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1 Octopamine and dopamine mediate waggle dance following
2 and information-use in honeybees

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26 **Abstract**

27 Honeybees can be directed to profitable food sources by following waggle dances performed
28 by other bees. Followers can often choose between using this social information or relying on
29 memories about food sources they have visited in the past, so-called private information. While
30 the circumstances that favour the use of either social or private information have received
31 considerable attention, still little is known about the neurophysiological basis of information-
32 use. We hypothesised that octopamine and dopamine, two biogenic amines with important
33 functions in reward signalling and learning, affect dance use in honeybees. We orally
34 administered octopamine and dopamine when bees collected food at artificial feeders and tested
35 if this affected interest in dance information about a new food source. We predicted that
36 octopamine reduces interest in dances and strengthens private information use *via* an increase
37 in the perceived value of the previously exploited resource. Since dopamine has been shown to
38 lower reward perception, we expected it to act in the opposite direction. Octopamine treated
39 foragers indeed followed 32% fewer dances than control bees and increased the use of private
40 information. Dopamine treated bees, on the other hand, followed dances 15% longer than
41 control bees, but surprisingly did not use social information more. Overall, our results suggest
42 that biogenic amine signalling affects interactions among dancers and dance followers and,
43 thus, information flow about high quality food sources.

44

45

46 **Introduction**

47 Social learning is learning that is influenced by other individuals or their products, either
48 through observation or interaction [1,2]. Honeybees, *Apis spp.*, use a unique form of social
49 learning, the waggle dance communication [3–7]. During their waggle dances, dancers attract
50 hive mates and provide them with information about the location and odour of a food source
51 [3,6–10]. Experienced foragers can decide to follow dances and decode this vector information
52 (social information) or to revisit food sources they remember from previous foraging trips
53 (private information) [11–16]. The dance follower's interest in social information can be
54 gauged by the number of waggle runs followed, with bees that decode waggle dances following
55 more waggle runs [11,13,14]. A third strategy, called scouting, is to ignore both social and
56 private information about foraging locations and search for a new food source independently
57 [6,7,17]. Empirical and theoretical studies suggest that the benefits of independent exploration,
58 social information and private information depend strongly on the spatiotemporal distribution
59 of food sources [18–22].

60 While social information-use has been studied extensively from a behavioural
61 ecological perspective [2,17,23–25], less is known about the molecular and neurophysiological
62 basis of the decision to use social *vs.* private information. Previous research suggests that, in
63 honeybee foragers, the perception of rewards is likely to play an important role in the use of
64 social and private information. When foragers experience that their food source is no longer
65 rewarding, they increase their dance following and social information use [13,14], whereas
66 foragers that experienced higher quality rewards in the past use private information more [26].
67 Likewise, when foragers exploit more distant and, thus, less profitable food sources, they are
68 more likely to use social dance information [16]. This suggests that neurophysiological
69 mechanisms of reward perception play an important role in the decision to use waggle dance
70 information *vs.* private information. Octopamine (OA) and dopamine (DA) are biogenic amines
71 that function as neuromodulators in the central nervous system of invertebrates and they play

72 important roles in reward signalling in honeybees [27–31]. They bind to specific membrane
73 proteins mainly belonging to the family of G-protein-coupled receptors in different parts of the
74 brain [32–35], such as the mushroom bodies and the antennal lobes, *i.e.* brain areas with
75 important functions in the processing and integration of information [36–38]. OA mediates the
76 reward information during reward learning and, if administered to honeybees, increases the
77 responsiveness of bees to sucrose [29,31,39,40] and to olfactory stimuli [29,30,41,42].
78 Additionally, oral or topical treatment of foragers with OA increases the motivation to perform
79 waggle dances, most likely by increasing the perceived value of rewards [27]. Interestingly,
80 some instances of OA signalling in the *Drosophila* mushroom bodies require DA neurons
81 [43,44]. In honeybees, DA has been found to reduce the response to sucrose rewards and
82 conditioned olfactory stimuli [29–31]. DA has various other effects, e.g. on avoidance learning
83 [45], scouting [46] and locomotion [47], which could directly or indirectly affect waggle dance
84 communication and the use of private information.

