1 **Bumblebees learn a relational rule but switch to a win-stay/lose-switch heuristic after**
2 **extensive training**
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15 **Running Head:** Switching strategy in bumblebees
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17

18 **Abstract**

19   Mapping animal performance in a behavioural task to underlying cognitive mechanisms
20 and strategies is rarely straightforward, since a task may be solvable in more than one manner.
21 Here, we show that bumblebees perform well on a concept-based visual discrimination task, but
22 spontaneously switch from a concept-based solution to a simpler heuristic with extended training,
23 all while continually increasing performance. Bumblebees were trained in an arena to find reward
24 on displays with shapes of different sizes where they could not use low-level visual cues. One
25 group of bees was rewarded at displays with bigger shapes and another group at displays with
26 smaller shapes. Analysis of total choices shows bees increased their performance over 30 bouts
27 to above chance. However, analyses of first and sequential choices suggest that after
28 approximately 20 bouts, bumblebees changed to a win-stay/lose-switch strategy. Comparing
29 bees' behaviour to a probabilistic model based on a win-stay/lose-switch strategy further supports
30 the idea that bees changed strategies with extensive training. Analyses of unrewarded tests
31 indicate bumblebees learned and retained the concept of relative size even after they had already
32 switched to a win-stay, lost-shift strategy. We propose that the reason for this strategy switching
33 may be due to cognitive flexibility and efficiency.
34
35 *Keywords:* abstract concepts, adaptive decision-making, animal cognition, behavioural analyses,
36 cognitive flexibility, cognitive offloading, law of least effort

1. Introduction

Cognitive flexibility reflects an individual's ability to adaptively alter their behavioural strategy in accordance with a changing environment [1]. A fundamental challenge for animal cognition researchers is to decipher which strategies an animal uses in solving any particular task [2]. Indeed, there is often multiple ways for an animal to solve a behavioural task.

Bees have been shown capable of learning various abstract relationships for example rules about target size (e.g. "pick the larger (or smaller) of two object sizes"), amongst myriad impressive cognitive abilities [3–5]. However, in some of these cases it may be that bees use a variety of different strategies to solve the tasks they are confronted with [5,6]. One recent study showed that some bees can solve a spatial concept learning task using a simple visual discrimination strategy through sequential scanning of stimuli rather than needing to compare stimuli based on an abstract rule, though other individuals may well follow such a rule [7]. In some numerical cognition tasks, honeybees may also use alternative cues that correlate with number, but are not in themselves numerical [8]. Bees' behavior in solving a delayed matching-to-sample task has been shown to be replicated by a model without any neural representations of the abstract concepts of sameness or difference [6]. Even the same individuals may have recourse to different solutions to the same task, depending on the extent of training. For example, with an increased number of training trials with a single pair of patterns, individual honeybees have been shown to have a greater generalized response to novel stimuli, i.e. the representation necessary to discriminate subsequent visual patterns changes with extended training [9]. All of these findings highlight the need for considering alternative strategies used by animals in cognitive tasks. This does not just concern the traditional dichotomy of "simple" versus "complex" solutions to such tasks. Different individuals may use different solutions that are equal in complexity, depending on their particular path to figuring out a solution.

Previous works have shown that honeybees are able to solve a task that appears to necessitate learning the concept of relative size and apply the rule to novel sizes within or outside the size range they were trained [10,11]. As with the examples above, bees may use more than one strategy to solve the same task, depending on the training protocol and context. Here, we test bumblebees to determine the strategies by which they cope with a relational rule learning task ("bigger-than"/"smaller-than") and examine their behaviour over time to reveal the cognitive strategies used over the course of training.

2. Material and methods

(a) Animals and experimental setup

Bumblebees (*Bombus terrestris audax*) from commercially available colonies (Agralan Ltd, UK), were housed in a wooden nest-box connected to a flight arena (100 cm x 75 cm x 30

74   cm). Bees were allowed access to a flight arena through an acrylic corridor (25 cm x 3.5 cm x 3.5

75   cm). Three plastic sliding doors located along the corridor allowed controlled access to the arena.

76   The arena was covered with a UV-transparent clear acrylic sheet. The stimuli were presented to

77   bees on the grey-coloured back wall of the arena.

78   Although there are no current requirements regarding insect care and use in research,

79   experimental design and procedures were guided by the 3Rs principles [12]. The bumblebees

80   were cared for on a daily basis by trained and competent staff, which included routine monitoring

81   of welfare and provision of correct and adequate food during the experimental period. Colonies

82   were provided with ~7 g irradiated commercial pollen (Koppert B.V., The Netherlands) every two

83   days. Bees from three colonies were used in this study.

84

85   **(b) Pretraining phase**

86   All bumblebee workers were recruited from a gravity feeder containing 30% (w/w)

87   sucrose solution placed in the centre of the arena. Outside of experiments, the colony was

88   provided with 30% (w/w) sucrose solution from a small gravity feeder placed inside the nest-box

89   during the evenings. Successful foragers on the arena gravity feeder were individually marked

90   with number tags, superglued to their thorax, for identification during the subsequent experiment

91   (Opalithplättchen, Warnholz & Bienenvoigt, Ellerau, Germany). Marked bees were pre-trained to

92   find 50% (w/w) sucrose solution from microcentrifuge tubes (5mm diameter) at the centre of each

93   of six white discs (7 cm diameter) on the grey-coloured back wall of the arena, horizontally 14 cm

94   from each other vertically 9.3 cm from each other (positioned as in figure 1). These discs were

95   made of paper and covered with transparent laminate to enable cleaning with 70% ethanol in

96   water (v/v). All stimuli were printed with a high-resolution printer.

97

98   **(c) Training phase**

99   When several number-tagged bees had learnt to find reward from the tubes located in the centre

100   of the display discs, one bee was randomly selected for the training phase. During the training

101   phase, an individual bee was trained on six discs on the back wall of the arena with the same

102   spacing as in training, each displaying one of two different sized shapes (figure 1*a* and *c*). During

103   30 training bouts (a bout was a bee's visit to the arena and landings on different stimuli until

104   satiated and subsequent return to her nest) one group of bees (n = 10) learned that the bigger of

105   the two shapes contained 30 μl 50% sucrose solution and the smaller contained 30μl saturated

106   quinine hemisulfate solution (bigger-than rule). Another group of bees (n = 8) learned the reverse

107   contingency (smaller-than rule).

108   Between training bouts, each disc was rotated randomly so that the position of a shape

109   varied across the six discs in relation to the central microcentrifuge tube containing sucrose

110   solution (figure 1*c*). The location, shape and colour of stimuli sets were changed between bouts.

