Bumblebees learn a relational rule but switch to a win-stay/lose-switch heuristic after extensive training

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

H.M. and L.C. conceived the study. H.M. designed and performed the experiment. H.M. and C.S. analysed data. H.M., C.S. and L.C. wrote the paper.

Keywords

Abstract

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

Contribution to the field

An animal's decisions in the wild are complex and must consider all variables to maximize benefits such as speed and accuracy and net energy gain over time. It may be that animals use a variety of different strategies to solve a particular task. Hence, the ability to monitor, evaluate their decision strategies and change cognitive strategies to cope with variabilities in the environment is a fundamental trait of intelligent behaviour. However, with the exception of a small number of primate studies, most explorations of animal decision-making have focused on analysing only subjects' choices towards a bias of the hypothesized strategy. We show that this approach can hide alternative strategies used by an animal in task solving designed to test a specific cognitive capacity. We designed a well-controlled concept learning task to understand how bees actually solved the task. Our results show that bees are capable of changing strategies during both the learning phase and within unrewarded tests. this indicates bees have the cognitive flexibility to evaluate both their experience and the costs of each strategy as they make decisions. These are arguably more impressive cognitive capacities than the ability to learn a relational rule, and encourage future efforts examining flexibility in cognitive strategy selection in animals.

Ethics statements

Studies involving animal subjects
Generated Statement: Ethical review and approval was not required for the animal study because 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 .

Studies involving human subjects
Generated Statement: No human studies are presented in this manuscript.

Inclusion of identifiable human data
Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://figshare.com/s/ebf002a9baa78bc1ef7b.

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Abstract

Mapping animal performance in a behavioral task to underlying cognitive mechanisms and strategies is rarely straightforward, since a task may be solvable in more than one manner. Here, we show that bumblebees perform well on a concept-based visual discrimination task, but spontaneously switch from a concept-based solution to a simpler heuristic with extended training, all while continually increasing performance. Bumblebees were trained in an arena to find reward on displays with shapes of different sizes where they could not use low-level visual cues. One group of bees was rewarded at displays with larger shapes and another group at displays with smaller shapes. Analysis of total choices shows bees increased their performance over 30 bouts to above chance. However, analyses of first and sequential choices suggest that after approximately 20 bouts, bumblebees changed to a win-stay/lose-switch strategy. Comparing bees' behavior to a probabilistic model based on a win-stay/lose-switch strategy further supports the idea that bees changed strategies with extensive training. Analyses of unrewarded tests indicate bumblebees learned and retained the concept of relative size even after they had already switched to a win-stay, lost-shift strategy. We propose that the reason for this strategy switching may be due to cognitive flexibility and efficiency.

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32 **1   Introduction**

33   Cognitive flexibility reflects an individual's ability to adaptively alter their behavioral strategy in
34   accordance with a changing environment (Wasserman and Zentall, 2006). A fundamental challenge
35   for animal cognition researchers is to decipher which strategies an animal uses in solving any
36   particular task (Shettleworth, 2001; Chittka Lars et al., 2012). Indeed, there are often multiple ways
37   for an animal to solve a behavioral task.
38
39   Bees have been shown capable of learning various abstract relationships for example rules about
40   target size (e.g. "pick the larger (or smaller) of two object sizes"), amongst myriad impressive
41   cognitive abilities (Giurfa et al., 2001; Perry and Barron, 2013; Skorupski et al., 2018). However, in
42   some of these cases it may be that bees use a variety of different strategies to solve the tasks they are
43   confronted with (Cope et al., 2018; Skorupski et al., 2018). One recent study showed that bees can
44   solve a spatial concept learning task using a simple visual discrimination strategy through sequential
45   scanning of stimuli rather than needing to compare stimuli based on an abstract rule, though some
46   individuals may well follow such a rule (Guiraud et al., 2018). In numerical cognition tasks,
47   honeybees may also use alternative cues that correlate with number, but are not in themselves
48   numerical (Vasas and Chittka, 2019). Bees' behavior in solving a delayed matching-to-sample task
49   has been shown to be replicated by a model without any neural representations of the abstract
50   concepts of sameness or difference (Cope et al., 2018). Even the same individuals may have recourse
51   to different solutions to the same task, depending on the extent of training. For example, with an
52   increased number of training trials with a single pair of patterns, individual honeybees have been
53   shown to have a greater generalized response to novel stimuli, i.e. the representation necessary to
54   discriminate subsequent visual patterns changes with extended training (Stach and Giurfa, 2005). All
55   of these findings highlight the need for considering alternative strategies used by animals in cognitive
56   tasks. This does not just concern the traditional dichotomy of "simple" versus "complex" solutions to
57   such tasks. Different individuals may use different solutions that are equal in complexity, depending
58   on their particular path to figuring out a solution.
59
60   Previous works have shown that honeybees are able to solve a task that appears to necessitate
61   learning the concept of relative size and apply the rule to novel sizes within or outside the size range
62   they were trained (Avarguès-Weber et al., 2014; Howard et al., 2017). As with the examples above,
63   bees may use more than one strategy to solve the same task, depending on the training protocol and
64   context. Here, we test bumblebees to determine the strategies by which they cope with a relational
65   rule learning task ("larger-than"/"smaller-than") and examine their behavior over time to reveal the
66   cognitive strategies used over the course of training.

