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Article:

MaBouDi, H., Roper, M., Guiraud, M. et al. (Submitted: 2021) Automated video tracking and flight analysis show how bumblebees solve a pattern discrimination task using active vision. bioRxiv. (Submitted)

<https://doi.org/10.1101/2021.03.09.434580>

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Automated video tracking and flight analysis show how bumblebees solve a pattern discrimination task using active vision

Running title: *Active scanning strategy in bumblebees*

HaDi MaBouDi^{1,2,3*}, Mark Roper^{3,4}, Marie Guiraud^{3,5}, James A.R. Marshall^{1,2}, Lars Chittka³

¹Department of Computer Science, University of Sheffield, Sheffield, UK

²Neuroscience Institute, University of Sheffield, Sheffield, UK

³School of Biological and Chemical Sciences, Queen Mary University of London, London, UK

⁴Drone Development Lab, Ben Thorns Ltd, Colchester, Essex, UK

⁵INSECT Lab, Zoology department, Stockholm University, Stockholm, Sweden

*Corresponding author: HaDi MaBouDi, h.maboudi@Sheffield.ac.uk

Summary statement

Automated video tracking and flight analysis is proposed as the next milestone in understanding mechanisms underpinning active vision and cognitive visual abilities of bees.

Abstract

Active vision, the ability of the visual system to actively sample and select relevant information out of a visual scene through eye and head movements, has been explored in a variety of animal species. Small-brained animals such as insects might rely even more on sequential acquisition of pattern features since there might be less parallel processing capacity in their brains than in vertebrates. To investigate how active vision strategies enable bees to solve visual tasks, here, we employed a simple visual discrimination task in which individual bees were presented with a multiplication symbol and a 45° rotated version of the same pattern ("plus sign"). High-speed videography of unrewarded tests and analysis of the bees' flight paths shows that only a small region of the pattern is inspected before successfully accepting a target or rejecting a distractor. The bees' scanning behaviour of the stimuli differed for plus signs and multiplication signs, but for each of these, the flight behaviour was consistent irrespective of whether the pattern was rewarding or unrewarding. Bees typically oriented themselves at $\sim\pm 30^\circ$ to the patterns such that only one eye had an unobscured view of stimuli. There was a significant preference for initially scanning the left side of the stimuli. Our results suggest that the bees' movement may be an integral part of a strategy to efficiently analyse and encode their environment.

Keywords

active vision, *Bombus terrestris*, cognitive strategy, flight analysis, scanning behaviour, visual recognition

38 Introduction

39 Bees are capable of memorising and discriminating a wide variety of visual patterns, including complex ones
40 that, for example, include different stripe orientations in each of four quadrants (Benard et al., 2006;
41 Srinivasan, 1994; Srinivasan, 2010; Stach et al., 2004; Turner, 1911; Von Frisch, 1914; Wehner, 1967). On the
42 other hand, there is a long history of observations that bees are incapable of discriminating some relatively
43 simple patterns (Avarguès-Weber et al., 2012; Hertz, 1929; Hertz, 1935; Horridge, 1996; Srinivasan, 1994; Von
44 Frisch, 1914). As one example, it was reported that honeybees (*Apis mellifera*) were not able to distinguish a
45 “plus pattern”, made up of a vertical and horizontal bar, from the same pattern rotated through 45° *i.e.*
46 multiplication symbol (Horridge, 1996; Srinivasan, 1994) in a Y-maze setup where the patterns were displayed
47 at a fixed distance from the bees’ decision point. Given the otherwise impressive capabilities of bees in
48 recognising complex visual patterns (Avarguès-Weber et al., 2011; Dyer et al., 2005; Srinivasan, 1994), the
49 difficulty in solving the plus versus multiplication sign discrimination task by bees is surprising. There is
50 evidence that the successes and failures of bees in discriminating visual patterns are not strictly related to
51 pattern complexity, but to the visual scanning procedures that bees use when examining and memorising the
52 patterns (Lehrer and Srinivasan, 1994).

53 In vertebrates, the repertoire of such active vision strategies is already well researched (Land, 1999; Land
54 and Nilsson, 2012; Yarbus, 2013). To scan visual targets, there can be large scale movement by the body or
55 head, or smaller scale movements of the eyes (saccades) (Juusola et al., 2017; Najemnik and Geisler, 2005;
56 Yang and Chiao, 2016). Such active vision is essential to obtain an accurate three dimensional representation
57 of the material world (Kagan, 2012; Martinez-Conde and Macknik, 2008; Martinez-Conde et al., 2013; Werner
58 et al., 2016). In some vertebrates, eye movements are also used as a sampling strategy, generating fine spatial
59 information and improving the encoding of high spatial frequency of natural stimuli (Anderson et al., 2020;
60 Kuang et al., 2012; Rucci and Victor, 2015). Some animals adopt a characteristic route during a visual task to
61 facilitate target recognition (Chittka and Skorupski, 2017; Dawkins and Woodington, 2000). For instance,
62 pigeons took stereotyped approach paths when learning to discriminate visual patterns (Dawkins and
63 Woodington, 2000; Theunissen et al., 2017). Interestingly, they failed at these tasks when they were prevented
64 from using their developed route. Also, characteristic head movements were observed in pigeons when
65 stabilizing the image for forward locomotion (Theunissen and Troje, 2017).

66 In insects with their miniature brains, and thus possibly more limited parallel processing, there might be an
67 even stronger need to acquire spatial information by sequential scanning than in large-brained animals
68 (Chittka and Niven, 2009; Chittka and Skorupski, 2011; MaBouDi et al., 2020; Spaethe et al., 2006). Indeed in
69 bumblebees, there is evidence that complex patterns cannot be discriminated when they are only briefly
70 flashed on a screen, preventing bees from sampling in a continuous scan (Nityananda et al., 2014).

71 Furthermore, bees exhibit defined sequences of movements in response to particular visual stimuli (Collett et
72 al., 1993; Guiraud et al., 2018; Lehrer and Srinivasan, 1994; MaBouDi et al., 2020; Werner et al., 2016).

73 Here we return to one of the pattern discrimination tasks that reportedly are challenging or impossible for
74 bees (Srinivasan, 1994) the plus versus multiplication sign discrimination task. We examine whether, and more
75 importantly, how bumblebees can solve it. By recording the bees' flight trajectories, and analysing their
76 scanning movements, we aimed to determine the strategies employed in solving this visual task, specifically
77 to investigate whether they are able to develop an active sampling strategy to solve the task if they are allowed
78 to fly as close to the patterns as they desired.

79 **Materials and Methods**

80 ***Animals and Experimental Setup***

81 Twenty bees from three colonies of bumblebees (*Bombus terrestris audax*, purchased from Agralan Ltd.,
82 Swindon, UK) were used during this study. Colonies were housed in wooden nest boxes (28 x 16 x 11 cm)
83 connected to a wooden flight arena (60 x 60 x 40 cm) via an acrylic tunnel (25 x 3.5 x 3.5 cm). The arena was
84 covered with a UV-transparent Plexiglas ceiling (Fig. 1A). Illumination was provided via high frequency
85 fluorescent lighting (TMS 24F lamps with HF-B 236 TLD ballasts, Phillips, Netherland; fitted with Activa daylight
86 fluorescent tubes, Osram, Germany); the flicker frequency of the lights was ~42kHz, which is well above the
87 flicker fusion frequency for bees (Skorupski and Chittka, 2010; Srinivasan and Lehrer, 1984). The walls of the
88 arena were covered with a Gaussian white and pink pattern (MATLAB generated); this provided good contrast
89 between the colour of the bees and the background, required for the video analysis. Sugar water was provided
90 at night through a mass gravity feeder and removed during the day when bees were performing experiments
91 to ensure motivation. Pollen was provided every two days into the colonies.

92

93 ***Stimuli***

94 The stimuli patterns were printed on laminated white discs (10 cm in diameter) to allow for cleaning (using
95 70% ethanol solution) in between training bouts and also tests. The training patterns consisted of two black
96 bars (1 x 10 cm) presented in two configurations: 1) Plus pattern: one vertical and one horizontal bar aligned
97 at their centre (\oplus). 2) Multiplication pattern: same as the plus pattern but rotated by 45° (\otimes). Additional
98 patterns were constructed for a transfer test; these only presented the top half of the training stimuli (Fig. 1B).
99 All patterns had 2 mm black margins around the outer circumference of the pattern. The centre of each disk
100 was attached to the back wall of the arena via the feeder made out of a small 0.5 ml Eppendorf tube without
101 the cap (5 mm in diameter), which contained 10 μ l of either 50% sucrose solution (w/w), saturated quinine
102 solution (0.12%), or sterilised water.

103 ***Training and test protocol***

104 Prior to the experiments, bumblebees could freely fly between the colony and a gravity feeder providing 30%
105 sucrose solution (w/w) placed in the centre of the flight arena. Successful foragers were individually marked
106 on the thorax with number labels (Opalithplättchen, Warnholz & Bienenvoigt, Germany) for identification
107 during the experiment. Marked bees were randomly selected and pre-trained to receive 50% sucrose solution
108 from eight white discs presented on the rear wall of the arena. These pre-training stimuli were 10 cm in
109 diameter with 2 mm wide black margins at the edges. After several bouts of pre-training, a forager that learned
110 to take the sucrose from the feeder at the centre of the white pattern was selected for the individual
111 experiment. During training, only the selected bee was allowed to enter the flight arena.

112 To improve the accuracy and the speed of learning, a differential conditioning protocol was used. Four
113 multiplication and four plus pattern stimuli were randomly affixed to set positions on the rear wall of the
114 arena. Each stimulus was 3-6 cm horizontally, and 5 cm vertically separated from the next stimulus, or arena
115 wall/floor/ceiling (Fig. 1B). One group of bees (n=10) was trained to receive 10 μ l 50% sucrose solution (w/w)
116 from the feeding tubes at the centre of the plus pattern stimuli, and to avoid the multiplication patterns that
117 contained 10 μ l saturated quinine solution. The second group (n=10) was trained on the reciprocal
118 arrangement, *i.e.* associate the multiplication pattern with a reward and avoided the plus pattern.

119 Bees were allowed to freely choose and feed from multiple stimuli, until they were satiated and returned to
120 their hive; empty tubes were refilled with 10 μ l of sucrose solution after the bee had left the correct stimulus
121 and made its next choice. A bout of training was completed once the bee returned to the hive. After each
122 bout, all feeding tubes were cleaned with soap and 70% ethanol and then rinsed with water. The patterns
123 were separately washed with 70% ethanol. Both tubes and patterns were air-dried in the lab before reuse.
124 The position of stimuli on the wall were randomly varied for each bout to prevent bees from using the location
125 of the reward when solving the task.

