

RESEARCH ARTICLE

Innate colour preference, individual learning and memory retention in the ant *Camponotus blandus*

Ayse Yilmaz^{1,*}, Adrian G. Dyer^{2,3}, Wolfgang Rössler¹ and Johannes Spaethe¹

ABSTRACT

Ants are a well-characterized insect model for the study of visual learning and orientation, but the extent to which colour vision is involved in these tasks remains unknown. We investigated the colour preference, learning and memory retention of *Camponotus blandus* foragers under controlled laboratory conditions. Our results show that *C. blandus* foragers exhibit a strong innate preference for ultraviolet (UV, 365 nm) over blue (450 nm) and green (528 nm) wavelengths. The ants can learn to discriminate 365 nm from either 528 nm or 450 nm, independent of intensity changes. However, they fail to discriminate between 450 nm and 528 nm. Modelling of putative colour spaces involving different numbers of photoreceptor types revealed that colour discrimination performance of individual ants is best explained by dichromacy, comprising a short-wavelength (UV) receptor with peak sensitivity at about 360 nm, and a long-wavelength receptor with peak sensitivity between 470 nm and 560 nm. Foragers trained to discriminate blue or green from UV light are able to retain the learned colour information in an early mid-term (e-MTM), late mid-term (l-MTM), early long-term (e-LTM) and late long-term (l-LTM) memory from where it can be retrieved after 1 h, 12 h, 24 h, 3 days and 7 days after training, indicating that colour learning may induce different memory phases in ants. Overall, our results show that ants can use chromatic information in a way that should promote efficient foraging in complex natural environments.

KEY WORDS: Vision, Cognitive capacity, Colour learning, Colour memory retrieval, Mid-term memory, Long-term memory

INTRODUCTION

Despite the importance of vision in the daily tasks of an ant, our knowledge about the use of chromatic information and spectral sensitivities in ants is currently rather sparse (Tsuneki, 1953; Marak and Wolken, 1965; Kiepenheuer, 1968; Kretz, 1979; Menzel and Knaut, 1973; Camlitepe and Aksoy, 2010; Aksoy and Camlitepe, 2012). Most behavioural and electrophysiological studies suggest dichromacy based on the presence of two types of spectrally distinct photoreceptors (PR) in the ants' retina that are maximally sensitive in the ultraviolet (UV, λ_{\max} =ca. 350 nm) and green (λ_{\max} =ca. 510 nm) range of the electromagnetic spectrum (Roth and Menzel, 1972; Menzel, 1973; Mote and Wehner, 1980; Labhart, 1986).

However, recent electrophysiological recordings from the retina of two Australian *Myrmecia* ants (*Myrmecia vindex* and *Myrmecia croslandi*; Ogawa et al., 2015) and molecular and phylogenetic analyses of visual opsin genes in *Camponotus rufipes* workers (Yilmaz et al., 2016) suggest the presence of an additional blue-sensitive PR, providing the physiological and molecular substrate for trichromatic colour vision. As several ant species were tested, the discrepancy among studies might partly be due to differences in the species-specific visual ecology and their visual navigation behaviour (Ogawa et al., 2015). However, whether both dichromacy and trichromacy are present within the ants, and how the colour vision system is linked to visual ecological requirements as well as cognitive challenges (processes such as evaluation, decision making, learning and memory) of different tasks is still not clear and remain to be investigated.

Visual cognitive capacities of a species are usually related to the visual requirements of its specific environment and behavioural challenges, like foraging. The learning and retrieval of visual cues during foraging trips, in addition to or in the absence of potential olfactory information, promote efficient navigation in an unpredictable environment (Collett et al., 1992; Steck et al., 2011; Fleischmann et al., 2016). Ants have recently become a promising insect model for studying various aspects of olfactory cognition; using both operant and classical conditioning paradigms, they have been shown to successfully recall olfactory information after a few minutes and up to three days, after conditioning (Dupuy et al., 2006; Josens et al., 2009; Guerrieri et al., 2011), which suggests the potential involvement of different memory types previously described for the honeybee (Menzel, 1999). Currently, however, visual studies have been confined to landmark-based memory, notably in the context of ant navigation and nest finding (Ziegler and Wehner, 1997; Judd and Collett, 1998; Bisch-Knaden and Wehner, 2003). No attempt, so far, has been made to analyse the visual memory dynamics of ants after associative colour learning. In this study, we performed behavioural experiments with controlled stimuli and computational modelling to test for the presence of colour vision, to analyse the putative spectral organization of the ants' visual system (dichromacy versus trichromacy) and to assess the individual colour learning and memory consolidation process under controlled laboratory conditions. We focused on the ant *Camponotus blandus* (Formicidae), a diurnal species (McClure et al., 2008), as a model for understanding the visual processing of ants, as *Camponotus* ants generally exhibit a rich behavioural repertoire coupled with sophisticated social organization (Josens et al., 2009; Guerrieri et al., 2011; Yilmaz et al., 2014, 2016).

So far, no information about the innate colour preference of ants, which might initially influence the learning performance of a forager, is available. We, thus, firstly tested colour-naïve foragers for potential phototactic responses and innate preferences towards the colour stimuli, which were subsequently used in the colour learning experiments. Secondly, we asked whether

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C. blandus workers can learn to discriminate monochromatic stimuli in an associative context. In order to assess the visual memory consolidation processes, we thirdly tested whether workers can store and retrieve the learned colour information in an early mid-term (e-MTM), late mid-term (l-MTM), early long-term (e-LTM) and late long-term (l-LTM) memory from where it can be retrieved 1 h, 12 h, 24 h, 3 days and 7 days after training.

MATERIALS AND METHODS

Animals

All tested workers originated from a *Camponotus blandus* (Smith 1858) colony, which was kept in a climate chamber (at 25°C, 50% relative humidity, and under a 12 h:12 h light:dark regime) at the University of Würzburg, Germany. For handling, subcolonies were established at least one month prior to the experiments starting, and consisted of ca. 120 larvae and 100 workers. Each subcolony was kept in a covered plastic box (20×20×10 cm) and connected to an artificial foraging arena through a silicon tube to allow the foragers to freely forage for food. Each foraging arena contained a vertical wooden stick in the centre from which motivated workers were taken or put back by allowing them to walk onto a piece of cardboard. The colonies were deprived of honey solution at least 20 days before the experiments started and a different subcolony was chosen every day to ensure highly motivated workers during the experiments. Only minor and medium-sized workers were used because individuals of these size categories usually behave as foragers in the genus *Camponotus* (Hölldobler and Wilson, 1990; Yilmaz et al., 2014). The compound eyes of *C. blandus* minor and media workers comprise ca. 800 ommatidia with a facet diameter of ca. 15 µm (F. Kozak and J.S., unpublished data).

Experimental setup and light stimuli

Freely walking foragers were trained and/or tested individually to discriminate a combination of different monochromatic light stimuli [365 nm (ultraviolet, UV), 450 nm (blue) and 528 nm (green)] in a Y-maze (Fig. 1A–C). The spectral stimuli were provided by three light-emitting diodes [(LEDs) 3.46×3.46 mm, OSRAM GmbH, Munich, Germany] mounted on a UV transparent Plexiglas plate of 5×5 cm and connected to a power supply (Fig. 1C). An ant placed at the starting point of the Y-maze could see both target (CS+, rewarded conditioned stimulus) and distractor (CS–, unrewarded conditioned stimulus) stimuli at the same time when she reached the decision point (Fig. 1C). In the phototactic response experiments only a single LED was presented and the alternative arm was dark. LEDs were positioned at a distance of 12.5 cm from the decision point and thus subtended a visual angle of ca. 23 deg (Fig. 1C). A radiometrically calibrated spectrophotometer (Jaz Spectrometer, Ocean Optics, Dunedin, FL, USA) was used to measure the photon numbers emitted by the LEDs. To confirm the use of chromatic information, the light intensities (*I*) of all stimuli were levelled or randomly changed (see Table S1, for all stimulus intensity pairs used during the experiments) by means of different combinations of neutral density filters (Roscolab Ltd, London, UK; Fig. 1B). Therefore, stimuli pairs emitted equal numbers of photons or differed by a factor of ca. 10 (*I*/10), 100 (*I*/100) or 1000 (*I*/1000, see Fig. S4) to test for true colour vision because exact receptor sensitivities are unknown (Quaranta, 1952; Kelber et al., 2003). Experiments were performed under dim light (<1 lx) conditions to eliminate the use of possible visual cues, and between 08:30 h and 13:00 h when *C. blandus* workers show highest foraging activity (McClure et al., 2008).

Phototactic response and colour preference

To determine the phototactic behaviour to any particular wavelength, freely walking colour-naïve foragers were allowed to choose between an arm where one of the monochromatic lights (365 nm, 450 nm and 528 nm) was presented and an empty arm in a dual-choice test without reinforcement. We then determined possible innate colour preferences; colour-naïve foragers were individually tested with different combinations of two monochromatic lights at various intensities in a dual-choice test without reinforcement. To exclude that the adaptation status of the eyes affects innate colour preference (strong UV preference, see Results), colour preference experiments were repeated with another group of ants, which were exposed (for 5 days) to an environmental illumination containing high UV radiation (relative UV content was ca. 5 times higher compared with standard D65 daylight) as a control (see Fig. S1). For each test, only the first decision of each forager was recorded. After the ants made a decision they were gently removed from the setup and kept in a separate box to prevent retesting.

