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et al.

Publication Date

2021

DOI

10.1016/j.jinsphys.2020.104177

Peer reviewed

1 | **Manuscript in preparation for the *Journal of Insect physiology***

2 | **Floral tea polyphenols can improve honey bee memory retention and olfactory**
3 | **sensitivity**

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46 | Abstract

47 | Animal-pollinated plants face a common problem, how their defensive anti-
48 | herbivore compounds may impair or alter pollinator behavior. Evolution has
49 | tailored multiple solutions, which largely involve pollinator tolerance or
50 | manipulation, to the benefit of the plant, not the removal of these compounds
51 | from pollen or nectar. The tea plant, *Camilla sinensis*, is famous for the
52 | caffeine and tea polyphenols (TP) that it produces in its leaves. However, these
53 | compounds are also produced in its nectar, which honey bees readily collect.
54 | We examined the effects of these compounds on bee foraging choices,
55 | learning, memory, and olfactory sensitivity. Foragers preferred a sucrose
56 | feeder with 100 µg or 10 µg TP/ml over a control feeder. Caffeine, but not TP,
57 | weakly increased honey bee learning. Both caffeine and TP significantly
58 | increased memory retention, even when tested 7 d after the last learning trial.
59 | In addition, TP generally elevated EAG responsiveness to alarm pheromone
60 | odors. These results demonstrate that not only caffeine, but other secondary
61 | plant compounds, can attract pollinators and influence their learning and
62 | memory.

63 |
64 | **KEYWORDS:** caffeine, *Camilla sinensis*, *Apis mellifera*, learning and memory,
65 | plant defensive compounds

67 | 1. Introduction

68 | Multiple plant species produce defensive compounds that deter herbivory
69 | (Sullivan et al., 2008, Harborne, 1993). Such chemicals are also consumed by
70 | pollinators, but there has evidently been little selective pressure for plants to
71 | exclude these compounds from nectar and pollen (Gegear et al., 2007; Irwin et

72 al., 2014; Stevenson et al., 2017; Jacobsen and Raguso, 2018; Jones and
73 Agrawal, 2016). In fact, plants can benefit from such compounds increasing
74 pollinator specialization, reducing nutrient degradation in nectar, decreasing
75 pollinator diseases, and reducing nectar robbing (Stevenson et al., 2017).
76 Through co-evolution (Jacobsen and Raguso, 2018), pollinators have also
77 adapted to these compounds (Jones and Agrawal, 2016). For example, the
78 Asian honey bee, *Apis cerana*, does not prefer to forage on the toxic, triptolide-
79 containing nectar of the thunder god vine, but will do so at times of relative
80 floral dearth and suffer relatively mild effects: decreased olfactory memory
81 after an acute exposure, but no learning or memory effects after chronic
82 exposure (Zhang et al., 2018).

83 Caffeine, common in *Coffea* and *Citrus* species, may increase plant fitness by
84 enhancing honey bee olfactory cognition (Wright et al., 2013, Sharma et al.,
85 1986) by improving learning in *Apis mellifera* (Couvillon et al., 2015; Mustard
86 et al., 2012; Si et al., 2005; Wright et al., 2013). Wright et al. (2013) reported a
87 range of natural caffeine levels (0.003 to 0.253 mM) and showed that acute
88 doses of 0.1 mM caffeine and higher enhanced memory. Moreover, a low
89 caffeine concentration in nectar can increase pollinator visitation
90 (Singaravelan et al., 2005). However, such cognitive effects have not been
91 documented for other secondary compounds, and we therefore sought to test
92 if tea polyphenols, another group of secondary compounds likely produced for
93 plant defense and found in tea nectar (Sharma et al., 1986), have similar
94 benefits for plants: attracting bee pollinators and enhancing their olfactory
95 memory.