126

127 After five bouts of training the bees were subjected to two tests, to evaluate if and how bees could recognize
128 and select the correct pattern. In the first test, the learning test, bees were presented with the same
129 multiplication and plus patterns used during training; this was to verify that bees had learned to associate the
130 correct pattern with the reward, and to control for any possible olfactory cues the bees may have used during
131 training. In the second test (transfer test), the bees were exposed to novel stimuli that only presented the top
132 half of the multiplication and plus patterns (see Fig. 1B). This was to determine if the bees could still recognize
133 the 'correct' pattern based only on the top half of the patterns. As during training, both tests provided four
134 correct and four incorrect stimuli, randomly positioned on the rear arena wall. All stimuli feeding tubes were
135 filled with 10 μ l of sterilized water (*i.e.* no reward or punishment). One to two refreshment bouts of training

136 (with reward and punishment) were conducted between tests to maintain the bees' motivation. The sequence
137 of the two tests was randomly chosen for each bee.

138

139 ***Video Analysis***

140 The arena was equipped with two cameras to record all activity of bees during tests. An iPhone 5 (Apple,
141 Cupertino, USA) with 1280x720 pixels and 240 fps (frames per second) was positioned above the arena
142 entrance tunnel viewing the rear stimuli wall, filming the bee's flight in front of the stimuli wall and patterns.
143 The second camera, a Yi sport camera (Xiaomi Inc. China) with 1280x720 pixels at 120 fps, was placed on the
144 top of the rear wall orientated downward to view the stimuli. The first 120 seconds of each test were recorded
145 and analysed.

146 To analyse bees' scanning behaviours in front of the stimuli, prior to their choices, a MATLAB algorithm was
147 developed that detected the bees automatically and then tracked the centroid of the bee bodies within each
148 frame as they flew through the arena. For each frame, the algorithm subtracted a background mask image to
149 find new candidate positions of the bee using MATLAB's blob detection function. The parameters of this
150 function were set to detect the blob with the same approximate size of a bee. In addition, an elliptic filter was
151 used in the frames from the top camera to extract the bees' body orientations. We utilised the MATLAB
152 smoothing function ('filter') to exclude any erroneous data points and correct trajectories. Examples of the
153 annotated flight paths and corresponding video recordings are shown in Figure 2 and Video S1.

154 Using the first frame of each video recording, we manually specified the x, y pixel position of each of the
155 eight pattern centres (i.e. entrances to the feeding tubes). After calculating the speed of each bee at each
156 point of the trajectory, a threshold rule was applied to the trajectories close to the feeding tube positions to
157 identify if the bees had landed, labelling the decision as either a correct/incorrect accept or rejection. This
158 'landing' threshold was determined by K-means clustering (MaBouDi et al., 2020a) of all bee speeds within the
159 specified region of the feeding tubes. For further analysis of flight speeds, distances from wall, orientation,
160 inspection times, areas of interest, and heat maps, we extracted the bees' trajectory data (using the above
161 procedure) from a cylindrical region in front of each stimulus, with a diameter 12 cm around the pattern centre
162 and 2 cm out from the stimuli wall. Bespoke MATLAB algorithms were developed to calculate and plot the
163 required datasets for each of these individual stimuli analyses (see examples: Figs. 2,3). Unfortunately one of
164 the learning test videos from Group 1 (trained to plus) was accidentally recorded at just 30 fps; we therefore
165 excluded it from the above flight analysis. This video was sufficient, however, for the behavioural results of
166 the choices and rejections of the bee to be extracted.

167

168 **Statistical analysis**

169 A generalised linear mixed model (GLMM) with Binomial distribution and link function “logit” was applied to
170 the bees’ choices recorded during the training phase to evaluate the effect of colony and group on bees’
171 performance and compare the learning rate between two groups of bees. To assess the bees’ performances
172 in the tests, we analysed the proportion of correct choices for each individual bee. The proportion of correct
173 choices was calculated by the number of correct choices divided by the bee’s total choices during the first 120s
174 of the test. A choice was defined as when a bee touched a microcentrifuge feeding tube with her antennae or
175 when she landed on a feeding tube. We then applied the Wilcoxon signed rank or Wilcoxon rank-sum tests to
176 compare the bees’ responses to the learning test and the transfer test.

177 **Results**

178

179 ***Bumblebees performance in a visual recognition task***

180 We first confirmed that bumblebees, when allowed to fly as close to the patterns as they desired, could
181 perform the simple visual discrimination task of identifying and associating either a plus or a multiplication
182 symbol with a reward (sucrose solution) and the other with quinine solution (penalty, bitter taste).

183 Bees in Group 1 recognised the plus patterns as rewarding above chance after just 20 choices (Wilcoxon
184 signed rank test; $z=2.04$, $n=10$, $p=0.04$; mean=59%). Conversely, Group 2 achieved the same performance on
185 the multiplication patterns after 30 choices (Wilcoxon signed rank test; $z=2.58$ $n=10$, $p=9.7e-3$). Nevertheless,
186 there was no notable difference in the learning rate between the two groups after 30 choices ($p=0.72$), and
187 the bees’ performance was not affected by colony ($p=0.17$) (see Table S1). The bees continued to increase in
188 performance during the 70 choices of the training (per block of 10 choices, see Fig. 1C); whereupon all bees
189 achieved $\geq 92\%$ (± 7.8 s.d.) correct choice performance. The results of a generalised linear mixed model
190 (GLMM) analysis confirmed that both groups of bees had learned to select the rewarding patterns significantly
191 above chance ($>50\%$) after training (Fig. 1C, $p=3.84e-10$). Additionally, the bees’ performance in the learning
192 tests indicated that both groups of trained bees successfully learned to discriminate the plus from the
193 multiplication symbol, and vice versa (Fig. 1D) (Wilcoxon signed rank test; $z=2.82$, $n=10$, $p=4.8e-3$ for Group 1;
194 $z=2.84$, $n=10$, $p=4.5e-3$ for Group 2); again there was no significant difference between the performance of
195 two groups in the learning test (Wilcoxon rank sum test; $z=0.23$, $n=20$, $p=0.81$). The bees’ performance in the
196 leaning test was similar to that seen during the last block of 10 choices of the training phase (Wilcoxon signed
197 rank test $z=1.32$, $n=20$, $p=0.18$).

198

199 During the learning tests, 6 out of the 10 bees in Group 1 (trained to plus) initially inspected (flight within 12
200 cm diameter of centre of pattern and 10 cm out from rear stimulus wall) the correct plus pattern. However,

201 an equal number of bees in Group 2 also inspected the plus first, in their case the incorrect pattern. Therefore,
202 as a whole, the bees' pattern selection from a distance (*i.e.* from arena entrance to stimuli wall) was no
203 different to chance (50% correct initial pattern inspections; χ^2 test, Chi-square statistics=0.8, df=18; p=0.37)
204 (Fig. 1E). In addition, during all the correct initial inspections, the bees still scanned the pattern before flying
205 to the feeder tube (see Fig. S1).

206

207 ***Bumblebee flight speeds and dynamics during the learning tests***

208 To explore how bees choose the correct patterns and reject the incorrect ones, we analysed the bees'
209 inspection behaviours, employing a custom algorithm to track the bee locations and body orientations within
210 each frame of the videos (Fig. 2A, B and Video S1; See Video analysis in Method section).

211 The bees' initial flight speed upon entering the flight arena and approaching the first inspected stimuli was
212 on median 0.20 (\pm 0.13 s.d.) ms^{-1} (Figs. 2C, S1). The speed reduced to a median of 0.11 (\pm 0.10 s.d.) ms^{-1} whilst
213 in front of stimuli; the highest proportion of flight speeds was less than 0.1 ms^{-1} (Fig. 2D). Bees' speed increased
214 to a median of 0.20 (\pm 0.24 ms^{-1}) whilst traversing between the presented patterns. Bees typically scanned the
215 patterns from a distance of 10 mm to 50 mm from the stimuli (Fig. 2E). The bees spent approximately 1.5 (\pm
216 0.5 s.d.) seconds in front of a stimulus, irrespective of whether this was a plus or multiplication, or the correct
217 or incorrect pattern (Fig. 3H). The flight speed when rejecting a pattern was on average three times that of
218 when the bee accepted a pattern and flew to the feeder. However, analysis of the flight trajectories (Fig.
219 3A,C,E) shows this was due to the bee accelerating away from the current pattern to the next. Interestingly,
220 the bees showed an overall tendency to scan the patterns with their bodies oriented at $\sim\pm 30^\circ$ relative to the
221 rear stimuli wall, keeping one or other eye predominantly aligned to the stimuli during the scans (Fig. 3F).
222 Conversely, when flying between the patterns, they mostly looked forward in the direction of their motion
223 with a much wider range of flight directions relative to the rear wall (see Discussion).

224

225 ***Bumblebees scanned specific regions of the patterns prior to making a decision***

226 As the bees did not appear to be making pattern selections from a distance (Fig. S1), we further analysed the
227 movements of the bees whilst directly in front of the patterns. In most instances (Group 1 trained to \oplus : 89.2%,
228 Group 2 trained to \otimes : 87%), the bees first traversed to, and then scanned, the lower part of the patterns
229 regardless of whether the target was rewarding or aversive (Fig. 2F). Each scan led to either a landing on the
230 feeding tube (an accept) or the bee flying to another stimulus without landing (a rejection). In Figure 2F, the
231 proportion of bees selecting each region of the patterns prior to a decision (accept or rejection) are plotted
232 for each group of trained bees. The highest proportion of interest was the bottom centre of the pattern with
233 correct choices of 54.5% within Group 1, and 39.7% of correct rejections in Group 2. However, this was similar
234 to the accumulated instances of lower left corner, lower right corner and both lower corners (summed totals

235 for correct choices Group 2: 47.5%, correct rejections Group 1: 35%). It should be noted that the bees showed
236 a consistent preference for the lower left corner, described further below. These preferences can be clearly
237 seen on the heat map representation of the accumulated bee positions during scanning (Fig. 3B,D).

238

239 Bees trained on the protocol with the plus pattern as rewarding (Group 1) would typically approach the lower
240 half of the stimulus (89% of inspections). If a plus was observed they would scan the lower centre of the
241 pattern (containing the vertical bar) and then fly directly to the pattern centre to access the feeding tube (see
242 Video S2). However, if the bees observed a multiplication they would usually scan the lower left corner of the
243 pattern, containing the oriented bar of the multiplication. Of these trails, over half consisted of a single corner
244 scan before the bees rejected the patterns. A scan of the whole pattern was clearly not required: the
245 inspection of a single diagonal pattern element was sufficient to ascertain that the pattern was not a plus sign.
246 In the remaining cases the bees would traverse to the opposite lower corner, then scan the remaining oriented
247 bar before rejection (Fig. 2F). On average, only 4.5% of such inspections did the bees only scan the right corner.
248 Bees trained on the multiplication pattern (Group 2) showed a slightly different behaviour. If the bees were
249 inspecting a multiplication stimulus, they would first approach the left or right lower section of the pattern
250 (see Videos S3, S4). We still observed the same preference for the left side inspections, double that of the
251 lower right side scans. However, there were far fewer instances of bees inspecting both corners before flying
252 to the feeding tube (Fig. 2F). When Group 2 bees (trained to the multiplication pattern) encountered a plus
253 pattern they would again scan the lower centre at the base of the vertical bar (see Video S3). In contrast to
254 the Group 1 bees accepting the multiplication symbol, these bees would also, on occasion, scan the lower left
255 corner where no oriented bar was present (Fig. 3C).

