

Current Biology

Merging of Long-Term Memories in an Insect

Highlights

- Bee memory is liable to an error involving the integration of two visual memories
- Bees chose a previously unseen stimulus combining features of prior learned stimuli
- Genuine memory merging, rather than a more simple generalization, occurred
- This is the first example of memory merging in a non-human animal

Authors

Kathryn L. Hunt, Lars Chittka

Correspondence

l.chittka@qmul.ac.uk

In Brief

False memories, in which people remember experiences that have never occurred, have rarely been explored in animal memory. Hunt and Chittka find that bumblebees, when sequentially trained to remember a b/w pattern and a unicolor stimulus, subsequently prefer a merged, but previously unseen, stimulus that combines features from both trained stimuli.



Merging of Long-Term Memories in an Insect

Kathryn L. Hunt¹ and Lars Chittka^{1,*}

¹Department of Biological and Experimental Psychology, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK

Summary

Research on comparative cognition has largely focused on successes and failures of animals to solve certain cognitive tasks, but in humans, memory errors can be more complex than simple failures to retrieve information [1, 2]. The existence of various types of “false memories,” in which individuals remember events that they have never actually encountered, are now well established in humans [3, 4]. We hypothesize that such systematic memory errors may be widespread in animals whose natural lifestyle involves the processing and recollection of memories for multiple stimuli [5]. We predict that memory traces for various stimuli may “merge,” such that features acquired in distinct bouts of training are combined in an animal’s mind, so that stimuli that have never been viewed before, but are a combination of the features presented in training, may be chosen during recall. We tested this using bumblebees, *Bombus terrestris*. When individuals were first trained to a solid single-colored stimulus followed by a black and white (b/w)-patterned stimulus, a subsequent preference for the last entrained stimulus was found in both short-term- and long-term-memory tests. However, when bees were first trained to b/w-patterned stimuli followed by solid single-colored stimuli and were tested in long-term-memory tests 1 or 3 days later, they only initially preferred the most recently rewarded stimulus, and then switched their preference to stimuli that combined features from the previous color and pattern stimuli. The observed merging of long-term memories is thus similar to the memory conjunction error found in humans [6].

Results

Bees were sequentially rewarded on two types of artificial flowers, one being homogeneously yellow and the other consisting of black and white (b/w) concentric circles (henceforth “black rings”). One group of bees was rewarded on “yellow” first and later on “black rings,” whereas the other group was given training in the opposite sequence (Figure 1; for more information, see the [Supplemental Experimental Procedures](#)). During subsequent tests, bees were given a choice between three types of flowers, two of which were the same as the previously entrained stimuli. The third type presented combined features of both these stimuli, i.e., yellow concentric circles (henceforth “hybrid: yellow rings”). Bees were tested immediately after training, 1 day after training, or 3 days after training. Memory for the last rewarded flower type was high for both training groups when tested in the short-term memory (STM) test (Figure 2A, “yellow” then “black rings”: 79.5% total group

choices were for the last entrained type, where chance expectation is 33.3%; Chi-square test for independence, $\chi^2 = 86.6$, $df = 2$, $p < 0.0001$; Figure 2B, “black rings” then “yellow”: 77% for the last entrained flower type, $\chi^2 = 81.5$, $df = 2$, $p < 0.0001$). In this test, which occurred within minutes after training, performance was governed by STM for the last rewarding stimulus, and the dominance of this memory remained high throughout the test (a Friedman test performed for individual preference revealed no significant change of choice values for the last rewarded stimulus over the four choice bins from the first five to the last five choices; “yellow” then “black rings”: $Fr = 6.167$, $p = 0.1$; “black rings” then “yellow”: $Fr = 1.8$, $p = 0.615$).

