The effect of cumulative experience on the use of elemental and configural visual discrimination strategies in honeybees

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Abstract

We addressed the question of whether the amount of individual experience determines the use of elemental or configural visual discrimination strategies in free-flying honeybees Apis mellifera. We trained bees to fly into a Y-maze to collect sucrose solution on a rewarded stimulus presented in one of the arms of the maze. Stimuli were colour disks, violet (V), green (G) or yellow (Y), which were of equal psychophysical salience for honeybees. Training followed an A, B+C design, followed by an AC versus BC test. Training consisted of six (3 A and 3 B+C), 20 (10 A and 10 BC) or 40 (20 A and 20 BC) acquisition trials. Elemental models of compound processing predict a preference for the non-trained stimulus AC while configural models predict a preference for the trained stimulus BC. Our results show that increasing the number of acquisition trials results in a change of the internal representation of stimuli. After six training trials, bees favoured an elemental strategy and preferred AC to BC during the tests. Generally, increasing the number of training trials resulted in an increase of the choice of BC. Thus, short training favoured processing of the compound as the sum of its elements (elemental account) while long training favoured its processing as being different from the sum of its elements (configural account). Additionally, we observed that the change in stimulus processing was also influenced by stimulus similarity. Colour perceptual similarity favoured configural processing with increasing experience.

Keywords: Learning; Compound processing; Elemental processing; Configural processing; Honeybee; Colour vision; Colour similarity

1. Introduction

Animals can learn to associate an originally neutral stimulus (conditioned stimulus, CS) with a meaningful stimulus (unconditioned stimulus, US). In the natural world, however, stimuli usually constitute compounds that enter in association with an US. In such a situation, animals need to filter the relevant from the non-relevant stimuli. Two main theoretical models have been proposed to account for the processing and learning of such compound stimuli. On the one hand, the elemental approach assumes that animals learn separately about each of the elemental stimuli that make up compounds [3,18,34]. An extension of this elemental approach, the unique-cue approach, assumes that a compound consists of its elements plus an additional, internally generated element that is unique to the compound, but that can otherwise be dealt with as an additional element [33,39]. On the other hand, the configural approach assumes that compound stimuli are represented and learned as new entities, which are different from but similar to their components [28,29,32]. Both approaches assume that stimuli within a compound are consistently represented either as elements, unique-cues or configurations from the outset of training. Conversely, in a review on classical conditioning studies performed in Russian laboratories, Razran [31] suggested that the amount of compound training might well lead to changes in internal stimulus representation. He reported that pairing of a compound stimulus consisting of the elements A and B with an US yielded a monotonous increase of the response to that compound and that occasional test trials with the elements (A, B) yielded an initial increase in responding to the test elements that declined with further training. Razran’s...
interpretation of this phenomenon was that a process, which he called ‘configuring’, changed the effective stimulus representation in the course of training [31]. Razran did not specify, however, which factors besides the amount of training influence ‘configuring’. He only mentioned that simultaneous compounds configure better than sequential compounds. Furthermore, he suggested that some stimuli would configure more than others. In the light of the theoretical positions on compound processing described above, one could argue that early in training, the compound is processed in an elemental manner while at the end of training, configural processing takes place. Nevertheless, it has to be mentioned that, to our knowledge, the basic finding reported by Razran [31] lacks consistent experimental support.

The question of changing internal stimulus representations can be addressed using the honeybee as a study case. Honeybees learn and memorize a great variety of stimuli associated primarily with their nest and their food sources, the flowers [22,25–27]. Classical and operant learning paradigms have been developed for honeybees, which allow addressing questions on the building of elemental and non-elemental associations [5,8–10,36]. Moreover, the accessibility of their nervous system allows searching and identifying the neural and molecular basis of such associations [15,26,27].