256

257 To control for the possibility that the upper region of patterns may have also influenced the bees' decisions,
258 we carried out a transfer test (see Method section), in which bees were confronted with only the top halves
259 of the patterns. None of the bees were able to recognize the correct pattern element, choosing equally both
260 types of novel patterns (Fig. 2G,E) (Wilcoxon signed rank test; $z=0.17$, $n=10$, $p=0.85$ for Group 1; $z=-0.05$, $n=10$,
261 $p=0.90$ for Group 2). We therefore concluded that bumblebees learned to only use the information of the
262 lower sections of these patterns for recognition (similar to what is seen in honeybees (Giurfa et al., 1999), see
263 Discussion).

264 These results demonstrate that bumblebees are able to learn specific features within a pattern to both
265 accept and reject stimuli. In addition, for the specific paradigm used in this experiment, bees did not make
266 their stimulus selection from a distance, only choosing to visit a feeder after close inspection of the presented
267 patterns.

268

269 **Discussion**

270

271 In this study, we aimed to explore the flight characteristics and active vision underpinning a simple visual
272 recognition task in bees. Such a task was failed by honeybees (*Apis mellifera*) when they were prevented from
273 viewing the stimuli up close (Srinivasan, 1994). Our results show that bumblebees (*Bombus terrestris audax*)
274 can discriminate these symbols within our flight arena design. However, they chose to inspect both the
275 rewarding and aversive stimuli from a distance of just 1 to 5 cm before making their decisions. There was no
276 indication that the bees chose their initial stimulus from a distance when entering the arena (with 50% of
277 initial inspections at the incorrect patterns (Fig. 1E)); even with the correctly chosen stimuli, the bees always
278 performed a scan of the pattern elements before landing on the feeder (Fig. S1). Our experimental paradigm
279 cannot confirm with certainty that bumblebees are unable to discriminate these simple patterns from a
280 distance; for that we would need to control for distance as done with the honeybee experiments (Horridge,
281 1996; Srinivasan, 1994). However, our experiment allowed us to carefully analyse the bees' scanning
282 behaviour of visual features and to extract useful insights into the active vision of bees.

283

284 In brief, our bumblebees had no difficulty in learning to identify and associate either the plus or
285 multiplication signs with reward, with all bees achieving over 90% accuracy after 70 trials (Fig. 1C). This
286 performance was preserved during the unrewarded learning tests (Fig. 1D). Our bespoke video analysis toolkit
287 allowed us to track the bee positions and body yaw orientations for every frame of each learning test. The
288 most notable, and consistent, characteristics observed were:

289 **Partial pattern inspection.** The bees primarily flew to, and scanned, the lower half of the patterns (Fig. 2F).
290 This suggests that the lower half was all the bees learned. Indeed, when exposed to a transfer test with only
291 the top half of the pattern available, bees failed to identify the correct halves of the training patterns (Fig. 2E).
292 A previous study showed that honeybees (*Apis mellifera*) trained in a Y-Maze using absolute conditioning
293 (where only the positive pattern and a secondary blank stimulus is provided) assigned more importance to the
294 lower half of the pattern to that of the top half (Giurfa et al., 1999). During tests with only the top half of the
295 training pattern and a novel pattern they failed to select the correct pattern half. Conversely, if bees were
296 presented with the lower half of the training pattern and again a novel pattern they could identify the correct
297 stimulus. In contrast, when trained using differential conditioning (using both rewarded and unrewarded
298 patterns), the honeybees learned the whole pattern; correctly identifying both bottom and top half patterns
299 during tests. However, in this instance, unlike in our study, the bees' choice was recorded from a distance (for
300 apparatus details, see (Horridge, 1996)) and bees' flights were not analysed systematically.

301 In a more recent study, in which the flight path of bees was also analysed, (Guiraud et al., 2018)
302 showed how honeybees (*Apis mellifera*) can solve a conceptual learning task of 'above and below' by scanning

303 the lower of two pattern elements presented on the stimuli; this provided sufficient information for the bees
304 to make a decision without needing to understand, or inspect, the relationship between the top and bottom
305 pattern elements.

306 ***Initial side preference.*** The bees had a significant preference for initially scanning the left side of the
307 multiplication pattern (Fig. 2F). This left side preference for visual objects, known as pseudoneglect, is also
308 seen in humans (Jewell and McCourt, 2000), and birds (Diekamp et al., 2005; Rugani et al., 2015). This
309 preference may allow an individual to always start its inspection of a stimulus at the same location, allowing
310 for consistent learning and recognition of natural stimuli; but it remains a curiosity as to why the left
311 preference was so prevalent amongst the bees tested (Fig. 2F). In humans and birds this lateralisation of spatial
312 attention may have evolved once in a common ancestor (Diekamp et al., 2005). However, since the visual
313 system of insects evolved largely independently from that of vertebrates, the left-side bias must have emerged
314 by convergent evolution. Its computational neural advantages in bees or vertebrates (if any), is not known.

315 ***Common body orientation during scans.*** The yaw orientation of the bees' bodies was most often at $\sim\pm 30^\circ$
316 to the stimuli during pattern inspections. In this manner, one or other of the bees' eyes would face the pattern,
317 with only a small proportion of the opposite eye having visual access to the pattern. There was no overall
318 preference for the left or right eye (with median orientations at $\sim -33^\circ$ and $\sim +27^\circ$ respectively) during scans
319 (Fig. 3F). In our previous modelling work (Roper et al., 2017), we showed that lateral connections from both
320 the left and right lobula to the bee mushroom bodies allowed for better pattern recognition during partial
321 occlusion of stimuli. However, this came at the expense of fine detail recognition. Therefore, and
322 counterintuitively, having one eye mostly obscured from the pattern may provide the mushroom bodies
323 (learning centres of the bee brain) with more distinct neural inputs. It may also allow bees to learn both the
324 pattern and location cues simultaneously whilst scanning a resource. Future work will be needed to see if this
325 behaviour is particular to the patterns used in this experiment, or a stereotypical behaviour.

326 ***Commonality in scan strategies is based on stimuli, not protocol.*** It may seem sensible, from the bees'
327 perspective, if trained on plus, only to inspect the lower centre of the pattern for the vertical bar. However,
328 both groups of trained bees initially approached and scanned the plus and multiplication in the same manner,
329 typically checking the lower left corner of the multiplication sign and the vertical bar of the plus. This might
330 suggest that the bees did not learn the relative position of the cues and simply searched for the first visual
331 item at the lower left of the pattern. However, the flight tracking analysis conflicts with this hypothesis, with
332 the inspection of the multiplication occasionally consisting of a scan of the adjacent bar of the multiplication,
333 and with the group trained on multiplication, after scanning the vertical bar of the plus they occasionally flew
334 to the lower left corner to presumably check for the multiplication signs oriented bar. We therefore assume
335 that the stimulus is directing the scanning behaviour of the bee, and in turn the bee is learning both rewarding

336 and aversive pattern features during training. Other experiments will be required to ascertain the particular
337 rules which dictate the bees' flight manoeuvres based on the 2D and 3D stimuli provided.

338 In the pioneering works of Karl Von Frisch, free-flying bees were trained to find sugar reward on certain black
339 or coloured patterns placed horizontally on a white background (Von Frisch, 1914). Later studies showed that
340 bees only used local cues corresponding to their approach direction when the stimuli were presented to them
341 horizontally (Wehner, 1967). Since bees were not able to capture global shapes, this might be the reason bees
342 could only recognise some simple patterns in the early studies. However, vertical presentation of stimuli was
343 developed to examine what diversity of visual features bees may use, such as orientation (Van Hateren et al.,
344 1990), radial or, bilateral symmetry (Giurfa et al., 1996; Horridge, 1996), or spatial frequency and ring-like
345 structures (Horridge and Zhang, 1995). To control the decision distance and understand which cues were
346 utilised by bees to recognise the target pattern, the Y-maze was introduced (Srinivasan and Lehrer, 1988).
347 Previous research has shown that honeybees and bumblebees can solve visual tasks by extracting the localised
348 or elemental features within the pattern (Giurfa et al., 1999; Guiraud et al., 2018). Bees may use different
349 parts of a stimulus to discriminate between correct and incorrect patterns, depending on the training protocol
350 employed or the specific patterns presented (Giurfa et al., 1999; Stach and Giurfa, 2005). Although the Y-maze
351 enabled researchers to control the cues that bees could see when making decisions about visual patterns from
352 a distance, it is a less useful paradigm to inspect the scanning strategies used by bees. Therefore, despite
353 several decades of research in bee vision, it is still debated why, and how, bees fail to recognise some simple
354 patterns while they show excellent recognition in other complex patterns (Avarguès-Weber et al., 2011; Dyer
355 et al., 2005; Srinivasan, 2010). We therefore used an experimental setup in which the bees' flight and scanning
356 behaviour could be examined while they were close to the targets that were to be discriminated.

357 In this study we showcase a new suite of tools for automatic video tracking of bees in free flight and during
358 their scanning manoeuvres, as well the algorithms needed to analyse and visualise the large amount of
359 positional and orientation data this tracking produces. In our previous work on 'above and below' conceptual
360 learning (Guiraud et al., 2018) we had to manually view and annotate 368 hours of video footage (46 hours of
361 video footage taken at 120 fps, watched at 1/8th speed). In contrast, here the only manual process was
362 providing a mask frame (without the bee present) per test, and marking the feeder positions within that frame.
363 With only a small number of test videos to process this was not an issue, but even here, recent advances in
364 making convoluted neural networks for pattern recognition accessible to non-programmers
365 (playground.tensorflow.org, runwayml.com), as well as the more research programmer-centric DeepLabCut
366 (Nath et al., 2019), allows researchers to provide a few dozen labelled mask frames and have these systems
367 process thousands of mask images for all the other videos (Egnor and Branson, 2016). Similarly, the ability to
368 visualise either individual flight paths (Fig. 3A) or combined heat maps of positional data (Fig. 3B) allowed us

369 to quickly identify behavioural aspects of interest. Histograms of velocity, distance and orientation can be
370 quickly generated, but more importantly the parameters defining the areas of interest can be modified and
371 processed in a matter of minutes. Previous studies have relied upon binary fixed decision lines (Avarguès-
372 Weber et al., 2012; Horridge, 1996; Horridge and Zhang, 1995; Srinivasan and Lehrer, 1988), with
373 experimenters manually recording these limited behavioural data. Our in-depth analysis on such a
374 straightforward pattern recognition task highlighted key behavioural characteristics, which can now influence
375 future work on active vision, this simply would not have been viable without these automated tools.