Colour learning and discrimination

Training procedure

To determine the colour learning and discrimination performances, individually marked foragers were trained in a differential conditioning procedure (Giurfa, 2004) to associate a monochromatic light stimulus with 20% (weight/weight) sucrose solution (CS+) and to discriminate it from a distractor stimulus, which was associated with water (CS–). In each trial, 3 µl of sucrose solution was provided as a reward. Ants choosing the arm associated with the CS– were allowed to taste the water, then were taken back to the starting point and allowed to make another choice until they found the reward. If an ant chose the arm associated with the CS+, she was allowed to drink and then gently taken and returned to the top of the wooden stick in the foraging arena. The training phase consisted of 21 consecutive visits (training and refreshment trials) to the Y-maze with inter-trial intervals between 4 min and 8 min, depending on the individual foraging speed (Fig. 1D, upper row). In each trial only the first choice of an individual ant was recorded.

Acquisition curves were calculated on the basis of the number of correct choices and the mean of three consecutive training blocks of seven trials. During training and testing, the positions of the target and distractor stimuli were changed between the two arms in a pseudorandom sequence (Dupuy et al., 2006; Avarguès-Weber et al., 2015). Additionally, the order of stimulus presentation was also randomized among respective ant groups. The floor of the Y-maze was cleaned with alcohol and immediately dried with a hairdryer after each visit to exclude possible olfactory cues.

The experiments comprised two different learning groups. In the first group, we tested whether ants can learn to discriminate between two (out of the three) monochromatic light stimuli based on chromatic information only, and irrespective of intensity changes. In this group, two subgroups of ants were trained with light stimuli with inverse contingencies, i.e. the rewarding and unrewarding wavelengths in one subgroup were reversed in the other subgroup. In the second group, we aimed to investigate whether ants actually learn the rewarded wavelength or, alternatively, simply learn to avoid UV, the innately preferred colour (see Results). In this group, ants were trained only to discriminate green or blue (CS+), respectively, from UV (CS–) and subsequently tested.

Test procedure

In the first learning group, where ants were tested whether they learn to discriminate between two stimuli irrespective of intensity

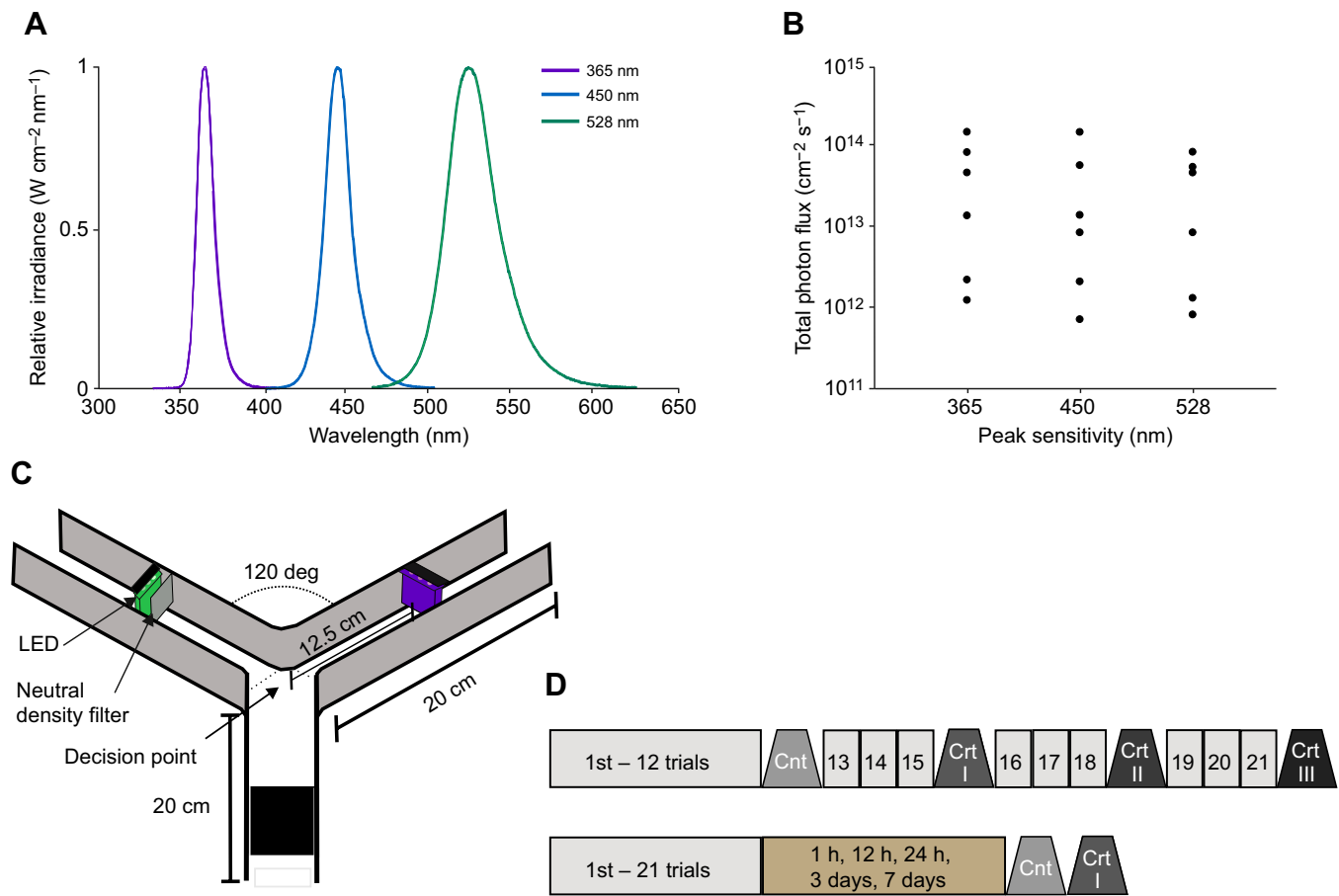


Fig. 1. Experimental setup and light stimuli used for the experiments. (A) Relative irradiance ($\text{W cm}^{-2} \text{nm}^{-1}$) of the three tested light-emitting diode (LED) stimuli (peak wavelength at 365 nm, 450 nm and 528 nm). (B) Total photon flux ($\text{cm}^{-2} \text{s}^{-1}$) of the monochromatic light stimuli generated by means of different combinations of neutral density filters, measured at the decision point. (C) Illustration of the Y-maze setup used for the experiments. (D) Protocols used for the learning (upper row) and memory (lower row) experiments. During the learning experiment, individuals were immediately tested after 12 conditioning trials in a sequence of several non-rewarded control and critical tests, during which the intensity of rewarded (CS+) and unrewarded (CS-) stimuli levelled or randomly changed (upper row). In the memory experiment, ants that completed 21 consecutive conditioning trials were put back to the colony and randomly tested in control and critical tests for memory retrieval 1 h, 12 h, 24 h, 3 days and 7 days after the last conditioning trial (lower row; for details of the tests, see Materials and methods). The training and refreshment trials between tests are represented by grey rectangles and control and critical tests by trapezoids in darker shades of grey (see Materials and methods for a detailed explanation). Cnt, control test; Crt, critical test.

changes, four unrewarded tests (control test and critical tests I–III) were performed, intermitted by refreshment trials (Fig. 1D, Table 1). In the control test, both stimuli were presented at the same intensity as during training. In critical test I and critical test II, the intensity of the CS+, and the intensities of both CS+ and CS- stimuli were decreased, respectively, by 1 log unit. In critical test III, the intensity of CS+ was decreased while the intensity of CS- was increased.

Thus, the brightness between target and distractor stimuli differed by two log units.

In the second learning group, where we tested whether ants actually learn the CS+ or, alternatively, simply learn to avoid UV, five unrewarded tests were performed (Table 1). Ants were firstly tested with CS+ versus CS- presented with the same intensity as during training (control test). Secondly, the intensity of CS+ was reduced by

Table 1. Different types of tests and intensity differences of stimuli in the learning and memory experiments

Experiment	Test type	Test conditions	Intensity levels
Learning experiments (first, second and third group), memory experiments	Control	CS+ (<i>I</i>) vs CS- (<i>I</i>)	Same
Learning experiments (first, second and third group), memory experiments	Critical I	CS+ (<i>I/10</i>) vs CS- (<i>I</i>)	1 log difference
Learning experiments – first group	Critical II	CS+ (<i>I/10</i>) vs CS- (<i>I/10</i>)	Same
Learning experiments – first group	Critical III	CS+ (<i>I/10</i>) vs CS- (<i>10xI</i>)	2 log difference
Learning experiments – second group	Critical II	CS+ (<i>I</i>) vs novel (<i>I</i>)	Same
Learning experiments – second group	Critical III	CS+ (<i>I/10</i>) vs novel (<i>I</i>)	1 log difference
Learning experiments – second group	Critical IV	CS- (<i>I</i>) vs novel (<i>I</i>)	Same
Learning experiments – third group (see Fig. S4)	Critical II	CS+ (<i>I/100</i>) vs CS- (<i>I</i>)	2 log difference
Learning experiments – third group (see Fig. S4)	Critical III	CS+ (<i>I/1000</i>) vs CS- (<i>I</i>)	3 log difference

I, intensity; *I/10*, intensity decreased by 1 log unit; *10xI*, intensity increased by 1 log unit; *I/100*, intensity decreased by 2 log units; *I/1000*, intensity decreased by 3 log units; CS+, rewarded conditioned stimulus; CS-, unrewarded conditioned stimulus.