A different picture emerged when bees were tested 1 or 3 days later. In this case, bees had to interrogate their long-term memory (LTM) for stimuli that had been rewarding in the past. LTM relies on different mechanisms and resides in different brain areas, and it is larger in capacity than STM [7]. We thus expected bees, in some way, to respond to both of the stimuli that had been previously associated with reward. For the group trained to the “black rings” first, 24 hr after training the mean percentage choice for the last rewarded flower type, “yellow,” was initially above chance (78%) for the first five choices of the test (Figure 2D) but was below chance (average 23%) during the remainder of the test ($Fr = 19.6$, $p = 0.0002$; Figure 2D). Conversely, the mean percentage choice for the “hybrid: yellow rings” flower type was initially below chance level (16%) during the first five choices of the test but was above chance (average 47%) for the last 15 choices ($Fr = 11.7$, $p = 0.0085$; Figure 2D). Three days after training, the same effect was seen: mean choices for “yellow” (last rewarded stimulus) were initially high at 60% but then fell to around 30% in the last ten trials ($Fr = 15.1$, $p = 0.0017$; Figure 2F). Over the same test, the preference for “hybrid: yellow rings”: increased from 34% in the first ten trials to about 50% in the last ten trials ($Fr = 10.9$, $p = 0.012$; Figure 2F). Thus, over the course of the LTM tests, bees switched their preference from the most recently rewarded pattern to a hybrid visual display that they had never seen before and that merged features of both previously rewarding flowers.

For the group trained to “yellow” first, the last rewarded flower type (“black rings”) remained the most preferred one throughout the tests both 1 and 3 days after training (Figures 2D and 2E). At both intervals, the moderate change observed over time for the last rewarded flower type was not significant (1 day: $Fr = 5.17$, $p = 0.16$; 3 days: $Fr = 2.03$, $p = 0.56$). At 1 day after training, there was a significant change in the preference for the “hybrid: yellow rings” flower type over the course of the test ($Fr = 8.15$, $p = 0.043$), but the choices for this pattern never exceeded those for the last visited rewarded type (“black rings”; Figure 2C). No change was seen in the preference for the “hybrid: yellow rings” type at 3 days after training ($Fr = 2.96$, $p = 0.4$). Thus, memory merging only occurred when bees were entrained to a b/w pattern followed by a single-colored stimulus. When this order was reversed, bees’ performance in an LTM test can be explained by recency/retroactive interference [8]. Here, newly learned information impedes the recall of prior learned information [9, 10]. Such retroactive interference, and suppression of information