3

111   The shapes used in training varied in size (small, medium, large), type (circle, rectangle, cross)
112   and colour (black, green, purple) (figure 1*a*). Only one type and colour of stimuli was presented to
113   a bee in each bout and only two of the three sizes were presented during one bout. The
114   dimensions of the shapes were as follows: small circle: Ø = 1.07 cm; medium circle: Ø = 1.97 cm;
115   large circle: Ø = 2.87 cm; small rectangle: 0.93 cm x 1.18 cm; medium rectangle: 1.79 cm x 2.92
116   cm; large rectangle: 2.3 cm x 3.94 cm; small cross: width of bars = 0.46 cm, length of bars = 1.3
117   cm; medium cross: width of bars = 0.6 cm, length of bars = 2.15 cm; large cross: width of bars =
118   0.96 cm, length of bars = 2.87 cm. Note that there was a large variability between physical
119   features of stimuli (figure *S1*). This variability ensured the bees were not able to solve the task by
120   associating an absolute size of stimuli with certain reinforcements. Several of stimuli were paired
121   with both positive and negative reinforcements during the training phase. For instance, middle
122   size stimuli were paired with the positive reinforcement in some training bouts while these were
123   paired with negative reinforcement in the rest of the training bouts. Further, the total area of the
124   middle rectangular was bigger than the total area of the big cross (see figure S1a). All of these
125   variations described ensured that low-level visual cues could not be used to solve the task.
126   Stimuli were cleaned between each training bout with 70% ethanol in water (v/v) to ensure odour
127   cues were not used to solve the task. After the daily experiment, all used microcentrifuge tubes
128   were washed with soap-water, then cleaned with 70% ethanol solution. Finally, they were rinsed
129   with water and air-dried in the temperature of the lab during the night.
130
131   **(d) Testing phase**
132   Following the training phase, each bee was tested in the same setup as in training in three
133   different scenarios, but with stimuli in the tests providing 30 µl of sterilized water (figure 1*b and c*).
134   Tests lasted 120 seconds, at which point the bee was gently removed from the arena by using a
135   cup and placed into the corridor until stimuli were changed for the refreshment bouts. Each test
136   was separated by two refreshment training bouts between tests to maintain the bee's motivation.
137
138   The sequence of the three tests were counterbalanced across bees. The learning test evaluated
139   performance by testing bees on one of the same sets of stimuli used during training, pseudo-
140   randomly chosen (i.e. a random number generator was used to generate a random sequence of
141   tests for each individual bee). The learning test used only the small- and bigger-sized training
142   shapes. The other two tests used either a novel shape and size (star) or a novel colour (yellow),
143   with the other properties pseudo-randomly chosen. The dimensions of the 5-poited stars were as
144   follows: small star: length of side of point = 0.5 cm; large star: length of side of point = 1.23 cm
145   (See figure S1). As in training, stimuli were cleaned between each bout during the testing phase
146   with 70% ethanol in water (v/v) to ensure odour cues were not used. Trained bees were removed
147   from the nest once the training and tests phases were finished.

148

(e) Statistical analysis and probabilistic model of learning curve

149

150   To evaluate bees' performance over bouts, the percentage of correct choices (choices

151 were defined as when a bee touched a microcentrifuge tube with her antennae or when she

152 landed on a microcentrifuge tube) was calculated from either all choices or from only the first or

153 second choices within each block of six bouts during training (total of five blocks). Using a

154 generalised linear mixed model (GLMM) for binary probability (correct or incorrect), the effect of

155 different factors such as colony, group of training and interaction between trial block and group of

156 bees in the bees' performance were calculated. The bee index was included in the model as

157 random factors. GLMMs were performed in MATLAB (MathWorks, Natick, MA, USA).

158   To determine whether bees used relative size information, rather than any other visual

159 cues, the choices of bees during the unrewarded tests were evaluated by a Wilcoxon signed rank

160 test. Further, a Kruskal-Wallis test was used to statistically evaluate and compare whether the

161 bees' performance or choice numbers in different blocks of bouts are from the same distribution.

162   To test if bees might use a win-stay/lose-switch strategy during training, we calculated the

163 conditional probabilities of each bee's second choice (c_2) given their first choice (c_1) at each block

164 of 10 bouts. A conditional probability, "Probability of B, given A ($P\{B|A\}$)", is a probability of an

165 event (B) occurring given that another event (A) has already occurred. The conditional probability

166 of a lose-switch strategy, i.e. a correct second choice after an incorrect first choice, is calculated

167 by $P\{c_2 = 1|c_1 = 0\} = P\{c_2 = 1, c_1 = 0\}/P\{c_1 = 0\}$ where $P\{c_2 = 1, c_1 = 0\}$ is the joint probability of

168 a correct second choice and an incorrect first choice and $P\{c_1 = 0\}$ is the probability of the first

169 incorrect choice. The conditional probability $P\{c_2 = 1|c_1 = 0\}$ at more than chance level indicates

170 that a bee switched to another presented size when they found the first choice was incorrect. In

171 the same way, we can calculate the conditional probability of a win-stay strategy, using

172 $P\{c_2 = 1|c_1 = 1\} = P\{c_2 = 1, c_1 = 1\}/P\{c_1 = 1\}$, i.e. the bee's second choice is the same size as

173 the first choice when their first choice was correct.

174

175 *Model of prediction of learning curve based on a bee's first two choices*

176   We propose a Markov stochastic model [13] to describe the learning curve of bees'

177 choices (total choices at each bout) based on the information of two first choices of bees. The

178 performance of the model at each bout is assumed as

$$Perf = P\{c_1 = p\} \sum_{k=2}^{N} \prod_{i=2}^{k} P\{c_{i+1} = q|c_i = p\}$$

179 $P\{c_1\}$ is the probability of the first choice at each bout and $P\{c_{i+1}|c_i\}$ is the conditional probability

180 of $(i + 1) - th$ choices given the of $i - th$ choices ($i \geq 1$) for when each choice in the sequential

181 choices is correct or incorrect. $p \ or \ q = 1$ if the choices are correct, otherwise $p \ or \ q = 0$. We

182 assume that the conditional probabilities of two sequential choices from the third choices are
183 equal to the conditional probability of the second choice given the first choice expressed by bees
184 at each bout of training. The sequence of possible events in which the probability of each event
185 depends only on the state achieved in the previous event will be stopped (N) when the simulated
186 bees collect all three positive reinforcements along with two, one or no incorrect choices within
187 each bout according to the average number of choices at each bout.
188
189 **3. Results**
190 **(a) Bees' overall performance increased over the 30 training bouts**
191 A multivariate statistical model, GLMM, applied to the performance of bees demonstrates
192 a significant increase in the proportion of correct choices made over the 180 choices of the
193 training phase (figure 2*a*, p = 0.018) irrespective of the shape, colour or position of patterns within
194 the stimuli. No significant differences were found between the learning curves of the two different
195 contingency groups (*i.e.* "bigger-than" rule versus "smaller-than" rule; p = 0.87). The output of the
196 GLM confirms that there was no significant difference between the different colonies of bees
197 during the training phase (p = 0.37). These results show that bees became better at solving either
198 contingency over training bouts.
199
200 **(b) Bees used a win-stay/lose-switch strategy after extensive training**
201 The typical analysis used to determine whether an animal has solved a particular task is
202 to calculate the animal's performance based on the number of correct and incorrect choices
203 throughout the training phase. At first inspection, bees' behaviour during training suggests they
204 learned to solve the concept-based task (figure 2*a*). However, a finer examination of their choices
205 suggests the involvement of another strategy in the later stages of training. If bees had only used
206 the concept of relative size throughout training, their first choices should reflect this by increasing
207 in accuracy throughout the 30 bouts. Although bees' average overall accuracy gradually
208 increased to 70% (significantly above chance level) over the 30 training bouts (figure 2*b*;
209 Wilcoxon signed rank test: z = 3.72, n = 18, p = 1.96e-4), their first-choice accuracy rose to 72%
210 (significantly above chance level: Wilcoxon signed rank test: z = 3.55, n = 18, p = 3.71e-4) over
211 the first 20 bouts and then decreased to chance level (54%) over the next 10 bouts (Wilcoxon
212 signed rank test: n = 18, z =1.25, n = 18, p = 0.21; figure 2*b*) and decreased significantly across
213 last two blocks of bouts (Wilcoxon signed rank test: z = 2.83, n = 18, p = 4.59e-3). Second-choice
214 accuracy was not different from chance level during the first two-thirds of the training phase
215 (Wilcoxon signed rank test: z = -0.67, n = 18, p = 0.49), but increased in the final third of the
216 training phase to 73.33%, significantly above chance level (figure 2*b*; Wilcoxon signed rank test: z
217 = 3.52, n = 18, p = 4.28e-4). These results suggest that bees changed to a win-stay/lose-switch
218 strategy after around 20 bouts of training, i.e. if they find reward at a stimulus they choose the

219   same type of stimulus next, or if no reward is found at a stimulus they choose a different type of
220   stimulus next.