96 In China, bees are potentially exposed to caffeine and TP in the nectar of tea
97 (*Camilla sinensis*), a widely cultivated crop (Sharma et al., 1986). *Camilla*

100 | *sinensis* flowers from August to February, a time of relative floral dearth.
101 | Although *C. sinensis* co-evolved with Asian honey bee species such as *A.*
102 | *cerana*, the introduced European species, *A. mellifera*, is now widespread in
103 | China where it is used for pollination and honey production (Yang, 2005). We
104 | therefore tested if TP can alter *A. mellifera* foraging preferences and if TP and
105 | caffeine can alter *A. mellifera* learning and memory and antennal
106 | responsiveness to odors, measured via electroantennograms (EAG). Honey
107 | bees will avoid inflorescences at which they detect alarm pheromones, signs of
108 | past danger (Wen et al., 2017). Such avoidance of dangerous inflorescences
109 | can decrease plant fitness (Romero et al., 2011). If TP increases bee sensitivity
110 | to bee alarm odors, an interesting side effect could arise, with plants suffering
111 | potentially decreased pollination but bees increasing their fitness via
112 | enhanced danger avoidance. We therefore tested if TP could increase honey
113 | bee antennal responsiveness to alarm pheromone components.

112

113 | 2. Materials and methods

114 | 2.1 Colonies and sites

115 | We used three (Exp 3 and Exp 4) or four (Exp 2) *Apis mellifera* colonies
116 | maintained at the apiaries of the Eastern Bee Institute of Yunnan Agricultural
117 | University, Yunnan, China (GPS coordinates: 25.128849N, 102.752200E).
118 | Experiments were conducted from August 2018 to February 2019. Colonies
119 | were in good condition, based upon standard inspection methods (Vincent et
120 | al., 2013) and engaged in natural foraging. Samples sizes are given in the
121 | figure legends and in Tables S1 and S2.

122

123 | 2.2 Experiment 1. Caffeine and TP natural percentage within the tea nectar

124 | **Sample collection**

125 | We collected *Camilla sinensis* tea nectar from Yunnan Agricultural University
126 | during its flowering season from November to December in 2018. We collected
127 | tea nectar from 8:00 a.m. to 10:00 a.m. with a microsyringe (10 ul, Shanghai
128 | Anting Co., Ltd. China) and obtained a total of >10 ml (10 tubes, 1 ml per tube,
129 | corresponding to the nectar contents of >100 flowers per tube), which was
130 | immediately stored at 4 °C at the end of each collection day.

131 |

132 | **Concentrations of caffeine and TP in tea nectar**

133 | We used an Agilent 1200-UV variable wavelength detector (at 280 nm) to
134 | measure caffeine and TP concentrations in natural tea nectar with HPLC (Zhou
135 | et al., 2013) and a TSK-GEL ODS-80TM (4.6 mmi × 250 nm) column_using_a
136 | semi-quantitative method. Mobile phase A consisted of CH₃CN (5% v/v) in a
137 | H₃PO₄ (0.261% v/v) solution. Mobile phase B was CH₃ON (40% v/v) in a H₃PO₄
138 | (0.261% v/v) solution. Elution gradient separation was performed as follows: 0-
139 | 20 min with 10% mobile phase B and 90% mobile phase A; 20-20.1 min with
140 | 22% B and 78% A; 20.1-26 min with 100% B and 0% A; 26-26.5 min with 100% B
141 | and 0% A; 26.5-27 min with 10% B and 90%[A](#); and finally held for an additional
142 | 5 min. The flow rate was 1 ml/min, and the injection volume was 2.0 ul for each
143 | analysis. We conducted 10 technical replicates: 10 different samples in 10
144 | different runs (total of 20 µl of nectar). Standards were purchased (DASF
145 | Biology Co., Ltd. Nanjing, China, Table 1, Fig. 1).

