information relating to the spectral sensitivity of the cone photopigments of the

77   horse is now available, data obtained from behavioral methods can also be compared

78   with the predicted effect of colors on the equine visual system. Using the values for

79   the spectral peaks of the cone photopigments of the horse (429 and 545 nm; Macuda,

80   2000), spectral sensitivity curves can be derived (Govardovskii *et al.*, 2000). These

81   can then be used to predict how easily the horse should be able to discriminate

82   individual colors from gray.

83   The aim of this study was to train horses to discriminate a range of different colors

84   from grays. Lightness cues were made irrelevant by mounting the colors randomly

85   within a chequerboard of mixed grays, similar to those used by von Frisch (1950)

86   when investigating the color sense of bees. A random chequerboard of mixed grays,

87   minus the color, was used as the negative stimulus. Stimuli were presented at ground

88   level to optimize discrimination accuracy (Hall *et al.,* 2003). The training phase

89   suggested that horses' discrimination learning performance was based on chromatic

90   information. Transfer tests, that presented familiar colors in a different sequence as

91   well as novel colors, were used to confirm that the discrimination was learned on the

92   basis of chromatic information. Finally, the reflectance spectrum of each of the colors

93   was measured and cone excitation ratios were calculated. The cone excitation ratios

94   for the colors and the grays were compared and the difference between them used as

95   an indication of the effect that each color had on the visual system of the horse. Thus

96   the effect of the colored stimuli on the photopigments of the horse could be compared

97   with the behavioral data. A reliable correlation between the cone excitation ratios and

98   the discrimination performance would in principle provide a means of predicting the

99   appearance of colors to the horse.

Methods

101   **Subjects**

102   The six subjects used were riding horses from the Brackenhurst Equestrian Centre,

103   Nottingham Trent University, where the study was carried out. They consisted of four

104   geldings and two mares whose ages ranged from 5-18 years, with a mean of 11.5

105   years. Their heights ranged from 1.52-1.65 meters, with a mean of 1.58 meters.

106   During the period that the study was carried out all of the horses were ridden

107   approximately six days a week for two hours a day. All of the horses had previously

108   been trained to perform a two choice black/white discrimination task to a criterion of

109   70% accuracy on four consecutive training sessions (Hall *et al.*, 2003). The same test

110   area and method of displaying the stimuli and delivering the reward were used in the

111   current study.

112   A veterinary surgeon examined the eyes of all six horses prior to the current study and

113   found no evidence of any ocular abnormality. When taking part in the study the horses

114   all wore head-collars from which the lead ropes had been removed.

Test area and apparatus

The test area was located in one half of an enclosed barn with a concrete floor, which was fenced off along the long side using galvanised wire mesh barriers (1.2 meters in height) and screened from view by sheeting to a height of 3 meters. A gap of 100 millimeters in this screening allowed the experimenter to view the subject performing the trials while remaining outside the test area. All of the training and testing was carried out in daylight via skylights in the roof. The test area was 5 meters wide and 10 meters long; a "starting" line of masking tape was placed on the floor 6.5 m from the end wall where the stimuli were displayed.

Two identical wooden boxes were placed on the floor against the wall, each 1.25 meters from the sidewall with a gap between the two boxes of 1.5 meters. The top flap of the box sloped forwards at an angle of 60° from the vertical and was hinged at the top to open inwards. Perspex sheets were mounted on the opening flaps of each box, behind which the stimulus cards could be slotted. The box was designed to allow the top to be left locked or unlocked, without being visually apparent. The horses could open the box by pushing the flap with their noses. The flap of the box displaying the positive stimulus was left unlocked, while the flap of the box displaying the negative stimulus was locked. A correct choice was rewarded by access to the food within the box. This consisted of a small piece of carrot, approximately 30 x 10 millimeters, placed in each of the stimulus boxes so that olfactory cues could not guide stimulus selection. During training and testing, both boxes were treated identically with respect to changing the stimulus cards, opening and shutting the flaps and removing or inserting the locking block, so auditory cues could not guide stimulus selection.

138 For further details of the test area, and design and dimensions of the stimulus boxes,

139 see Hall *et al.* (2003).

140 **The stimulus cards**

141 A range of fifteen colors was produced for training and a further six were produced

142 for the transfer tests. In both cases, horses were required to select chromatic from

143 achromatic stimuli, where lightness could not be used as a cue. The stimulus cards

144 measured 375 mm square and were subdivided into a chequerboard pattern of eight

145 different grays within which was mounted a colored panel in the case of the chromatic

146 stimuli, or not in the case of the achromatic stimuli. The gray and colored stimuli were

147 printed, cut to size and glued onto a piece of card. The whole card was then laminated

148 to prevent damage when being inserted behind the Perspex panel on the front of the

149 stimulus box. See Figure 1 for the design and dimensions of the stimulus cards.