The classical olfactory conditioning of the proboscis extension reflex (PER) [4,37] has been used to show that bees can learn to associate odour compounds with the presence or absence of reward, in a non-elemental way (i.e. treating the compound as being different from the simple sum of its elements) [5,8–10,17]. The spontaneous response of harnessed honeybees to sucrose solution delivered to their antennae is a reflexive extension of the proboscis to reach and suck the sucrose. In the paradigm of olfactory conditioning of the PER [4,37], hungry, harnessed honeybees are conditioned to originally neutral olfactory stimuli (CS) by pairing the presentation of odours to the antennae with contact of the antennae with sucrose solution (US). After forward pairing of odour and sucrose, an association is formed, which enables the odour to release the proboscis extension response in a following test. In this context, bees can solve discrimination tasks, which go beyond mere elemental processing. For example, they can master biconditional discrimination and negative patterning discriminations [5,8–10,17]. Biconditional discrimination [5,17] requires discriminating four binary compounds made from four different elements A, B, C and D such that ambiguity is created at the elemental level (AB+, CD+, AC−, BD−; with ‘+’ indicating reinforcement and ‘−’ absence of reinforcement). Negative patterning [8,9] implies learning to discriminate two elements A and B reinforced from their non-reinforced joint presentation AB despite ambiguity at the level of the elements (A+, B+, AB−).

The conditioning of free-flying bees to visual targets has been also used to address the question of elemental versus configural processing of visual compounds [36]. In such a paradigm, each bee is pre-trained to fly towards the training/test place and reinforced with sucrose solution whenever it chooses the appropriate visual target at that place. The associations built in this context link visual stimuli, such as colours or patterns (CS), and the sucrose reward (US), but may also link the response of the animal (e.g. touching or landing on the targets) and the US. Using this approach, it was shown that free-flying bees could solve both negative patterning and biconditional discrimination tasks [36].

In the present work, we used the conditioning of free-flying bees to visual stimuli (colour elements and compounds) to address the question of whether increasing individual experience results in a change of the internal representation of stimuli, visible through a change from elemental to configural choice strategies. To answer these questions, we conceived a summation design allowing to distinguish between elemental (elemental and unique-cue approaches) and configural processing.

2. Materials and methods

2.1. Apparatus

Experiments were conducted during the summer to avoid competition with the nectariferous flora, which renders conditioning of free-flying bees difficult.

Individually marked honeybees *Apis mellifera* from a hive 40 m from the laboratory were trained to enter a wooden Y-shaped maze [12], to collect 50% sucrose solution at a coloured stimulus presented vertically on one of the back walls (20 cm × 20 cm) of the maze (Fig. 1). The background of both back walls was achromatic (grey) to the bees. The apparatus was placed in the laboratory close to an open window. The maze was covered with an ultraviolet-transparent ceiling in order to leave the spectral properties of the coloured stimuli unaffected for the bees’ eyes. Bees entered the maze through a sliding door leading to a frontal wall with a central hole, 5 cm in diameter. The bees accessed the decision chamber of the maze by flying through the hole. Only there could they see for the first time both arms of the maze simultaneously. In one of the arms, termed positive, a coloured stimulus was presented against the grey background. A bee entering that arm received a reward of sucrose solution when it landed on that stimulus. The solution was provided in the middle of the rewarding stimulus (i.e. in the middle of the back wall) by a plastic micropipette 3 mm in diameter that was invisible from the decision point. The alternative arm, termed negative, displayed the grey background alone with a dummy micropipette in its centre and offered no reward. Thus, bees were trained to distinguish between the presence and the absence of a coloured stimulus. The positive and the negative arms were exchanged in a pseudo-random way to ensure that the bees did not associate the reward with a particular side of the labyrinth. Only one bee at a time was admitted to the apparatus. The stimuli were frequently
replaced by fresh ones to prevent the bees from using odour cues.

2.2 Stimuli

The stimuli and background used in the experiment were cut from high-quality HKS-N coloured cardboards (Hostmann-Steinberg, Celle; K+E Druckfarben, Stuttgart; H. Schminke and Co., Erkrath; Germany). An HKS-92N cardboard provided the grey background. The elemental stimuli were colour discs, 8 cm in diameter, cut from HKS-1N, HKS-36N and HKS-54N papers. They were yellow, violet and green for humans, respectively. The spectral reflectance of the stimuli and background used was measured with a flash photometer (SR01, Gröbel UV-Elektronik, resolution 1 nm) and calibrated against a BaSO4 white standard. Fig. 2a shows the spectral reflection curves of stimuli and background. This information is processed by the bee...
photoreceptors. The honeybee sees colours from 300 to 700 nm and has a trichromatic colour vision [7,24] based on the existence of three photoreceptor types maximally sensitive in the ultraviolet (S or UV receptor; \( \lambda_{\text{max}} = 344 \) nm), blue (M or blue receptor; \( \lambda_{\text{max}} = 436 \) nm), and green regions of the spectrum (L or green receptor; \( \lambda_{\text{max}} = 544 \) nm) [1,23,30]. The three receptor signals, however, do not provide the colour coding stage in the honeybee brain. They are fed into and evaluated by two spectrally opponent coding systems: one type is UV−Blue+ Green− (type A; with ‘+’ indicating here neuronal excitation and ‘−’ inhibition) and the other is UV− Blue+ Green− (type B) [2,19]. Thus, any spectral stimulus \( S \) possesses an unequivocal locus \( (A_S, B_S) \) in the resultant bidimensional colour space with the adapting background at its origin. Such a honeybee colour space is the Colour Opponent Coding (COC) space [2]. \( A_S \) and \( B_S \) are defined as:

\[
A_S = -9.86E_{\text{uS}} + 7.70E_{\text{bS}} + 2.16E_{\text{gS}} \\
B_S = -5.17E_{\text{uS}} + 20.25E_{\text{bS}} - 15.08E_{\text{gS}}
\]

where \( E_{\text{uS}} \), \( E_{\text{bS}} \) and \( E_{\text{gS}} \) represent the excitations of the UV, blue and green receptor types, respectively, produced by a stimulus \( S \). Fig. 2b represents the loci of the colour stimuli employed in our experiments in the COC space of the honeybee. The COC space allows reading the perceptual difference between colours according to the City-Block metric [2]. The distance between stimulus \( S \) and its background (origin of the space) constitutes a measure of the chromatic contrast of the stimulus. The colours used as stimuli were chosen to avoid positional biases within the maze. A bee's crossing into one arm of the maze was recorded as a choice during acquisition. The choice of the positive arm presenting the grey background alone in the negative arm of the maze. Sucrose was delivered in the centre of the disc. When they were rewarded on a compound, two adjacent coloured discs were presented simultaneously and aligned horizontally. Compounds presenting two adjacent colour discs were obviously twice as larger as single discs. Sucrose was delivered at the contact point between the two discs. Vertical alignment was avoided as bees assign different weights to the upper and lower visual field, with the lower visual field being more relevant [14]. Therefore, presenting one disc above the other could make the bees ignoring the upper disc of the compound. In presenting a compound, care was taken to randomise the side (left or right) of each element from one presentation to the next.

### 2.3. Procedure

Only a single marked bee was allowed in the apparatus at any time. When the bee entered the maze, a frontal door was closed to avoid other bees to enter the maze and interfere with its choices. In all cases the experimenter’s position was behind the maze to avoid interferences with the arrival and choices of the bees. Moreover, to avoid a bias in the bees’ choices determined by seeing the experimenter’s position before entering the maze, the experimenter stood always aligned with the central hole that allowed access to the decision chamber. Each bee was trained with an A+, BC+ schedule and received equal number of A+ and BC+ trials. During each conditioning trial, either A+ or BC+ was presented. Bees confronted with the A+ situation saw a single coloured disc on the grey background in the positive arm and the grey background alone in the negative arm of the maze. In the BC+ situation, they saw a compound made from two elemental coloured discs, B and C, on the grey background in the positive arm and the grey background alone in the negative arm. Bees were thus trained to choose both the reinforced elemental stimulus A and the reinforced compound BC. The sides of the reinforcement and of the coloured discs were alternated in a pseudo-random sequence (the same situation was not presented more than two consecutive times) to avoid positional biases within the maze. A bee’s crossing of the imaginary line leading from the decision chamber into one arm of the maze was recorded as a choice during acquisition. The choice of the positive arm presenting the reinforced stimulus (A or BC) was scored as correct while the choice of the negative arm presenting the grey background alone was scored as incorrect. In the latter case, the bee was immediately tossed away from the maze by hand. The bee had then to re-enter through the entrance hole and make a new decision until the correct stimulus was chosen. In that way, wrong decisions were penalised and choice performance improved. Only the first choice (i.e. first entrance