146 | *C. sinensis* polyphenols in can differ according to the plant part analyzed
147 | and consist of a mixture of several compounds including gallic acid (GA),
148 | epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate
149 | (EGCG), and 1,4,6-tri-O-galloyl-β-D-glucose (GG), and epicatechin gallate (ECG)

150 | , and Epigallocatechin (EGC) (Morikawa et al., 2013; Lin et al., 2003). Based upon
151 | the TP concentrations measured in natural *C. sinensis* nectar, we created a
152 | ~~synthetic-artificial~~ synthetic TP solution containing the same relative proportions of each
153 | TP compound (GA, SG8050; EC, SE8100; EGCG, E8120; ECG, IE0130. Solarbio, ≥98.0%
154 | purity, China), ~~excepting~~ with the exception of caffeine. We created , and the 10 and 100
155 | ~~ug/ml total TP solutions~~ bracket to bracket the (one is higher and one is lower) the
156 | naturally occurring concentrations of TP (see Table 1). ~~with these compounds (GA,~~
157 | ~~SG8050; EC, SE8100; EGCG, E8120; ECG, IE0130. Solarbio, ≥98.0% purity, China)~~ These
158 | ~~in the same concentrations found in natural tea nectar (Table 1), . However, the synthetic~~
159 | ~~TP solutions~~ did not contain ~~was~~ but without EGC (because we did not detect
160 | measurable EGC levels in natural tea nectar) or and without caffeine (because we
161 | wished to test the effects of TP compounds separately from caffeine).

162

163 | 2.3 Experiment 2. Choice preference test on honey bees

164 | We bioassayed TP nectar preferences with ~~three~~ four colonies of *A. mellifera*.
165 | We trained bees to a grooved plate feeder (5.0 cm diameter and 6.5 cm high)
166 | with a circle of green paper placed underneath to facilitate visual orientation.
167 | We trained bees by placing the feeder on a plastic stool 100 m from the focal
168 | colony, capturing departing foragers from the focal colony with a 20 ml glass
169 | vial, releasing them at the feeder, and marking bees that fed with a numbered
170 | bee tag (Opalith-Zeichenplättchen) affixed to the thorax with shellac. We
171 | repeated this training procedure until 20 bees from the focal colony reliably
172 | and repeatedly visited the feeder. An observer at the focal colony verified the
173 | return of our numbered bees. All unmarked bees from focal or other colonies
174 | were captured with aspirators. We trained on one day and tested on the
175 | subsequent day. Once our marked bees began foraging again at the training

176 location, we captured all but one forager with an aspirator to ensure that each
177 bee made an individual choice in the absence of other bees. This holding
178 aspirator was kept in the shade to keep the bees in good condition. We then
179 waited for the focal forager to leave the feeder, cleaned the stool with 100%
180 ethanol, and set out two identical clean feeders 20 cm apart at the same
181 location. After analyzing natural tea nectar, we measured an average of $19.1 \pm$
182 $0.56 \mu\text{g/ml}$ of TP compounds (excluding caffeine, Table 1). In our choice
183 bioassay, we therefore chose to test three different total TP concentrations: 0
184 $\mu\text{g/ml}$ (control), 10 $\mu\text{g/ml}$ (low TP), and 100 $\mu\text{g/ml}$ (high TP, not field-
185 realistic). The feeders offered the following paired choices (all in 30% sucrose
186 solution w/w): 0 vs. 10 $\mu\text{g/ml}$ TP, 0 vs 100 $\mu\text{g/ml}$ TP, or 10 vs 100 $\mu\text{g/ml}$ TP. We
187 tested 20 bees per choice type per colony and used four different colonies
188 (total of 240 bees).

189 Once the focal forager returned, it would often sample both feeders, but we
190 only scored a choice if it fed >10 s on one feeder. Between each trip, we set
191 out clean feeders and swapped their positions to avoid site biases. We assayed
192 the choice of each focal bee over 10 trips to the feeder array and then removed
193 it with a separate aspirator. We then cleaned the stool again and replaced the
194 array with a clean set of feeders, released a marked bee from the holding
195 aspirator, and used it as the next focal bee.