85 We hypothesized that OA would reduce the use of new social information and
86 strengthen the use of private information by increasing the perceived value of a currently
87 exploited food source. As a result, we expected a decrease in the interest in waggle dances by
88 OA-treated foragers. DA effects are more difficult to predict since DA signalling seems to also
89 complement OA signalling in *Drosophila* during reward learning [43,44]. But due to the
90 contrasting effects of DA on sucrose responsiveness and extinction in honeybees, we suspected
91 that treatment with DA reduces the use of private information about previous foraging sites and
92 increase interest in waggle dances advertising new food sources. To test these predictions, we
93 trained bees to collect sucrose solution with or without biogenic amines and then exposed these
94 foragers to dances for an alternative, unknown food source. We quantified the interest of trained
95 foragers in these alternative dances and recorded whether they used private information or
96 social information provided by the dance when deciding which feeder to visit.

97

98 **Materials and Methods**

99 Experiments took place from August to October 2016. We used three colonies (H1-H3) of *Apis*
100 *mellifera carnica* housed in glass-walled observation hives in a hut situated on the campus of
101 the University in Mainz, Germany. The colonies consisted of 2000-3000 workers, a queen,
102 brood, pollen and honey reserves.

103

104 *(a) Experimental procedure*

105 One hive at a time was studied and two trials per hive were performed (one with OA, one with
106 DA; 6 to 14 days between the two trials. The order of the trials was randomised for each hive).
107 Each trial lasted 3-4 days and consisted of 1-2 days of training, followed by a treatment day and
108 the test day. We used standard training procedures [7] to simultaneously train two groups of
109 50-60 foragers to two feeders (unscented 0.8 M sucrose solution – a sugar concentration that
110 induced bees to perform waggle dances) at a distance of 150 m from the hive and 7 m from
111 each other (Fig. 1). One group was trained to a feeder with a blue underlay (TF_a) and the other
112 group to a feeder with a yellow underlay (TF_b). Colours were randomly assigned for each trial.
113 The distance of 7 m between the two feeders and the two different colours made sure that trained
114 foragers would visit just one of the two feeders. Afterwards, usually on the same day, we trained
115 a third group of 10-20 foragers to a third feeder (DF, dance feeder) 160 m from the TFs and
116 150 m from the hive (Fig. 1). All trained foragers were individually marked with numbered tags
117 of different colours glued to the notum (Opalithplättchen). On the day after training, all feeders
118 provided 0.3 M of identically scented sucrose solution (5µl essential oil per 100 ml sucrose
119 solution; Primavera Life GmbH, Oy-Mittelberg, Germany). For each hive, we used a different
120 odour: sage for H1, jasmine for H2 and peppermint for H3. On this treatment day, sucrose
121 solution was provided for 60 min, from about 12.00 to 13.00 h. The sugar concentration was
122 lower in order to prevent the recruitment of more bees, but make sure trained bees returned to
123 their feeder. The duration of 60 min allowed foragers to learn the association between location,

124 reward and scent and to form a long-term memory [48]. The number and time of each visit were
125 noted for all marked bees during the 60-min treatment time.

126 Additionally, at one TF (either a or b) we added 2 mg/ml of biogenic amine (octopamine
127 or dopamine hydrochloride, Sigma Aldrich) during the treatment period. This concentration has
128 induced behavioural changes in previous studies [27,45,48]. The other TF served as a control
129 (untreated bees). All solutions (training, treatment and test) also contained 1.75 mg/ml ascorbic
130 acid (Sigma Adrich) to reduce oxidation of the biogenic amines [31]. Orally administering
131 biogenic amines has been shown to have similar effects on behaviour as other administration
132 methods, such as topical application [27,40,49,50]. The exact routes of biogenic amines from
133 the crop to the brain remain to be investigated. Gmeinbauer & Crailsheim [51], for example,
134 found that glucose solution consumed by bees after flight quickly appeared in the haemolymph,
135 suggesting a rapid transfer from the crop to the open circulatory system. This would explain
136 why the feeding of biogenic amines leads to rapid changes in biogenic amine titres in the head
137 [52] and in reward perception [40].

138 While experimentally administered biogenic amines are metabolised and cleared
139 relatively quickly from the brain, probably within a couple of hours [29,52], we expected that
140 our treatment would affect the perception of, and learning about food sources during treatment
141 [29,31], which is likely to have long-term effects. Long-term memory can affect foraging
142 decisions in honeybees for several days [48].