67 **2   Material and methods**

68 **2.1   Animals and experimental setup**

69   Bumblebees (*Bombus terrestris audax*) from commercially available colonies (Agralan Ltd, UK),
70   were housed in a wooden nest-box connected to a flight arena (100 cm x 75 cm x 30 cm). Bees were
71   allowed access to a flight arena through an acrylic corridor (25 cm x 3.5 cm x 3.5 cm). Three plastic
72   sliding doors located along the corridor allowed controlled access to the arena. The arena was
73   covered with a UV-transparent clear acrylic sheet. The stimuli were presented to bees on the grey-
74   colored back wall of the arena. Colonies were provided with ~7 g irradiated commercial pollen
75   (Koppert B.V., The Netherlands) every two days. Bees from three colonies were used in this study.
76

2.2 Pretraining phase

All bumblebee workers were recruited from a gravity feeder containing 30% (w/w) sucrose solution placed in the center of the arena. Outside of experiments, the colony was provided with 30% (w/w) sucrose solution from a small gravity feeder placed inside the nest-box during the evenings. Successful foragers on the arena gravity feeder were individually marked with number tags, superglued to their thorax, for identification during the subsequent experiment (Opalithplättchen, Warnholz & Bienenvoigt, Ellerau, Germany). Each day of experimentation, marked bees were pre-trained to find 50% (w/w) sucrose solution from microcentrifuge tubes (5mm diameter) at the center of each of six white discs (7 cm diameter) on the grey-colored back wall of the arena, horizontally 14 cm from each other vertically 9.3 cm from each other (positioned as in Figure 1). These discs were made of paper and covered with transparent laminate to enable cleaning with 70% ethanol in water (v/v). All stimuli were printed with a high-resolution printer.

2.3 Training phase

Each day, after several number-tagged bees had learnt to find reward from the tubes located in the center of the display discs, one bee was randomly selected for the training phase, and assigned at random to one of two groups to be trained either to the "larger-than" or "smaller-than" relational rule learning task. During the training phase, an individual bee was trained on six discs (three of one size and three of a different size, but of the same type and color) on the back wall of the arena with the same spacing as in pretraining, each displaying one of two differently sized shapes (Figure 1A and C). During each training bout, bees were able to freely land on any stimulus and left the arena when they had fed to satiation. A bout was considered a bee's visit to the arena, landing on different stimuli until she filled her crop and subsequently returned to her nest. Inter-trial intervals were usually between 5-10 minutes. Only bees that completed the entire training phase and tests in one day were included in the results. Four bees were excluded from the analysis process. During 30 training bouts, one group of bees (n = 10) learned that the larger of the two shapes contained 30 µl 50% sucrose solution and the smaller contained 30µl saturated quinine hemisulfate solution (larger-than rule). Another group of bees (n = 8) learned the reverse contingency (smaller-than rule).

Between training bouts, each disc was rotated pseudo-randomly so that the position of a shape varied across the six discs in relation to the central microcentrifuge tube containing sucrose solution (Figure 1C). The location, shape and color of stimuli sets were changed between bouts. The shapes used in training varied in size (small, medium, large), type (circle, rectangle, cross) and color (black, green, purple) (Figure 1A). Only one type and color of stimulus was presented to a bee in each bout and only two of the three sizes were presented during one bout. The dimensions of the shapes were as follows: small circle: Ø = 1.07 cm; medium circle: Ø = 1.97 cm; large circle: Ø = 2.87 cm; small rectangle: 0.93 cm x 1.18 cm; medium rectangle: 1.79 cm x 2.92 cm; large rectangle: 2.3 cm x 3.94 cm; small cross: width of bars = 0.46 cm, length of bars = 1.3 cm; medium cross: width of bars = 0.6 cm, length of bars = 2.15 cm; large cross: width of bars = 0.96 cm, length of bars = 2.87 cm. Note that there was a large variability between physical features of stimuli (Supplementary Figure 1). This variability ensured the bees were not able to solve the task by associating an absolute size of stimuli with certain reinforcements. Several of stimuli were paired with both positive and negative reinforcements during the training phase. For instance, medium size stimuli were paired with the positive reinforcement in some training bouts while these were paired with negative reinforcement in the rest of the training bouts. Further, the total area of the medium rectangular was larger than the total area of the large cross (see Supplementary Figure 1A). All of these variations described ensured that low-level visual cues could not be used to solve the task. Stimuli were cleaned between each

3

141 training bout with 70% ethanol in water (v/v) to ensure odor cues were not used to solve the task.
142 After the daily experiment, all used microcentrifuge tubes were washed with soap-water, then
143 cleaned with 70% ethanol solution. Finally, they were rinsed with water and air-dried at room
144 temperature during the night.
145
146 **2.4   Testing phase**
147
148 Following the training phase, each bee was tested in the same setup as in training in three different
149 scenarios, but with stimuli in the tests providing 30 μl of sterilized water (Figures 1B and C). Tests
150 lasted 120 seconds, at which point the bee was gently removed from the arena by using a cup and
151 placed into the corridor until stimuli were changed for the refreshment bouts. Each test was separated
152 by two refreshment training bouts between tests to maintain the bee's motivation.
153 The sequence of the three tests were counterbalanced across bees. The learning test evaluated
154 performance by testing bees on one of the same sets of stimuli used during training, pseudo-randomly
155 chosen (i.e. a random number generator was used to generate a random sequence of tests for each
156 individual bee). The learning test used only the small- and larger-sized training shapes. The other two
157 tests used either a novel shape and size (star) or a novel color (yellow), with the other properties
158 pseudo-randomly chosen. The dimensions of the 5-pointed stars were as follows: small star: length of
159 side of point = 0.5 cm; large star: length of side of point = 1.23 cm (See Supplementary Figure 1). As
160 in training, stimuli were cleaned between each bout during the testing phase with 70% ethanol in
161 water (v/v) to ensure odor cues were not used. Trained bees were removed from the nest once the
162 training and tests phases were finished.
163
164 **2.5   Statistical analysis and probabilistic model of learning curve**
165
166 To evaluate bees' performance over bouts, the percentage of correct choices (choices were defined as
167 when a bee touched a microcentrifuge tube with her antennae or when she landed on a
168 microcentrifuge tube) was calculated from either all choices or from only the first or second choices
169 within each block of six bouts during training (total of five blocks). Using a generalized linear mixed
170 model (GLMM) for binary probability (correct or incorrect), the effect of different factors such as
171 colony, group of training and interaction between trial block and group of bees in the bees'
172 performance were calculated. The bee index was included in the model as random factors. GLMMs
173 were performed in MATLAB (MathWorks, Natick, MA, USA).
174
175 To determine whether bees used relative size information, rather than any other visual cues, the
176 choices of bees during the unrewarded tests were evaluated by a Wilcoxon signed rank test. Further,
177 a Kruskal-Wallis test was used to statistically evaluate and compare whether the bees' performance
178 or choice numbers in different blocks of bouts are from the same distribution.
179
180 To test if bees might use a win-stay/lose-switch strategy during training, we calculated the
181 conditional probabilities of each bee's second choice (c_2) given their first choice (c_1) at each block of
182 10 bouts. A conditional probability, "Probability of B, given A ($P\{B|A\}$)", is a probability of an event
183 (B) occurring given that another event (A) has already occurred. The conditional probability of a
184 lose-switch strategy, i.e. a correct second choice after an incorrect first choice, is calculated by
185 $P\{c_2 = 1|c_1 = 0\} = P\{c_2 = 1, c_1 = 0\}/P\{c_1 = 0\}$ where $P\{c_2 = 1, c_1 = 0\}$ is the joint probability
186 of a correct second choice and an incorrect first choice and $P\{c_1 = 0\}$ is the probability of the first
187 incorrect choice. The conditional probability $P\{c_2 = 1|c_1 = 0\}$ at more than chance level indicates
188 that a bee switched to another presented size when they found the first choice was incorrect. In the