150 Four different arrangements of the eight different grays could be displayed either way

151 up and resulted in eight different configurations on the achromatic cards. For each of

152 the colors tested, a set of four different stimulus cards was constructed, which could

153 also be displayed either way up, thus allowing the colored panel to appear in any of

154 the eight positions within the card. The other seven positions contained seven of the

155 same grays as those on the distracter cards.

156 *The colors*

157 The colored stimuli were produced using the computer graphics programme Paint

158 Shop Pro 5 (version 5.01). The colors were specified by additive mixtures of red,

159 green and blue using proportions of 0 – 255 of each color. Specifications included the

160 hue, saturation and lightness for each color, as well as a value for the grayscale

161 equivalent. A range of fifteen colors was produced for the initial training and a further

162   six for the final test. The stimulus colors were printed using a Hewlett Packard laser

163   jet 4500 color printer with a resolution of 600 x 600 dots per inch.

164   The fifteen colors were presented in a sequence that related to their appearance to the

165   trichromatic human. They were arranged according to their spectral appearance and

166   presented to the subjects in one of the two counterbalanced orders (either starting with

167   the short or long wavelength colors). The colors were numbered 1-15 with a "C"

168   preceding these numbers. C1 was at the short wavelength end of the spectrum and

169   C15 at the long wavelength end.

170   Discrimination ability with six novel colors was tested in the same way in the transfer

171   tests. The print specifications for the training (C1-C15) and novel (NC1-NC6) colors

172   are shown in Table 1.

173   *The grays*

174   The grays were produced using the grayscale option of the same computer graphics

175   program and a black and white laser printer (Hewlett Packard 4000 N). The vertical

176   resolution rating of the printer was 600 dpi (dots per inch), the same as the color

177   printer. Only 256 different shades of gray are available in Paint Shop Pro 5 (version

178   5.01) resulting in a reduction in variation once colors are converted to grayscale. This

179   meant that several colors had the same value in grayscale. The perceived lightness of

180   the colors would depend upon the spectral sensitivity of the visual system, and may

181   not be equivalent to the match generated by the graphics program. However, given the

182   random location of the colors within seven different shades of gray, the possibility

183   that these lightness cues could still be used effectively was minimal.

184   The print specifications (percentage of black ink) for each of the eight grays used in

185   training, and in the eight new grays used in the transfer tests are shown in Table 2.

186 **Design**

187 *Color discrimination training*

188 The horses were divided into two groups, each consisting of two geldings and one

189 mare that were tested in one of two counterbalanced sequences. The first group

190 started their training with C1 and the second group with C15. The performance of

191 each subject for each of the fifteen colors was assessed using four separate measures:

192 number of trials to criterion; accuracy (the percentage of correct choices made at the

193 first attempt); errors (the percentage of repeated incorrect choices made at each of the

194 trials during the session); and latency of approach to the stimulus (measured in

195 seconds). The approach to the stimulus was timed from the release of the horse at the

196 starting line to when the stimulus box was touched. Latency of approach to each color

197 was calculated for correct and incorrect choices separately. A comparison of

198 performance was made using the correct choice latencies for each color. These scores

199 were then compared with the scores for incorrect choices.

200 *Transfer tests*

201 The fifteen pre-trained colors were then randomly arranged into five sets (S1-S5) each

202 containing three of the colors. The learning criterion for each set was the same as

203 during the training sessions (ten consecutive correct choices within one session) and

204 once this had been reached for all five sets, the novel color sets were tested.

205 The six novel colors (that were mounted within the novel grays and paired with the

206 novel achromatic stimuli) were arranged into two sets (NS1 and NS2). NS1 consisted

207 of a novel green (NC1), yellow (NC2) and brown (NC3), NS2 of a novel blue (NC4),

208 purple (NC5) and pink (NC6). The ability of the horses to discriminate these novel

209   sets of colors was then tested. The first group 1 started with NS1; the second group

210   started with NS2. See Table 3 for the order of presentation of the sets of colors.

211   **Procedure**

212   *Color discrimination training*

213   Color discrimination training sessions were carried out 2-3 times / week and consisted

214   of sixteen presentations of the paired stimuli, each presentation being referred to as

215   one trial. The left/right position of the positive stimulus was varied randomly, up to a

216   maximum of three consecutive choices on one side to avoid spatial cues from

217   becoming more important than visual cues. Within each training session equal

218   numbers of left and right presentations of the positive stimulus were included. The

219   design of the stimulus cards meant that the horses could not use brightness cues to

220   make the discrimination. The stimuli were always presented at the same angle and

221   were all illuminated from above.