221   To help evaluate the possibility that bees switched strategies part way through training,
222   we calculated the conditional probabilities (Materials and Methods) for 1) a correct second choice
223   after a correct first choice (win-stay), and 2) a correct second choice after an incorrect first choice
224   (lose-switch). Both of these two conditional probabilities increased over bouts (figure 2*c*; Kruskal-
225   Wallis test, chi-sq > 12.94, df = 53, p < 1.55e-3), most notably rising to significantly above
226   chance level in the last third of training (Wilcoxon signed rank test: z = 2.18, n = 18, p = 0.02 for
227   win-stay and z = 3.63, n = 18, p = 2.74e-4 for lose-switch), again suggesting that bees had
228   changed to a win-stay/lose-switch strategy.

229

230   **(c) Modelling a win-stay/lose-switch strategy**
231   To further examine whether bees switched strategies during training, we utilised a probabilistic
232   model based on a win-stay/lose-switch strategy. Within our model, we used bees' overall and
233   conditional performance (figures 2*b* and 2*d*) and initial first and second choices to predict bees'
234   subsequent choices in each bout (Materials and Methods). Figure 2*d* shows that our model
235   predicts the bees' performance in the last 10 bouts (i.e. no difference between the model's
236   performance and bee's performance; Wilcoxon signed rank test: z = -1.41, n = 18, p = 0.15). In
237   contrast, our model's predicted performance was significantly poorer than the performance of
238   bees in the first 20 bouts (Wilcoxon signed rank test: z > 2.32, n = 18, p < 0.01 for both first two
239   blocks). The ability of our model to predict the behaviour of our bees in the later stages of training
240   but not the initial stages supports the hypothesis that bees changed to a win-stay/lose-switch
241   strategy within the last 10 bouts of training.

242

243   **(d) Bees retained the concept of relative size after having switched strategies**
244   So far, our analyses and model results suggest that bees used a win-stay/lose-switch
245   strategy only after extensive training. Bees seemed to have used a different strategy during the
246   initial blocks of training bouts. Their increased performance to above chance level, suggests they
247   were discriminating the stimuli based on size. To ensure that bees' initial strategy had actually
248   been a relative size rule, we measured bees' performance directly after training in unrewarded
249   tests. Because the tests were unrewarded, bees could not solve the task based on a win-
250   stay/lose-switch strategy. Bees' performance on the learning test was above chance level
251   (Wilcoxon signed rank test: z = 3.73, n = 18, p = 1.87e-4), as was their performance on the novel
252   shape transfer test (Wilcoxon signed rank test: z = 3.51, n = 18, p = 4.46e-4), and on the novel
253   colour transfer test (z = 3.03, n = 18, p = 2.41e-3 for novel colour; figure 3*a*). Note that the
254   variability in different shape sizes and resulting overlap between sizes across shapes prevented

255   bees from associating a general size with reward (figure *S1*). These results suggest that the bees

256   had at some point during training learned to solve the task based on the concept of relative size.

257   Because animals vary in their learning and performance, we posited that if bees had

258   learned and retained a relative size rule, how well they performed in training before changing

259   strategies should reflect how well they perform (i.e. remember the relational rule) during the

260   learning test. In line with this, there was a positive correlation between the average of first choice

261   accuracy in the second third of the training phase (prior to strategy change) and bees'

262   performance in the learning test (figure 3*b;* Spearman correlation: rho = 0.58, n = 18, p = 0.01).

263   Although bees seemed to have changed strategies after extensive training, the results of the

264   unrewarded tests show that bees had learnt the relative size rule during training, retained the rule

265   even after having changed strategies late in training, and therefore resorted to the relative size

266   rule strategy during the tests.

267   Note that the performance of bees in the learning test was significantly poorer than the

268   last bout of the training phase (figure 3*c*; Wilcoxon signed rank test: z = 3.31, n = 18, p = 9.30e-

269   4). This suggests that bees began the learning test using a win-stay/lose-switch strategy. This

270   makes sense because they had just been using a win-stay/lost-switch strategy during training

271   and had no knowledge that the test was unrewarded. Further, bees' performances on the second

272   half of choices during each of the tests was better than their performance on the first half, (figure

273   3*d*; Wilcoxon signed rank test: z = 1.82, n = 18, p = 0.03 for Learning test; z = 0.57, n = 18, p =

274   0.28 for Novel shape; z = 1.05, n = 18, p = 0.14 for Novel colour), indicating that bees had

275   reverted to the retained relative size strategy.

276   Why would bees change strategies if they were already performing above chance level?

277   We hypothesized that bees might change strategies if the new strategy was more efficient, i.e. it

278   took them less effort to locate all three rewarding discs (discs were not refilled during training). In

279   support of this, the number of total choices by bees decreased from an average of 7.1 choices

280   per bout at the beginning of training to an average of 5.1 choices per bout at the end of training

281   (figure 4; Kruskal-Wallis test, chi-sq = 22.70, df = 53, p = 1.17e-5), indicating that bees' efficiency

282   increased during training across a change in strategy.

283

284   **4. Discussion**

285   We demonstrate and corroborate previous findings [11,14] that bees can learn a relative

286   size rule, but after extensive training opted to use a simpler strategy. Because there can often be

287   more than one way of processing the same stimuli to solve a cognitive task, it is useful to

288   examine individual strategies and over extended periods to explore if multiple strategies might be

289   at play. In our paradigm, we prevented bees from using low-level visual cues. Initial increases in

290   performance suggested that bees learned the task and later performance on unrewarded tests

291   verified that bees had learnt and retained a relational rule, as was previously demonstrated in

292   honeybees [11,14]. However, statistical analyses showed that after extensive training, bees

293   began to use a win-stay/lose-switch strategy based on whether or not they were rewarded on

294   each stimulus. Bees' performance calculated by their first choices or by multiple sequential

295   choices revealed a strategy of decision making that had been hidden within the gross calculation

296   by total choices. Averaging all choices in a training bout or test is common within bee cognition

297   and within other animal research communities. We suggest that interpretations of any animal

298   cognition study involving multiple choices include analyses of first and sequential choices to

299   investigate potential alternative strategies.

300

301   Theoretical and empirical work maintain that animals tend to follow the "law of least effort"

302   [15,16], whereby subjects choose strategies that minimise the costs in obtaining desirable

303   outcomes [15,17]. In comparative cognition research, animals may use strategies different to

304   those we intend a specific paradigm to test and still perform well on the behaviour we are

305   measuring [2,7,8,18,19]. Most studies have focused on the idea that animals opt to minimize

306   physical work, but this idea extends to cognitive effort as well [20–22]. The ability to change

307   decision-making strategies with the changing demands of the environment is essential to

308   adaptive behaviour, and therefore survival. Lloyd and Dayan [23] proposed that constant

309   monitoring of information to promptly assess and predetermine decision-making strategies would

310   be too costly for animals to maintain. Similarly, commitment for extended periods of time to one

311   strategy without the ability to adjust could be deleterious [23]. These authors suggested, with

312   support from computational models, that temporal commitment to certain strategies with

313   intermittent interruption to assess costs and switch strategies would be more advantageous for

314   real world scenarios. Bumblebees in our study seem to follow a similar overall approach, as they

315   first learn an abstract concept (relative size) and stick with this rule for approximately 20 bouts, at

316   which point they change to a new strategy (see figure S2 for the individual difference between

317   bees). A decrease in the number of choices taken to find all rewarding stimuli (figure 4) indicate

318   that bees may have changed strategies to become more efficient. Further studies are needed to

319   check the role of efficiency in strategy selection in animals. Further studies should involve

320   videotaping the behaviour of bees during the training and test phases, so that one can make

321   some direct inferences about time invested, mechanisms of inspecting stimuli and the efficiency

322   of decisions.