1 log unit (critical I). To investigate which type of associations (CS+/sucrose or CS-/water) mediated the ants' decision during the acquisition, the CS+ (blue or green) and CS- (UV) was replaced by a novel stimulus in subsequent critical tests (critical II–IV, Table 1).

For both learning groups, each test started when the ant entered the Y-maze and lasted for 1 min. First choices and the time an individual ant spent in each arm were recorded. Stimuli positions in the arms were randomized between tests. After each test, the ants were allowed to perform refreshment trials where the rewarded and unrewarded stimuli of constant intensity were presented again. For each ant, the whole procedure took ca. 3–4 h and was completed on the same day.

Visual (colour) memory

To test how long ants can maintain the learned association in memory, individual foragers were trained to discriminate green or blue (CS+) from the innately preferred colour, UV (CS-) for 21 training trials according to the training procedure described above (Fig. 1D, lower row). Ants that completed 21 consecutive learning trials were put back to the colonies and randomly assigned to one of five experimental groups, which were subsequently tested for memory retrieval 1 h, 12 h, 24 h, 3 days or 7 days after conditioning, considering early mid-term (e-MTM), late mid-term (l-MTM), early long-term (e-LTM) and late long-term (l-LTM). Memory retrieval was tested under two conditions, by presenting CS+ and CS- at the same intensity (control test), and with CS+ reduced by 1 log unit (critical test I). Ants were not rewarded between the two retention tests and the order of the tests was randomized among individuals. Similar to the colour learning tests, two variables, first choice and the time spent in the corresponding arm during the first 1 min, were recorded.

Computer modelling of colour vision system

We used a principle outlined by Kemp et al. (2015) of first applying the simplest biologically relevant model to evaluate physiologically plausible explanations of perceived colour vision, and then progressively building upon modelling outcomes to derive solutions consistent with the observed behavioural responses by the ants. The modelling thus considered ranges of previously observed PR sensitivities in ants (Briscoe and Chittka, 2001), formal definitions for colour vision (Quaranta, 1952; Kelber et al., 2003) and current behaviourally informed colour spaces for insects (Kelber et al., 2003). We first tested to see if a monochromatic visual system could explain the results by employing a Stavenga vitamin A1 visual nomogram, and tested at 10 nm steps a range of alpha-band peak sensitivities between 340 nm and 620 nm, with a common beta-band sensitivity of 340 nm (Stavenga et al., 1993). Each of the respective PRs were weighted to have equivalent photon catch considering spectrally neutral stimulation (Neumeyer, 1981; Stojcev et al., 2011), and the respective stimuli irradiance values being converted to relative photon flux values (Kemp et al., 2015). We integrated (integral 300–700 nm) spectral photon catches of putative monochromatic PR and respective UV, blue and green stimulus intensity data values at 10 nm steps. We used the ratios of respective stimuli at all intensities (range 3 log units) to test if any monochromatic visual system could explain the results. We additionally considered if a multiple receptor system driven by differing positive/negative phototactic responses (Menzel, 1979; Goldsmith, 1990) could explain results using wavelength-specific behaviours, which included developing control behavioural experiments to establish phototactic responses to respective stimuli at various intensities (see 'Phototactic response and colour preference', above).

We next considered a dichromatic explanation for the observed behaviour. As all tested insects that have colour vision possess a UV PR (Briscoe and Chittka, 2001), and the ants in our current study exhibited strong innate preferences for the UV stimulus plus a capacity to learn this stimulus in a dynamic way (i.e. as CS+ or CS-) compared with the longer wavelength blue and green stimuli, we assumed the presence of a UV receptor at a wavelength of 360 nm, which is close to the wavelength maxima reported for other ant species (Briscoe and Chittka, 2001; Ogawa et al., 2015). We then considered a second primary PR with peak sensitivity in the range 390–560 nm, and the relative Euclidean colour distance that is predicted in a dichromatic colour space (Kelber et al., 2003; Kemp et al., 2015) for the respective UV versus blue, UV versus green and blue versus green stimuli combination experienced by some of the groups of tested ants.

Finally, we considered how a trichromatic visual system would likely enable colour perception of the respective UV versus blue, UV versus green and blue versus green stimuli combinations. We used a chromaticity diagram based on PR excitations as a generalized representation of colour opponency in Hymenoptera, termed colour hexagon (Chittka, 1992; Chittka et al., 1992). This colour space has also been applied more broadly to trichromatic visual systems, such as in primates (Rowe and Jacobs, 2007). The advantage of this model is that it has been well calibrated for colour perceptual judgments considering hymenopteran bees in differential conditioning experiments where there is improvement in learning over multiple trials (Dyer and Neumeyer, 2005; Spaethe et al., 2014), as was observed in our experiments, allowing for behaviourally informed analyses of how the respective UV versus blue, UV versus green and blue versus green stimuli combinations would likely be perceived by a trichromatic insect. We considered the two available sets of trichromatic receptors recently reported for diurnal (370 nm, 470 nm and 510 nm) and nocturnal (370 nm, 450 nm and 550 nm) ants (Ogawa et al., 2015), which are reasonably consistent with data for trichromatic PR maxima in other Hymenoptera (Peitsch et al., 1992; Briscoe and Chittka, 2001).

Statistical analyses

First choices of the ants during the phototactic response and colour preference tests were compared with expected random visits using a binomial and χ^2 test, respectively. Learning performances of ants were compared between the three consecutive blocks of seven trials by means of ANOVA for repeated measurements followed by a *post hoc* Bonferroni test using arcsine square-root-transformed data. First choices in control and critical tests of learning and memory retrieval experiments were compared with the innate colour preference of the respective wavelength using binomial test. One-sample *t*-test after arcsine square-root transformation was conducted to compare the relative time spent in each arm with a theoretical proportion of 50%. We compared acquisition between memory groups by using a two-way ANOVA for repeated measurements. All statistical calculations were performed with SPSS 22 (IBM, Chicago, IL, USA). The significance level for all analyses was set to $P < 0.05$. Graphs and figures were compiled in SigmaPlot 12 (Systat Software, Inc., San Jose, CA, USA) and edited using COREL DRAW X7 (Corel Corporation Ltd., Ottawa, Canada).

RESULTS

Phototactic response

Camponotus blandus foragers showed a clear positive phototactic behaviour towards all tested monochromatic stimuli presented at high- [UV (*I*) versus dark: $P = 0.001$; blue (*I*) versus dark: $P = 0.001$;

green (*I*) versus dark: $P < 0.001$, Fig. 2A] and low-intensity levels [UV (*I/10*) versus dark: $P < 0.001$; blue (*I/10*) versus dark: $P = 0.004$; green (*I/10*) versus dark: $P = 0.004$, Fig. 2B]. From these results, we conclude that ants can easily detect and are attracted by each of the wavelengths at high- and low-intensity levels when the stimulus subtends a visual angle of 23 deg.

Colour preference

The results obtained from wavelength preference tests showed that freely walking colour-naïve foragers exhibit a strong innate preference for UV (365 nm) over blue (450 nm) and green (528 nm) stimuli presented at the same intensity [UV (*I*) versus blue (*I*): $\chi^2 = 25.920$, $P < 0.001$; UV (*I*) versus green (*I*): $\chi^2 = 15.680$, $P < 0.001$, Fig. 3A,B]. Interestingly, the preference still persists when the stimuli intensities differed by a factor of 10 [UV (*I/10*) versus blue (*I*): $\chi^2 = 22.533$, $P < 0.001$; UV (*I*) versus blue (*I/10*): $\chi^2 = 19.200$, $P < 0.001$; UV (*I/10*) versus green (*I*): $\chi^2 = 22.533$, $P < 0.001$; UV (*I*) versus green (*I/10*): $\chi^2 = 10.800$, $P = 0.001$, Fig. 3A,B]. In contrast, ants did not show a significant preference for either blue or green light when presented simultaneously [blue versus green: $\chi^2 = 0.320$, $P = 0.572$; blue (*I*) versus green (*I/10*): $\chi^2 = 0.133$, $P = 0.715$; blue (*I/10*) versus green (*I*): $\chi^2 = 3.333$, $P = 0.068$, Fig. 3C].

We additionally tested the colour preference of ants that were exposed to an environmental illumination containing high UV radiation (Fig. S1). We found no difference between ants exposed to one of the illumination regimes, indicating that the adaptation status of the visual system did not significantly affect the colour preference of *C. blandus* foragers for the tested stimuli (statistical data provided in Fig. S2).