196 During the trial, we continued to remove all other bees, only counted
197 choices made in the absence of all other bees at the feeder, rotated the
198 feeders 180° after each choice to exclude potential side bias, and replaced the
199 feeders with clean ones after each choice to remove olfactory cues. The feeder
200 monitor sat directly behind and between the feeders, allowing bees to fly
201 unimpeded from the nest to the array.

202

203 | **2.4 Experiment 3. Learning and memory in honey bees**

204 | **Sample collection**

205 | **We used aspirators to collect returning foragers from the entrances of three**
206 | **colonies between 9:30 a.m. to 11:30 a.m. on sunny days (sample sizes in Table**
207 | **S1). We individually fed each bee with 15 µl of 30% (w/w) pure sucrose solution**
208 | **with a micropipette and then caged them (no more than 100 individuals with**
209 | **one colony per cage) in wood cages (20 cm X 20 cm X12 cm) in an incubator**
210 | **overnight (25°C, 65% relative humidity). Following standard protocols (Giurfa**
211 | **and Sandoz, 2012), all bees were starved overnight to facilitate successful**
212 | **conditioning.**

213

214 | **Classical olfactory conditioning**

215 | **To prepare bees for PER, we placed each bee in a clean glass vial on ice for**
216 | **approximately 5 min until movement significantly diminished. ~~We~~ To restrain the**
217 | **bees for PER, we ~~then placed bees them~~ in 0.5 ml plastic centrifuge tubes that had**
218 | **holes cut from their tips, allowing only the bee heads, mouthparts, and antennae to**
219 | **emerge - (Gong et al., 2016). Bees were ~~still~~ able to move their heads and**
220 | **proboscises and were trained 5 h later. Olfactory learning and memory were**
221 | **tested with a PER conditioning assay (Bitterman et al., 1983). During each**
222 | **trial, bees were exposed to a continuous air flow of 0.5 L min⁻¹ through a**
223 | **syringe (60 ml, inner diameter of 3 mm). The olfactory conditioned stimulus**
224 | **(CS) was 5 µl of hexane (Sigma-Aldrich, St Louis, MO, USA) dispensed onto a**
225 | **filter paper (1 cm × 1 cm) inside a syringe (Gong et al., 2018). Hexane is**
226 | **typically not used as a conditioning odor for honey bees because it lacks the**
227 | **salience of some other odors (Wright and Smith, 2004). However, preliminary**

228 trials with our setup showed that 80% of control bees learned to associate
229 hexane with food reward after 2-3 trials, the same level of learning exhibited
230 by honey bees to other pure odorants (Matsumoto et al., 2012; Tan et al.,
231 2015).

232 During acquisition training, the CS was paired with the unconditioned
233 stimulus (US; 30% w/w pure unscented sucrose solution in a micropipette tip)
234 as the reward. We lightly tapped one antenna with the US to elicit PER and
235 then allowed the bee to feed. The US was presented 3 s after CS and
236 overlapped with the CS for 2 s. A bee showing learning would extend its
237 proboscis during the presentation of the CS only (response scored as all or
238 none). We placed a fan 12 cm behind the bee and vented all odors out a
239 window. We conditioned each bee six times with an inter-trial interval of 10
240 min, which facilitates honeybee olfactory learning (Menzel, 2001). During the
241 memory tests, we exposed trained bees at each memory test time point to the CS
242 alone (hexane) or to a novel odor (nonanal), none of which were rewarded
243 (Menzel, 1999), such that half of the bees received the hexane followed by
244 nonanal and half received nonanal followed by hexane. We calculated the
245 Discrimination Index (DI) = response to the CS - response to novel_odor. In
246 total, we tested bee's memory at 1 h, 5 h, 24 h and 7 d after the last learning
247 trial.