222   At the start of each session, the horse was led into the barn, the doors were closed and

223   the horse was positioned behind the starting line, directly facing the stimulus boxes. It

224   was released by the handler (from either side randomly, to control for directional

225   influences) and allowed to approach the boxes to make its selection. A correct choice

226   was rewarded by access to the carrot via the unlocked flap before the subject was

227   caught and led behind the screens. The number of trials that the horse made a correct

228   selection at the first attempt was calculated as a percentage of the total number of

229   trials and resulted in an accuracy score. An incorrect choice resulted in the horse

230   being caught by the handler before it could try the correct box and being led back to

231   the starting line to try again. If an incorrect choice was made, the same presentation

232   was repeated until the horse made the correct choice. The initial choice would be

233   scored incorrect and repeated errors with the same stimulus presentation were counted

234   up within any one trial. The number of error runs (on first or subsequent attempts)

235   was calculated as a percentage of the total number of runs (whether correct or

236   incorrect) in that session. Thus the error scores were not simply the obverse of

237   accuracy scores and reflected perseverance in making an incorrect choice.

238   At the end of each trial the horse was led behind the screens while the experimenter

239   re-positioned the stimuli according to the pre-arranged semi-random order. When no

240   change of stimulus position was required the cards were removed and replaced in the

241   same box to control for possible auditory cues. After a period of 30 seconds, the horse

242   was led back to the starting line to commence the next trial.

243   Training with each color continued until a learning criterion of ten consecutive correct

244   choices had been made within one training session, the next color then being

245   introduced in the following session. The probability of making ten correct choices by

246   chance was ($p<0.001$), and training with each color continued until this criterion had

247   been reached.

248   *Transfer tests*

249   The basic test procedure was exactly the same as that used in training. Controls for

250   spatial cues and the random positioning of the colored panel within the positive

251   stimulus were the same. Each session consisted of up to twenty trials, with the colors

252   appearing randomly (each color would be presented at least five times during each

253   session unless the learning criterion had been reached prior to that; colors would not

254   be repeated more than twice consecutively). The same measures of performance that

255   had been used in training were recorded throughout the testing phase (trials to

256   criterion, accuracy, errors and latency to approach). The colors included in each set

257   and the order in which they were tested is shown in Table 3.

258   *Cone excitation values of the colored stimuli*

259   The spectral reflectance of each of the colors mounted on the stimulus cards was

260   measured under the experimental conditions using a Minolta CS1000 spectro-

261   radiometer. Spectral radiance values (radians) for wavelengths between 380 and 780

262   nm were recorded in 1 nm steps. In order to minimize the effect of minor variations in

263   environmental conditions two separate readings were taken for each color at each

264   wavelength and the mean value calculated. The latter was then used in subsequent

265   calculations. The data was downloaded onto Excel files and line graphs to show the

266   reflectance spectrum for each of the colors were then plotted. Given the range of light

267   visible to the human and the spectral sensitivity of the horse's photoreceptors, only

268   the radiance values for wavelengths of between 380 and 720 nm were used.

269   The spectral sensitivity of an A_1-based photopigment was modeled using the process

270   detailed in Govardovskii *et al.* (2000) for the peak sensitivities of the photoreceptors

271   of the horse (429 and 545 nm; Macuda, 2000). The nomogram produced by this

272   process gives the spectral sensitivity of the cones over the same range of wavelengths

273   as used to measure the spectral radiance of the colored stimuli. The resultant cone

274   fundamental spectral sensitivity curves were produced in Matlab, using the

275   Psychtoolbox GovardovskiiNomogram function (Brainard, 1997).

276   To assess the effect that each of the colored stimuli had on each type of cone

277   photopigment, the spectral energy values of the former were multiplied with the

278   sensitivity values of the latter. In Matlab, the Psychtoolbox EnergyToCones function

279   (Brainard, 1997) was applied to the energy value for each wavelength in discrete

280   steps, resulting in cone excitation values at every wavelength. This series of values

281   was integrated to obtain the cone excitation value for each stimulus.

282   To obtain the relative effect of each color on the two cone photopigments of the horse,

283   the cone excitation values were plotted on a graph in Excel (values on the x-axis for

284   the M-L cones and on the y-axis for the S cones), and the ratio of y/x calculated. The

285   cone excitation values for the achromatic (grays, black and white) stimuli were also

286   plotted and the ratio of y/x calculated for each stimulus. The ratios were consistent to

287   two decimal places, and the mean was calculated for use in subsequent calculations (y

288   = 0.3354x). The difference from the achromatic excitation ratio was calculated for

289   each of the chromatic stimuli, this value then being used as a measure of the perceived

290   chromaticity of the stimulus represented by that point.

291   Because of possible differences in the contribution to the perception of brightness

292   made by each of the two cone types (Stockman and Sharpe, 1999), those values that

293   represented relatively greater excitation of the M-L cones were considered separately

294   from those that represented greater excitation of the S cones. Correlation between

295   these ratio differences and the behavioral scores obtained was then investigated.