Colour learning

In the first learning group (Fig. 4), the performance of foragers was clearly influenced by their innate wavelength preference during the learning phase; the proportion of the correct choices during acquisition was very high, even from the very beginning (first trial) of the training when UV (365 nm) was the rewarded stimulus (choice rate for UV within first choices: UV versus blue: 87.5%; UV versus green: 100%; Fig. 4A,C, see also Fig. S3). However, despite their strong preference for UV, workers could reliably learn to discriminate blue (450 nm, CS+) or green (528 nm, CS+) stimuli from the innately preferred UV (CS-) during the three blocks of training [blue (CS+) versus UV (CS-): $F_{2,14} = 11.855$, $P = 0.001$; green (CS+) versus UV (CS-): $F_{2,14} = 17.511$, $P < 0.001$, Fig. 4A,C].

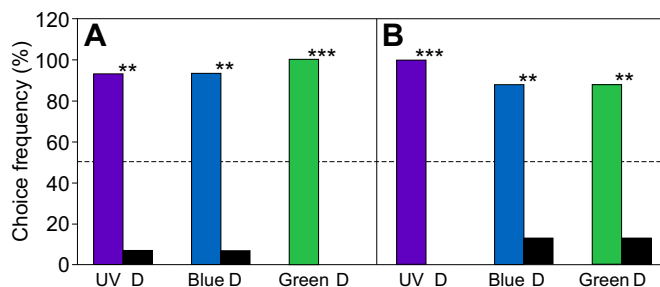


Fig. 2. Phototactic response of individual foragers. Ants could choose between an arm where one of three tested wavelengths [ultraviolet (UV), 365 nm; blue, 450 nm; green, 528 nm] was presented against a dark arm (black bar). (A) High- and (B) low-stimulus intensities (for detailed intensity levels, see Table S1). The dashed line in the graph indicates random choice. Significant differences are marked with asterisks. $N = 16$ individuals were tested for each wavelength in each test condition. ** $P < 0.01$; *** $P < 0.001$. D, dark arm of the Y maze.

During the control and critical tests, ants could discriminate between CS+ and CS- in the UV versus blue and UV versus green combination, independently of which wavelength was rewarded. They chose the correct arm more often than expected compared with their innate preference (14% for blue and 20% for green) when blue and green was presented as CS+ [UV (CS-) versus blue (CS+): control (Cnt): $P < 0.001$; critical (Crt) I: $P < 0.001$; Crt II: $P < 0.001$; Crt III: $P < 0.001$; UV (CS-) versus green (CS+): Cnt: $P < 0.001$; Crt I: $P < 0.001$; Crt II: $P < 0.001$; Crt III: $P < 0.001$, Fig. 4A,C]. However,

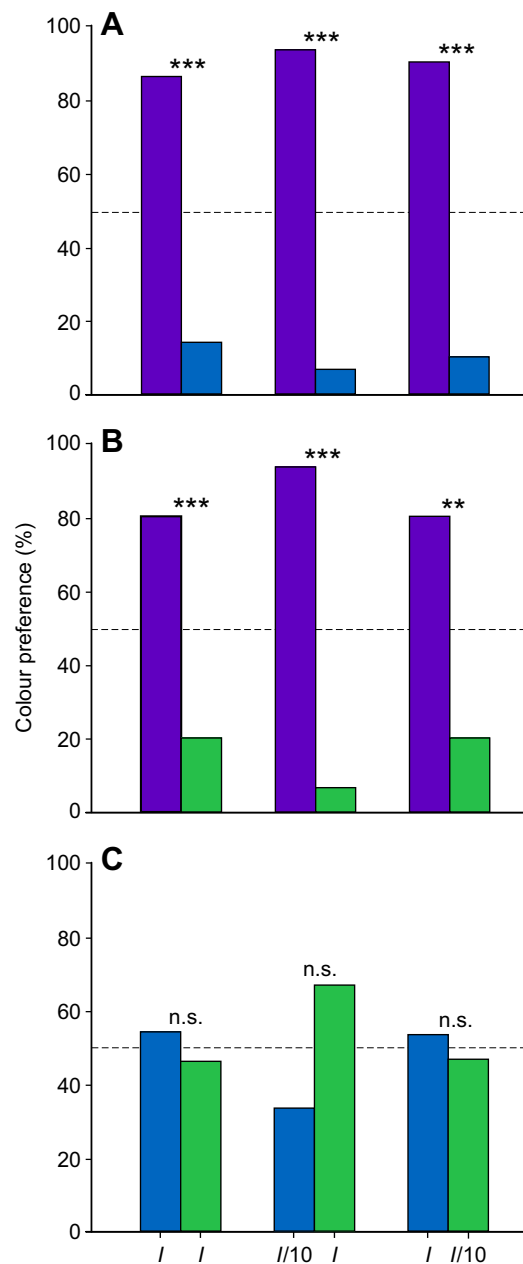


Fig. 3. Innate colour preference of colour-naïve foragers for colour pairs of varying intensities. (A) Ultraviolet (UV) versus blue, (B) UV versus green and (C) blue versus green light pairs were tested. Significant differences are marked with asterisks. Intensity was adjusted so that the two stimuli emitted equal numbers of photons (*I/I*; $N = 50$) or one stimulus intensity was reduced by a factor of 10 (*I/10*; $N = 30$). The dashed lines in the graphs indicate random choice. n.s., not significant; ** $P < 0.01$; *** $P < 0.001$. UV wavelength, purple bar; blue wavelength, blue bar; green wavelength, green bar.

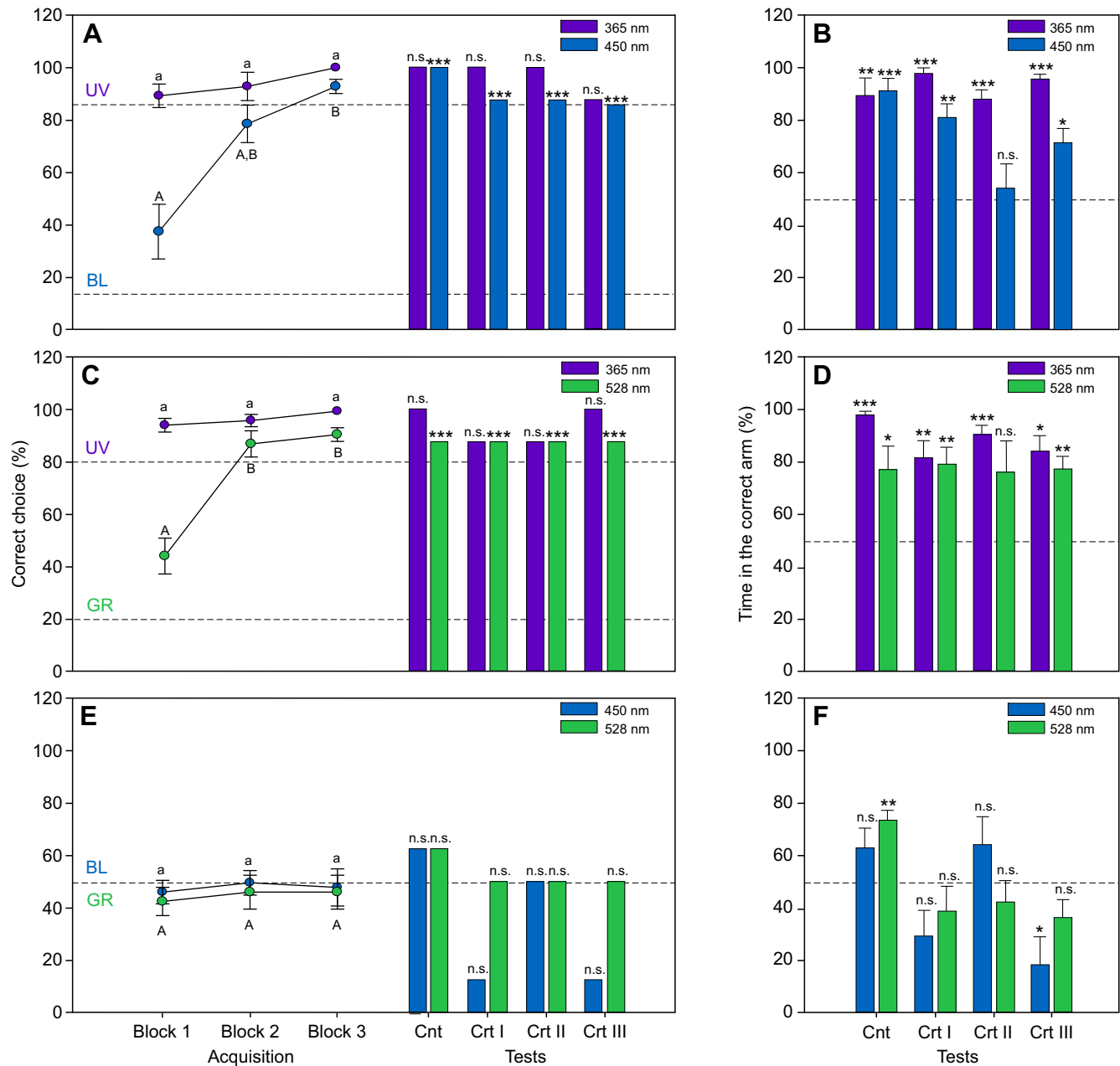


Fig. 4. Visual learning and discrimination performances of foragers tested in a differential learning task with training stimuli of varying intensities (first learning group). (A,C,E) The learning performance (learning curve with circles) and choice frequencies (bars) of the ants. (B,D,F) The relative time spent in the arm where the rewarding wavelength was presented. Stimuli pairs emitted equal numbers of photons (during training, control and critical test II) or differed by 1 log (critical test I) or 2 log units (critical test III). The dashed lines in the graphs in the left-hand panels indicate the innate preference levels for the rewarded wavelengths determined in the preference tests (see Fig. 3). The dashed lines in the graphs in the right-hand panels indicate the random choice level. Significant differences (from the innate preferences in A, C and E, and the 50% level in B, D and F) are marked with asterisks. $N=8$ individuals were trained and tested for each group. BL, blue; Cnt, control test; Crt, critical test; GR, green; n.s., not significant; UV, ultraviolet; * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Error bars show standard errors. Different letters indicate significant differences between blocks.