*Correspondence: l.chittka@qmul.ac.uk



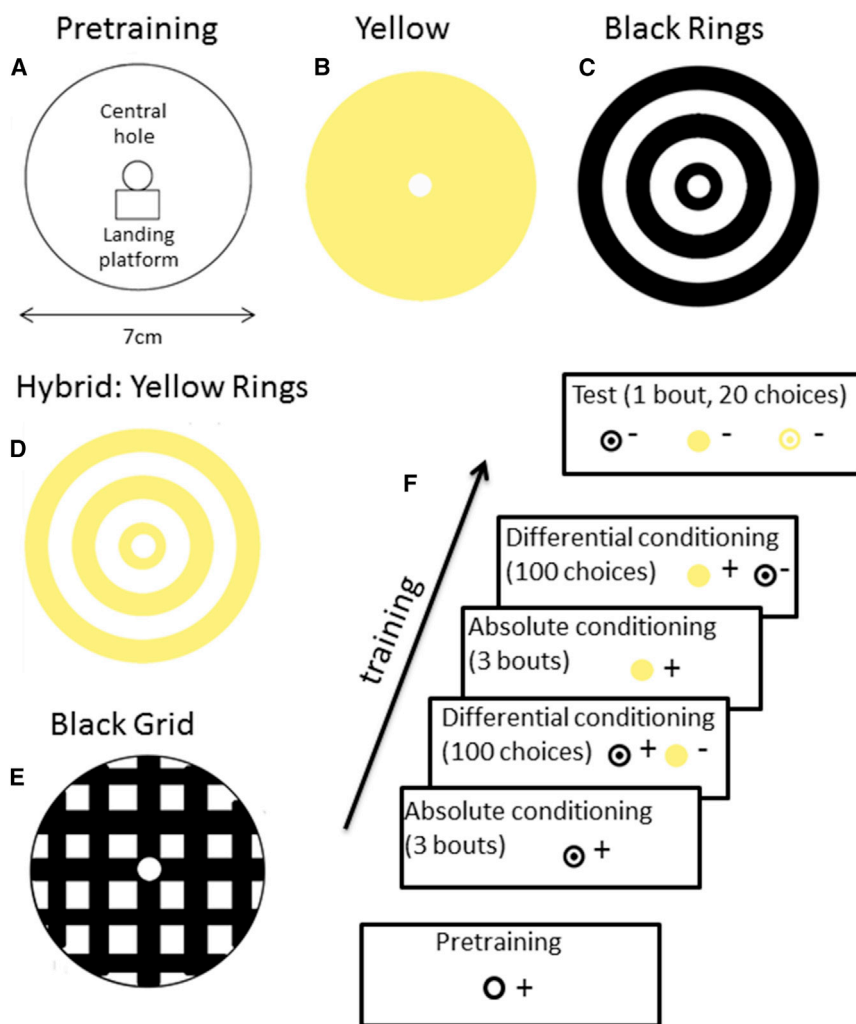


Figure 1. Basic Types of Artificial Flowers, and Training Schedule

(A) Example artificial flower ($\varnothing = 7$ cm), showing the central hole ($\varnothing = 0.5$ cm) enabling the experimenter to insert a reward droplet from outside the arena by means of an electronic pipette. Beneath the hole in front of the artificial flower target, a Perspex landing platform ($1.5\text{cm} \times 1.5\text{cm} \times 1\text{cm}$) is attached with Velcro, allowing rapid detachment and re-attachment after cleaning. All flower types had this basic design.

(B–E) Single-colored flowers were circular and yellow (experiment 1; B) or blue (experiment 2; not shown). Ringed flower types had a colored black (C), yellow (D), or blue (not shown) ring around the central hole (width of 4.5 mm) and two white and two colored rings toward the periphery, each with a stripe width of 7 mm. “Black grid” flowers (E) and “blue grid” flowers (not shown) had 7 mm stripes on a white background, as shown.

(F) Example training schedule, shown here for bees first trained to “black rings” then “yellow” flowers. +, reward (sucrose solution); –, no reward. Other groups of bees were trained with the reversed order, with “blue” instead of “yellow,” or with “black grids” instead of “black rings.” See also [Figure S1](#).

acquired earlier in reversal learning tasks, has been shown in various species of bees [11–13].

Control: Memory Merging or Generalization

The “hybrid: yellow rings” flower shares the yellowness with the previously rewarded “yellow” flowers. It is thus possible that preference for the hybrid flowers was in part a result of generalization for the feature of yellowness, without an effect of the training to the “black rings” flower type. In theory, an additional preference for pattern dissectedness [14, 15] might have driven bees to prefer the “yellow rings” flowers of the previously rewarded “yellow” flowers.

To exclude this possibility, we modified the experimental procedure to include a control group using a b/w flower pattern not contained in the “yellow rings” pattern. In this case, a group of bees was first trained to a pattern displaying vertical and horizontal black lines (henceforth “black grid”) and subsequently to “yellow” flowers. During tests 24 hr later, bees were shown the flower types “yellow” and “black grid” in addition to the “yellow rings” type used in the memory merging test above.

In this control experiment, bees simply preferred the stimulus on which they were last rewarded, i.e., “yellow,” throughout the entire 20 choices, though there was a significant decrease in preference for this target over the course of

the test ($Fr = 9.6$, $p = 0.022$; [Figure 3A](#)). However, no preference for the “yellow rings” flower type over the previously rewarded flower types was observed in any phase of the test, and the moderate increase in preference for this flower type was not significant ($Fr = 7.8$, $p = 0.051$). Comparing the control with the memory merging result (in which bees had first been rewarded on “black rings” and subsequently “yellow”), there was a significant difference of the choice values in the second half of the test (last ten choices: $\chi^2 = 23.8$, $df = 2$, $p = 0.001$). This lack of a switch of preference to the “yellow rings” flower in this test, in contrast to the experiment above, also shows that there is no inherent attractiveness of this flower type (e.g., by way of its contour length or dissectedness). These results support the occurrence of memory merging from features of both patterns rewarded during training when LTM is utilized, rather than a generalization from just one of the rewarded patterns.

Experiment 2: Memory Merging for Different Stimulus Combinations

To further explore the generality of our finding that memory merging occurs when bees are entrained first to b/w patterns and then to single-colored stimuli, we tested bees on two more combinations of colors and patterns. One group of bees was trained on “black rings” first ([Figure 3B](#)), and another on “black grid” flowers ([Figure 3C](#)). Both groups were subsequently rewarded on homogeneously blue-colored targets, following the same reversal learning schedule as above. In tests 1 day later, bees were given a choice between four target types: both of the flower types that had been rewarded earlier, as well as blue concentric rings and blue grids. Our evaluation of the experiment above revealed that if preferences changed