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192 same way, we can calculate the conditional probability of a win-stay strategy, using
193 $P\{c_2 = 1|c_1 = 1\} = P\{c_2 = 1, \ c_1 = 1\}/P\{c_1 = 1\}$, i.e. the bee's second choice is the same size as
194 the first choice when their first choice was correct.
195
196 *Model of prediction of learning curve based on a bee's first two choices*
197
198 We propose a Markov stochastic model (Gagniuc, 2017) to describe the learning curve of bees'
199 choices (total choices at each bout) based on the information of two first choices of bees. The
200 performance of the model at each bout is assumed as

201
$$Perf = P\{c_1 = p\} \sum_{k=2}^{N} \prod_{i=1}^{k} P\{c_{i+1} = q|c_i = p\}$$

202 $P\{c_1\}$ is the probability of the first choice at each bout and $P\{c_{i+1}|c_i\}$ is the conditional probability
203 of $(i + 1) - th$ choices given the of $i - th$ choices $(i \geq 1)$ for when each choice in the sequential
204 choices is correct or incorrect. $p \ or \ q = 1$ if the choices are correct, otherwise $p \ or \ q = 0$. We
205 assume that the conditional probabilities of two sequential choices from the third choices are equal to
206 the conditional probability of the second choice given the first choice expressed by bees at each bout
207 of training. The sequence of possible events in which the probability of each event depends only on
208 the state achieved in the previous event will be stopped (N) when the simulated bees collect all three
209 positive reinforcements along with two, one or no incorrect choices within each bout according to the
210 average number of choices at each bout.

3   Results

3.1   Bees' overall performance increased over the 30 training bouts

214 A multivariate statistical model, GLMM, applied to the performance of bees demonstrates a
215 significant increase in the proportion of correct choices made over the 180 choices of the training
216 phase (Figure 2A, p = 0.018) irrespective of the shape, color or position of patterns within the
217 stimuli. No significant differences were found between the learning curves of the two different
218 contingency groups (*i.e.* "larger-than" rule versus "smaller-than" rule; p = 0.87). The output of the
219 GLMM confirms that there was no significant difference between the different colonies of bees
220 during the training phase (p = 0.37). These results show that bees became better at solving either
221 contingency over training bouts.

3.2   Bees used a win-stay/lose-switch strategy after extensive training

223 The typical analysis used to determine whether an animal has solved a particular task is to calculate
224 the animal's performance based on the number of correct and incorrect choices throughout the
225 training phase. At first inspection, bees' behavior during training suggests they learned to solve the
226 concept-based task (Figure 2A). However, a finer examination of their choices suggests the
227 involvement of another strategy in the later stages of training. If bees had only used the concept of
228 relative size throughout training, their first choices should reflect this by increasing in accuracy
229 throughout the 30 bouts. Although bees' average overall accuracy gradually increased to 70%
230 (significantly above chance level) over the 30 training bouts (Figure 2B; Wilcoxon signed rank test: z
231 = 3.72, n = 18, p = 1.96e-4), their first-choice accuracy rose to 72% (significantly above chance level:
232 Wilcoxon signed rank test: z = 3.55, n = 18, p = 3.71e-4) over the first 20 bouts and then decreased to
233 chance level (54%) over the next 10 bouts (Wilcoxon signed rank test: n = 18, z =1.25, n = 18, p =
234 0.21; Figure 2B) and decreased significantly across last two blocks of bouts (Wilcoxon signed rank

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236   test: $z = 2.83$, $n = 18$, $p = 4.59e-3$). Second-choice accuracy was not different from chance level
237   during the first two-thirds of the training phase (Wilcoxon signed rank test: $z = -0.67$, $n = 18$, $p =$
238   0.49), but increased in the final third of the training phase to 73.33%, significantly above chance
239   level (Figure 2B; Wilcoxon signed rank test: $z = 3.52$, $n = 18$, $p = 4.28e-4$). These results suggest that
240   bees changed to a win-stay/lose-switch strategy after around 20 bouts of training, i.e. if they find
241   reward at a stimulus they choose the same type of stimulus next, or if no reward is found at a
242   stimulus they choose a different type of stimulus next.

243

244   To help evaluate the possibility that bees switched strategies part way through training, we calculated
245   the conditional probabilities (Materials and Methods) for 1) a correct second choice after a correct
246   first choice (win-stay), and 2) a correct second choice after an incorrect first choice (lose-switch).
247   Both of these two conditional probabilities increased over bouts (Figures 2C and 2D; Kruskal-Wallis
248   test, chi-sq > 12.94, df = 53, $p < 1.55e-3$), most notably rising to significantly above chance level in
249   the last third of training (Wilcoxon signed rank test: $z = 3.68$, $n = 18$, $p = 2.27e-4$, chance level = 0.4
250   for win-stay and $z = 3.33$, $n = 18$, $p = 8.40e-4$, chance level = 0.6 for lose-switch), again suggesting
251   that bees had changed to a win-stay/lose-switch strategy. Note that the chance levels of the
252   conditional probability of correct second choice in a win-stay and lose-switch strategy were 0.4 and
253   0.6, respectively, because after first choosing correctly, only two of the five remaining stimuli were
254   correct, and after a first incorrect choice, three of the remaining five stimuli were correct.