their UV preference (80% and 86% when compared with green and blue, respectively) did not change when UV light was presented as CS+ [UV (CS+) versus blue (CS−): Cnt: $P=0.299$; Crt I: $P=0.299$; Crt II: $P=0.299$; Crt III: $P=0.689$; UV (CS+) versus green (CS−): Cnt: $P=0.168$; Crt I: $P=0.503$; Crt II: $P=0.503$; Crt III: $P=0.168$, Fig. 4A,C].

For all tests, the ants also spent significantly more time in the arm in which the previously rewarded colour was presented [UV (CS+) versus blue (CS−): Cnt: $t_7=5.201$, $P=0.001$; Crt I: $t_7=12.920$, $P<0.001$; Crt II: $t_7=6.293$, $P<0.001$; Crt III: $t_7=10.758$, $P<0.001$;

UV (CS−) versus blue (CS+): Cnt: $t_7=6.227$, $P<0.001$; Crt I: $t_7=4.563$, $P=0.003$; Crt III: $t_7=2.976$, $P=0.021$; UV (CS+) versus green (CS−): Cnt: $t_7=15.841$, $P<0.001$; Crt I: $t_7=3.892$, $P=0.006$; Crt II: $t_7=6.974$, $P<0.001$; Crt III: $t_7=3.479$, $P=0.01$; UV (CS−) versus green (CS+): Cnt: $t_7=2.837$, $P=0.025$; Crt I: $t_7=3.917$, $P=0.006$; Crt III: $t_7=4.135$, $P=0.004$, Fig. 4B,D], except for critical test II where the blue and green stimuli were presented as rewarded wavelengths and tested against UV [UV (CS−) versus blue (CS+): Crt II: $t_7=0.062$, $P=0.952$; UV (CS−) versus green (CS+): Crt II: $t_7=1.796$, $P=0.116$, Fig. 4B,D].

Ants, however, failed to distinguish between blue and green, irrespective of whether the rewarded wavelength was blue or green [blue (CS+) versus green (CS-): $F_{2,14}=0.127$, $P=0.882$; blue (CS-) versus green (CS+): $F_{2,14}=0.140$, $P=0.870$]. For these colour combinations, the proportion of correct choices did not differ from the 50% chance level [first block: blue (CS+) versus green (CS-): $\chi^2=1$, $P=0.607$; blue (CS-) versus green (CS+): $\chi^2=2$, $P=0.572$; second block: blue (CS+) versus green (CS-): $\chi^2=2$, $P=0.572$; blue (CS-) versus green (CS+): $\chi^2=3.250$, $P=0.197$; last block: blue (CS+) versus green (CS-): $\chi^2=0.750$, $P=0.945$; blue (CS-) versus green (CS+): $\chi^2=2$, $P=0.736$]. The ants also failed to show evidence of learning considering all control and critical tests independent of whether the rewarded wavelength was blue or green [blue (CS+) versus green (CS-): Cnt: $P=0.727$; Crt I: $P=0.07$; Crt II: $P=1.00$; Crt III: $P=0.07$; blue (CS-) versus green (CS+): Cnt: $P=0.727$; Crt I: $P=1.00$; Crt II: $P=1.00$; Crt III: $P=1.00$, Fig. 4E]. Additionally, workers did not spend more time in the arm in which the previously rewarded colour was presented [blue (CS+) versus green (CS-): Cnt: $t_7=1.557$, $P=0.163$; Crt I: $t_7=-2.231$, $P=0.061$; Crt II: $t_7=0.913$, $P=0.392$; blue (CS-) versus green (CS+): Crt I: $t_7=-1.348$, $P=0.220$; Crt II: $t_7=-1.046$, $P=0.330$; Crt III: $t_7=-1.855$, $P=0.106$, Fig. 4F] except for control and critical test III where the green and blue stimuli were presented as rewarded wavelengths, respectively [blue (CS-) versus green (CS+): Cnt:

$t_7=5.740$, $P=0.001$; blue (CS+) versus green (CS-): Crt III: $t_7=-3.266$, $P=0.014$, Fig. 4F].

In the second learning group, the results revealed that all ants could successfully learn to discriminate blue or green (CS+) from UV (CS-) [blue (CS+) versus UV (CS-): $F_{2,18}=20.115$, $P<0.001$; green (CS+) versus UV (CS-): $F_{2,18}=34.770$, $P<0.001$, Fig. 5A,C]. They also chose the correct arm in control and critical test I where the intensity of the rewarded stimulus was levelled or decreased by a factor of 10, respectively [blue (CS+) versus UV (CS-): Cnt: $P<0.001$; Crt I: $P<0.001$; green (CS+) versus UV (CS-): Cnt: $P<0.001$; Crt I: $P<0.001$, Fig. 5A,C]. However, ants failed to choose the correct arm when the unrewarded stimulus (UV) was replaced by a novel stimulus (green or blue) in critical test II and critical test III for both colour combinations [blue (CS+) versus UV (CS-): Crt II: $P=0.754$; Crt III: $P=0.344$; green (CS+) versus UV (CS-): Crt II: $P=0.754$; Crt III: $P=0.754$, Fig. 5A,C]. Interestingly, ants given the choice between UV and a novel stimulus (Crt IV) chose the novel stimulus [blue (CS+) versus UV (CS-): Crt IV: UV: $P=0.107$; novel (green): $P<0.001$; green (CS+) versus UV (CS-): Crt IV: UV: $P=0.221$, novel (blue): $P<0.001$, Fig. 5A,C], indicating that they learned to avoid the UV light but not the CS+ stimulus. When comparing the percentage of time spent in any arm, workers spent significantly more time in the arm in which the previously rewarded colour was presented, in both control and critical tests I [blue (CS+)