143 On the test day, the day after the treatment, DF foragers were allowed to collect 1.8 M
144 sucrose solution for 60 to 180 min (approx. 12.00-15.00 h) at the DF, whereas both TFs
145 remained empty. This sucrose concentration made sure DF bees were likely to perform waggle
146 dances advertising the DF location. The sucrose solution at the DF contained the same scent as
147 during training. During this test period, 5-10 DF dancers made repeated foraging trips and
148 performed waggle dances inside the hive. Meanwhile, TF foragers following these dances could
149 decide whether to decode the dances advertising the DF, *i.e.* use social information, or use

150 private information to fly to the TFs. Previous studies have shown that experienced foragers are
151 attracted to dancers carrying a familiar scent, which made it likely that a large proportion of TF
152 foragers interacted with DF dancers [7,14]. The arrival times of all bees at all feeders were
153 noted. At the same time, we filmed the “dance floor” [6] to record DF dances and the dance-
154 following behaviour of TF foragers with high-definition video cameras.

155 A waggle dance usually consists of many waggle runs (range: 1 to >100) [6,7]. While
156 waggle dances are frequently attended by both social and private information users, bees that
157 attempt to decode dances follow more waggle runs [11,13,14]. We defined dance following as
158 directing the head towards a dancer and being within a distance of one antenna length during
159 the waggle run phase [53,54]. If a bee stopped dancing for at least 5 seconds we considered this
160 dance to have ended [53,54]. We analysed the time, the number of dances TF foragers followed
161 as well as the number of waggle runs they followed.

162

163 *(b) Statistical analyses*

164 Statistical analyses were performed in R 3.2.3 (<https://www.r-project.org/>). The data was
165 analysed using generalized linear mixed-effects models (GLMM) for Poisson and binomial
166 distribution. For normally distributed data we used linear mixed-effects models (LME). R fitted
167 these models with the packages “lme4” and “nlme” [55,56]. In the case of zero-inflation or
168 overdispersion (estimated with the “Dharma” package), we used GLMMs for zero-inflated data
169 with the “glmmADMB” (Poisson distribution) and the “glmmTMB” (negative binomial
170 distribution, nb) functions [57] (see electronic supplementary material for details on final
171 models). As random effects, we chose “hive” and “trial” to account for any hive or day effects.
172 Occasionally, models failed to converge. In this case, we used only “trial” as a random effect
173 because “trial” effects were stronger. We tested for differences in the number of dances
174 followed, the number of waggle runs followed, the visited test feeder (DF or TF) and the
175 recruitment probability between the two treatments (OA, DA) versus the control. Interactions

176 between two fixed-effects were tested by comparing a model with and a model without the
177 interaction using a likelihood ratio test (LRT) [58]. By means of a survival analysis for a
178 constant hazard with exponential distribution [58] (“survival” package), we compared the time
179 of leaving the hive between the three treatment groups.

180

181 **Results**

182 During the six trials (two trials per hive), DF dancers performed 678 dances and a total of
183 10,789 waggle runs (Table 1). Overall, 259 bees were trained to the TF (5.24 ± 3.79 visits
184 during the treatment time) and of those, 84% followed DF dances. Of this latter group, 40%
185 were recruited to the DF by the end of the test period, whereas the remaining 60% exclusively
186 visited the TF (Table 1).

187

188 *(a) Dance-following behaviour*

189 Overall, TF foragers followed 4.7 ± 5.1 dances with an average number of 5.9 ± 2.5 waggle
190 runs per dance (Table 1). Bees that were recruited to the DF followed dances ~20% longer than
191 bees visiting only the TF feeder (6.70 ± 2.72 vs. 5.6 ± 1.78 waggle runs per dance) (LME: $t =$
192 2.25 , $p = 0.026$), but there was no difference in the number of dances followed (nb GLMM: z
193 $= -1.73$, $p = 0.08$) or the total number of waggle runs followed (LME: $t = -1.25$, $p = 0.21$).