### Table 1

<table>
<thead>
<tr>
<th>Colour stimulus</th>
<th>Chromatic co-ordinates</th>
<th>Chromatic contrast (COC units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HKS-52N</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HKS-36N</td>
<td>0.17</td>
<td>3.56</td>
</tr>
<tr>
<td>HKS-54N</td>
<td>1.75</td>
<td>1.80</td>
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The back walls on which the stimuli were presented were placed at a distance of 15 cm from the middle point of the decision chamber. Thus, each colour disc subtended a visual angle of 30° to the bee’s eye at that point. Such an angular size ensured that bees relied only on the chromatic information of the stimuli for discrimination. It has been shown that when stimuli have an angular subtense larger than 15°, the choice behaviour of bees is only governed by differences in the chromatic properties of the stimuli [12,13].
into one of the arms of the labyrinth was recorded per foraging bout.

Green, violet and yellow were used as A, B and C in a balanced way, i.e. three groups were obtained: (1) A = green, B = violet, C = yellow; (2) A = yellow, B = green, C = violet; (3) A = violet, B = yellow, C = green. Within each group, bees were trained either with 6 (3 A+ and 3 BC+), 20 (10 A+ and 10 BC+) or 40 (20 A+ and 20 BC+) reinforced trials. Thus, a total of nine independent groups of bees were trained and tested in our experiments. Three reinforcement trials on a coloured disc are usually enough to generate a stable colour memory in free-flying honeybees [20, 21].

After training, each bee was tested in a dual choice test situation in which two compounds, AC and BC, were presented simultaneously, one in each arm of the maze. The compound BC was already known to the bees from the training while the compound AC was a novel one, although its elements were already used during training. Both stimuli were fresh (i.e. they were made from new discs that the bees had not touched before) and non-rewarded. Each test lasted 2 min and was repeated twice, firstly with AC in the left arm and BC in the right arm of the maze, and secondly with the reversed situation. In such a way, positional biases were avoided. During each test, we recorded the number of flights towards the test stimuli that ended with a contact of the bee’s antennae with the stimulus surface (‘touches’) and also the number of landings (‘landings’) on both compound test stimuli. A touch that ended as landing was only scored as a landing. Touches and landings were added and from them, relative choice frequencies were calculated for each test stimulus. Between tests, each bee received a refreshment training with A+ and with BC+. In such training, both A+ and BC+ were presented twice, the order and side of presentation of A and BC were randomised. Refreshment was necessary to keep the bees’ motivation high until the second test and to verify, for instance, that after a long training (40 trials), fatigue or overconditioning were not affecting the results recorded in the tests. The results of the two tests were pooled after testing for homogeneity.

2.4. Predictions

The elemental approach predicts summation of the associative strengths of the compound’s elements. In training A+, A should reach an associative strength $V_A$ of 1 and in training BC+, the compound reaches an associative strength $V_{BC}$ of 1. After the summation principle of elemental theories, each of the equal salient elements B and C of the compound reaches therefore an associative strength of 0.5 ($V_B = V_C = 0.5$). During tests the bees were confronted with AC versus BC. On the basis of the summation principle, bees should prefer the novel compound AC to the known compound BC.

\[ V_{AC} = 0.5 + 0.5 = 1 \]
\[ V_{BC} = 0.5 + 0.5 = 1 \]

For AC: $V_A = 1.0; V_C = 0.5; V_{AC} = 1.5$  \hspace{1cm} AC > BC

For BC: $V_B = 0.5; V_C = 0.5; V_{BC} = 1.0$  \hspace{1cm} BC > AC

The unique-cue approach also predicts that in training A+, A should reach an associative strength $V_A$ of 1 and in training BC+, the compound reaches an associative strength $V_{BC}$ of 1. In the case of BC, the value of 1 would be supported by the associative strengths of B, C, and the unique-cue Q. One can thus assign a theoretical associative strength of 0.33 to each of these three elements, assuming again equal salience. During tests the bees were confronted with AC versus BC. On the basis of the summation principle, bees should prefer again the novel compound AC to the known one BC.

\[ V_{AC} = 0.33 + 0.33 + 0.33 = 1 \]
\[ V_{BC} = 0.33 + 0.33 = 0.66 \]