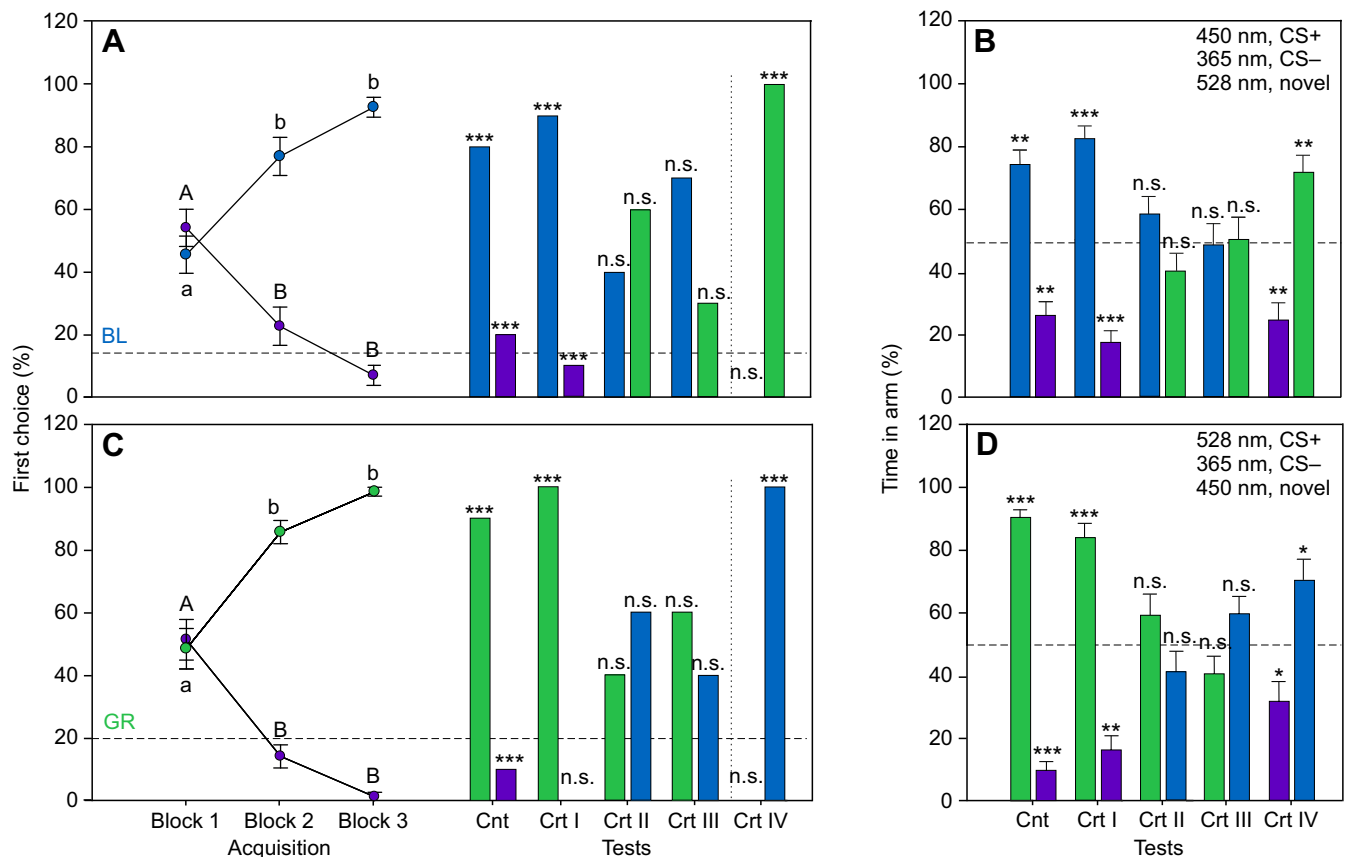


Fig. 5. Visual learning and discrimination performances of foragers tested in a differential learning task with novel stimuli (second learning group). (A,C) The learning performance (learning curve with circles) and choice frequencies (bars) of the ants. (B,D) The relative time spent in the arm where the rewarding, unrewarding or novel stimuli were presented. Stimulus pairs emitted equal numbers of photons (training, control and critical tests II, IV) or the intensity of the rewarded wavelength was lowered by 1 log unit (critical tests I and III). The dashed lines in the graphs in the left-hand panels indicate the innate preference levels for the rewarded wavelengths determined in the preference tests (see Fig. 3). The dashed lines in the graphs in the right-hand panels indicate random choice. Significant differences are marked with asterisks. $N=10$ individuals were trained and tested for each experimental group. BL, blue; CS+, rewarded conditioned stimulus; CS-, unrewarded conditioned stimulus; Cnt, control test; Crt, critical test; GR, green; n.s., not significant; UV, ultraviolet; * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Error bars show standard errors. Different letters indicate significant differences between blocks.

versus UV (CS⁻): Cnt: CS⁺ (*I*): $t_9=4.095$, $P=0.003$; Crt I: CS⁺ (*I/I*): $t_9=6.169$, $P<0.001$, Fig. 5B; green (CS⁺) versus UV (CS⁻) Cnt: CS⁺ (*I*): $t_9=9.219$, $P<0.001$; Crt I: CS⁺ (*I/I*): $t_9=5.524$, $P<0.001$, Fig. 5D]. However, they did not spend more time in the correct arm in critical tests II and III where the CS⁻ (UV) was replaced by a novel (N) stimulus [blue (CS⁺) versus UV (CS⁻): Crt II: CS⁺ (*I*): $t_9=1.516$, $P=0.164$; N (*I*): $t_9=-1.672$, $P=0.129$; Crt III: CS⁺ (*I/I*): $t_9=-0.170$, $P=0.869$; N (*I*): $t_9=0.047$, $P=0.964$; green (CS⁺) versus UV (CS⁻): Crt II: CS⁺ (*I*): $t_9=1.291$, $P=0.229$; N (*I*): $t_9=-1.421$, $P=0.189$; Crt III: CS⁺ (*I/I*): $t_9=-1.760$, $P=0.112$; N (*I*): $t_9=1.615$, $P=0.141$, Fig. 5C,D]. For both stimulus pairs, ants given the choice between the CS⁻ (UV) and the novel stimulus spent more time in the arm where the novel stimulus was presented [blue (CS⁺) versus UV (CS⁻): Crt IV: CS⁻ (*I*): $t_9=-3.952$, $P=0.003$; N (*I*): $t_9=3.838$, $P=0.004$; green (CS⁺) versus UV (CS⁻): Crt IV: CS⁻ (*I*): $t_9=-2.992$, $P=0.015$; N (*I*): $t_9=2.864$, $P=0.019$, Fig. 5C,D].

To exclude the possibility that ants simply preferred UV because their eyes are much more sensitive to short-wavelength light, an additional group of ants (third learning group) was trained to discriminate between blue versus UV and subsequently tested in control tests where the intensity of the rewarded wavelength (blue or UV) were decreased by a factor of 100 and 1000, respectively (Fig. S4). The results revealed that ants can still discriminate between blue and UV, even if the intensity of the rewarded

wavelength was reduced by 3 log units (statistical data provided in Fig. S4).

Visual (colour) memory

In memory experiments, the learning performances of ants during the training did not differ among different memory groups [blue versus UV: $F_{3,26}=0.431$, $P=0.732$; green versus UV: $F_{1,20}=0.251$, $P=0.622$]; therefore, results were pooled. Foragers trained with green or blue (CS⁺) versus UV (CS⁻) were able to reliably learn the rewarded stimulus [blue (CS⁺) versus UV (CS⁻): $F_{2,118}=239.027$, $P<0.001$; green (CS⁺) versus UV (CS⁻): $F_{2,98}=121.923$, $P<0.001$, Fig. 6A,C]. Their responses to the unrewarded stimulus (UV) decreased gradually along the three consecutive blocks of seven trials [blue (CS⁺) versus UV (CS⁻): first to second block: $P<0.001$; second to third block: $P<0.001$; green (CS⁺) versus UV (CS⁻): first to second block: $P<0.001$; second to third block: $P<0.001$, Fig. 6A, C]. Ants could recall the learned colour information within tested time intervals and chose the correct arm in control and critical tests more often than expected compared with their innate colour preference (14% for blue and 20% for green) [blue (CS⁺) versus UV (CS⁻): 1 h: Cnt: $P<0.001$; Crt I: $P<0.001$; 12 h: Cnt: $P<0.001$; Crt I: $P<0.001$; 24 h: Cnt: $P<0.001$; Crt I: $P<0.001$; 3 days: Cnt: $P<0.001$; Crt I: $P<0.001$; 7 days: Cnt: $P<0.001$; Crt I: $P<0.001$; green (CS⁺) versus UV (CS⁻): 1 h: Cnt: $P<0.001$; Crt I: $P<0.001$; 12 h: Cnt:

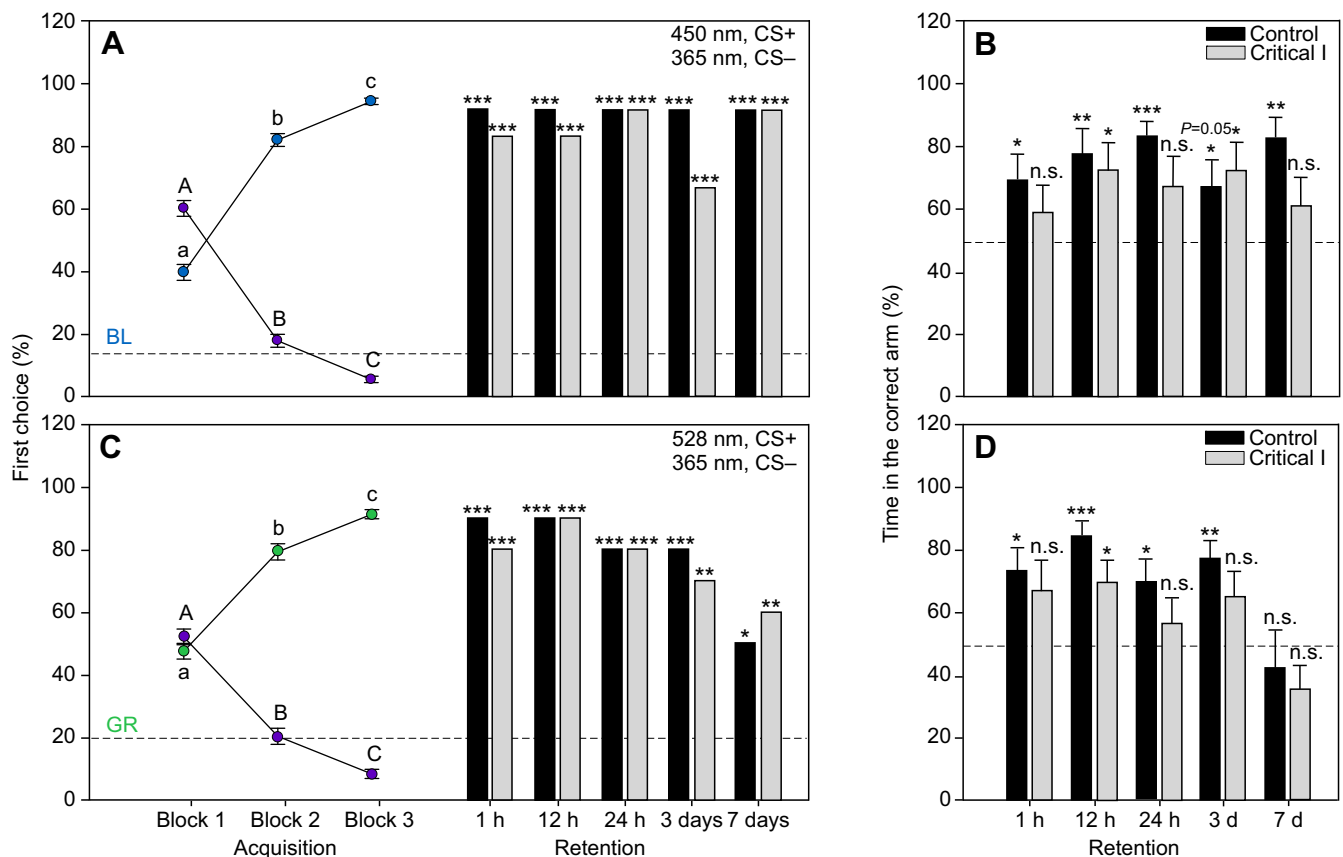


Fig. 6. Memory retrieval of foragers trained to discriminate blue (450 nm, CS⁺) or green (528 nm, CS⁺) from UV (365 nm, CS⁻). (A,C) Learning performance (learning curve with circles) and choice frequencies (bars) of the ants. (B,D) Relative time spent in the arm where the rewarding stimuli were presented. Ants were tested for retention 1 h (early mid-term memory), 12 h (late mid-term memory), 24 h (early long-term memory), 3 days and 7 days (late long-term memory) after the last acquisition trial. The dashed lines in the graphs in the left-hand panels indicate the innate preference levels for the rewarded wavelengths determined in the preference tests (see Fig. 3). The dashed lines in the graphs in the right-hand panels indicate random choice. Significant differences are marked with asterisks. $N=12$ and $N=10$ individuals were trained and tested for blue versus UV and green versus UV, respectively. BL, blue; CS⁺, rewarded conditioned stimulus, CS⁻, unrewarded conditioned stimulus; GR, green; n.s., not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Error bars show standard errors. Different letters indicate significant differences between blocks.

$P < 0.001$; Crt I: $P < 0.001$; 24 h: Cnt: $P < 0.001$; Crt I: $P < 0.001$; 3 days: Cnt: $P < 0.001$; Crt I: $P = 0.001$; 7 days: Cnt: $P = 0.033$; Crt I: $P = 0.006$, Fig. 6A,C]. Seven days after the last conditioning trial, the ants still chose the arm with the previously learned rewarded colour (Fig. 6). The retention, however, gradually declined and showed its lowest level after 7 days in the green versus UV group (Fig. 6C) whereas in the blue versus UV group, memory retrieval stayed constant even after one week (Fig. 6A).

The time spent in the arm providing the CS+ was significantly longer in control tests in both experimental conditions [blue (CS+) versus UV (CS-): 1 h: Cnt: $t_{11} = 2.486$, $P = 0.03$; 12 h: Cnt: $t_{11} = 3.505$, $P = 0.005$; 24 h: Cnt: $t_{11} = 5.510$, $P < 0.001$; 3 days: Cnt: $t_{11} = 2.202$, $P = 0.05$; 7 days: Cnt: $t_{11} = 4.652$, $P = 0.001$; green (CS+) versus UV (CS-): 1 h: Cnt: $t_9 = 2.868$, $P = 0.019$; 12 h: Cnt: $t_9 = 5.655$, $P < 0.001$; 24 h: Cnt: $t_9 = 2.482$, $P = 0.035$; 3 days: Cnt: $t_9 = 3.766$, $P = 0.004$, Fig. 6B,D], except for the 7 day group when the ants trained with green as a rewarded wavelength [7 days: Cnt: $t_9 = 0.678$, $P = 0.515$, Fig. 6B,D]. In critical test I where the intensity of the rewarded wavelength was decreased by 1 log unit, the percentage of the time spent in the correct arm did not differ from the 50% theoretical level 1 h, 24 h and 3 days and 7 days after post-conditioning when the rewarded wavelength was green [1 h: Crt: $t_9 = 1.495$, $P = 0.169$; 24 h: Crt: $t_9 = 0.421$, $P = 0.683$; 3 days: Crt: $t_9 = 1.900$, $P = 0.09$; 7 days: Crt: $t_9 = -1.910$, $P = 0.088$, Fig. 6], and 1 h, 24 h and 7 days after post conditioning when the ants were trained with blue as a rewarded wavelength [1 h: Crt: $t_{11} = 0.824$, $P = 0.428$; 24 h: Crt: $t_{11} = 1.722$, $P = 0.113$; 7 days: Crt: $t_{11} = 1.476$, $P = 0.168$, Fig. 6].

Computer modelling of colour vision system

For the putative PRs with an alpha-band peak sensitivity in the range of 340–620 nm, some combinations of the wide range of stimulus intensities we tested (Fig. 1B) always produced a case where for the UV versus blue stimuli a monochromatic visual system would fail to reliably choose the CS+ stimulus based solely on intensity cues. For the UV versus green stimuli there was also always a case for putative PRs with peak sensitivity in the range 400–620 nm that a monochromatic visual system would fail to reliably choose the CS+ stimulus based solely on intensity cues. However, the green stimulus had insufficient intensity to stimulate a single monochromatic short-wavelength receptor, even when intensity differences were modulated by up to 3 log units (data not shown). Nevertheless, the ants demonstrated successful discrimination between all combinations of UV versus blue stimuli intensities, which cannot be mediated by a monochromatic visual system.

We thus next considered the Euclidean colour distance in a dichromatic colour space for a visual system based on a primary UV alpha-band PR at 360 nm, and a second long-wavelength sensitive receptor between 390 nm and 560 nm. For blue versus green stimuli the model predicts poor colour resolution (=small colour distances, Fig. 7; dashed line). For UV versus blue and UV versus green stimuli, the model predicts relatively good colour resolution (colour distance between 0.4 and 0.8), and in particular for long-wavelength-sensitive receptor positions > 470 nm, the model predicts UV versus green stimuli should be learned better than the UV versus blue stimuli, assuming colour similarity judgments exist in ants (Fig. 7). We indeed found that ants learned the green versus UV task faster than the blue versus UV task (Fig. S5), suggesting that the most likely position for the LWS (long wavelength-sensitive) receptor in the ants would be in the range of 470–560 nm. For all the tested putative dichromatic receptor combinations (and in contrast to a trichromatic system, see below), the green and blue

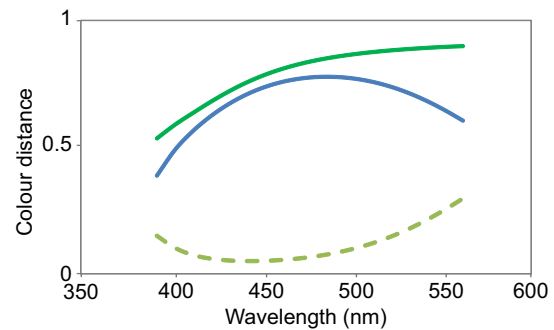


Fig. 7. Euclidean relative colour distance in a dichromatic plane. Plots of relative colour distance in a dichromatic colour space considering a fixed ultraviolet (UV) receptor with a wavelength maximum at 360 nm and a second long-wavelength receptor with a peak in the range 390–560 nm. The predicted colour distance between the UV and green stimuli used in the behavioural experiments (green line) or the UV and blue stimuli (blue line) is relatively large, whereas between the blue and green stimuli (dashed line) the model predicts a small colour distance for all modelled ranges of dichromatic vision. The relative predictions are consistent with observed ant behaviour (see Results).

stimuli are predicted to be learned poorly, or not at all, which is consistent with the observed behaviour in our experiments (Fig. 4).

To test how a trichromatic colour vision would judge stimuli similarity, the colour distance between stimuli was calculated using an Euclidean metric in the hexagon colour space (Chittka, 1992). For a 370 nm, 450 nm and 550 nm trichromatic visual system (corresponding to the PR types found in *M. vindex*), the colour distances would be 0.59 (UV versus blue), 0.52 (UV versus green) and 0.54 (blue versus green) hexagon units. For a 370 nm, 470 nm and 510 nm trichromatic visual system (corresponding to *M. croslandi*), the colour distances were 0.60 (UV versus blue), 0.50 (UV versus green) and 0.31 (blue versus green) hexagon units. By comparison, honeybees, bumblebees and stingless bees (Dyer and Chittka, 2004; Dyer and Neumeyer, 2005; Spaethe et al., 2014) all discriminate between colours at ceiling level when hexagon distances are greater than 0.20; predicting that all our LED stimuli combinations should have been well discriminated, if the ants were trichromatic.

DISCUSSION

In the present study, we could show that colour-naive *C. blandus* foragers reveal a positive phototactic response to all tested wavelengths, and a strong preference for the UV over the green or blue stimuli, independent of intensity differences. Colour learning and discrimination performances of individual ants together with computer modelling analysis suggest dichromacy, i.e. the existence of two spectrally distinct PRs in the retina of *C. blandus* foragers most sensitive in the UV and long-wavelength (green) range of the light spectrum. Furthermore, foragers were able to retain the learned colour information in an e-MTM, l-MTM, e-LTM and l-LTM from where it can be retrieved 1 h, 12 h, 24 h, 3 days and 7 days after training, showing for the first time that colour learning induces different memory phases in ants. Overall, our findings provide a better understanding of the ants' colour vision system and present new insights into the colour cognitive capacities of ants, which are probably related to the ecological needs in their natural environments.