194 OA-treated foragers followed 3.4 ± 5.7 dances and 27.5 ± 34.3 waggle runs in total, the
195 control group followed 5.0 ± 5.2 dances and 30.2 ± 26.4 waggle runs. DA-treated foragers
196 followed 5.3 ± 3.8 dances and 34.7 ± 24.4 waggle runs in total (Fig. 2a, b). OA-treated foragers
197 followed significantly fewer DF dances than control bees (Poisson GLMM: $z = -3.1$, $p =$
198 0.0017). Considering only the bees that followed at least one dance, OA-treated foragers also
199 followed fewer waggle runs (Poisson GLMM: $z = -2.4$, $p = 0.016$) compared to the control
200 group. We found no difference in the number of dances followed between DA-treated foragers
201 and control bees (Fig. 2a) (Poisson GLMM: $z = 1.42$, $p = 0.14$). However, DA-treated foragers

202 that followed dances followed significantly more waggle runs in total (Fig. 2b) (Poisson
203 GLMM: $z = 5.6$, $p < 0.0001$). We found no differences between the treatment groups in the
204 average number of waggle runs followed per dance (LME, OA vs. C: $t = 0.36$; $p = 0.72$; DA
205 vs. C: $t = 1.37$; $p = 0.17$).

206 We also tested whether there was an interaction between treatment and the
207 number of treatment visits. Indeed, these two factors significantly interacted in their effects on
208 the number of dances followed (Poisson GLMM: LRT = 11.93; $p = 0.003$) and the total number
209 of waggle runs followed (Poisson GLMM: LRT = 19.4; $p < 0.0001$). We, therefore, analysed
210 the effect of feeder visits for each treatment group separately. The number of treatment visits
211 had no effect on the number of dances followed in control- and DA-foragers (Poisson GLMM,
212 control: $z = 0.04$, $p = 0.97$; DA: $z = -0.97$, $p = 0.33$), but we found a positive relationship
213 between treatment visit number and the number of dances followed in OA-treated bees ($z =$
214 1.98 , $p = 0.048$). Likewise, treatment visits did not affect the total number of waggle runs
215 followed in control and DA-treated bees (Poisson GLMM, control: $z = 0.27$, $p = 0.79$; DA: $z =$
216 -1.24 , $p = 0.22$), but we again found a positive effect of the number of treatment visits in OA-
217 treated bees (nb GLMM: $z = 3.1$, $p = 0.002$).

218

219 *(b) Feeder visitation probability*

220 The DF was visited by 33% of OA-foragers, 54% of control foragers and 45% of DA-foragers
221 (Fig. 3). Of all bees visiting either feeder, OA-treated bees were significantly less likely to visit
222 the DF than control bees (binomial GLMM: $z = -2.6$, $p = 0.0085$), but significantly more likely
223 to visit only the TF (binomial GLMM: $z = 2.5$, $p = 0.011$). OA-foragers also visited the TF
224 more often than control bees (Poisson GLMM: $z = 2.7$, $p = 0.0080$). Conversely, the probability
225 to visit the DF or the TF did not differ between DA-foragers and control group foragers
226 (binomial GLMM: DF: $z = -0.6$, $p = 0.54$; TF: $z = 0.7$, $p = 0.47$). Also the number of visits of
227 the TF did not differ between these two groups (Poisson GLMM: $z = -1.0$, $p = 0.30$).

228 With a survival analysis, we studied the temporal dynamics of the arrival times at the
229 TF during testing. In this analysis, we included all bees that visited a feeder during the 60-
230 minute treatment period (including those that did not visit a feeder during the testing). Again,
231 more OA-treated visited the TF than control bees (Fig. 4) (survival analysis for exponential
232 response: $z = -1.6$, $p < 0.001$) and this effect seems especially clear at the beginning of the test
233 period. A larger number of DA-bees visited the TF than control bees (survival analysis for
234 exponential response: $z = -0.8$, $p < 0.001$). This difference became apparent after approximately
235 20 minutes (Fig. 4).

236

237 **Discussion**

238 We found that oral treatment of honeybee foragers with octopamine and dopamine affected
239 dance following behaviour and information-use. Foragers treated with OA followed fewer
240 waggle dances and, if they followed dances, they followed fewer waggle runs compared to
241 control bees. This is consistent with our prediction that OA-treated bees are less interested in
242 new social information. Despite experiencing that the food source they exploited in the past
243 (TF) was not presently rewarding, these bees mostly relied on their private information and
244 inspected this feeder more often than control bees. Site-fidelity is well-known in honeybees,
245 even if the visited foraging site does not currently offer rewards [13,14,26]. A possible
246 explanation for an increased use of private information by bees treated with octopamine is that
247 OA increased the reward perception of bees collecting food at the TF during the treatment
248 period. OA plays a crucial role in reward signalling and has been shown to increase
249 responsiveness to sucrose, learning and retrieval of information in honeybees [27,29,31,40].