376

377 **Acknowledgements**

378 We thank Olivia Brookes for her help in collecting the preliminary data.

379

380 **Competing Interests**

381 All authors declare no conflict of interest.

382

383 **Funding**

384 This study was supported by the Human Frontier Science Program (HFSP) grant (RGP0022/2014), and the
385 Engineering and Physical Sciences Research (EPSRC) Programme Grant Brains on Board (EP/P006094/1).

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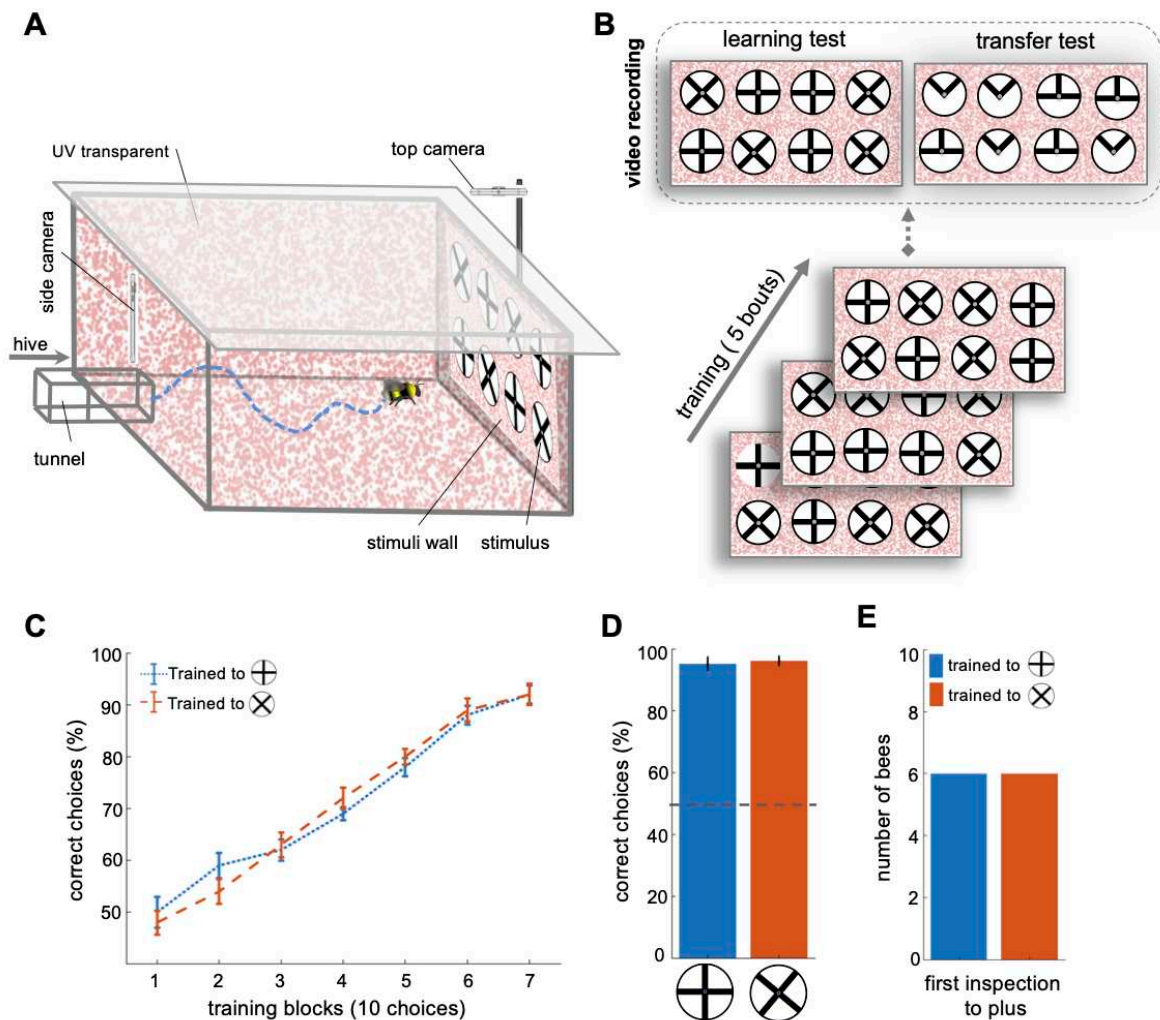
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486 **Figure legends**

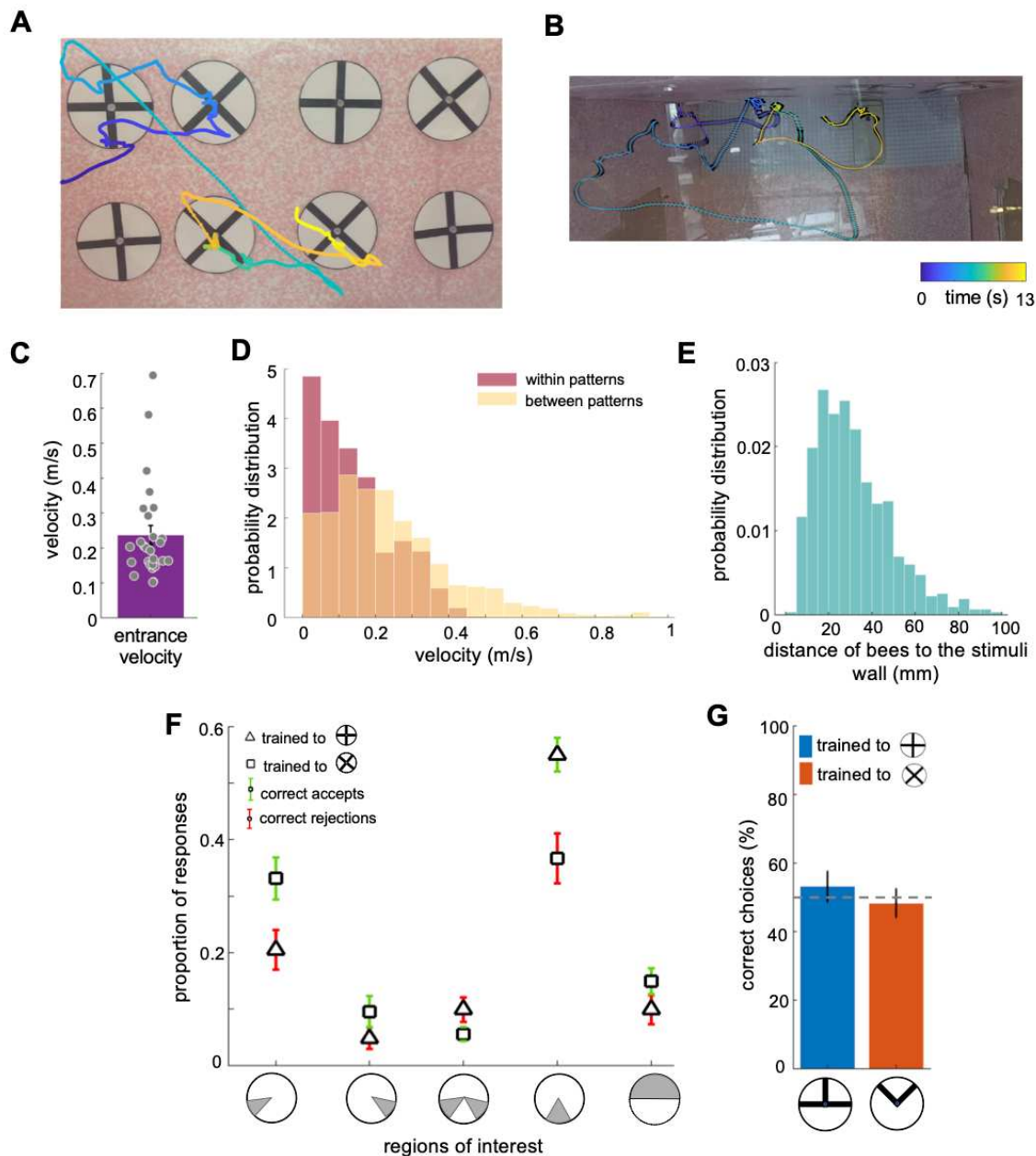


487

488 **Figure 1. Bees' performance in a pattern recognition task.** (A) Schematic view of the flight arena. During the
 489 training and test experiment, an individual bumblebee was allowed to enter the flight arena through the
 490 tunnel. She then learned to fly toward the rear wall where eight patterns were displayed. She gradually learned
 491 to recognize the correct pattern (a plus or multiplication depending on the protocol) containing a sucrose
 492 solution in its centre. The flight arena was equipped with two cameras to record the bees' flight dynamics. The
 493 side camera was placed on the top of the entrance viewing the rear wall and the top camera was placed at the
 494 top of the rear wall to record bees' flight from above. (B) Training and testing protocol. The training and test
 495 patterns were constructed from 10 cm diameter white discs with 2 mm margins. Two black shapes, plus and
 496 multiplication, were presented to the bees during the training. Each pattern was attached via its centre to the
 497 rear wall of the flight arena by a microcentrifuge tube (5 mm diameter). Each bee was subjected to five training
 498 bouts, in which she entered the flight arena and was presented with eight patterns (4 plus and 4 multiplication
 499 symbols). One group of bees (n=10) was positively reinforced with the plus containing 10 µl of sucrose
 500 (w/w) and negatively reinforced with the multiplication containing 10 µl quinine. Another group (n=10) was

Active scanning strategy in bumblebees

501 trained on the reciprocal protocol. The bees were free to sample the rewarding and unrewarding patterns
502 multiple times (refilled after departing), and return to the nest box when satiated. The place of patterns was
503 randomly changed before each bout of the training phase. Following training, each bee's performance was
504 examined with two tests; here the positive and negative reinforcement were replaced with sterile distilled
505 water. In the learning test, the bee was presented with the same patterns as during training. In the second
506 test, the bee was confronted with the novel patterns that only displayed the upper half of the plus and
507 multiplication symbols to the bees. One or two refreshment training bouts were used between tests to keep
508 the bee motivation high. Bees' flight paths were recorded for the initial 120 seconds via the two cameras. **(C)**
509 The learning curves of two groups, blue: Group 1 (trained to plus rewarding), orange: Group 2 (trained to
510 multiplication rewarding). Both groups of bees similarly learned to distinguish between patterns. **(D)** The
511 performance of bees during the unrewarded learning test shows that all bees had successfully learned to
512 distinguish between patterns ($p < 4.8e-3$). **(E)** The number of first pattern inspections, upon entering the flight
513 arena, that were of the plus symbol. The bees did not fly to the correct pattern from a distance (> 5 cm) more
514 than chance (accumulated results: 10/20 correct initial visits). Blue: Group 1 (trained to plus rewarding),
515 orange: Group 2 (trained to multiplication rewarding).
516