323

324   In this light, our results support the idea that animals can adaptively weigh the costs of

325   cognitive effort across decision-making approaches and choose the less cognitively demanding

326   strategy [24]. This interpretation requires that the win-stay/lose-switch strategy was actually

327   simpler than the relative size rule. Indeed, the win-stay/lose-switch heuristic is cognitively less

328   demanding than any relational rule, simply because it is based only on the outcome of the

329   previous choice, and therefore could be solved using working memory alone [25]. Accordingly,
330   bees could have stored the visual template of the first stimulus in working memory and, if the first
331   choice was correct, subsequently chosen a stimulus that had more overlap with the stored
332   template, or if the first choice was incorrect, subsequently chosen a stimulus with less overlap
333   (template hypothesis; [26]). The win-stay/lose-shift strategy has been broadly observed and
334   explored in bees foraging strategies and flower constancy amongst variable rewarding species of
335   flowers [27–31]. This type of sequential matching/non-matching to sample strategy has been
336   shown to be solvable with a simple computational model based on the known neural circuitry of
337   the bee brain, without requiring any higher-order abstract concept [6]. Learning and applying an
338   abstract concept like relative size rule requires a substantial abstraction process to different
339   stimuli that must work independent of the physical characteristics of stimuli [32]. In mammals, it is
340   assumed that higher cognitive functions processed in the prefrontal cortex or analogous
341   structures are essential for rule learning [33,34]. In insects, it has been proposed that rule
342   learning occurs in the mushroom bodies, high-level sensory integration centres [35,36]. In
343   contrast to rule-learning, bees can use a simple associative mechanism to remember the
344   previously visited stimulus in order to make decisions about a subsequent stimulus. Therefore,
345   the effort required in a win-stay/lose-switch type mechanism is likely to be lower than an abstract
346   rule because bees can learn to recognize and associate a stimulus with reward without using
347   their mushroom bodies [37,38]. For example, honeybees with inactivated mushroom bodies can
348   perform some odour learning tasks as well as control bees [37,39]. Further, a realistic
349   computational model of olfactory information processing in the bee brain shows that two parallel
350   odour pathways with different functions provide the flexibility necessary for comparing multiple
351   olfactory stimuli during associative and non-associative discrimination tasks [38].

352

353   Although our results indicate that bees switched to a win-stay/lose-switch heuristic, it is
354   unclear why bees would learn the relative size concept first if the win-stay/lose-switch strategy is
355   cognitively simpler. We speculate that this strategy may have been initially favoured simply to
356   reduce the load on long-term memory and to speed up the decision-making process so as to
357   avoid the quinine containing discs. During pretraining, bees only received reward from white
358   disks. When training began, all of the discs suddenly contained coloured shapes and the bees
359   found not only reward but also aversive quinine. Because of this abrupt and dramatic change,
360   bees' priority may have been to learn to avoid the quinine containing discs. To accomplish this
361   quickly, they could have extracted a set of elementary visual features to avoid in the first bout of
362   training. During the next bouts, instead of switching to a new strategy relying on working memory,
363   they stuck with identifying and avoiding the template for the quinine containing discs. Over the
364   next trials, they learned to generalise and group visual features across stimuli in a manner
365   consistent with the concept of relative size [32,40]. Because constant monitoring of how well they

10

366   were doing would be too costly [23], it took them some time to assess their performance and try

367   out a new strategy. Of course, further analysis of bees' behaviour during the training and test

368   phases are required to uncover the true mechanisms underlying bees' strategy selections.

369

370   As a result of bees learning a relative size rule early in training, we would have expected to

371   see an improvement on second choice performance from the first 10 bouts to the second 10

372   bouts in the training phase similar to the bees' improvement on first choices (figure 2*b*). However,

373   bees' performance on second choices was not significantly different from chance level within 20

374   bouts of training. We are unable to say from our data why this was the case, but speculate that

375   motivation and attention may play a role – once bees found reward, they might have been less

376   likely to fly back within the arena to view stimuli head on to properly view and assess stimuli, and

377   rather flew directly to a nearby disc to check for food, which statistically would be more likely to

378   be unrewarding (because of the remaining five discs only two would be rewarding). This type of

379   motivational-based exploration may also account for why bees eventually changed to a win-

380   stay/lose-switch strategy. Figure S2 shows a large variability between individuals in second

381   choice performance, and therefore individual differences in motivation and attention may have

382   played a part in why second choice performance was lower than expected [41,42]. However,

383   many of the bees did show an improvement in their second choices from the first 10 bouts to the

384   second ten bouts. Analyses of sequential choices in future studies of animal cognition will help

385   resolve these questions.

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388   **Authors' contributions**

389   H.M. and L.C. conceived the study. H.M. designed and performed the experiment. H.M. and C.S.

390   analysed data. H.M., C.S. and L.C. wrote the paper.

391

392   **Competing interests**

393   The authors declare no completing financial interests.

394

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399

400   **Acknowledgments**

402

Conflict of interest statement

All authors declare they have no conflicts of interest.

Ethical statement

There are currently no international, national or institutional guidelines for the care and use of bumblebees in research. However, experimental design and procedures were guided by the 3Rs principles. Bumblebees were cared for on a daily basis by trained and competent staff, which included routine monitoring of welfare and provision of correct and adequate food during the experimental period.

Supplemental information

Information includes two figures and one data file.