255

256   It may have been that after a first choice, bees simply chose the stimulus nearest to that first choice.
257   To determine whether a bee's stimulus choice was based on physical closeness to their previous
258   choice, we also evaluated the spatial pattern of their landings. Bees were more likely to choose
259   stimuli further away than those closest to their previous choice (Supplementary Figure 3; Wilcoxon
260   signed rank test: $z > 3.72$, $n = 18$, $p < 1.95e-4$). Later in training, bees' second choices were further
261   away from their first choice compared to earlier in training (Supplementary Figure 3; Kruskal-Wallis
262   test, chi-sq = 7.84, df = 53, $p = 0.01$). These results indicate that bees did not make their second
263   choice by visiting an adjacent stimulus, but rather searched for specific types of stimuli, following
264   either a relational rule or win-stay/lost-switch strategy.

265
266   **Modelling a win-stay/lose-switch strategy**
267   To further examine whether bees switched strategies during training, we utilized a probabilistic
268   model based on a win-stay/lose-switch strategy. Within our model, we used bees' overall and
269   conditional performance (Figures 2B and 2E) and initial first and second choices to predict bees'
270   subsequent choices in each bout (Materials and Methods). Figure 2E shows that our model predicts
271   the bees' performance in the last 10 bouts (i.e. no difference between the model's performance and
272   bee's performance; Wilcoxon signed rank test: $z = -1.41$, $n = 18$, $p = 0.15$). In contrast, our model's
273   predicted performance was significantly poorer than the performance of bees in the first 20 bouts
274   (Wilcoxon signed rank test: $z > 2.32$, $n = 18$, $p < 0.01$ for both first two blocks). The ability of our
275   model to predict the behavior of our bees in the later stages of training but not the initial stages
276   supports the hypothesis that bees changed to a win-stay/lose-switch strategy within the last 10 bouts
277   of training.

278   **3.3   Bees retained the concept of relative size after having switched strategies**

279   So far, our analyses and model results suggest that bees used a win-stay/lose-switch strategy only
280   after extensive training. Bees seemed to have used a different strategy during the initial blocks of
281   training bouts. Their increased performance to above chance level, suggests they were discriminating
282   the stimuli based on size. To ensure that bees' initial strategy had actually been a relative size rule,

    6

283 we measured bees' performance directly after training in unrewarded tests. Because the tests were
284 unrewarded, bees could not solve the task based on a win-stay/lose-switch strategy. Bees'
285 performance on the learning test was above chance level (Wilcoxon signed rank test: z = 3.73, n =
286 18, p = 1.87e-4), as was their performance on the novel shape transfer test (Wilcoxon signed rank
287 test: z = 3.51, n = 18, p = 4.46e-4), and on the novel color transfer test (z = 3.03, n = 18, p = 2.41e-3
288 for novel color; Figure 3A). Note that the variability in different shape sizes and resulting overlap
289 between sizes across shapes prevented bees from associating a general size with reward
290 (Supplementary Figure 1). These results suggest that the bees had at some point during training
291 learned to solve the task based on the concept of relative size.
292
293 Because animals vary in their learning and performance, we posited that if bees had learned and
294 retained a relative size rule, how well they performed in training before changing strategies should
295 reflect how well they perform (i.e. remember the relational rule) during the learning test. In line with
296 this, there was a positive correlation between the average of first choice accuracy in the second third
297 of the training phase (prior to strategy change) and bees' performance in the learning test (Figure 3B;
298 Spearman correlation: rho = 0.58, n = 18, p = 0.01). Although bees seemed to have changed
299 strategies after extensive training, the results of the unrewarded tests show that bees had learnt the
300 relative size rule during training, retained the rule even after having changed strategies late in
301 training, and therefore resorted to the relative size rule strategy during the tests.
302
303 Note that the performance of bees in the learning test was significantly poorer than the last bout of
304 the training phase (Figure 3C; Wilcoxon signed rank test: z = 3.31, n = 18, p = 9.30e-4). This
305 suggests that bees began the learning test using a win-stay/lose-switch strategy. This makes sense
306 because they had just been using a win-stay/lost-switch strategy during training and had no
307 knowledge that the test was unrewarded. Further, bees' performances on the second half of choices
308 during each of the tests was better than their performance on the first half, (Figure 3D; Wilcoxon
309 signed rank test: z = 1.82, n = 18, p = 0.03 for Learning test; z = 0.57, n = 18, p = 0.28 for Novel
310 shape; z = 1.05, n = 18, p = 0.14 for Novel color), indicating that bees had reverted to the retained
311 relative size strategy.
312
313 Why would bees change strategies if they were already performing above chance level? We
314 hypothesized that bees might change strategies if the new strategy was more efficient, i.e. it took
315 them less effort to locate all three rewarding discs (discs were not refilled during training). In support
316 of this, the number of total choices by bees decreased from an average of 7.1 choices per bout at the
317 beginning of training to an average of 5.1 choices per bout at the end of training (Figure 4; Kruskal-
318 Wallis test, chi-sq = 22.70, df = 53, p = 1.17e-5), indicating that bees' efficiency increased during
319 training across a change in strategy.
320
321 **4   Discussion**
322 We demonstrate and corroborate previous findings (Avarguès-Weber et al., 2014; Howard et al.,
323 2017) that bees can learn a relative size rule, but in our study they opted to use a simpler strategy
324 after extensive training. Because there can often be more than one way of processing the same stimuli
325 to solve a cognitive task, it is useful to examine individual strategies and over extended periods to
326 explore if multiple strategies might be at play. In our paradigm, we prevented bees from using low-
327 level visual cues. Initial increases in performance suggested that bees learned the task and later
328 performance on unrewarded tests verified that bees had learnt and retained a relational rule, as was
329 previously demonstrated in honeybees (Avarguès-Weber et al., 2014; Howard et al., 2017). However,
330 statistical analyses showed that after extensive training, bees began to use a win-stay/lose-switch