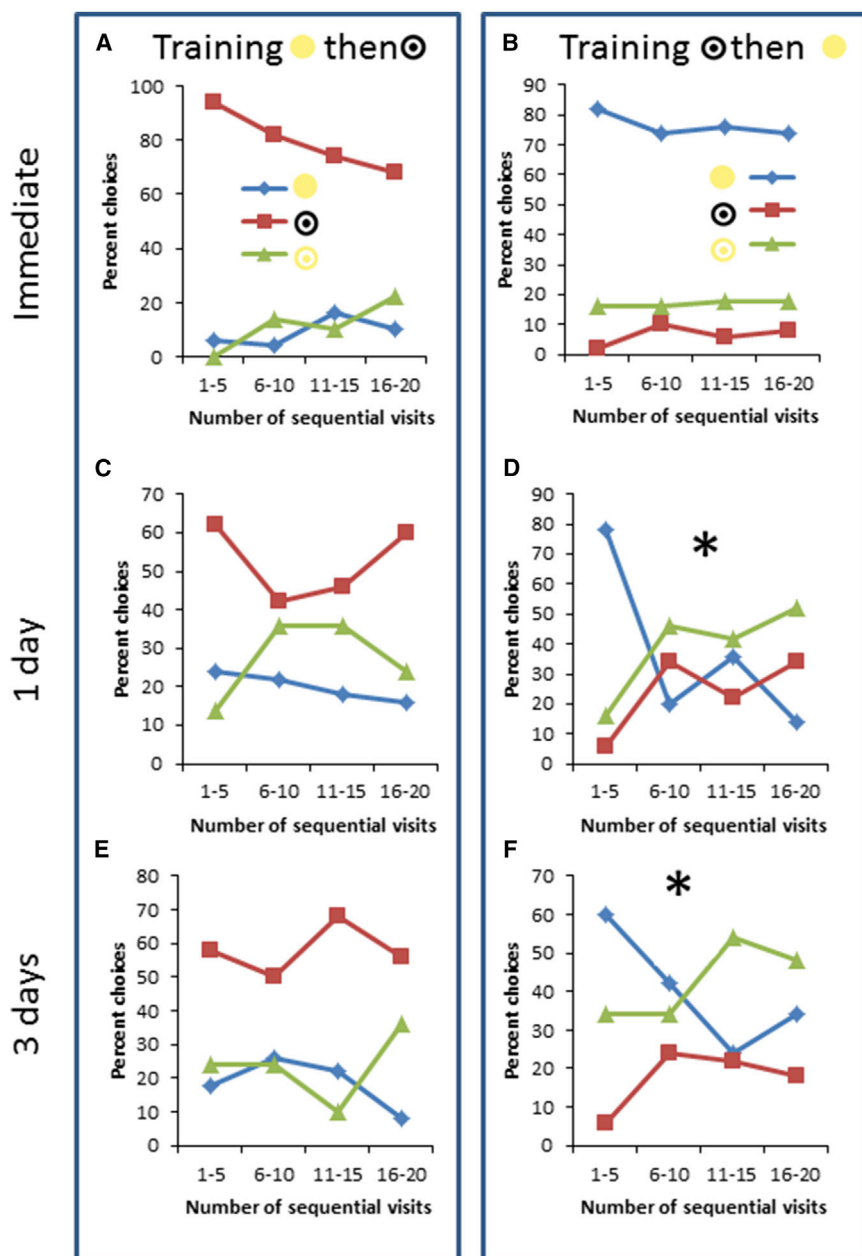


Figure 2. Choice Values in Tests for Two Types of Entrained Flower Types, as well as a Pattern that Merges Features from Both Flower Types

(A, C, and E) Training to “yellow” then “black rings.” At all time intervals (STM test within minutes, A; LTM tests at 1 day and 3 days, C and E), bees consistently preferred the most recently rewarding color.

(B, D, and F) Training to “black rings” then “yellow.” In the STM test (B), bees again preferred the recently rewarded stimulus (“yellow”); in LTM tests (D and F), this was also initially the case, but bees then shifted their preference to hybrid: “yellow rings”—the stimulus that contained features from both previously encountered flower types.

Each panel contains data from ten bees, and each data point consists of 50 choices. Asterisks indicate significant changes over the course of the test in the preference for *both* the last rewarding flower types *and* the hybrid flower type (Friedman test). In none of the six groups were there significant changes for the stimulus that had been rewarded first, before the reversal learning phase (Friedman test, $p > 0.05$ in all cases).

stimulus “blue grid” over all other types ($\chi^2 = 29.64$, $df = 3$, $p < 0.0001$) in the second half of the test (Figure 3C).