296   **Data analysis**

297   *Color discrimination training*

298   The ability of the horses to discriminate each of the fifteen colors was assessed by

299   calculating the mean number of trials taken to reach the learning criterion (ten

300   consecutive correct choices, $p < 0.001$), the mean accuracy and error percentages and

301   mean latency of approach. A Kolmogrov-Smirnov (Lilliefors) test was carried out in

302   SPSS 9.0 for Windows to evaluate whether the data was normally distributed. The

303   distribution of the accuracy and error percentages did not vary significantly from the

304   normal distribution (accuracy=0.119, df=15, p=0.2, errors=0.19, df=15, p=0.148), but

305   the distribution of the number of trials to criterion and latencies did (number of trials

306   to criterion=0.269, df=15, p=0.005, latency=0.226, df=15, p=0.038). Consequently,

307   although parametric tests were used to analyze the accuracy and error percentages,

308   non-parametric equivalents were used for the trials to criterion and latencies.

309   The effect of color on the four measures of performance was assessed using the one-

310   way repeated measures analysis of variance for the parametric data and the Friedman

311   test for the non-parametric data.

312   A comparison between the two groups of subjects was made to assess the possible

313   effect that the order of presentation of the colors had on their training. The mean

314   scores for each measure were calculated and compared using the t-test (unrelated) and

315   Mann Whitney U test. All analyses were two-tailed.

316   *Transfer tests*

317   The same measures (trials to criterion, accuracy, errors and latency) were used to

318   evaluate performance. Shifts in performance from the pre-trained to novel color sets

319   were assessed using the t-test (related) and Wilcoxon test.

320   *Cone excitation values of the colored stimuli*

321   The differences between the cone excitation values for the chromatic and achromatic

322   stimuli did not vary significantly from the normal distribution (C1-C7 = 0.245, df = 7,

323   p = 0.2; C8-C15 = 0.161, df = 8, p = 0.2). The behavioral scores and the cone

324   excitation values were compared using the Pearson correlation coefficient for the

325   parametric data and the Spearman's Rank correlation coefficient for the non-

326   parametric data.

327 **Results**

328 *Color discrimination training*

329 All of the six horses successfully completed the color discrimination training,

330 reaching the learning criterion of ten consecutive correct choices ($p < 0.001$) for all

331 fifteen colors. Some variation in performance was found with individual colors. The

332 color C7 (blue-green) took the greatest number of trials and C13 (orange) the least

333 (see Figure 2). However, presenting the colors in the opposite order to the two groups

334 controlled for any effect that order of training might have had, and these differences in

335 relation to color were not found to be significant (trials to criterion: $\chi^2_{14} = 15.338$, p =

336 0.355; accuracy: $F_{14, 70} = 1.085$, p = 0.386; errors: $F_{14, 70} = 1.014$, p = 0.45; latency:

337 $\chi^2_{14} = 2.832$, p = 0.999).

338 There was no significant difference between the groups trained with the different

339 sequences of the colored stimuli for any of the measures used (trials to criterion: U =

340 99, $N_1 = N_2 = 15$, p = 0.575; accuracy: $t_{28} = -0.289$, p = 0.775; repeated errors: t_{28} =

341 0.040, p = 0.968; latency: U = 101.5, $N_1 = N_2 = 15$, p = 0.648).

342 *Transfer tests*

343 The only significant difference in performance when transferring from the color

344 discrimination training to the color set testing was in the speed at which the stimuli

345 were approached (z = -2.201, N-ties = 6, p = 0.028). Although on average the first

346 color set was approached significantly more slowly than the final color trained, no

347 drop in performance was found for any of the other measures (trials to criterion: z = -

348 0.405, N-ties = 5, p = 0.686; accuracy: t = -0.643, df = 5, p = 0.548; errors: t = 1.002,

349 df = 5, p = 0.362).

350   However, it took a significantly greater number of trials to reach the criterion for this

351   first set than for the second set ($z = -2.023$, N-ties $= 5$, $p = 0.043$). No significant

352   difference was found between any of the other consecutively tested sets, regardless of

353   which colors they consisted of. See Figure 3 for the mean number of trials taken to

354   reach the criterion for the last color trained and each of the color sets. No significant

355   overall difference was found in the accuracy percentages for the seven sets of colors;

356   the improvement shown in set 2 as compared with set 1 was only marginal ($t = -2.323$,

357    $df = 5$, $p = 0.068$). The number of repeated errors made varied according to the set of

358   colors being tested ($F_{(6,30)} = 2.631$, $p = 0.036$), but when consecutive sets were

359   compared, the only significant difference found was again between sets 1 and 2 ($t =$

360   3.280, $df = 5$, $p = 0.022$), with fewer errors being made in the latter. No significant

361   differences in the speed of approach were found with any of the sets of colors.