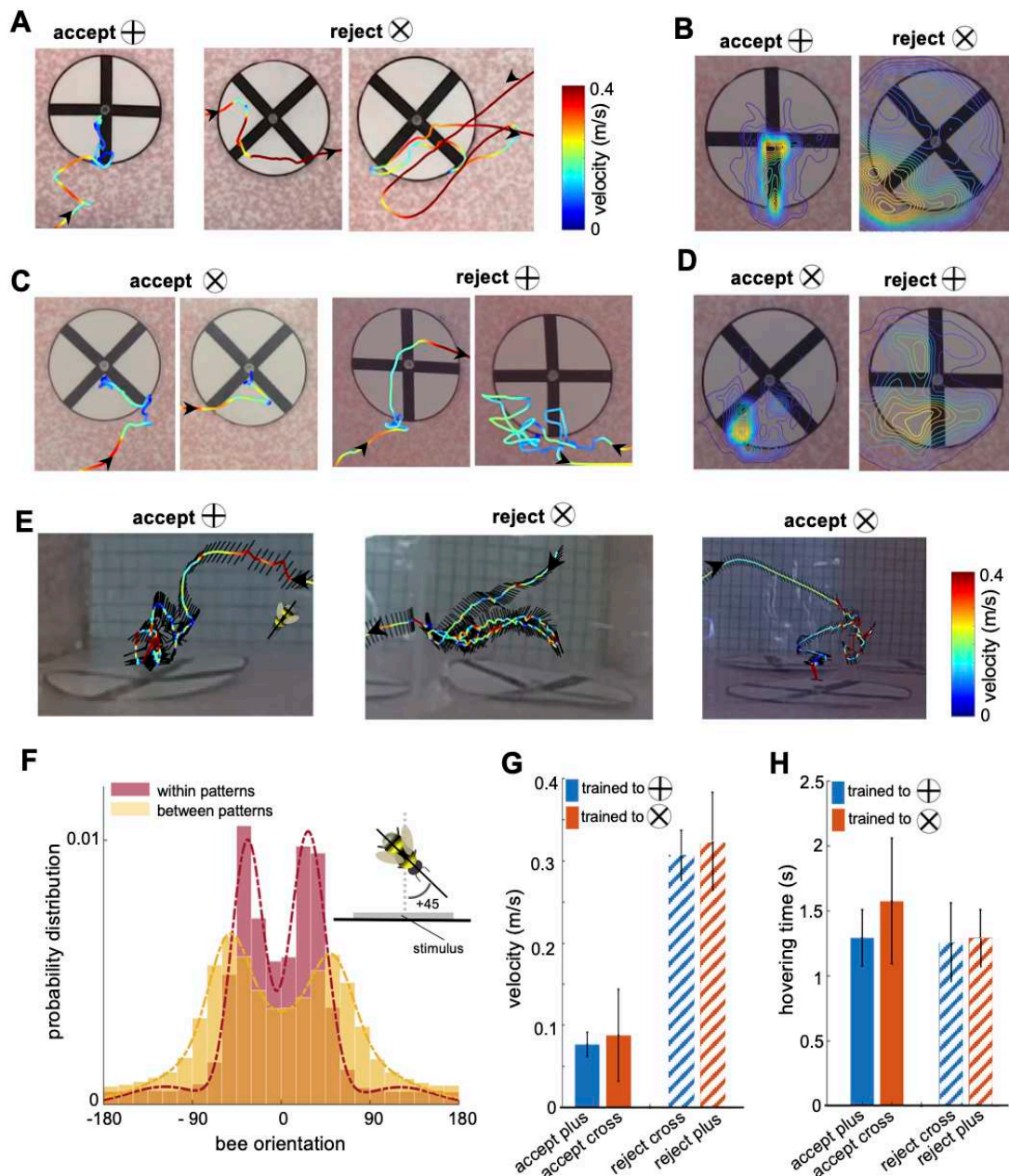
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519 **Figure 2. Bees' flight analysis demonstrates an efficient strategy of scanning in bumblebees** (A) An example
 520 of a flight path showing the activity of a bee during part of the learning test; presented bee trained to select
 521 the plus and avoid the multiplication. Each point on the flight path corresponds to a single video frame with
 522 an interval of 4 ms between frames which was recorded from the front camera (left panel) and top camera
 523 (right panel). The bee sequentially inspected each pattern, correctly landed on multiplication and avoided the
 524 plus. The colour map changes from blue to the yellow with increasing time (See Video S1). The black lines in
 525 the left panel exhibit bee's body yaw orientations. (B) Distribution of average entrance flight speed toward
 526 the wall in the learning tests (See Fig. S1). Filled dots: speed of each individual bee. (D) Probability distribution
 527 of the bees' speed in two conditions; when they were inspecting patterns (Red), and when they were flying
 528 between patterns (Yellow). This indicates that they scanned patterns slower when they accepted them than

Active scanning strategy in bumblebees

529 when they flew to another pattern. **(E)** Probability distribution of the bees' distance from the stimuli wall whilst
530 inspecting patterns. **(F)** From video analysis, the proportion of scanned regions (mean \pm s.e.m.) of bees'
531 inspections before the correct accept or correct rejection (x-axis: regions of interest are highlighted in grey).
532 Triangles: inspection proportion (mean \pm SEM) of Group 1 bees (trained to plus rewarding), squares: Group 2
533 bees (trained to multiplication), green error bars: correct accepts; red error bars: correct rejections. **(G)** The
534 performance of bees during the novel test; bees equally chose both upper half-patterns ($p>0.85$),
535 demonstrating that they did not learn the upper half of the patterns during training. Grey dashed lines=chance
536 level (50%). Bar: mean performance (error bars: \pm s.e.m.).



537

538 **Figure 3. Bee scanning strategy in a pattern recognition task.** (A) The flight paths of one example of
 539 acceptance and two examples of rejection behaviours of a bee trained to plus; the bee accepted the plus
 540 pattern after scanning the lower half of the vertical bar, while she rejected the multiplication pattern after
 541 scanning one or both diagonal bars. Line colour: flight speed 0.0 - 0.4 ms⁻¹ (See Videos S2, S3). (B) Group 1
 542 (trained to plus) probability maps (heat-maps) of bees' locations per frame in front of plus and multiplication
 543 type stimuli during all learning tests. The yellow colours show most visited regions. (C & D) same analysis as
 544 A, B for Group 2 bees trained to discriminate multiplication from plus. This indicates that bees typically
 545 scanned the lower half of the pattern with a lower speed to Group 1 bees, prior to their decisions (See Videos
 546 S4, S5). (E) Three examples of bees' flight paths shown from the top camera; black lines show bees' body
 547 orientation during the flight, and arrows designate the start and ending time of scanning. (F) probability

548 distribution of the bees' body yaw orientation perpendicular to the rear stimuli wall in two conditions: when
549 they were inspecting patterns (red) and when they were flying between patterns (yellow). Inset figure exhibits
550 one example of bee's orientation with $+45^\circ$. This suggests the bees viewed the patterns at a median $\sim\pm 30^\circ$
551 whilst scanning, with one or other eye having a predominant view. On the other hand, when they flew to
552 another pattern the body orientation was more parallel to the flight direction with a wider distribution of
553 orientations relative to the stimuli wall, resulting in a median of $\sim\pm 50^\circ$ perpendicular to the stimuli wall. The
554 dashed lines show the Gaussian mixture distribution models were fitted to each distribution (flights within
555 patterns: $\mu_1 = +27, \mu_2 = -33$; flights between patterns: $\mu_1 = +51, \mu_2 = -55$). **(G)** mean flight speed (\pm
556 s.e.m.) of scanning flight prior to decisions (accept and rejection) for both groups of bees. Blue: Group1
557 (trained to plus); orange: Group 2 (trained to multiplication). **(H)** inspection time (i.e. the time spent hovering
558 in front of a pattern) for each symbol type for both groups of bees; inspection times of bees in front of both
559 pattern types were equal regardless of their decision or training protocol.

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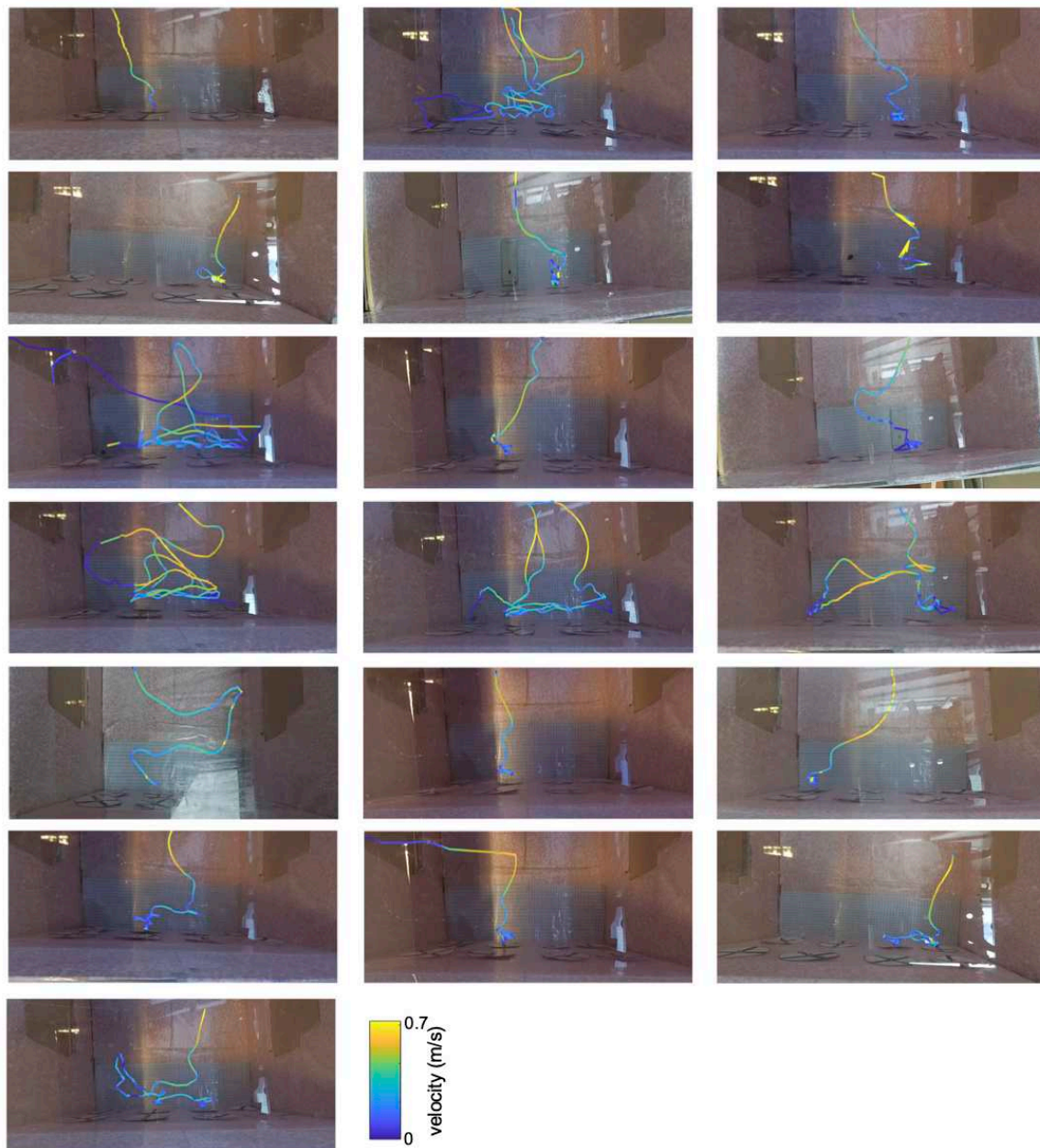
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563

564 **Appendices**

565 **Supplementary Figures**



566

567 **Figure S1. Bees' flight paths upon first entering the arena during learning tests.** In ten instances the bees
568 initially inspected the correct stimulus, scanned the pattern and visited the feeder. In the remaining nine flights
569 the bees initially inspected the incorrect pattern, then rejected the pattern and flew to another, usually
570 adjacent pattern. One video is missing where the footage was only recorded at 30 fps; this bee initially
571 inspected the incorrect pattern, and again rejected the stimulus. The bees' first inspection appears to be
572 random with 50/50 correct pattern selections from the arena entrance; this suggests bees have to scan the
573 stimuli before making decisions. Line colour from blue to yellow: flight speed 0.0 - 0.7 ms⁻¹.