250 Octopamine could also directly reduce the use of social information. Boulay et al. [59]
251 found that OA negatively affects social interactions in ants. Low levels of OA brain titres, on
252 the other hand, are associated with an increased motivation to engage in trophallaxis, which
253 represents an important mechanism of social learning in ants and honeybees [60–62]. Thus,

254 OA-treatment might have reduced dance following by reducing the motivation of bees to
255 interact with hivemates. This is consistent with the findings that OA-treatment increases
256 scouting, *i.e.* the search for food without following dances [46], and that scouts have higher
257 tyramine titres, a precursor of octopamine, than recruits [63]. Thus, OA might not only
258 strengthen the use of private information by increasing the perceived value of the reward offered
259 at the TF, but also reduce social information-use by lowering the motivation to engage in social
260 interactions, such as following waggle dances. The negative effects of OA on dance following
261 are also consistent with the observation that older and more experienced foragers appear to rely
262 more on private information and follow dances less [11,64]: OA titres change with age and are
263 higher in older bees [65–67]. Surprisingly, OA-treated foragers showed more interest in dances
264 if they visited the OA-feeder more often during the treatment period. It could, thus, be that the
265 OA-treatment has a weaker inhibitory effect on foragers that are more motivated to forage, *i.e.*
266 those that performed more visits during the treatment time. For instance, a larger dose of OA
267 could induce molecular mechanisms that attenuate OA signalling in the brain, thereby reducing
268 signalling when OA titres are very high [68]. More research is needed to better understand the
269 relationship between experience, communication behaviour and biogenic amine signalling.

270

271 While DA-treated bees did not follow more dances overall, those bees that did follow
272 dances followed significantly more waggle runs than control bees (Fig. 2). Interestingly, despite
273 their increased interest in dances, dopamine treated bees were not more likely to be recruited to
274 the advertised feeder, suggesting that an increased interest in waggle dances does not
275 necessarily increase the decoding and use of social information. On the contrary, we found
276 evidence that DA increased the use of private information. A survival analysis that included all
277 treated bees found that DA-treated bees were significantly more likely than control bees to visit
278 the training feeder (Fig. 4). In other words, while DA caused bees to follow dances more
279 thoroughly, it may also have increased their use of private information. These contradictory

280 effects are puzzling but could be explained by the diverse and complex roles DA plays in the
281 insect brain. Felsenberg et al. [69], for example, demonstrated that there are different subsets
282 of dopaminergic neurons in *Drosophila* mushroom bodies (see also [70]). One subset
283 neutralizes or extinguishes previously gained memory whereas the other subset reconsolidates
284 the original memory. Furthermore, DA signalling is involved in both aversive and reward
285 learning in fruit flies and is suspected to signal the nutritive value of a reward, while OA signals
286 sweetness [43,44]. Much less is currently known about the role of DA in reward signalling in
287 honeybees [44]. Distinct functions of DA together with the discrete compartmentalization of
288 dopaminergic neurons in the mushroom bodies [33,69,71] might explain the complex effects
289 on information use we found. Disentangling these effects would require a much more targeted
290 way of treating honeybee foragers, e.g. by injecting dopamine into specific parts of the brain
291 and the mushroom bodies.

292 It is possible that there are distinct types of information-users, *i.e.* private information
293 users that consistently persist at familiar feeding sites [14,72,73] and social information users
294 that have a high propensity to abandon their food source if it is below a certain threshold and
295 follow dances to find better ones. Scouting bees, *i.e.* bees that have a high propensity to search
296 for new food sources without following dances, differ substantially in their brain gene
297 expression and learning performance compared to non-scouting bees [46,63]. The probability
298 to follow one of these three strategies seems to be influenced by biogenic amines in complex
299 ways (see also [63]). We currently have a limited understanding of how biogenic amines affect
300 the use of different types of information, but social insects are excellent model systems that can
301 help us uncover the role of biogenic amines in individual decision-making and the coordination
302 of foraging activities of colonies.