362   The introduction of the novel sets of colors was not found to have any effect on the

363   measures taken to assess performance. It was immaterial as to whether the novel set

364   introduced first consisted of a brown, green and yellow stimulus, for the first group, or

365   a purple, blue and pink stimulus, for the second group (trials to criterion: $z = -0.674$, p

366    $= 0.5$, two-tailed; accuracy: $t = 0.116$, $df = 4$, $p = 0.48$; errors: $t = -0.377$, $df = 4$, $p =$

367   0.725; latency: $z = -1.091$, $p = 0.275$, two-tailed). Once the horses had learned to

368   select the chromatic from the achromatic stimulus, regardless of the specific color,

369   they were able to apply this rule to all of the pre-trained colors equally easily, as well

370   as to the two sets of novel colors.

371   *Correlation with cone excitation values*

372   It was found that those colors that differed most from the S to M/L cone excitation

373   ratio of the achromatic stimuli (and as such were predicted as being the most colorful

374   to the horse) were also most easily discriminated from grays. For colors C8 – C15

375   there was a highly significant negative correlation between the cone excitation ratio

376   difference and the number of trials to criterion (rho = -0.857, N = 8, p = 0.007).

377   The greater the difference in the cone excitation ratio from the grays, the greater the

378   accuracy (r = 0.872, N = 8, p = 0.005), the fewer the repeated errors (r = -0.883, N =

379   8, p = 0.004), and the faster the selection was made (rho = -0.792, N = 8, p = 0.019).

380   For colors C1 – C7, although the correlation between the cone excitation ratio

381   difference and the trials to criterion was not significant (rho = -0.679, N = 7, p =

382   0.094), there was a highly significant positive correlation with the accuracy scores (r =

383   0.923, N = 7, p = 0.003) and a negative one with both the repeated errors (r = -0.880,

384   N = 7, p = 0.009) and the latencies (rho = -0.775, N = 7, p = 0.041).

385   The relationship between the cone excitation ratio differences and the mean number

386   of trials taken to reach the learning criterion the fifteen training colors is shown in

387   Figure 4.

Discussion

389   All of the horses in the study demonstrated the ability to use chromatic information to

390   discriminate between stimuli across the spectrum. They successfully reached the

391   learning criterion for all of the colors, including five shades of green. As was

392   previously found in the study by Grzimek (1952), horses can discriminate greens from

393   grays when the stimuli are presented at ground level, as can the fallow deer

394   (Birgersson *et al.*, 2001). Like the fallow deer, the horse is a selective grazer and the

395   ability to use the color of plants, as well as olfactory, gustatory and tactile signals, as

396   an additional cue in their choice of food, may be an advantage. The recognition of

397 familiar conspecifics, of prime importance in a herd animal such as the horse, would

398 also be enhanced.

399 It was clearly demonstrated in the current study that the horses were using chromatic

400 cues to make their selection by the way in which they applied this rule of "color is

401 correct" in the transfer tests. Generalization to novel stimuli occurred equally well

402 when the set contained a new green, brown and yellow, as when it contained a new

403 blue, pink and purple. Neither combination resulted in a decrease in discriminative

404 ability. It is also worth noting that the inclusion of the color that had caused the most

405 problems in the initial color discrimination training (C7) in set 5 did not result in any

406 decrease in performance either. Once the colors had been trained, they appeared

407 equally easy for the horses to discriminate from grays.

408 However, in agreement with previous studies, although all the middle wavelength

409 colors (greens) were successfully discriminated from grays eventually, more trials

410 were required to reach the learning criterion for one particular color. Performance

411 with C7 (blue-green) was less consistent and more training was required (before the

412 criterion was reached) than for any of the other colors. The study by Pick *et al.* (1994)

413 resulted in the single subject failing to reach the same criterion (of ten consecutive

414 correct choices) when discriminating green from gray. Despite the use of an

415 alternative criterion of 70% correct responses, Macuda and Timney (1999) also

416 concluded that chromatic discrimination was inconsistent with green and yellow. It is

417 likely that the green, C7, was close to the neutral point of the horse and appeared less

418 colorful to the horse than the other shades of green. However, the reflectance spectra

419 of the colors in the current study consisted of a broad range of wavelengths, as is the

420 case with most natural objects, so some chromatic information was still available to

421 the horse. The dominant wavelength (496 nm) of the green that could not be reliably

422 discriminated from gray in the study by Pick *et al.* (1994) was also close to the neutral

423 point location (480 nm) identified in the horse (Geisbauer *et al.*, 2004) although that

424 used by Macuda and Timney (1999) was not (530 nm).