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Figures

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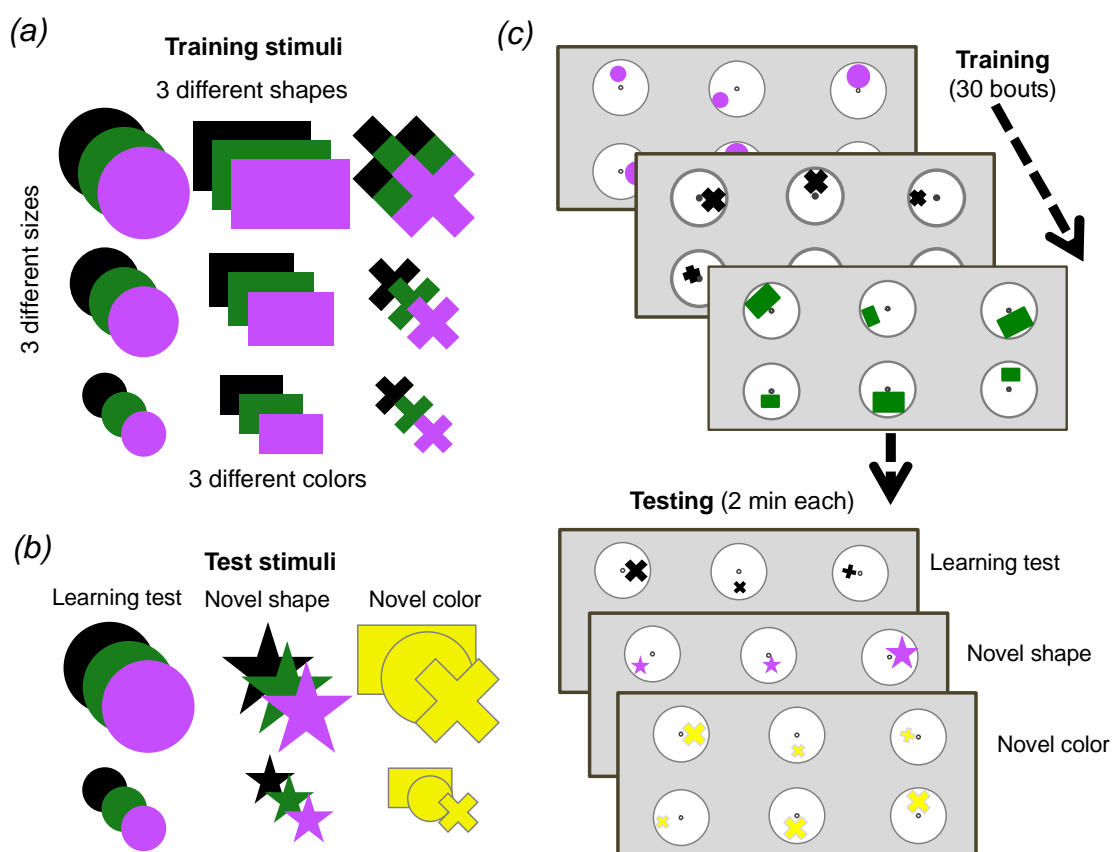
Figure 1. Training and testing protocol. *(a)* Stimuli options used during training. *(b)* Stimuli options used for each of the three different unrewarded tests. *(c)* Training and test protocol. Bees were trained for 30 bouts (visits to the arena before returning to the hive). All stimuli in (*a*) were used randomly across bouts during training. Only two of the possible three sizes of shapes were presented during a single bout. Only one of the possible three colours and one of the possible three shapes were presented each bout. One group of bees (n = 10) was trained to find 50% sucrose solution at the centre of the stimulus containing the bigger of the three shapes and bitter quinine solution at the smaller of the three shapes. Another group (n = 8) were trained on the opposite contingency. Once training was complete, bees were subjected to three unrewarded tests (with one or two reminder/training bouts between each test to keep bees motivated). All tests used small and large sized shapes. The learning test used one randomly chosen type and colour used during training. The novel shape test used one randomly chosen colour used during training but always a star shape that had not been used during training. The novel colour test used one randomly chosen shape used during training but always coloured yellow, which had not been used during training.

16

Switching strategy in bumblebees
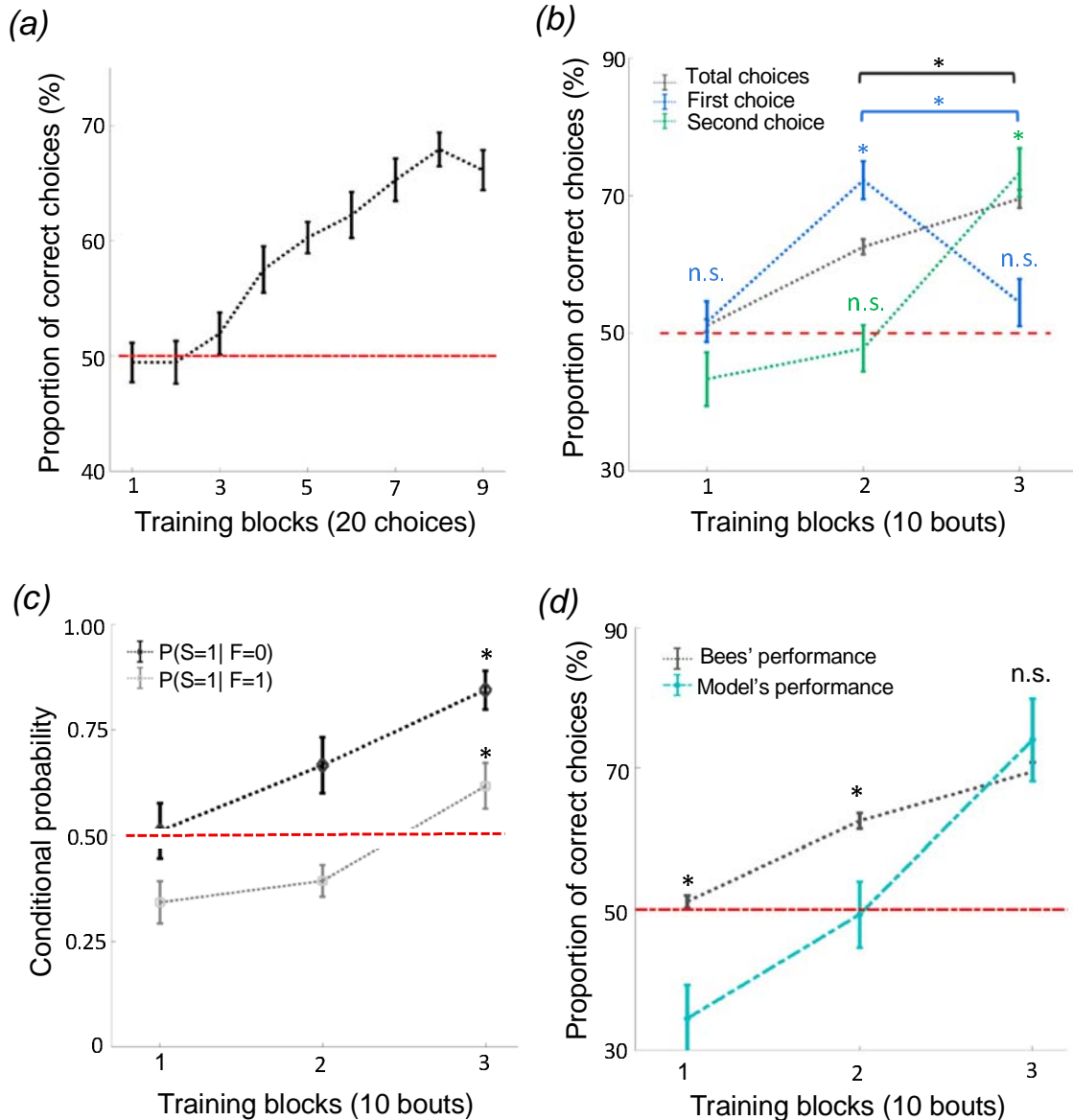
Figure 2. Bees use a win-stay/lose-switch strategy after extensive training.

(a) There was a significant increase in the number of correct choices over the 180 conditioned choices (p = 0.018). *(b)* Bees' performance over 3 blocks of 10 training bouts during the relative size discrimination task. Performance increased gradually over bouts when considering total number of choices in each bout (black line; p = 1.96e-4). Bees' first choice performance increased significantly from the first to the second block of training bouts to 72.22% (p = 3.71e-4) but then dropped to chance level from the second to third block of training bouts (blue line; p = 0.79). Second choice performance was near chance for the first two blocks of training bouts (p > 0.49), but then increased significantly during the third block of training bouts (green line; p = 4.28e-4). These results indicate that bees changed to a win-stay/lose-switch strategy after extensive training. Vertical lines = standard error of the mean. Red dashed line = chance level

17

543   performance (50%). *(c)* The average conditional probabilities of a bee's second choice within

544   each bout being correct given the outcome of the bee's first choice of the bout (either correct or

545   incorrect). Both conditional probabilities increased to above chance during the second and third

546   blocks of bouts (p = 0.02 for win-stay and p = 2.75e-4 for lose-switch) *(d)* Our win-stay/lose-

547   switch model's performance matches our bees' performance on the task during the last block of

548   10 bouts during training (p=0.15), again suggesting that after extensive training bees changed to

549   a win-stay/lose-switch strategy. (Vertical lines = standard error of the mean). Red dashed line =

550   chance level (50%).