For AC: $V_A = 1.0; V_Q = 0.33; V_{AC} = 1.33$  \hspace{1cm} AC > BC

For BC: $V_B = 0.33; V_C = 0.33; V_{BC} = 1.0$  \hspace{1cm} BC > AC

The configural approach predicts the opposite result. Again, both stimuli BC+ and A+ reach the same level of associative strength during training, but BC is learned as a fixed configuration $A^{BC}$. When bees are tested with AC versus BC, they should prefer the known configuration $A^{BC}$ to the novel one $A^{BC}$ due to its higher similarity to the trained stimulus. This can be explained by calculating the associative strengths of AC and BC, respectively. According to Pearce’s configural approach [28, 29], the associative strengths of AC and BC are calculated as:

\[ V_{AC} = A^{BC} (V_A) + BC^{AC} (V_{BC}) \]
\[ V_{BC} = A^{BC} (V_B) + BC^{BC} (V_{BC}) \]

where $V_A$ and $V_{BC}$ are the associative strengths of the trained stimuli A+ and BC+, which are set to 1, and $\tau$ the similarity values, which represent the training-to-test similarity between A and AC ($\tau_{A^{AC}}$), between BC and AC ($\tau_{BC^{AC}}$), between A and BC ($\tau_{A^{BC}}$), and between BC and BC ($\tau_{BC^{BC}}$). Similarity values can vary between 0 and 1 and are estimated by:

\[ \tau_{AC} = \frac{P_{com}}{P_{test}} \]

where $P_{com}$ is the number of elements that are common between the training and the test situation and $P_{test}$ and $P_{com}$ are the number of elements present during test and training, respectively. In our case $A^{BC} = 0; BC^{AC} = 0.25; V_A = 0.5; V_{BC} = 1.0$. Thus:

\[ V_{AC} = A^{BC} (V_A) + BC^{AC} (V_{BC}) = 0.5 \times 1 + 0.25 \times 1 = 0.75 \]
\[ V_{BC} = A^{BC} (V_B) + BC^{BC} (V_{BC}) = 0 \times 1 + 1 \times 1 = 1 \]

Thus, the experimental design of this experiment allows distinguishing qualitatively between elemental (elemental and unique-cue) and configural approaches, as their predictions are exactly the opposite (elemental: AC > BC; configural: BC > AC). Furthermore, as we varied the amount...
of training, it is possible to look for potential changes in processing from elemental to configural in the sense of Razran’s configuring [31].

2.5. Variables and statistics

During the acquisition phase, the choice of the positive arm of the labyrinth presenting the reinforced stimulus (A+ or BC+) was scored as correct while the choice of the negative arm presenting the grey background alone was scored as incorrect for each individual bee (1: correct; 0: incorrect). This was done until completing the corresponding training schedule with 6, 20 or 40 reinforcements. During the tests, the absolute number of choices of AC and BC (touches + landings) were recorded and relative choice frequencies were calculated.

In all of our analyses, we collapsed across subgroups of bees trained with different colours for A, B and C because we had no a priori reasons for assuming differences between these subgroups. Planned contrasts [35] were used for focused comparisons in the statistical evaluation of the competing hypotheses. Such contrasts constitute an appropriate choice because they involve a priori planned comparisons of two or more means. When constructing contrasts, cell means are orthogonally weighted (by a set of lambdas) to correspond to the hypothesised order of their effect. This allows a more focused examination of the data than that resulting from an interaction term in conventional analysis of variance. Additionally, we performed one-sided t-tests to analyse for each training length whether or not the choice performance at the end of training was above chance.

In order to evaluate the predictions of the elemental and the configural approaches (consistent preferences across training length, either for AC or BC), we computed an analysis of variance comparing the preference for BC between the three training lengths.

3. Results

Fig. 3a illustrates for the three training lengths (6, 20 or 40 reinforcements), the percentage of correct choices in the last A+ and the last BC+ trial (columns) for the three bee groups. The performance recorded during the refreshment trials between tests is also shown (circles on top of bars). This information is relevant as it shows that even when the performance in the last acquisition trial (A+ or BC+) was not indicative of learning (see, for instance, first column left), bees had indeed learned the task. Moreover, in the case of a long training (40 trials), refreshment trials were all above chance, thus showing that after the 40th acquisition trial no fatigue or overconditioning was affecting the performance of the bees in the tests. Fig. 3b shows the percentage of BC choices recorded in the tests for the same groups and training lengths.