425 In addition to the possible effect of stimulus position on the results of earlier studies,

426 discrepancies in previous findings may also be partially explained by individual

427 differences in the horses used. Most features of visual ability are unlikely to be unique

428 to individual animals and the results gained from a few can be applied to the species

429 as a whole. All of the horses in the current study successfully learned to discriminate

430 all of the colors presented, but some variation between individuals was noted and the

431 effect of previous experience was clear. Individual variation was also found in the five

432 horses tested by Smith and Goldman (1999): three horses successfully reached the

433 learning criterion of 85% correct responses with green and yellow, but another

434 performed at chance levels only with these colors. In an early study into color vision

435 in the horse, Grzimek (1952) used only two subjects, as did Macuda and Timney

436 (1999). Pick *et al.* (1994) trained a single horse of nineteen years of age. Just as color

437 vision varies in humans (Dartnall *et al.*, 1983), so it may in horses.

438 The correlation found between the cone excitation values for each of the colors and

439 the results of the color discrimination training was stronger for colors C8 – C15 (the

440 medium-long wavelength colors that would appear green, yellow or red to the

441 trichromatic human) than for C1 – C7 (the short wavelength colors that would appear

442 blue or purple). For example, yellow (C12) and orange (C13) were the two colors that

443 varied the most from the achromatic stimuli in the proportion to which they excited

444 the M/L cone (545 nm) photopigment. The latter color was the easiest to discriminate

445 overall.

446 One of the consequences of dichromacy in the human is the inability to discriminate

447 between certain colors, most commonly between certain shades of red and green

448 (Jameson and Hurvich, 1978). The cone excitation data would provide a way of

449 predicting likely difficulties with pair-wise discriminations. The proximity of C3, C4

450 and C5 suggest that blues may be difficult to discriminate from purple. Given that

451 C10 and C13 vary to the same extent from the achromatic stimuli, the horse may also

452 have difficulty discriminating between lime green and orange.

Conclusions and implications

454 The color discrimination training and testing provided a clear demonstration of the

455 ability of the horse to use chromatic information across the spectrum, including those

456 wavelengths perceived as greens and yellows. The high correlation between the

457 behavioral data and the calculated effects of the colors used on the cone

458 photopigments of the horse suggests that cone excitation calculations could be used to

459 predict the ease with which colors can be discriminated by the horse, as well as

460 providing further evidence of dichromacy in this species. Such predictions would also

461 provide some basis for the comparison of horse color perception with that of the

462 trichromatic human, and thus an improved understanding of equine behavior.

Acknowledgements

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515   **Figure captions**

516   **Figure 1.** Design of the stimulus cards, with the dimensions of the individual panels.

517   **Figure 2**. Mean number of trials taken to reach the learning criterion of 10

518   consecutive correct choices for each color (C1-C15). Variation is shown as +1

519   standard deviation. The values for groups 1 and 2 were combined to show

520   performance in relation to the individual colors, regardless of the order in which they

521   were trained.

522   **Figure 3.** Mean number of trials taken to reach the learning criterion of 10

523   consecutive correct choices for the final color trained (C1 / C15), for each of the pre-

524   trained color sets (S1 – S5) and for the novel color sets (NS1 and NS2). Variation is

525   shown as +1 standard deviation.

526   **Figure 4.** Correlation between the extent to which the cone excitation ratios of each

527   of the trained colors (C1-C15) differed from that of the grays, and the mean number

528   of trials taken to reach the learning criterion (of ten consecutive correct choices) with

529   each color. The correlation of the short wavelength colors is shown separately from

530   that of the medium-long wavelength colors. Trend lines are included to illustrate the

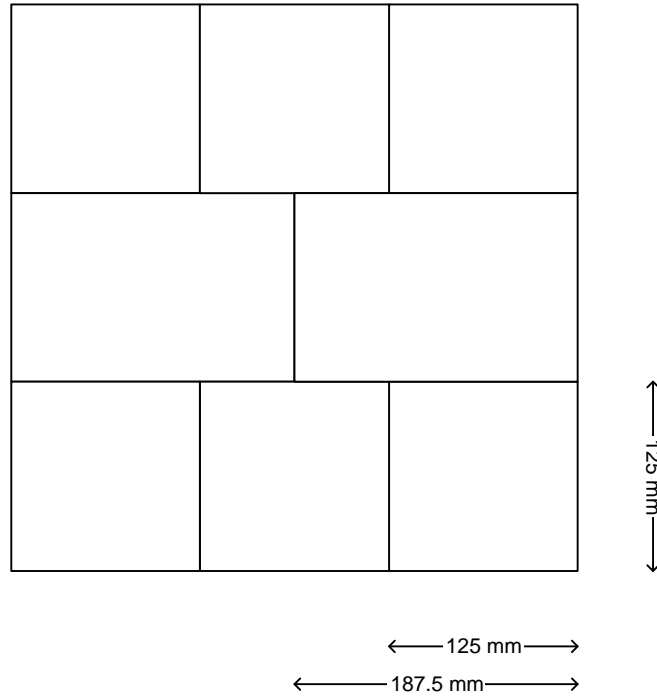531   relative extent of the correlation of the two groups of colors.