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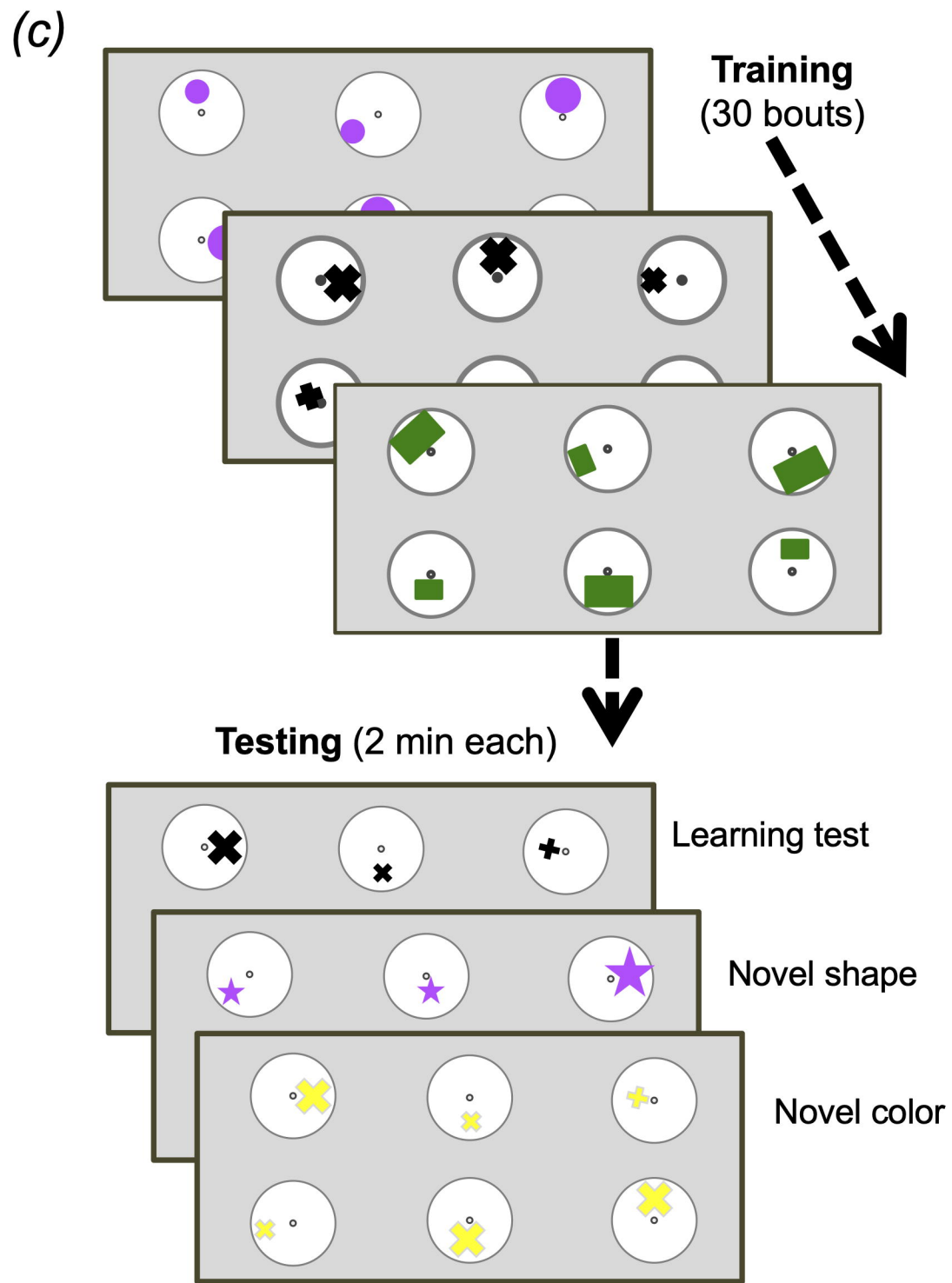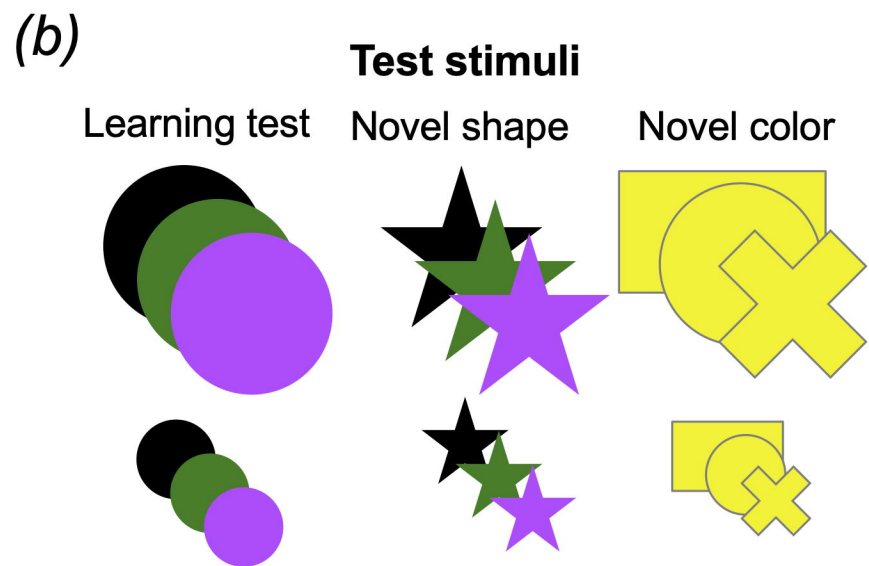
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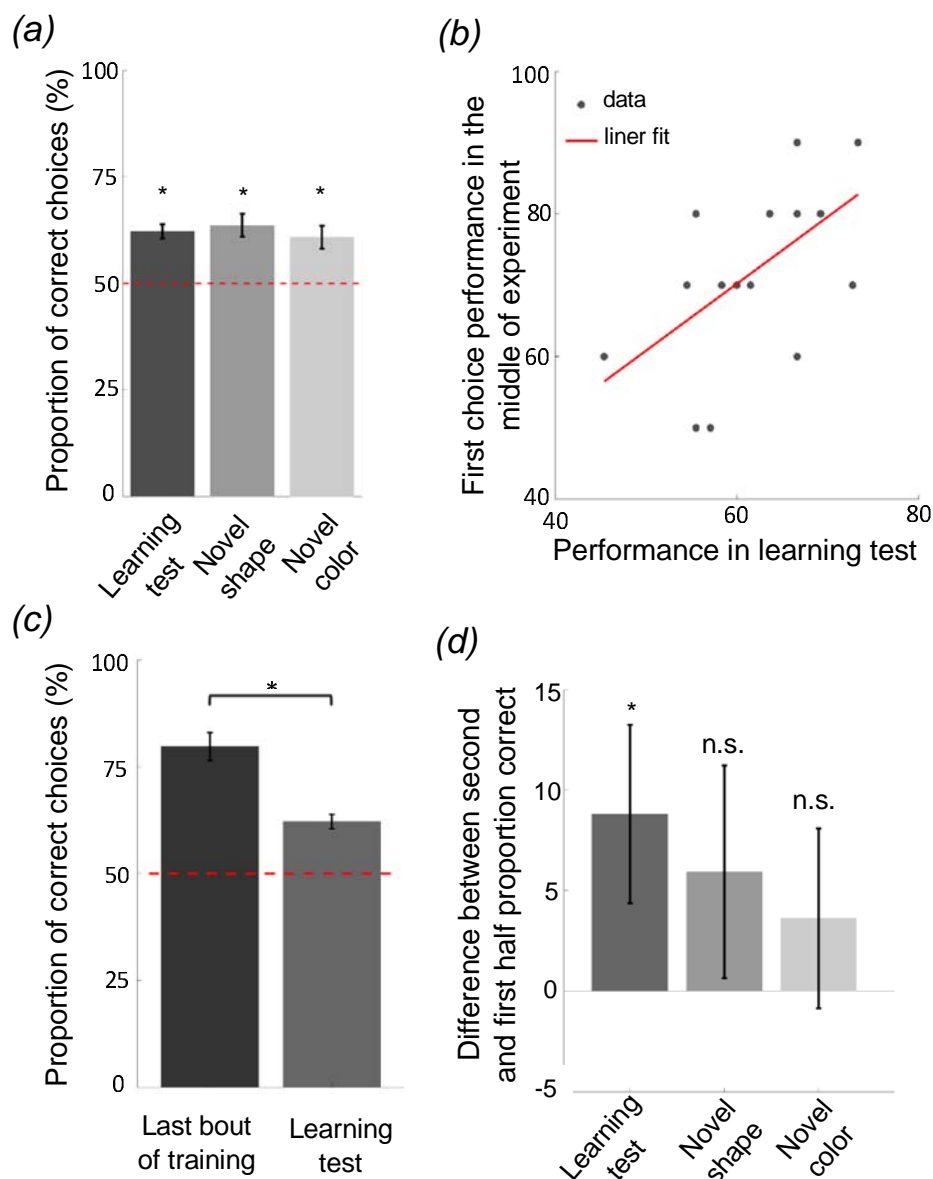
Figure 3. Bees learn and retain a relative size rule. *(a)* The performance of bees during each of the three unrewarded tests shows that they learned and retained the concept of relative size (p <2.41e-3). *(b)* The scatter plot displays the correlation between the performance of bees in the learning test and their first choice performance prior to changing strategies, during the second block of 10 bouts (rho = 0.58, p = 0.01). The red solid line = line of best fit. (*c and d*) The significant drop in performance from the last bout of training to the learning test (p=9.30e-4) (*d*) and the difference in performance between the second and first half of choices during