By means of planned contrasts, we first examined whether or not different lengths of training (6, 20, 40 trials) led to different associative strengths at the end of training. Such an analysis is a precondition for further interpretation of the test data because differences in associative strength at the end of training may influence the choice of AC and BC during the tests. We calculated a score characterising the amount of learning for each individual bee. A value of 0 was assigned if the bee made a wrong decision at the end of training both in the last A+ and the last BC+ trial; a value of 1 was assigned if the bee made one correct decision in the last A+ and the last BC+ trial, and a value of 2 was assigned if the bee chose correctly in both the last
A+ and the last BC+ trial. The appropriate set of lambdas coding the training lengths of 6, 20 and 40 trials was −16, −2 and 18. The planned contrast of the amount of learning reached at the end of acquisition across groups showed that different training lengths did not result in different associative strengths of the training stimuli (F < 1). In addition, by means of one-sided t-tests, we analysed, for each training length, whether or not the choice performance at the end of training was above chance (score of 1). For all three groups the score was significantly higher than 1 (length 6: t26 = 2.05, P < 0.03; length 20: t20 = 3.20, P < 0.003; length 40: t23 = 3.87, P < 0.001). Thus, these results show that at the end of training the choice performance of bees was above chance. Furthermore, the planned contrasts excluded the possibility that differences in the tests between groups with different amount of training trials were simply due to differences in the associative strength gained by the training stimuli.

We next investigated the preference for BC over AC in the final test. In case of elemental processing across the three different lengths of training, bees should consistently prefer AC to BC (the relative choice frequency of BC should be smaller than 50); in case of configural processing the bees should consistently prefer BC to AC. If, however, as Razran [31] suggested, configuring takes place as a consequence of training, a change from elemental to configural processing should be observed along the three different training lengths. The preference for BC should be lower for the group with the shortest training (6 trials) and should increase with increasing training length (20 and 40 trials). This result would be consistent with a change in the internal stimulus representation along training. The analysis of variance comparing the preference for BC between the three training length groups showed significant differences (F2,39 = 25.54, P < 0.001). The t-tests comparing the preference for BC against chance behaviour (50%) for each training length showed a significant preference for BC in the group with 6 trials (t26 = 13.75, P < 0.001) and no significant preferences in the groups with 20 trials (t20 = 0.90, NS) and 40 trials (t23 = 1.47, NS). Thus, only the group with six trials showed a significant preference indicating elemental processing. Across length of training, however, this preference changed significantly. Thus, there was no support for assuming that consistent representations in terms of elements, unique cues or configurations are present and maintained from the outset of training.

In a last analysis we evaluated Razran’s idea of configuring [31], a change from elemental to configural processing with increasing amount of training. We computed a planned contrast using lambdas of −16, −2 and 18 (coding 6, 20 and 40 trials). This contrast was significant, t26 = 6.09, P < 0.001, indicating that the preference changed indeed from elemental to configural processing with increasing amount of training.

In order to further elaborate Razran’s suggestion that configuring might be influenced by properties of stimuli forming the compound [31], we analysed our elemental colour stimuli in terms of their perceptual similarity. Such an analysis is possible because colour vision in the honeybee is well known (see Section 2 and reviews in refs. [12,24]). The perceptual distance between a given pair of stimuli can be read off from the colour opponent coding space of the honeybee (Fig. 2b). According to its metric, the City-Block metric [2], the perceptual distance D between two colour stimuli S1 and S2 is calculated as the sum of the absolute differences in excitation of the two opponent coding neurones:

\[ D(S_1, S_2) = |A_1 - A_2| + |B_1 - B_2| \]

where A1, A2, B1 and B2 define the co-ordinates of S1 (A1, B1) and of S2 (A2, B2) in the honeybee colour space with the adapting background at its origin (see Section 2, for calculation of the A, B co-ordinates).

For any two colours, it is thus possible to calculate the perceptual difference between them. Applying these calculations to our stimuli yield the following results (in COC units): D(Violet, Green) = 7.08; D(Violet, Yellow) = 6.67; D(Green, Yellow) = 2.77. These values show that despite their similar salience against the grey background employed (see Table 1), green and yellow were more similar to each other than violet to green, and violet to yellow. The latter two pairs were similar in their perceptual difference. If the consequence of cumulative experience is to change from elemental to configural processing, similarity between elements of a compound may play an important role in such a modification. The more similar the elements are, the more configuring would take place.