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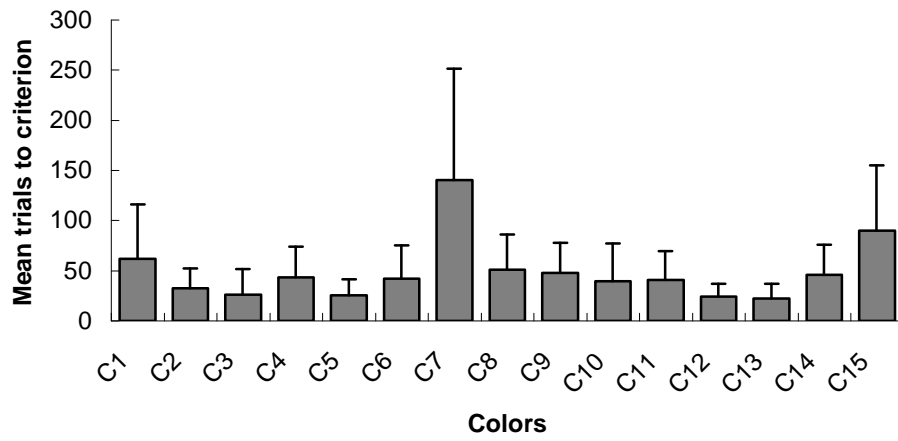
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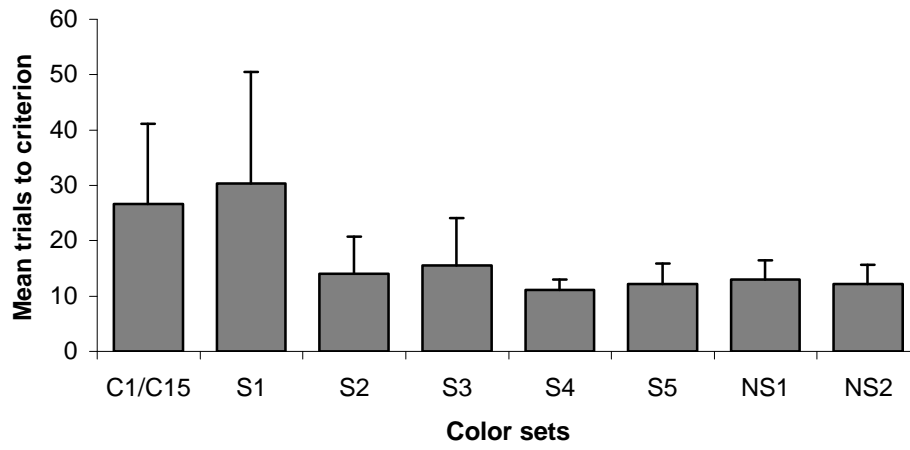539 **Figure 1.**

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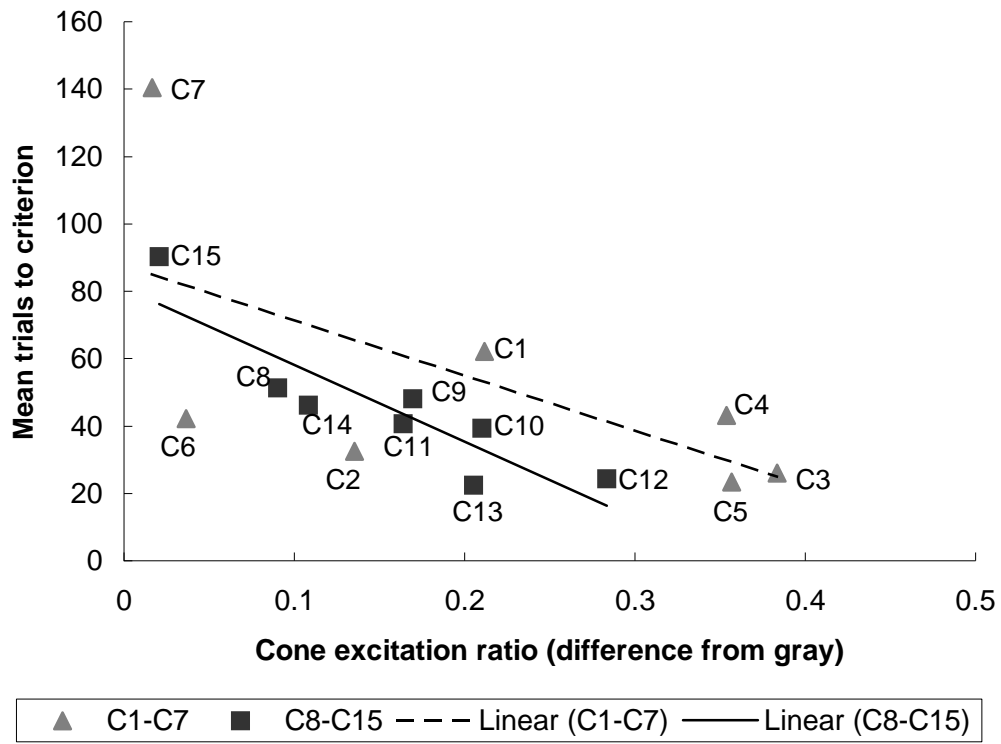542