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332 strategy based on whether or not they were rewarded on each stimulus. Bees' performance calculated
333 by their first choices or by multiple sequential choices revealed a strategy of decision making that
334 had been hidden within the gross calculation by total choices. Averaging all choices in a training bout
335 or test is common within bee cognition and within other animal research communities. We suggest
336 that interpretations of any animal cognition study involving multiple choices include analyses of first
337 and sequential choices to investigate potential alternative strategies.
338
339 Theoretical and empirical work maintains that animals tend to follow the "law of least effort" (Hull,
340 1943; Froment et al., 2014), whereby subjects choose strategies that minimize the costs in obtaining
341 desirable outcomes (Hull, 1943; Mobbs et al., 2018). In comparative cognition research, animals may
342 use strategies different to those we intend a specific paradigm to test and still perform well on the
343 behavior we are measuring (Shettleworth, 2001; Pfungst, 2010; Chittka et al., 2012; Guiraud et al.,
344 2018; Vasas and Chittka, 2019). Most studies on the "law of least effort" have focused on the idea
345 that animals opt to minimize physical work, but this idea extends to cognitive effort as well (Elner
346 and Hughes, 1978; Kool et al., 2010; LeDoux, 2012). The ability to change decision-making
347 strategies with the changing demands of the environment is essential to adaptive behavior, and
348 therefore survival. Lloyd and Dayan, 2018 proposed that constant monitoring of information to
349 promptly assess and predetermine decision-making strategies would be too costly for animals to
350 maintain. Similarly, commitment for extended periods of time to one strategy without the ability to
351 adjust could be deleterious (Lloyd and Dayan, 2018). These authors suggested, with support from
352 computational models, that temporal commitment to certain strategies with intermittent interruption
353 to assess costs and switch strategies would be more advantageous for real world scenarios.
354 Bumblebees in our study seem to follow a similar overall approach, as they first learn an abstract
355 concept (relative size) and stick with this rule for approximately 20 bouts, at which point they change
356 to a new strategy (see Supplementary Figure 2 for the individual difference between bees). A
357 decrease in the number of choices taken to find all rewarding stimuli (Figure 4) indicate that bees
358 may have changed strategies to become more efficient. Further studies are needed to check the role of
359 efficiency in strategy selection in animals. Further studies should involve videotaping the behavior of
360 bees during the training and test phases, so that one can make some direct inferences about time
361 invested, mechanisms of inspecting stimuli and the efficiency of decisions.
362
363 In this light, our results support the idea that animals can adaptively weigh the costs of cognitive
364 effort across decision-making approaches and choose the less cognitively demanding strategy (Risko
365 and Gilbert, 2016). This interpretation requires that the win-stay/lose-switch strategy was actually
366 simpler than the relative size rule. Indeed, the win-stay/lose-switch heuristic is cognitively less
367 demanding than any relational rule, simply because it is based only on the outcome of the previous
368 choice, and therefore could be solved using working memory alone (Nowak and Sigmund, 1993).
369 Accordingly, bees could have stored the visual template of the first stimulus in working memory and,
370 if the first choice was correct, subsequently chosen a stimulus that had more overlap with the stored
371 template, or if the first choice was incorrect, subsequently chosen a stimulus with less overlap
372 (template hypothesis; (Dittmar et al., 2010)). The win-stay/lose-shift strategy has been broadly
373 observed and explored in bees foraging strategies and flower constancy amongst variable rewarding
374 species of flowers (Greggers and Menzel, 1993; Chittka et al., 1997; Menzel, 2001; Raine and
375 Chittka, 2007; Real, 2012). This type of sequential matching/non-matching to sample strategy has
376 been shown to be solvable with a simple computational model based on the known neural circuitry of
377 the bee brain, without requiring any higher-order abstract concept (Cope et al., 2018). Learning and
378 applying an abstract concept like relative size rule requires a substantial abstraction process to
379 different stimuli that must work independent of the physical characteristics of stimuli (Zentall et al.,

380 2008). In mammals, it is assumed that higher cognitive functions processed in the prefrontal cortex or
381 analogous structures are essential for rule learning (Wallis et al., 2001; Miller et al., 2003). In insects,
382 it has been proposed that rule learning occurs in the mushroom bodies, high-level sensory integration
383 centers (Chittka and Niven, 2009; Menzel, 2012). In contrast to rule-learning, bees can use a simple
384 associative mechanism to remember the previously visited stimulus in order to make decisions about
385 a subsequent stimulus. Therefore, the effort required in a win-stay/lose-switch type mechanism is
386 likely to be lower than an abstract rule because bees can learn to recognize and associate a stimulus
387 with reward without using their mushroom bodies (Devaud et al., 2015; MaBouDi et al., 2017). For
388 example, honeybees with inactivated mushroom bodies can perform some odor learning tasks as well
389 as control bees (Devaud et al., 2015; Carcaud et al., 2016). Further, a realistic computational model
390 of olfactory information processing in the bee brain shows that two parallel odor pathways with
391 different functions provide the flexibility necessary for comparing multiple olfactory stimuli during
392 associative and non-associative discrimination tasks (MaBouDi et al., 2017).