Innate colour preference

The distinct innate preference for UV light might be due to a relatively higher overall sensitivity of the UV PRs, which has been shown for honeybees (*Apis mellifera*; Von Helversen, 1972) and

also some ant species (*Cataglyphis bicolor*; Labhart, 1986). The higher sensitivity of the UV PRs has been suggested to be a prerequisite to compensate for the low proportion of UV light in the natural light spectrum (Laughlin, 1976) and to keep the UV signal reliable at low ambient light intensities (Ogawa et al., 2015). However, adaptation status of the eyes exposed to a higher amount of UV light did not cause any changes in ant colour preferences. Therefore, we assume that the UV preference is not caused by differences at the receptor level but rather generated at a later stage in a more central neuropil (see also Gao et al., 2008).

UV perception might be essential for ant orientation as UV radiation is associated with the open sky and polarized light information (Menzel, 1979; Pichaud et al., 1999). *Camponotus blandus* ants forage frequently in open habitats, also on pioneer tree foliage such as *Cecropia* (McClure et al., 2008). Compared with the understory, the higher amount of UV light in the canopy (Möller, 2002; Differt and Möller, 2016) might provide an intriguing directional cue (Schultheiss et al., 2016), specifically for naive foragers, facilitating an efficient and successful foraging trip by increasing the probability of finding rich food sources at particular foraging places. UV preference is a common trait among insects (Menzel, 1979), which is probably related to the innate response towards open space. In moths, UV light has been suggested to release innate responses associated with foraging behaviour (Telles et al., 2016). Similarly, it has been suggested that innate colour preferences of insects are related to their ecological needs allowing colour-naïve foragers to locate the most profitable food sources of particular colours with higher efficiency (Giurfa et al., 1995; Raine and Chittka, 2007). However, one should be careful when comparing the innate preferences for monochromatic lights and natural flower colours, which are usually broad-band-reflecting colours, as there might be differences in perceiving and processing the two stimuli types (Dyer et al., 2016).

Individual colour learning

Ants have long been considered to be an exception among the trichromatic hymenopterans for having only two spectrally distinct PRs with sensitivities in the UV and green range of the light spectrum (Menzel and Knaut, 1973; Menzel and Blakers, 1975; Labhart, 1986; Camlitepe and Aksoy, 2010; Aksoy and Camlitepe, 2012). However, recent molecular (Yilmaz et al., 2016) and electrophysiological (Ogawa et al., 2015) studies challenged this assumption and suggested that there might be some ant species that exhibit the ancient state of trichromatic colour vision and possess an additional blue-sensitive PR. In the present study, however, colour learning and discrimination experiments supported dichromacy, i.e. the existence of only two spectrally distinct PR types in the retina of *C. blandus* foragers. Additionally, the results of the computer modelling analysis also found highest support for dichromacy in *C. blandus*, suggesting a PR with a peak sensitivity at approximately 360 nm, and a second long-wavelength-sensitive PR with peak sensitivity between 470 nm and 560 nm. The combination of a UV and long-wavelength PR might be optimal for object detection and identification. It has been suggested that ants use a UV–green contrast mechanism during navigation (Labhart, 1986), providing the best contrast between natural objects and the sky (Möller, 2002). Furthermore, green (LW1, long wavelength opsin gene that is expressed in the compound eye) and UV opsin mRNAs are expressed at much higher levels than the blue opsin in the compound eyes of *C. rufipes*, also supporting the prominent function for these two PR types in visual orientation (Yilmaz et al., 2016). In this case, blue opsin has been suggested to be co-expressed with green and UV

opsins to give rise to a broad-band receptor (Yilmaz et al., 2016) as recently found in various insects (Arikawa et al., 2003; Hu et al., 2014).

In the conditioning experiments, foragers highly preferred the UV light during the first training trials and learned it much faster than blue and green, which is probably influenced by the innate preference for UV light. However, the ants could learn to override this preference with increasing visual experience. Insects have been shown to learn innately preferred colours faster (Menzel, 1967; Giurfa et al., 1995; Kinoshita et al., 1999) but alter their innate preferences through experience (Kelber, 1996; Weiss, 1997; Satoh et al., 2016). This flexibility is probably important for an efficient foraging activity in the field and to allocate their foraging efforts towards profitable food sources, which might vary over space or time (Weiss, 1997; Kinoshita et al., 1999).

In our study, *C. blandus* foragers, with a supposed UV–green dichromatic visual system could also learn to discriminate between UV and blue wavelengths, independent of intensity changes. It is likely that the excitation of long-wavelength PRs by blue light (together with UV PRs) facilitates colour processing at the neuronal level by antagonistic colour opponent mechanisms. Similarly, the responses of ants to blue stimuli during phototactic experiments might be due to the excitation of the long-wavelength PR. In our experiments, it might not be the only possibility that ants simply learn to avoid UV light during the learning process. It is also possible that the blue and green stimuli appeared perceptually indistinguishable due to the principle of univariance, even though there were intensity differences in respective stimuli (Rushton, 1972; Garcia et al., 2015). The principle of univariance indicates that one individual PR class cannot differentiate between a change in wavelength and a change in intensity because it only pools all available photons in a probabilistic way as described by its relative sensitivities per wavelength. Thus, the perception of changes in wavelength information consistent with colour vision can only be enabled by a brain comparing the responses across different types of receptors, through an opponent neural network (Dyer et al., 2011). In our current study, this phenomenon of visual perception (Rushton, 1972) plausibly explains why the ants generalized the blue and green stimuli despite large variations in the respective stimulus intensities. This interpretation that the ants thus only have dichromatic vision is also consistent with our modelling results that putative trichromatic visual systems based on reported values for other ants would easily discriminate our stimuli. This interpretation remains true for our 528 nm stimulus, which because of the principle of univariance would strongly stimulate a long-wavelength PR with a 550 nm alpha-band if one was indeed present in a trichromatic visual system.

Visual (colour) memory formation

Camponotus blandus foragers were able to retrieve the learned colour information up to seven days after training. The ants learned the respective stimuli in a dynamic way, independent of intensity differences, and formed complex memories based upon conditioning experience. To the best of our knowledge, our results constitute the first demonstration of colour memory in ants. These results indicate that ants possess similar memory capabilities as flower-visiting honeybees and bumblebees in which colour learning leads to different memory phases (Wittstock and Menzel, 1994; Dyer and Garcia, 2014; Lichtenstein et al., 2015). In a natural context, dynamics of memory formation of a species have been suggested to be associated with specific foraging requirements (Menzel, 1999, 2001). Central-place foragers may visit the same food sites repeatedly, at varying intervals up to several months so long as it is

profitable, or they may travel between different places at short intervals during one foraging trip (Menzel and Muller, 1996; Menzel, 1999, 2001). The retrieval and utilization of chromatic cues might be essential for the foragers for the reliable detection of landmarks or celestial orientation. Such a capacity facilitated by colour processing would avoid issues of changing brightness over time, which can cause stimuli to appear completely different under varying light conditions (Möller, 2002; Differt and Möller, 2016). Using colour perception to localize the nest or food sources independent of achromatic cues, a mid- to long-term memory would be necessary to enable multiday adventures. Similarly, the possible use of coloured snapshot memories (Cheng et al., 1986; el Jundi et al., 2016) by *C. blandus* might be plausible as they are foraging during the day (McClure et al., 2008) when illumination is good and should allow chromatic vision (Kelber and Roth, 2006). In the same line, the decline in memory strength after seven days (in green versus UV experiments) suggests an adaptive flexibility during foraging to increase foraging efficiency, allowing exploitation of new food sources. In honeybees, there is also evidence that colour memory may decline over a period of about one week if the respective target and distractor stimuli are perceptually similar (Dyer and Garcia, 2014).

At higher neuronal levels, the mushroom bodies (MBs) might play an important role in associative colour learning and memory formation. Previous studies have shown that long-term storage of information after appetitive (honeybees, Hourcade et al., 2010) and aversive (ants, Falibene et al., 2015) learning leads to structural modifications in the synaptic architecture of the olfactory input region (lip) of the MBs. Recently, in the search for possible changes in the MB calyx in relation to fine colour discrimination learning, Sommerlandt et al., (2016) suggested a promising approach to combine learning experiments with neuronal analysis within subjects to investigate the colour memory trace. Additionally, a recent computational study revealed that neural architecture of the desert ant's MB has the potential to store a high number of independent images, and to distinguish them from highly similar images obtained when visually searching off-route (Ardin et al., 2016). Therefore, it might be promising in future studies to investigate the neuronal mechanism underlying visual (colour) learning and memory in ants to allow for a better understanding of the visual navigational adaptations of social insects to their species-specific environment.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.Y., W.R., J.S.; Methodology: A.Y., A.G.D., J.S.; Validation: A.Y.; Investigation: A.Y.; Resources: W.R., J.S.; Writing - original draft: A.Y.; Writing - review & editing: A.Y., A.G.D., W.R., J.S.; Visualization: A.Y.; Supervision: J.S.; Funding acquisition: W.R., J.S.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.158501.supplemental>

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