303

304

305

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311

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492 **Tables**

493 **Table 1: Dancing and dance following behaviour.** Data shown are sample size or the mean
 494 \pm StDev. DF = dance feeder; TF = training feeder. ¹Number of TF foragers that followed DF
 495 dances. ²Average number of DF waggle runs followed per dance by TF foragers. ³ Number of
 496 DF dances followed per TF forager. ⁴Number of TF foragers recruited to the DF. ⁵Number of
 497 visits of the TF by TF foragers during testing. Note that the values for “TF foragers” include
 498 both treatment and control foragers in a given trial.

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Hive	Trial	Dances to DF	Waggle runs performed	Trained to TF	Bees followed ¹	Waggle runs followed	Waggle runs/dance ²	Dances followed ³	Recruited ⁴	Visits to TF ⁵
1	OA	60	1040	48	40	626	6.7 \pm 3.0	2.6 \pm 1.7	15	1.7 \pm 1.1
1	DA	79	1447	36	31	644	5.5 \pm 2.1	3.5 \pm 2.8	12	2.6 \pm 2.0
2	OA	182	2706	42	31	1673	4.8 \pm 1.3	10.9 \pm 8.3	13	3.0 \pm 1.8
2	DA	102	979	35	28	578	6.8 \pm 3.3	3.4 \pm 2.4	17	2.7 \pm 1.7
3	OA	114	2717	40	34	849	6.5 \pm 3.0	3.7 \pm 2.2	12	2.1 \pm 1.3
3	DA	141	1900	58	57	2440	5.4 \pm 1.6	7.9 \pm 4.4	15	4.1 \pm 2.5

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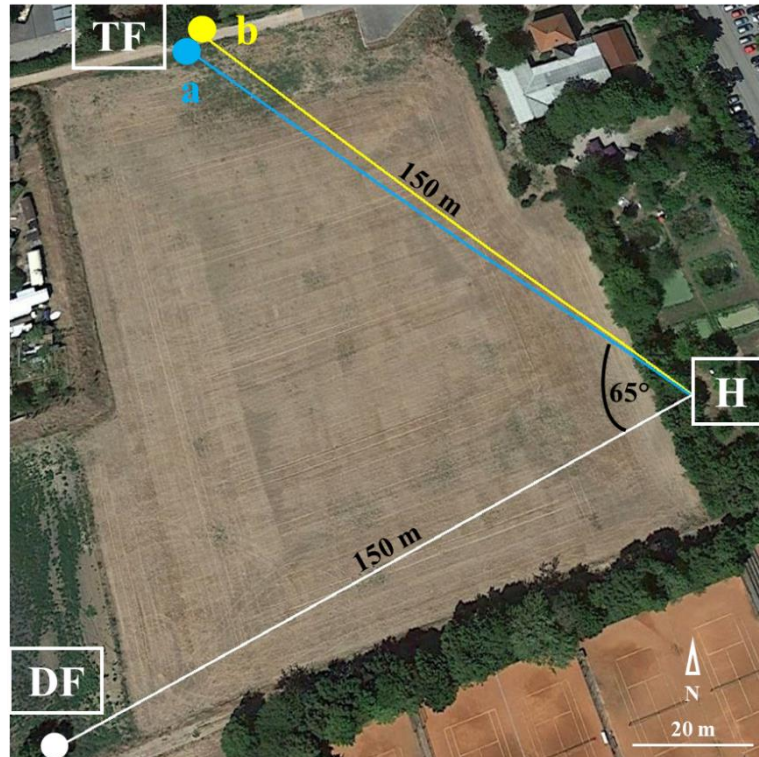
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511 **Figures**

512 **Fig. 1**



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514 **Figure 1. Experimental set-up.** Location of the hive (H), dance feeder (DF) and training
515 feeders (TF). The distance between DF and TF was 160 m. Picture taken from Google Earth
516 (49°59'15.63" N, 8°14'07.20" E).