394 Although our results indicate that bees switched to a win-stay/lose-switch heuristic, it is unclear why
395 bees would learn the relative size concept first if the win-stay/lose-switch strategy is cognitively
396 simpler. We speculate that this strategy may have been initially favored simply to reduce the load on
397 long-term memory and to speed up the decision-making process so as to avoid the quinine-containing
398 discs. During pretraining, bees only received reward from white disks. When training began, all of
399 the discs suddenly contained colored shapes and the bees found not only reward but also aversive
400 quinine. Because of this abrupt and dramatic change, bees' priority may have been to learn to avoid
401 the quinine containing discs. To accomplish this quickly, they could have extracted a set of
402 elementary visual features to avoid in the first bout of training. During the next bouts, instead of
403 switching to a new strategy relying on working memory, they stuck with identifying and avoiding the
404 template for the quinine containing discs. Over the next trials, they learned to generalize and group
405 visual features across stimuli in a manner consistent with the concept of relative size (Zentall et al.,
406 2008; Avarguès-Weber and Giurfa, 2013). Because constant monitoring of how well they were doing
407 would be too costly (Lloyd and Dayan, 2018), it might have taken them some time to assess their
408 performance and try out a new strategy. Further analysis of bees' behavior during the training and
409 test phases are required to uncover the true mechanisms underlying bees' strategy selections.

411 As a result of bees learning a relative size rule early in training, we would have expected to see an
412 improvement on second choice performance from the first 10 bouts to the second 10 bouts in the
413 training phase similar to the bees' improvement on first choices (Figure 2B). However, bees'
414 performance on second choices was not significantly different from chance level within 20 bouts of
415 training. We are unable to say from our data why this was the case, but speculate that motivation and
416 attention may play a role – once bees found reward, they might have been less likely to fly back
417 within the arena to view stimuli head on to properly view and assess stimuli, and rather flew directly
418 to a nearby disc to check for food, which statistically would be more likely to be unrewarding
419 (because of the remaining five discs only two would be rewarding). This type of motivational-based
420 exploration may also account for why bees eventually changed to a win-stay/lose-switch strategy.
421 Supplementary Figure 2 shows a large variability between individuals in second choice performance,
422 and therefore individual differences in motivation and attention may have played a part in why
423 second choice performance was lower than expected (Muller et al., 2010; Carere and Locurto, 2011).
424 However, many of the bees did show an improvement in their second choices from the first 10 bouts
425 to the second ten bouts. Analyses of sequential choices in future studies of animal cognition will help
426 resolve these questions.

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5 Acknowledgments

6 Author Contributions

H.M. and L.C. conceived the study. H.M. designed and performed the experiment. H.M. and C.S. analyzed data. H.M., C.S. and L.C. wrote the paper.

7 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.

8 Funding

9 Conflict of Interest

All authors declare they have no conflicts of interest.

10 Supplementary Material

Information includes three figures.

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11 Data Availability Statement

The datasets for this study can be found in the link https://figshare.com/s/ebf002a9baa78bc1ef7b