Discussion

Bees trained on two visually distinct artificial flower types show high levels of memory retention for the most recently rewarded stimulus initially, but 1 and 3 days after learning, an initial preference for the last entrained stimulus is quickly succeeded by a preference for a hybrid flower that combines the two learned visual features, color and pattern. The observed changes of preference over the course of testing cannot be explained by absence of reward in the tests. This might result in gradual weakening of the preference for the previous stimulus in conjunction with generalization to similar stimuli or simple exploration of alternative stimuli. Conversely,

in the course of the experiment at all, they did so roughly halfway through the test (or, indeed, earlier). We therefore simplified the analysis to compare preferences solely in the first half and the second half of the test.

In the first half of the test (first ten choices), bees sequentially trained on “black rings” then “blue” preferred the most recently rewarding stimulus (“blue”) as well as the merged stimulus (“blue rings”) over the other types (“black grid,” “blue grid”); Chi-square test for independence, $\chi^2 = 19.76$, $df = 3$, $p = 0.0002$; Figure 3B). However, they preferred the merged stimulus “blue rings” in the second ten choices of the test over the stimuli previously rewarding. The distribution of choices was significantly different from chance ($\chi^2 = 22.41$, $df = 3$, $p < 0.0001$). Bees trained first on “black grid” then “blue” initially preferred “blue” over the other types ($\chi^2 = 23.68$, $df = 3$, $p < 0.0001$), but they switched their preference to the merged

in all cases in which bees were trained to a b/w pattern and then a single-colored stimulus, the change in preference observed over the course of the test was specific and reproducible and resulted in a preference for the merged stimulus only.

Bumblebee memory therefore seems to be susceptible to an error specific to the integration of multiple memories in a similar way as human memory does in certain experimental settings. The “memory conjunction error” in which partial information from multiple memories is combined to create a “hybrid memory” containing elements of multiple memories is known to effect human memory for pictures of faces, nonsense words, and simple sentences [6, 16]. Here we have shown that, when presented with an artificial flower displaying a combination of features previously learned, bees mistakenly select this merged stimulus in later test phases, rather than their memory simply degrading over time.