574

A

UV transparent

top camera

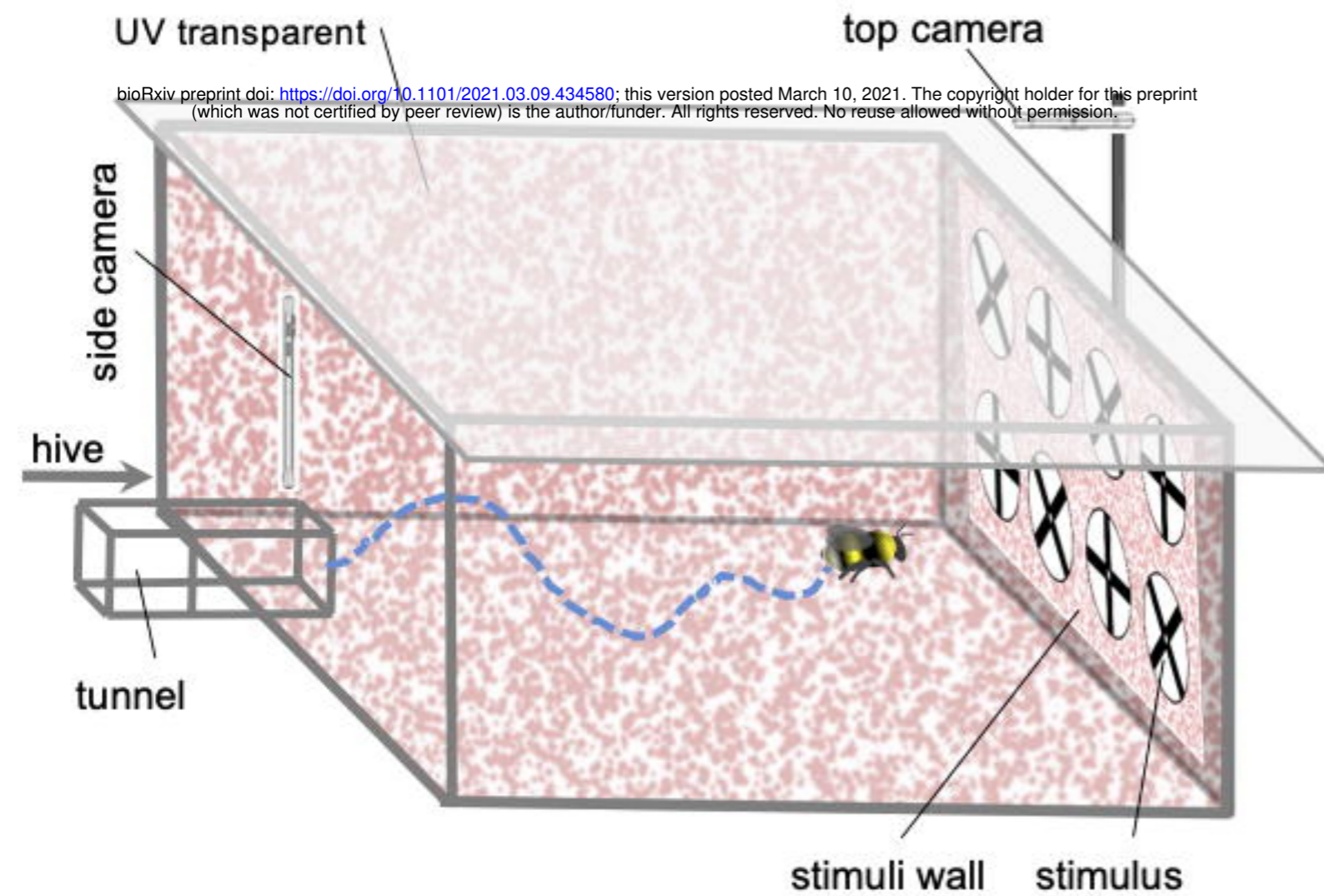
bioRxiv preprint doi: <https://doi.org/10.1101/2021.03.09.434580>; this version posted March 10, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