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545   **Figure 2.**

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550   **Figure 3.**

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554 **Figure 4.**

555 **Table 1:** Print specifications of the colored stimuli (Paint Shop Pro 5)
556

	COLOR	R, G, B	HUE	SATUR-ATION	LIGHT-NESS	GRAYSCALE EQIVALENT (= R, G, B)
C1	Violet	255, 0, 255	213	255	128	170, 170, 170
C2	Indigo	128, 0, 128	213	255	64	85, 85, 85
C3	Purple	128, 0, 255	191	255	128	128, 128, 128
C4	Blue	0, 0, 255	170	255	128	85, 85, 85
C5	Powder blue	0, 128, 255	149	255	128	128, 128, 128
C6	Cyan	0, 255, 255	128	255	128	170, 170, 170
C7	Blue-green	0, 128, 128	128	255	64	85, 85, 85
C8	Greenish	0, 255, 128	106	255	128	128, 128, 128
C9	Green	0, 255, 0	85	255	128	85, 85, 85
C10	Lime green	128, 255, 0	64	255	128	128, 128, 128
C11	Khaki	128, 128, 0	43	255	64	85, 85, 85
C12	Yellow	255, 255, 0	43	255	128	170, 170, 170
C13	Orange	255, 128, 0	21	255	128	128, 128, 128
C14	Red	255, 0, 0	0	255	128	85, 85, 85
C15	Pink-purple	255, 0, 128	234	255	128	128, 128, 128
NC1	New green	135, 200, 0	56	255	100	158, 158, 158
NC2	New yellow	236, 237, 139	43	186	188	225, 225, 225
NC3	New brown	156, 100, 46	21	139	101	110, 110, 110
NC4	New blue	64, 200, 255	140	255	160	165, 165, 165
NC5	New purple	135, 5, 173	203	241	89	62, 62, 62
NC6	New pink	255, 128, 128	0	255	192	166, 166, 166

557

558 Print specifications for colors C1-C15 and NC1-NC6. Each color was specified by

559 additive mixes of red, green and blue (R, G, B) using proportions of 0-255 of each

560 color, as shown in the third column. Each different hue is labeled numerically in Paint

561 Shop Pro 5, as shown in column 4. Specifications also include the saturation

562 (maximum 255), lightness and grayscale equivalent of each color, as shown in the

563 final three columns.

564

565 **Table 2:** Specifications of the gray stimuli (Paint Shop Pro 5)
566

GRAY	GRAYSCALE (= R, G, B)	% BLACK INK
G1	85, 85, 85	67
G2	100, 100, 100	61
G3	114, 114, 114	55
G4	128, 128, 128	50
G5	142, 142, 142	45
G6	156, 156, 156	40
G7	170, 170, 170	34
G8	184, 184, 184	28
NG1	62, 62, 62	76
NG2	88, 88, 88	66
NG3	110, 110, 110	57
NG4	135, 135, 135	47
NG5	158, 158, 158	38
NG6	165, 165, 165	36
NG7	166, 166, 166	35
NG8	225, 225, 225	12

567

568

569 The different shades of gray, selected from the grayscale option of the same computer

570 graphics programme (Paint Shop Pro 5). Specifications shown include both these

571 grayscale values (equal proportions of red, green and blue) and the percentage of

572 black ink in each of the grays. The grayscale equivalents to the colored stimuli were

573 limited, so additional intermediary values were also included.

574
575

576 **Table 3.** The component colors and order of presentation of the color sets used in the

577 transfer tests.

578

COLOR SET	COMPONENT COLORS	ORDER OF PRESENTATION	
		Group 1	Group 2
S1	C4, C9, C14	1	1
S2	C3, C10, C13	2	2
S3	C6, C11, C12	3	3
S4	C2, C8, C15	4	4
S5	C1, C5, C7	5	5
NS1	NC1, NC2, NC3	**6**	**7**
NS2	NC4, NC5, NC6	**7**	**6**

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