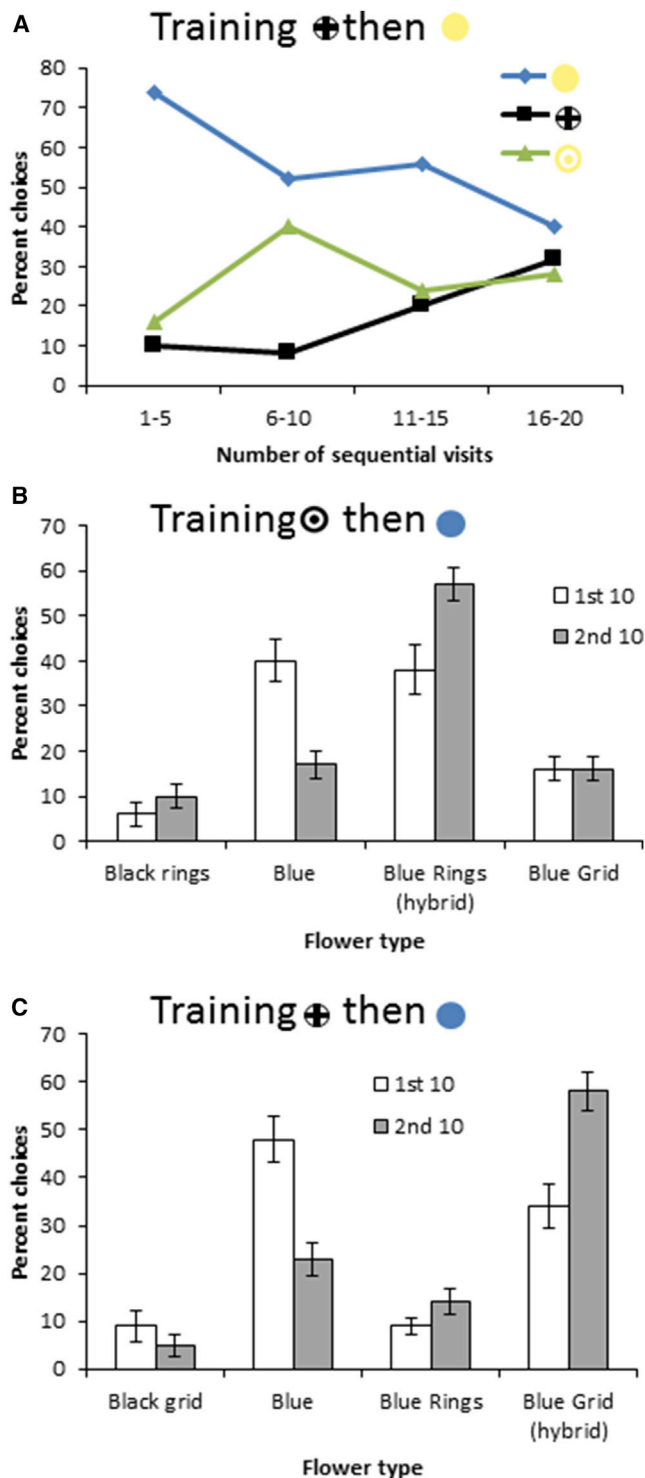


Figure 3. Memory Merging versus Generalization

Choice percentages \pm SE 24 hr after training for bees trained to “black grid” then “yellow” flowers (in sequential bins of five choices; A), “black rings” then “blue” (B), and “black grids” then “blue” (in bins of the first ten and second ten choices; C). $N = 10$ individuals in all groups. (A) shows that bees do not simply prefer the “yellow rings” stimulus as a result of stimulus generalization from the previous “yellow” stimulus (cf. results in Figure 2D); if bees had earlier been rewarded on a pattern (“black grid”) whose features were not contained in the “yellow rings” pattern, this latter pattern was not preferred. (B) shows bees’ preference for a merged stimulus after training to “black rings” then “blue” in the second (not the first) ten choices of the

This depended on the sequence of training of the two stimuli, however. Memory merging only occurred when the colored (yellow or blue) stimulus followed the b/w pattern. Conversely, when the b/w pattern followed the color training, bees simply preferred the last entrained stimulus at all testing intervals. This highlights the potential importance of the order of artificial flower presentation in a reversal learning paradigm for both experimental design and data interpretation. A possible explanation might be differences in the strengths of memory traces for different artificial flower types: colors versus patterns. It may be that the memory traces for the b/w-patterned flower type were stronger than for the unicolor flower type and as such were more resistant to both memory decay and manipulation. In humans, it is known that stronger memory traces are less prone to the effects of suggestibility [17]. However, if there was such a difference, it was not apparent in the training phase, in which bees performed equally well when trained to “yellow” or “black rings” first and were equally good at reverse learning both respective associations. Hence, the reason that memory merging occurs only for one of the two training sequences is not clear.

One potential explanation for the formation of such a memory conjunction error is that the failure occurs at retrieval [18] and involves “processing fluency” [19]. In this argument, during recall or recognition, source monitoring, which is the set of processes that make attributions about memory origins [20, 21], is low and as a result a misattribution occurs due to “remembering” on the basis of the feeling of familiarity [18]. These “memories” are therefore not based on the recollections of the specific details of items to be recalled/recognized, but rather on a more general feature that applies not only to the items to be recalled, but also to other items, which results in their erroneous retrieval. It is known that human memory utilizes the cognitive processes of categorization and generalization, in which a large number of items can be stored and recalled based on just a few exemplars, in order to economize and gain efficiency [22, 23]. It has been proposed that memory errors caused by misattribution (one of which is the memory conjunction error) may therefore simply be inevitable byproducts of the adaptive cognitive ability to form general concepts [3, 5, 24]. Many non-human animal species, including bees, can categorize visual patterns by shared properties, and indeed, bees appear to have the ability to form simple spatial concepts [25–29]. These abilities might come with undesirable side effects, such as misattribution errors.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and one figure and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.01.023>.

Author Contributions

L.C. conceived the study. K.H. and L.C. designed and carried out the experiments. Both authors were involved in writing all versions of the manuscript and have agreed to the final content.

test; again, only the stimulus combining both pattern and color (“blue rings”) was preferred, not the “blue grid” pattern. (C) shows that the reverse choice pattern was observed when bees were first rewarded on the “black grid” and later on the “blue” flowers, where, in the second half of the test, bees again preferred the merged (and previously unfamiliar) stimulus “blue grid” over all the other types.

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