In order to evaluate this hypothesis, we computed a planned contrast collapsed across all three lengths of training with lambdas of 1.2, 1.6 and −2.8 (reflecting the dissimilarity of the elements forming the compound in the three different subgroups). This contrast was significant (t26 = −2.18, P < 0.02), thus showing that the tendency to switch from elemental to configural processing increased with increasing similarity of the stimuli forming the compound along training lengths. The same planned contrasts were performed separately for training lengths 6, 20 and 40; there was no significant effect for length 6 (t26 = −1.28, NS) but significant differences were found for length 20 (t20 = −2.56, P < 0.01) and length 40 (t23 = −1.85, P < 0.04). This means that with short training there was no significant impact of stimulus similarity; processing was elemental in all cases. With longer training, however, similarity had a significant impact. Enhanced similarity between elements (in the present case, a lower perceptual distance between the two colour elements of the compound) promoted configuring. Note, however, that such a conclusion applies only in case of adoption of configural processing, i.e. after an increased cumulative experience with the stimuli. After few (six) learning trials, no difference was observed between the different colour pairs as elemental processing mediates choice behaviour.

\[ D(S_1, S_2) = |A_1 - A_2| + |B_1 - B_2| \]
4. Discussion

In the present work, we addressed the question of whether the amount of individual experience determines the use of elemental or configural visual discrimination strategies in free-flying honeybees. Our results show that increasing the number of acquisition trials results in a change of the internal representation of stimuli, visible through a change between elemental and configural choice strategies. We used three colour stimuli presented in an A+, B+ - training, followed by a test in which AC was confronted with BC. The latter was explicitly trained while the former was not. The elemental approach (elemental and unique-cue) predicts a preference for AC despite the fact that it was never trained; the configural approach predicts a preference for the trained stimulus BC. For both approaches this preference should be stable regardless of training length. Razran’s idea of configuring predicts elemental processing with short training, and increasing configural processing with increasing amount of training. We found that after short training (six trials), bees consistently preferred AC to BC in the tests and thus applied an elemental strategy. With increasing amount of training, however, this preference changed towards configural processing as suggested by Razran [31]. These conclusions are not confounded by differences in associative strength of stimuli at the end of the initial acquisition, as the first analysis in the Section 3 showed. Thus, our results support the claim that internal stimulus representations may undergo significant changes throughout acquisition. Moreover, we showed that perceptual similarity between stimuli within a compound significantly affects their processing as an elemental or a configural entity. More similar colours promoted configural processing.

Tasks that raise ambiguity at the level of the elements are usually employed to determine whether or not animals are capable of non-elemental learning and processing. Examples of such tasks are the biconditional discrimination (AB+, CD+, AC−, BD−) and negative patterning (A+, B+, AB−). Both can be solved by free-flying bees in a visual discrimination context [36]. The task proposed to the bees in the present experiments is unambiguous (A+, BC+) and may thus retard adoption of a non-elemental strategy. Using a task that is ambiguous at the level of the elements may thus aid adoption of a non-elemental strategy. Moreover, we showed that perceptual similarity between stimuli within a compound significantly affects their processing as an elemental or a configural entity. More similar colours promoted configural processing.

Our results have to be interpreted in the natural context in which bees learn visual discrimination problems opportunistically as they did in our experiments. While foraging, bees do not visit different flower species at random but usually restrict their visits to a single or a small number of species within a foraging bout, a behaviour that is generally termed flower constancy [16]. For reviews see refs. [6,38]. Bees can visit hundreds of flowers of the species exploited in a single foraging bout to collect minute quantities of nectar and/or pollen [40]. Learning and memory capabilities underlie such a selective foraging choice [22,25,27]. Flowers are learned and recognised according to specific signals, such as colour, size, shape, patterns, odour and texture among other characteristics. However, differently rewarding flowers may partially overlap in their stimulus composition. In that sense, non-elemental processing resulting from a repeated exposure to the exploited flower may allow identifying and discriminating a particular rewarding flower species from non-rewarding species having overlapping elements. Our results indicate that bees ‘working on line’, i.e. following one flower type, will treat flower stimuli in a non-elemental way with increasing cumulative experience and will thus reduce their error rate in foraging choice by decreasing generalisation on the basis of components.

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