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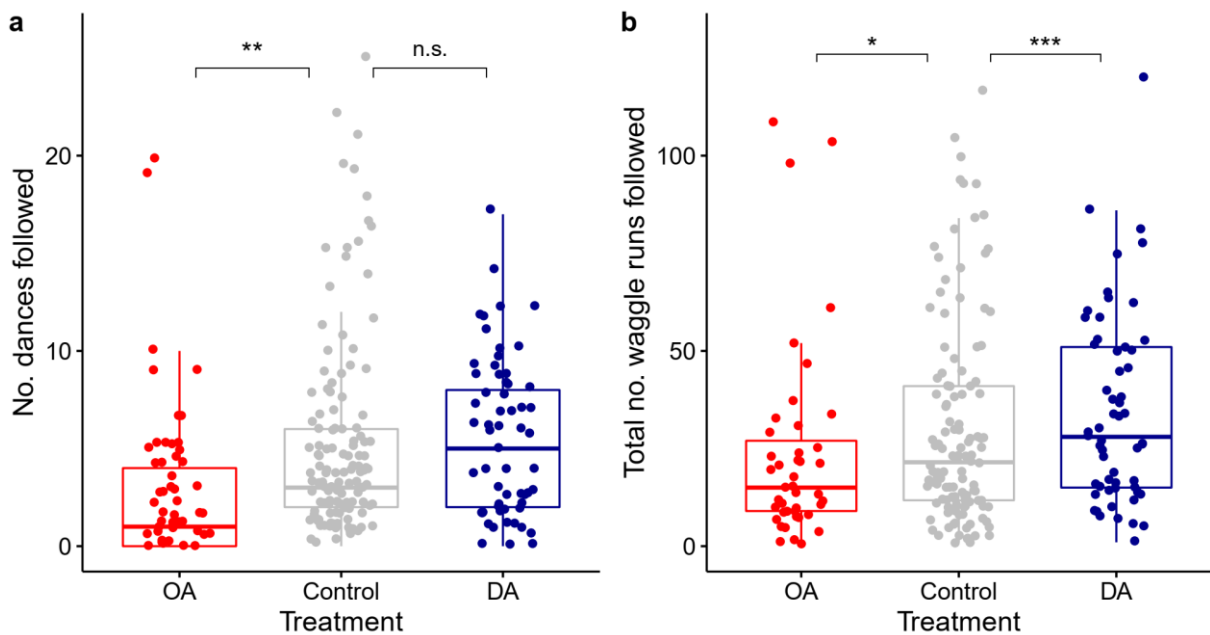
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525 **Fig. 2**



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527 **Figure 2. Effect of biogenic amine treatment on dance following behaviour.** a) The number
528 of waggle dances bees followed after oral treatment with octopamine (OA), control solution
529 and dopamine (DA). **b)** The effect of OA, control solution and DA on the total number of
530 waggle runs followed by TF bees that followed at least one dance. Boxplots show medians,
531 interquartile ranges (top line 75% quartile, bottom line 25% quartile) and whiskers show the
532 5% and 95% percentile). n.s. = $p > 0.05$, * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$). Control bees
533 from both trials per colony are combined. Dots represent individual bees.

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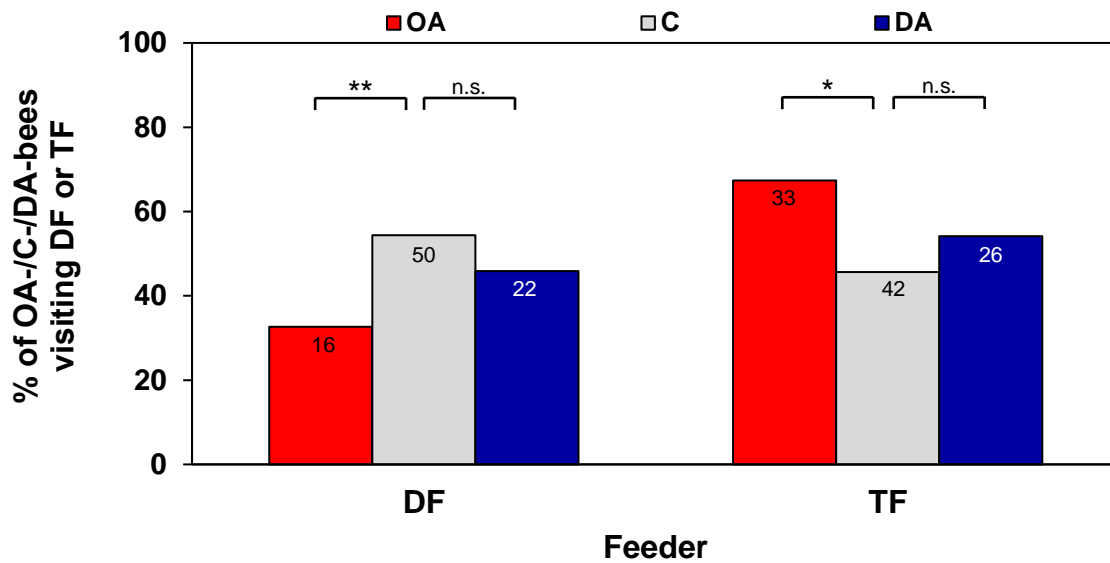
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543 **Fig. 3**