each of the tests (p = 0.03 for learning test; p = 0.28 for novel shape transfer test; p = 0.14 for novel colour transfer test) suggest that bees had begun the tests with the win-stay/lose-switch strategy. Bars = mean. Vertical lines = standard error of the mean. Red dashed line = chance level (50%).
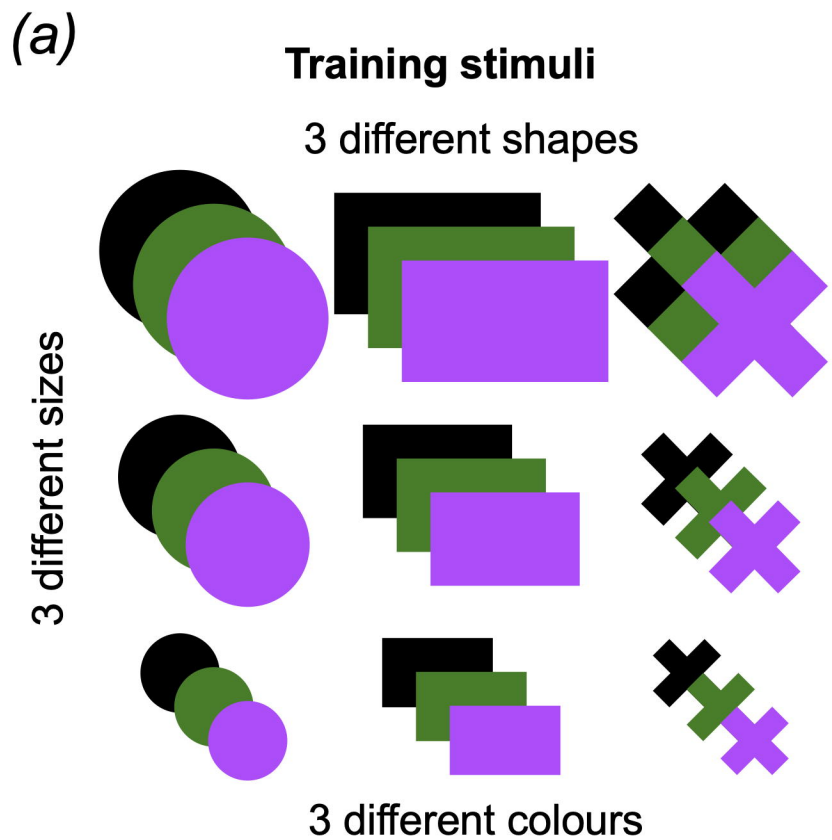
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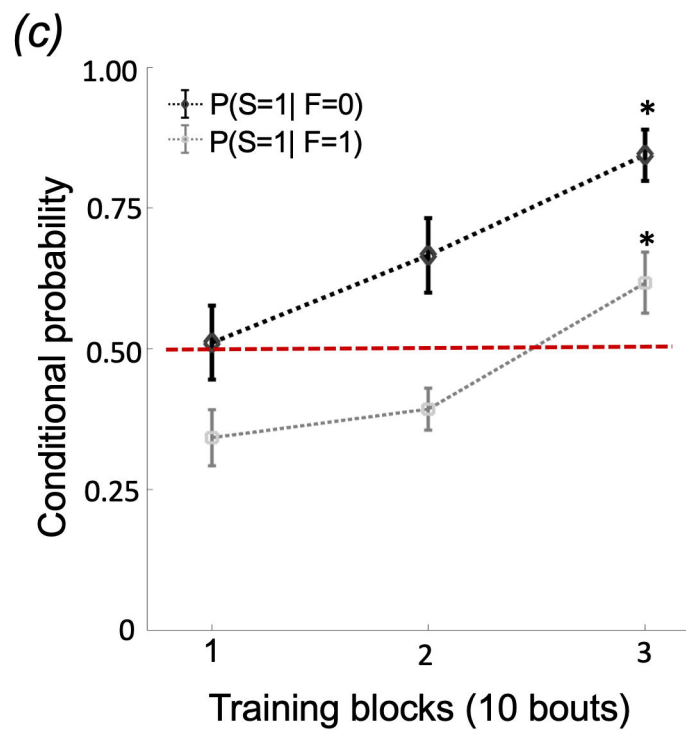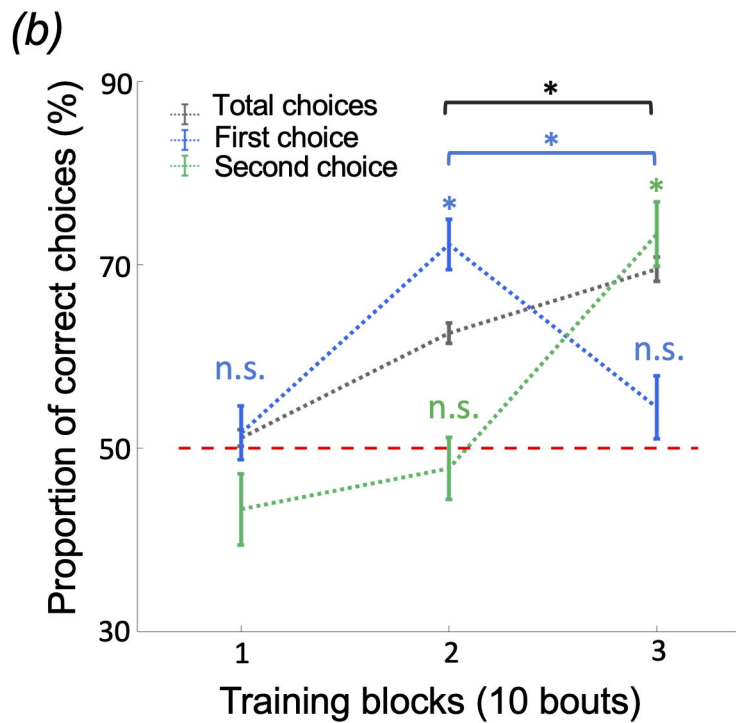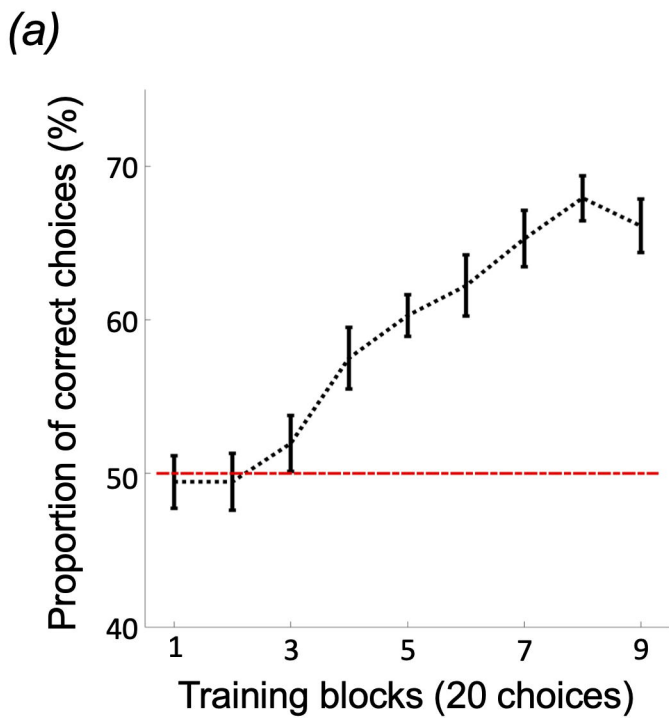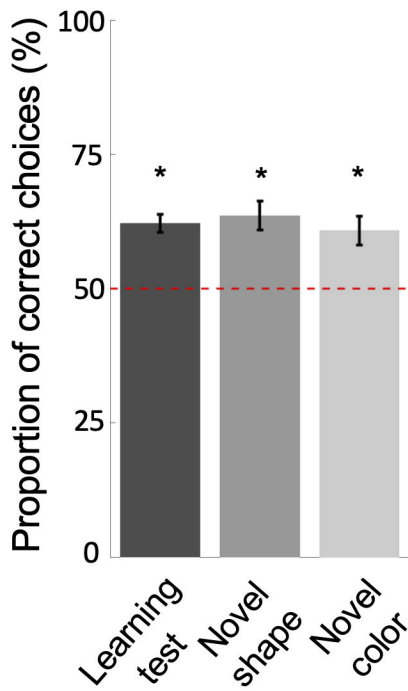
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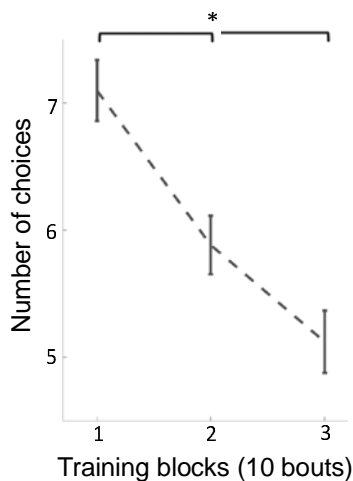
Figure 4. The average of number of choices on stimuli (correct and incorrect) over 3 blocks of 10 training bouts. Over training bouts, bees made fewer choices to visit all three available rewarding stimuli (p = 1.17e-5), indicating that bees continually increased efficiency on solving the task during training. Vertical lines = standard error of the mean.