side camera

hive

tunnel

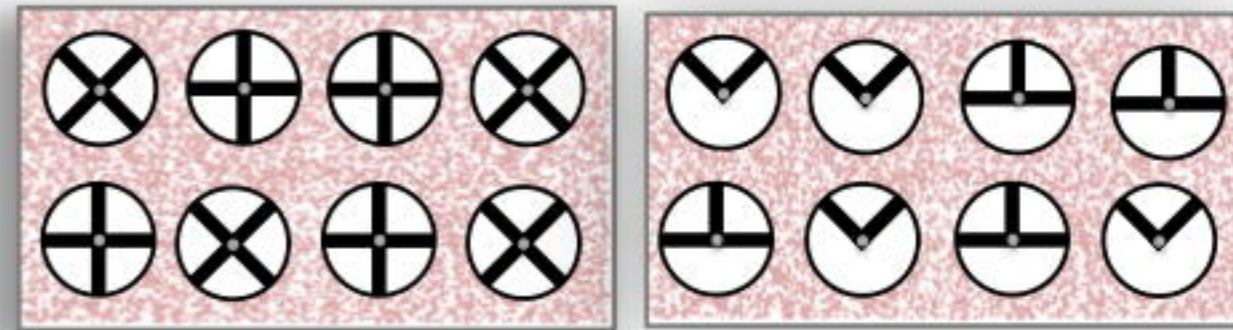
stimuli wall stimulus

**B**

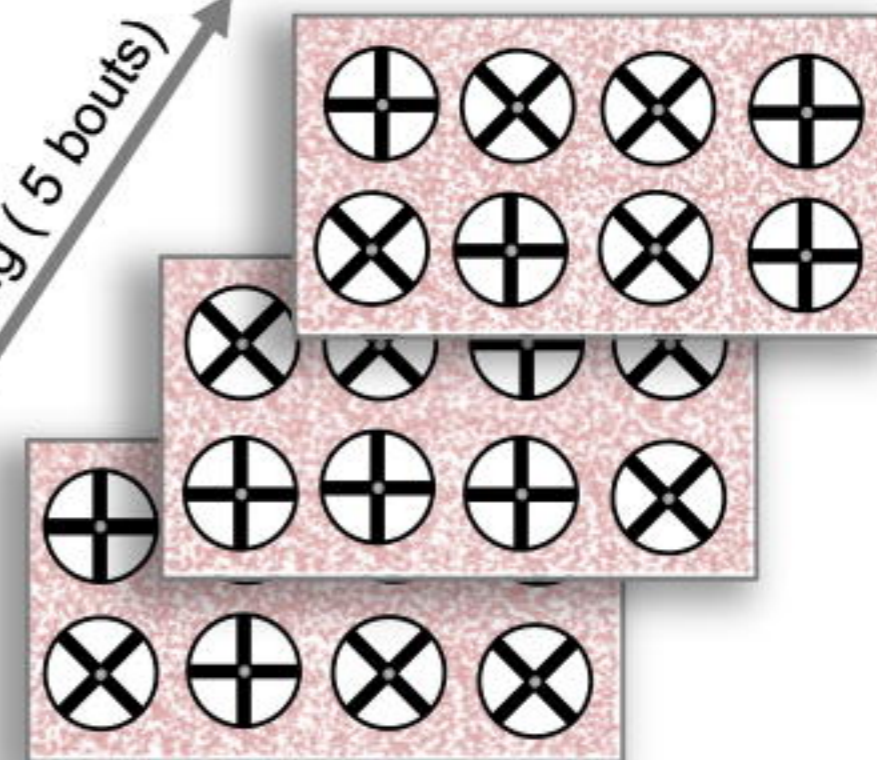
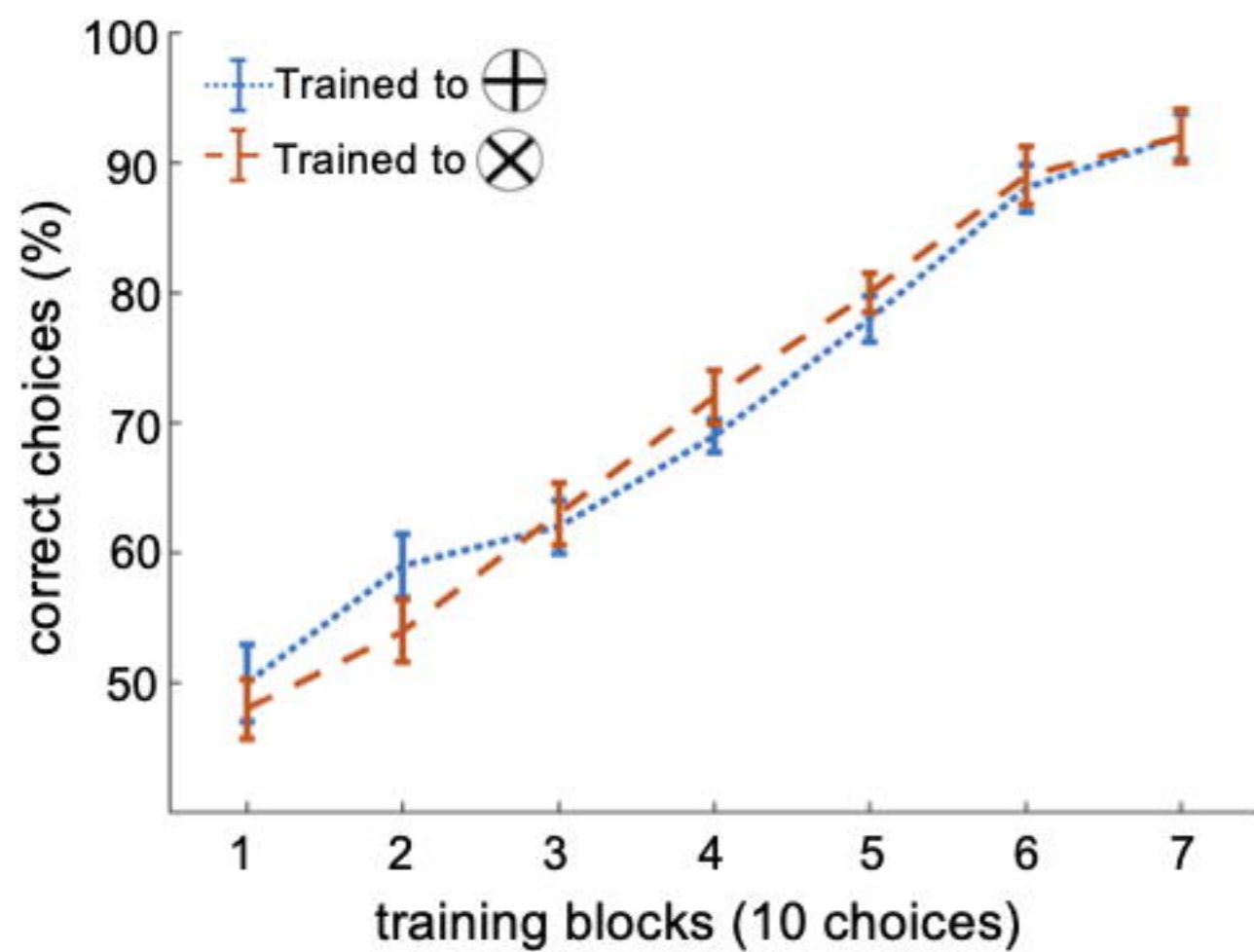
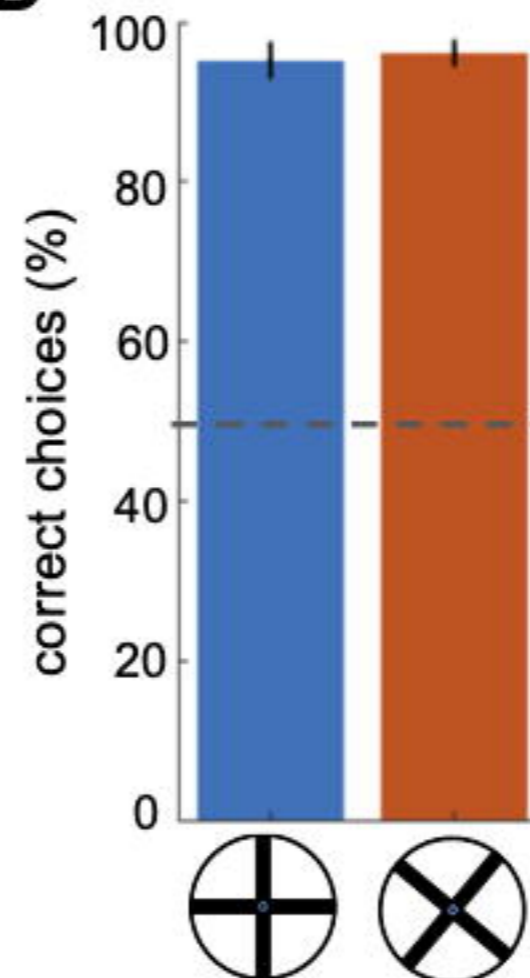
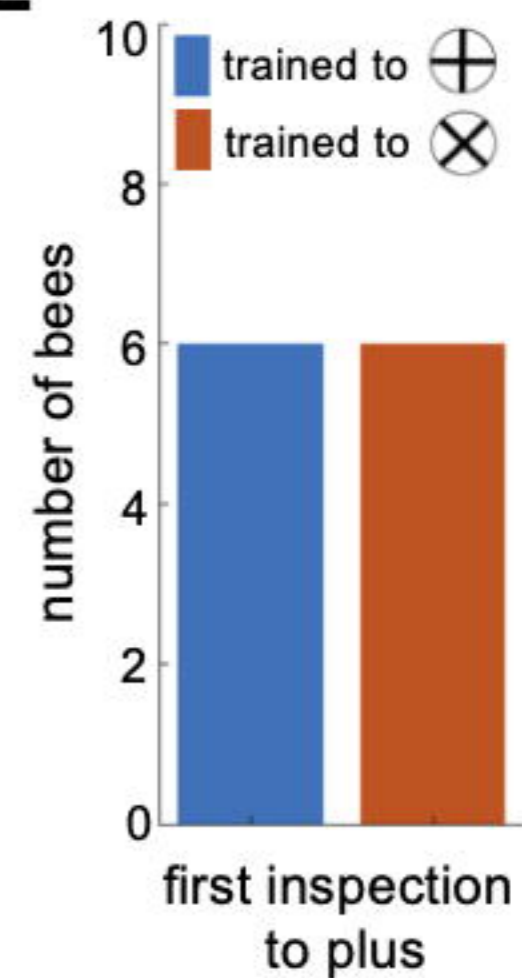
learning test