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545 **Figure 3: Effect of biogenic amine treatment on visitation probability.** The percentage of
546 bees that visited the dance feeder at least once, *i.e.* was recruited (DF, left) or exclusively visited
547 the training feeder, *i.e.* only used private information (TF, right) after oral treatment with
548 octopamine (OA), control solution (C) and dopamine (DA). Numbers in bars represent the
549 number of bees.

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559 **Fig. 4**

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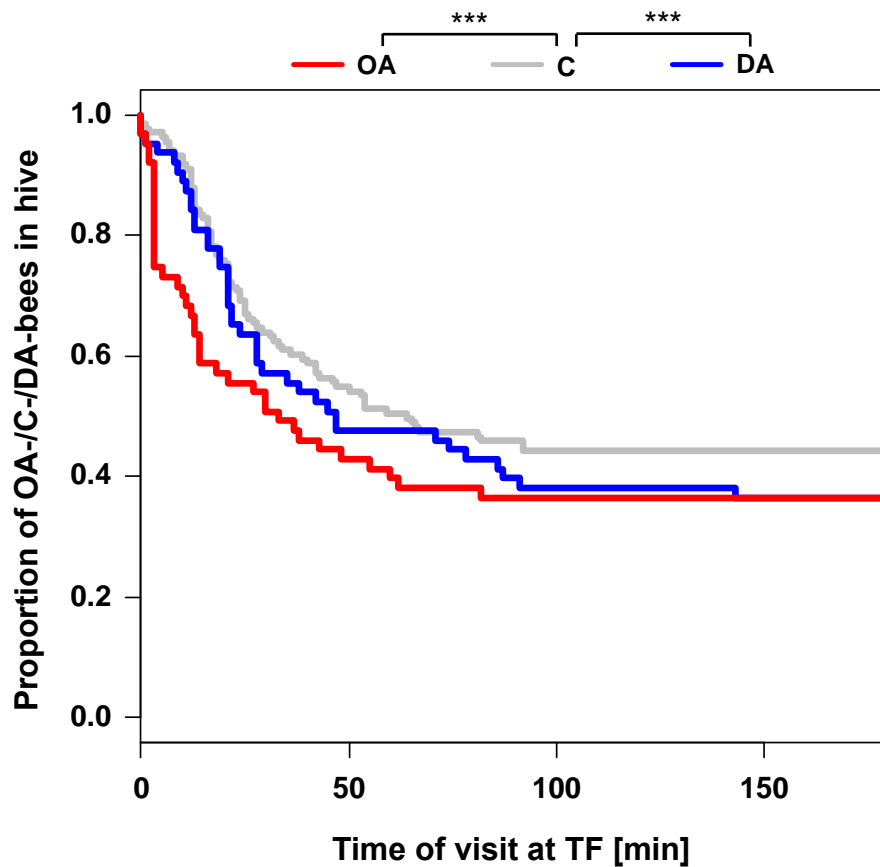
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573 **Figure 4: Proportion of bees not yet visiting the training feeder (TF) during the testing**

574 **period.** The first visit of a bee at the TF counted as the beginning (time = 0 min). A survival

575 analysis suggests that there are differences in the temporal dynamics when comparing

576 octopamine-treated foragers (OA, n = 62) vs. bees that were fed with a control solution (C, n =

577 134) and when comparing dopamine-treated bees (DA, n = 63) with control bees.

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