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552 **Figure captions**
553
554 **Figure 1. Training and testing protocol**. **(A)** Stimuli options used during training. **(B)** Stimuli
555 options used for each of the three different unrewarded tests. **(C)** Training and test protocol. Bees
556 were trained for 30 bouts (visits to the arena before returning to the hive). All stimuli in (A) were
557 used randomly across bouts during training. Only two of the possible three sizes of shapes were
558 presented during a single bout. Only one of the possible three colors and one of the possible three
559 shapes were presented each bout. One group of bees (n = 10) was trained to find 50% sucrose
560 solution at the center of the stimulus containing the larger of the three shapes and bitter quinine
561 solution at the smaller of the three shapes. Another group (n = 8) were trained on the opposite
562 contingency. Once training was complete, bees were subjected to three unrewarded tests (with one or
563 two reminder/training bouts between each test to keep bees motivated). All tests used small and large
564 sized shapes. The learning test used one randomly chosen type and color used during training. The
565 novel shape test used one randomly chosen color used during training but always a star shape that
566 had not been used during training. The novel color test used one randomly chosen shape used during
567 training but always colored yellow, which had not been used during training.
568
569 **Figure 2. Bees use a win-stay/lose-switch strategy after extensive training.**
570 **(A)** There was a significant increase in the number of correct choices over the 180 conditioned
571 choices (p = 0.018). **(B)** Bees' performance over 3 blocks of 10 training bouts during the relative size
572 discrimination task. Performance increased gradually over bouts when considering total number of
573 choices in each bout (black dashed line; p = 1.96e-4). Bees' first choice performance increased
574 significantly from the first to the second block of training bouts to 72.22% (p = 3.71e-4) but then
575 dropped to chance level from the second to third block of training bouts (blue dash-dotted line; p =
576 0.79). Second choice performance was near chance for the first two blocks of training bouts (p >
577 0.49), but then increased significantly during the third block of training bouts (green dotted line; p =
578 4.28e-4). These results indicate that bees changed to a win-stay/lose-switch strategy after extensive
579 training. Vertical lines = standard error of the mean. Red dashed line = chance level performance
580 (50%). **(C and D)** The average conditional probabilities of a bee's second choice within each bout
581 being correct given the outcome of the bee's first choice of the bout (either correct or incorrect). Both
582 conditional probabilities increased to above chance during the second and third blocks of bouts (p =
583 2.27e-4 for win-stay and p = 8.40e-4 for lose-switch) **(E)** Our win-stay/lose-switch model's
584 performance matches our bees' performance on the task during the last block of 10 bouts during
585 training (p=0.15), again suggesting that after extensive training bees changed to a win-stay/lose-
586 switch strategy. (Vertical lines = standard error of the mean). Red dashed line = chance level.
587
588 **Figure 3. Bees learn and retain a relative size rule. (A)** The performance of bees during each of
589 the three unrewarded tests shows that they learned and retained the concept of relative size (p <2.41e-
590 3). **(B)** The scatter plot displays the correlation between the performance of bees in the learning test
591 and their first choice performance prior to changing strategies, during the second block of 10 bouts
592 (rho = 0.58, p = 0.01). The red solid line = line of best fit. **(C and D)** The significant drop in
593 performance from the last bout of training to the learning test (p=9.30e-4) (D) and the difference in
594 performance between the second and first half of choices during each of the tests (p = 0.03 for
595 learning test; p = 0.28 for novel shape transfer test; p = 0.14 for novel color transfer test) suggest that
596 bees had begun the tests with the win-stay/lose-switch strategy. Bars = mean. Vertical lines =
597 standard error of the mean. Red dashed line = chance level (50%).
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605   **Figure 4. The average of number of choices on stimuli (correct and incorrect) over 3 blocks of**
606   **10 training bouts.** Over training bouts, bees made fewer choices to visit all three available rewarding
607   stimuli (p = 1.17e-5), indicating that bees continually increased efficiency on solving the task during
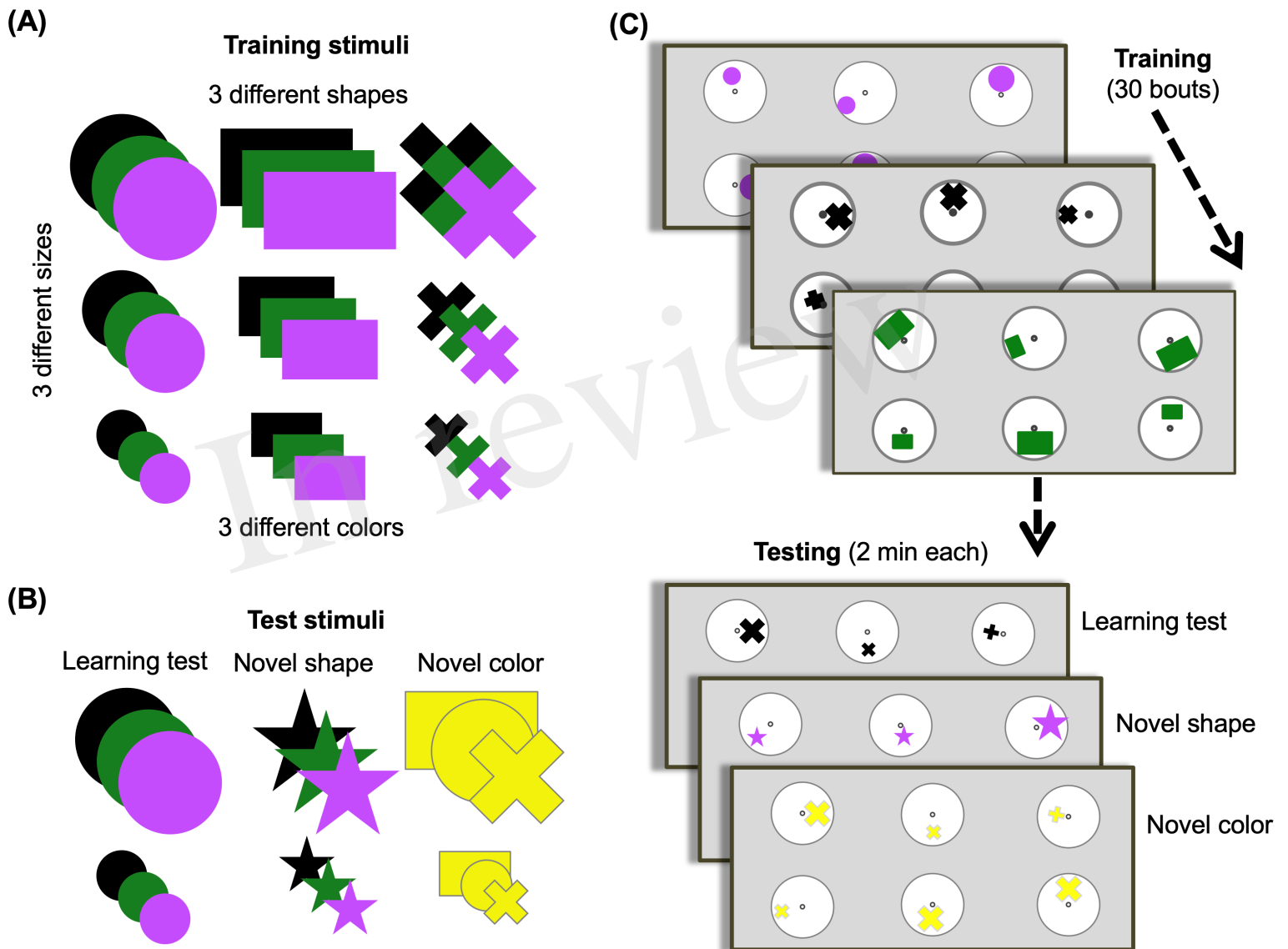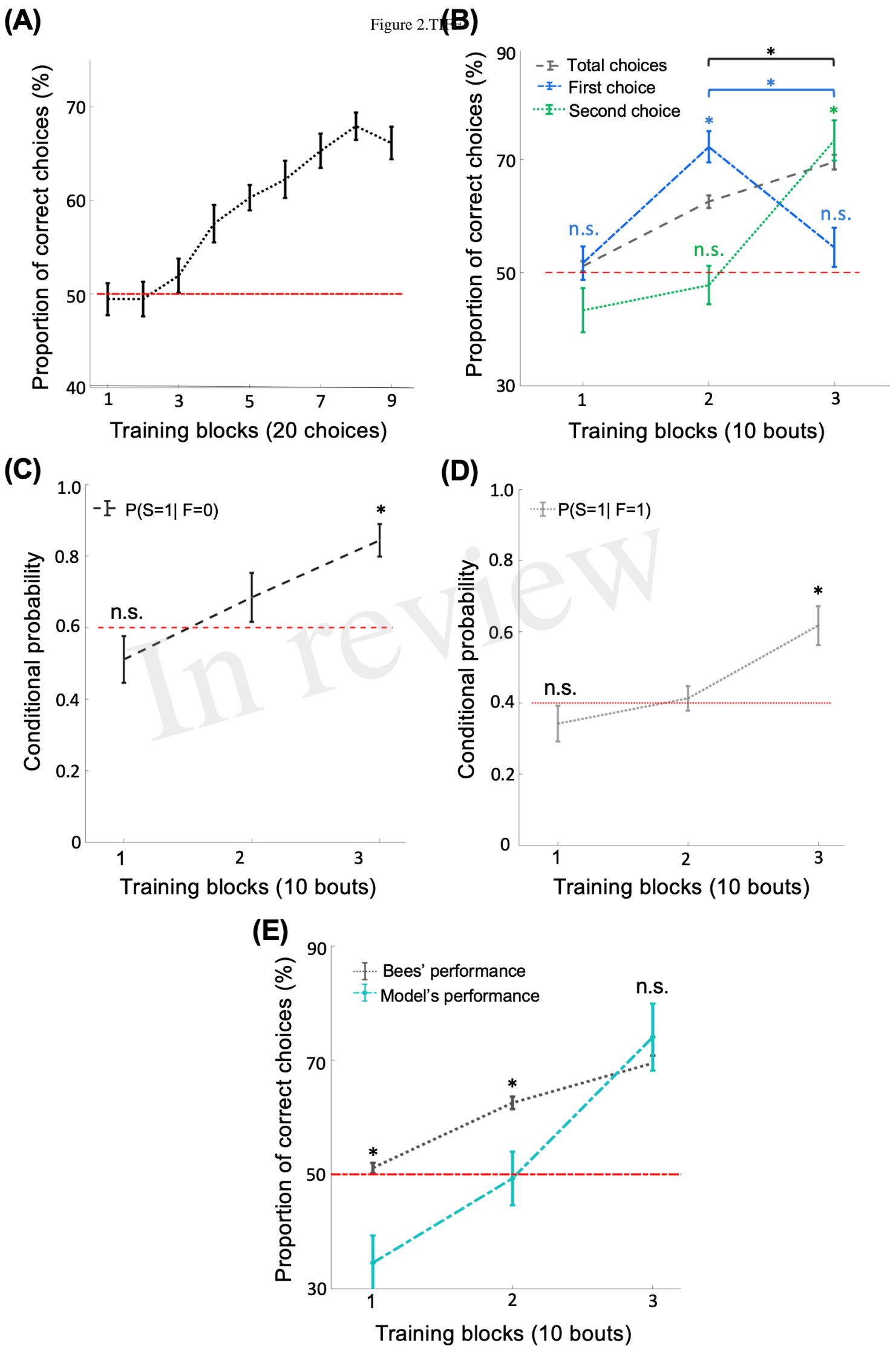608   training. Vertical lines = standard error of the mean.