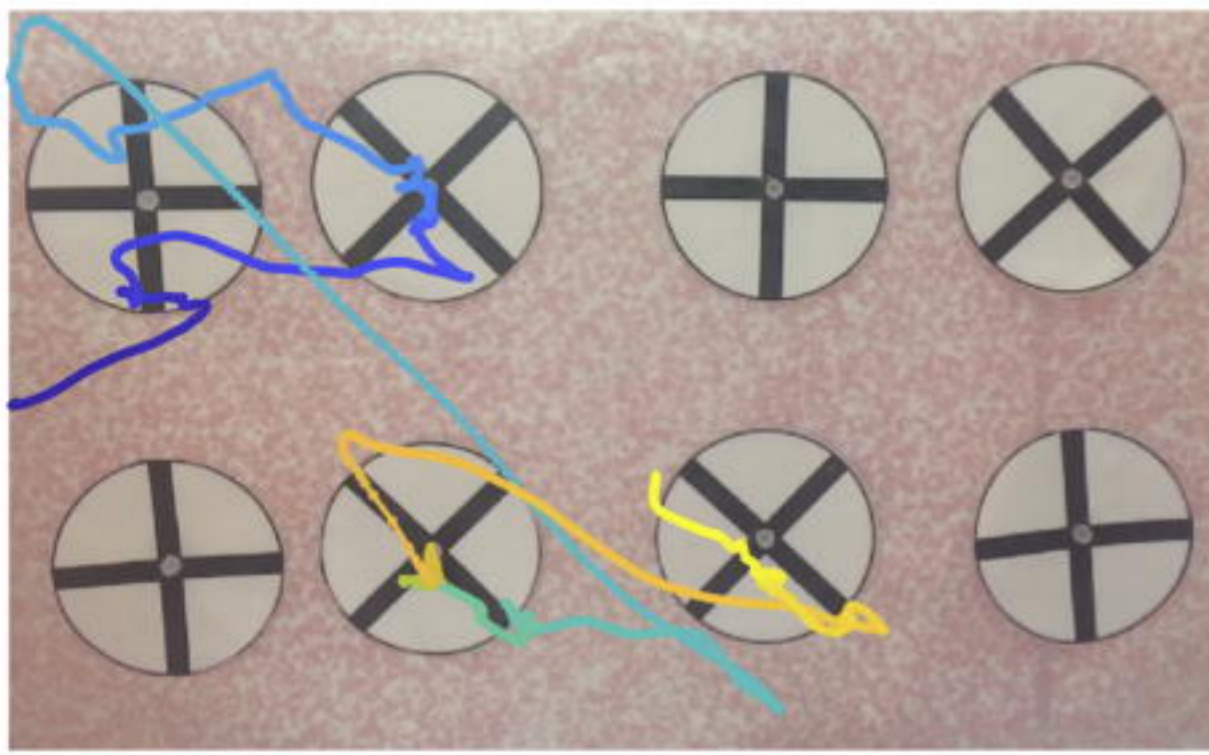
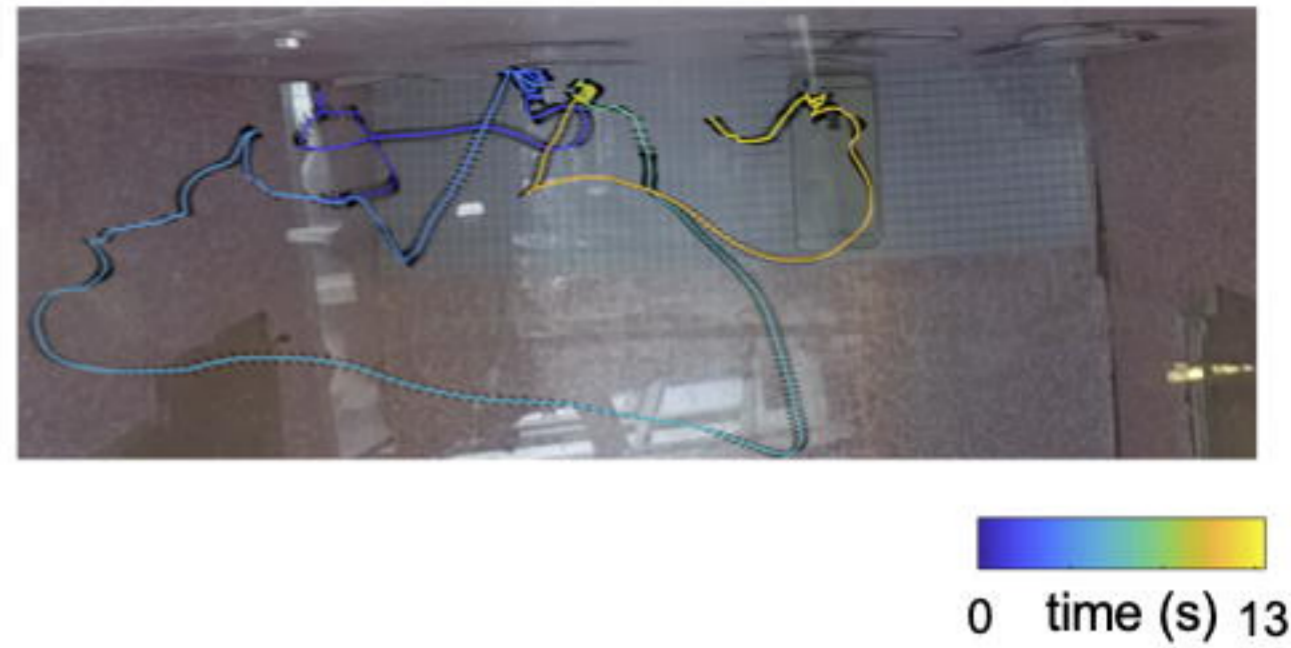
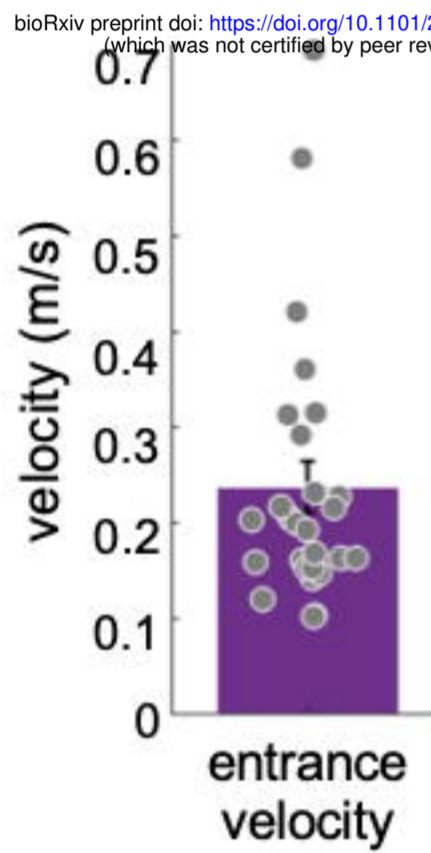
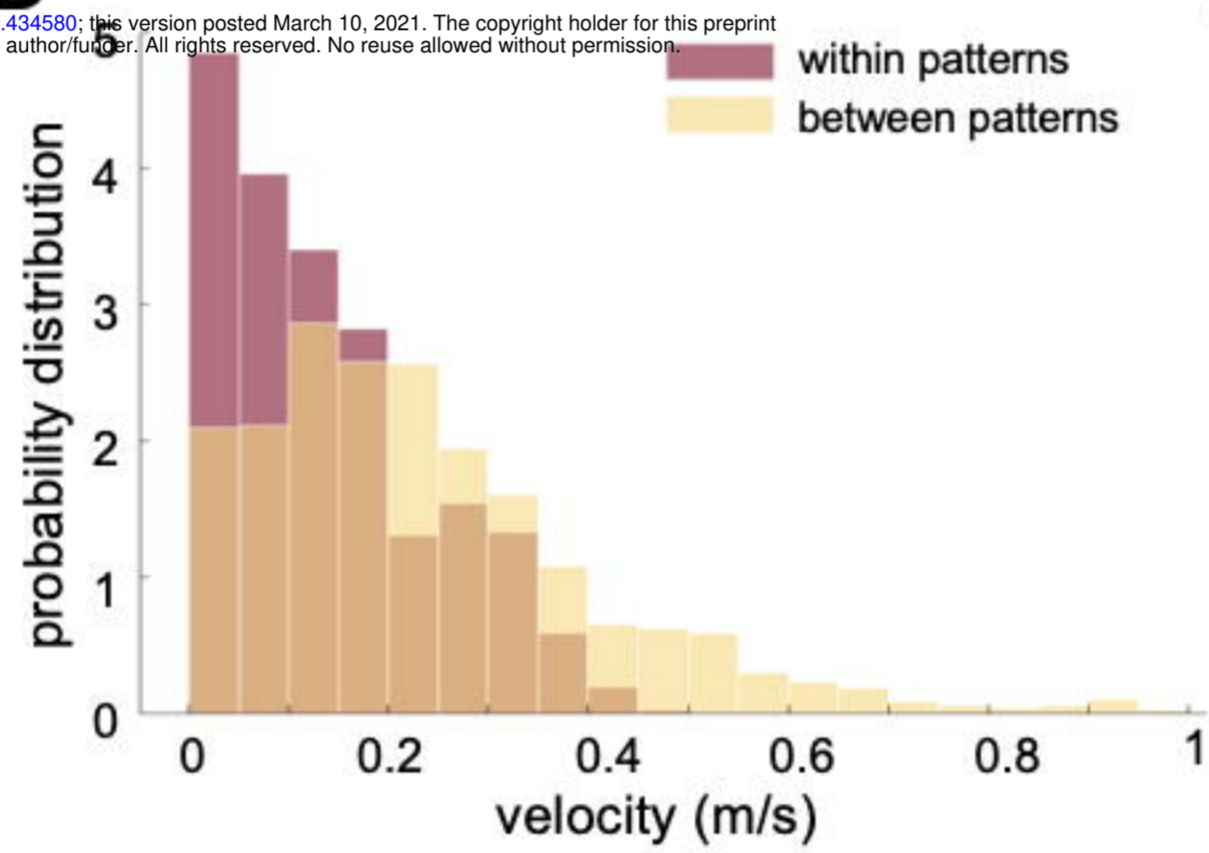
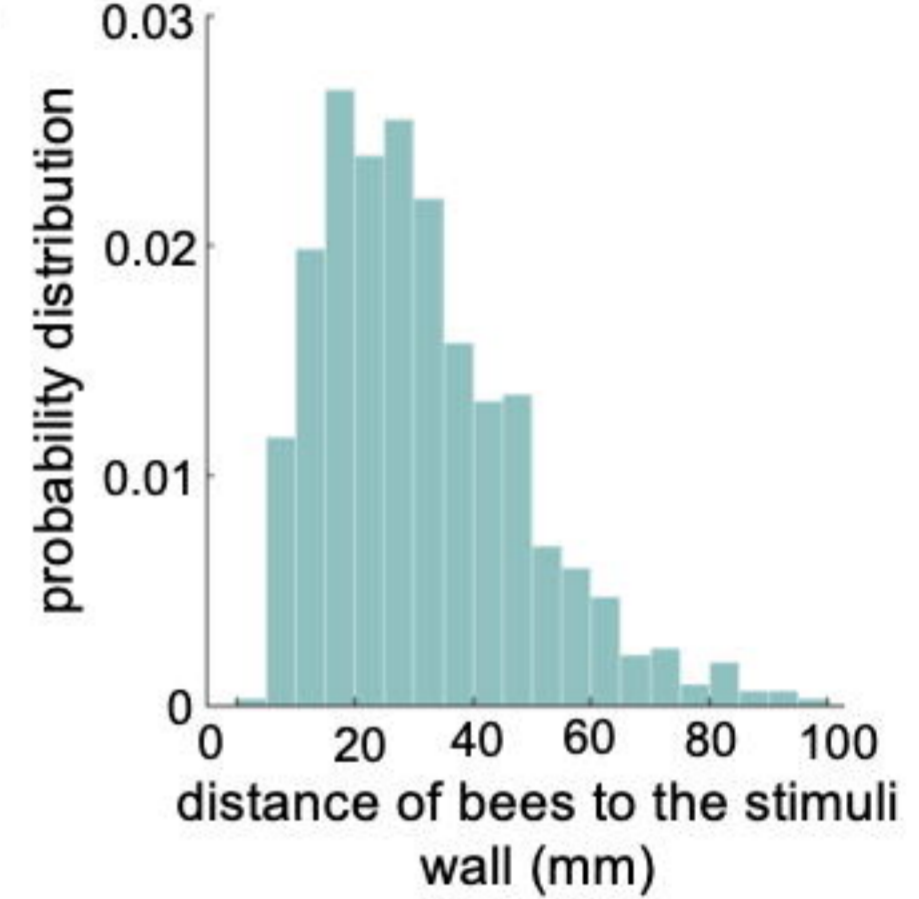
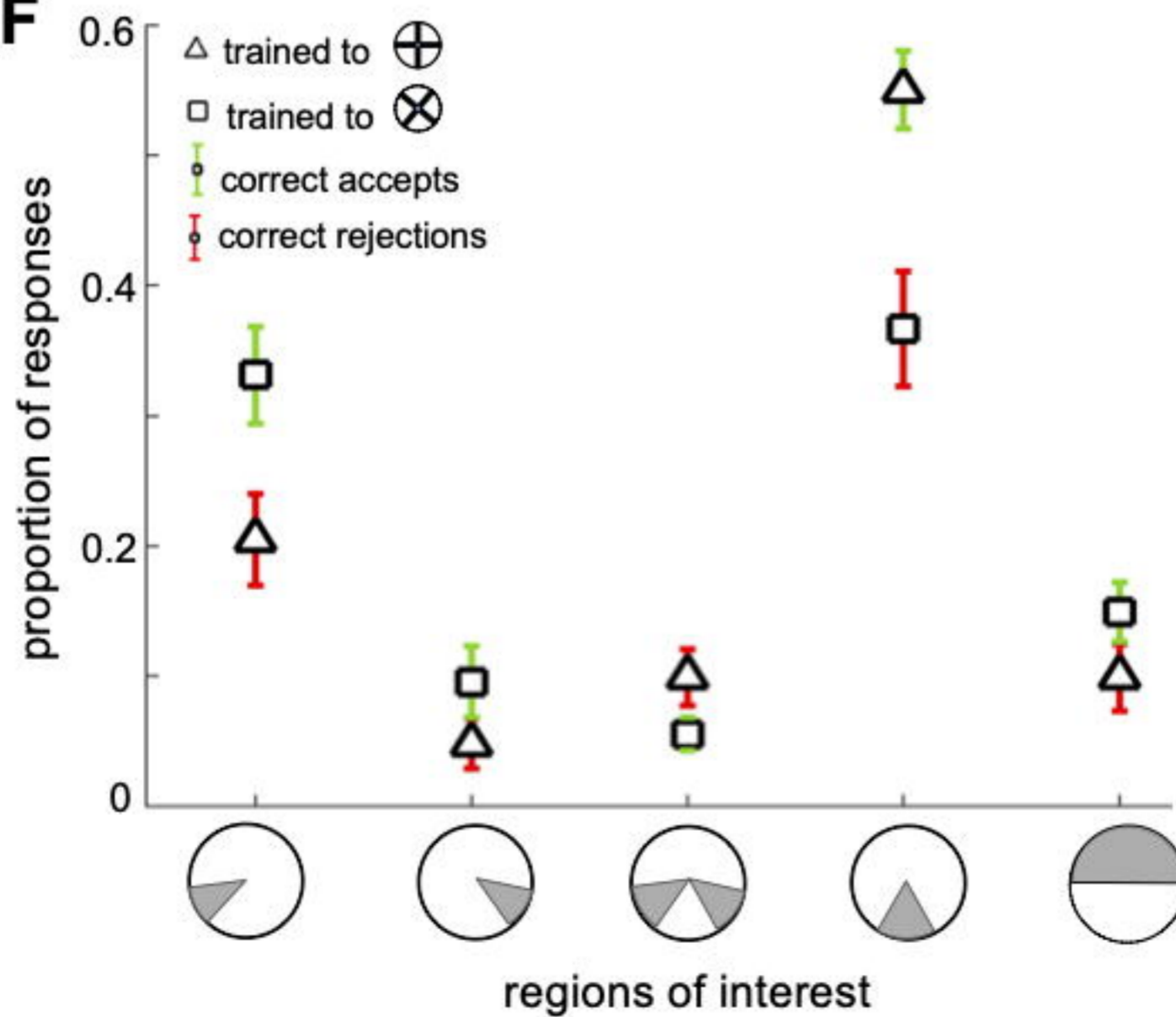
transfer test

video recording



training (5 bouts)

**C****D****E**

A**B****C****D****E****F****G**