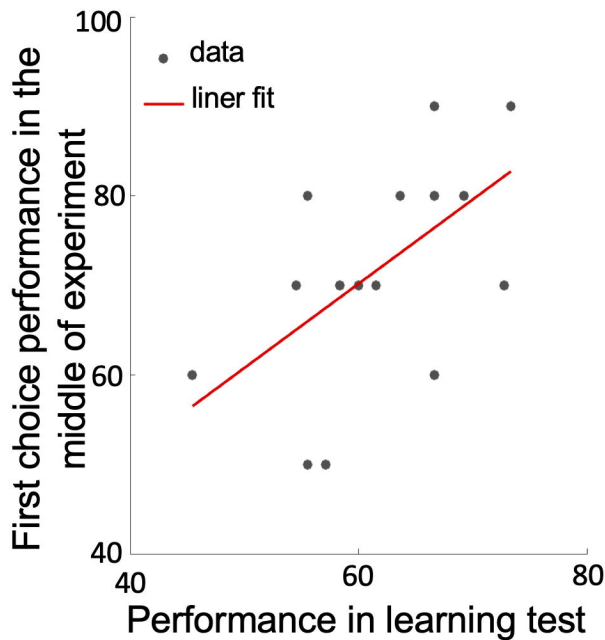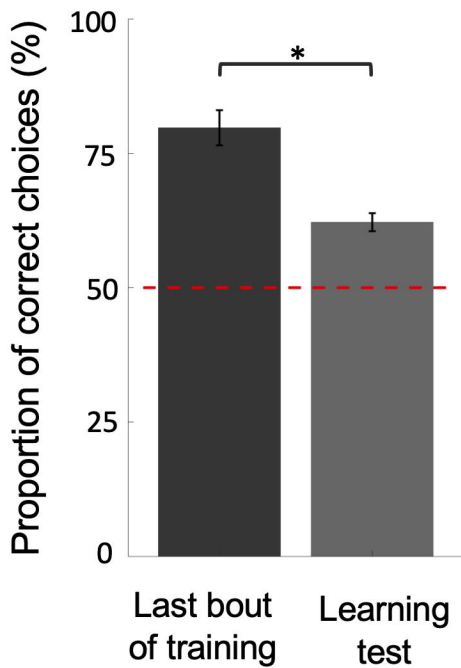
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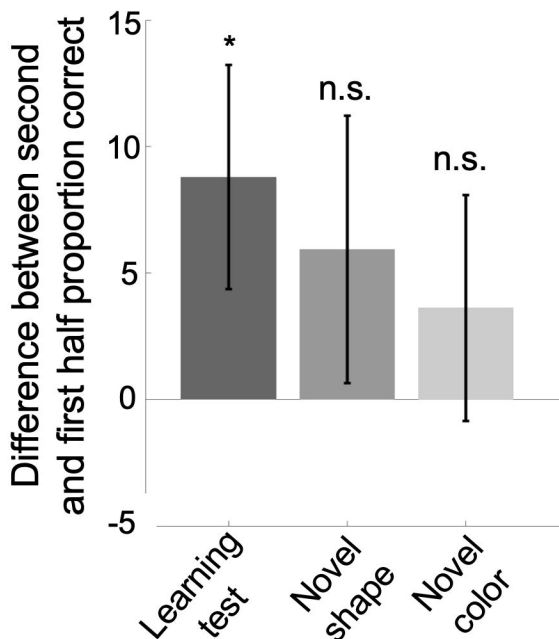*(a)* **Training stimuli**

3 different shapes

3 different sizes

3 different colours

(b) **Test stimuli**

Learning test   Novel shape   Novel color

(c) **Training** (30 bouts)

Testing (2 min each)

Learning test

Novel shape

Novel color

(a) Proportion of correct choices (%) — Learning test, Novel shape, Novel color

(b) First choice performance in the middle of experiment vs Performance in learning test
- data
- liner fit

(c) Proportion of correct choices (%) — Last bout of training, Learning test

(d) Difference between second and first half proportion correct — Learning test, Novel shape, Novel color