Figure 1.TIFF

Figure 2. The

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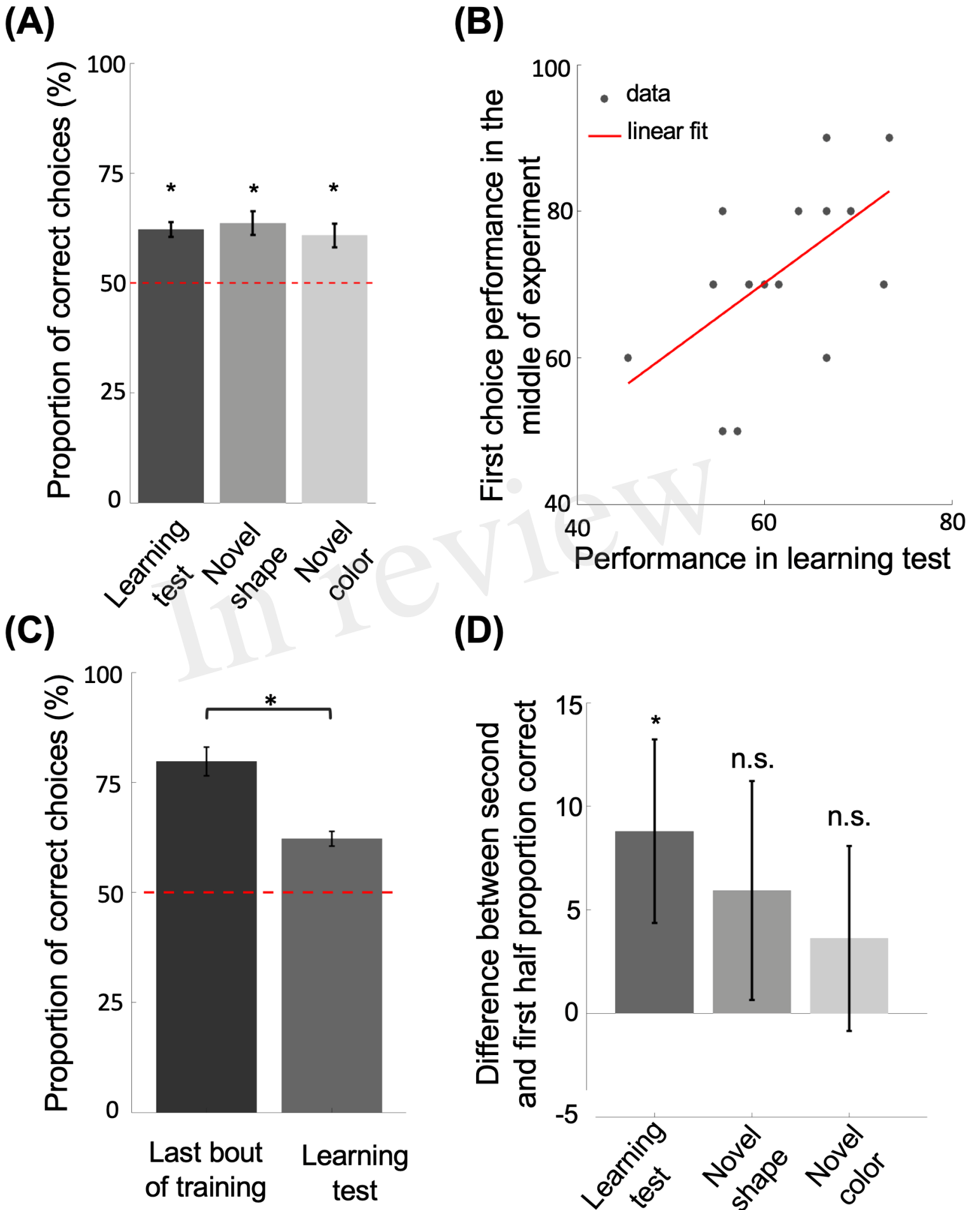
Figure 4.TIFF