249 *Treatments*

250 We dissolved caffeine (CAS ID 58-08-2, Toronto Research Chemicals, Cat.
251 No., C080100, ≥98.0% analytical purity, Canada) or artificial tea polyphenols_
252 (described above) prepared in (30% w/w, analytical grade sucrose and distilled
253 water) to make our test solution, the actual concentrations of the different_TP_

254 components is shown in Table 1. In these learning experiments, we tested the
255 efforts of two concentrations of caffeine (10 µg/ml and 100 µg/ml) and the same
256 concentrations for TP. We chose these concentrations of caffeine because
257 Wright et al. (2013) reported that honey bees can show improved learning and
258 memory ability after collecting *Citrus* and *Coffea* nectar with a caffeine
259 concentration <1 mM (194.19 µg/ml). The same concentrations were used for
260 TP because they represent a wide range: a low TP concentration (10 µg/ml) and
261 a higher TP concentration (100 µg/ml). As controls, we used separate groups of
262 bees that were only fed pure 30% sucrose solution (w/w) containing now
263 caffeine or TP. We first made the higher concentration solutions and then
264 diluted them 10x with pure 30% sucrose solution (w/w) to obtain the lower
265 concentration solutions.

266 We removed bees from the incubator on the morning of second day,
267 harnessed them for our PER experiments, and allowed them to sit in the test
268 environment for 5 h to acclimate. Bees were then individually fed once with a
269 micropipette providing 10 µl of a treatment. We then tested bees either 2 h
270 after this acute exposure (testing short-term effects) or, with separate groups
271 of bees, 1 d after exposure (testing longer term effects). ~~For the 1 d bees, we~~
272 ~~exposed them to the treatment and then fed them to satiation with 30% pure sucrose at~~
273 ~~9 pm of that day and kept them in long-term effect test bees, after exposure treatment,~~
274 ~~we fed another meal of normal sucrose at 9: 00 p.m. and kept in the incubator (-25°C,~~
275 ~~65% relative humidity) overnight. humidified box overnight.~~

276 With each bee, we also conducted an unrewarded memory test 7 d after the
277 last learning trial. To do this, we removed bees from their PER stands after the
278 24 h memory test (see above) and placed them inside wood boxes (inside the
279 incubator at 25°C, 65% relative humidity). We fed each bee with 5 µl of 30%

280 sucrose solution twice per day (at 9:00 a.m. and again at 9:00 p.m.). On the
281 sixth day, we fed the bees in the morning, but did not feed them in the evening
282 to ensure that they would be hungry for the 7 d memory test. This test
283 consisted of with one presentation of the conditioned odor, hexane, and one
284 presentation of the novel odor, nonanal, (both non-rewarded, presentation
285 order alternated for half of the bees) on the following morning (7 d after the
286 last learning trial).

287

288 2.5 Experiment 4. Effect of TP on honey bee antennal responses (EAG)

289 To test if TP could influence *A. mellifera* antennal response to alarm
290 pheromone compounds, we recorded electroantennograms (EAG) of each bee
291 to the same primary alarm compounds in honey bee sting alarm pheromone:
292 isopentyl acetate (IPA), octyl acetate (OA), and benzyl acetate (BA) (Koeniger
293 et al., 1979; Blum et al., 1978). We purchased our test compounds from
294 Jingchun Biological Technology, Shanghai, China. After capturing honey bee
295 foragers from entrances of three different colonies (sample sizes in Table S2),
296 we then put them into cages and fed them different concentrations of TP
297 concentration (0 µg/ml, 10 µg/ml and 100 µg/ml) in 30% (w/w) sucrose solutions.
298 We fed bees a single dose (in 10 µl) of TP and tested their EAG responses 2 h
299 later.

300 In a preliminary test, we compared the responses of freshly dissected left
301 and right antennae but found no difference between the responses and
302 thereafter only used the left antennae. We cut off this antenna and placed it
303 inside a glass electrode filled with insect Ringer's solution. The antenna was
304 placed 1 cm away from the outlet of a polytetrafluoroethylene (PTFE) tube (1
305 cm inner diameter, 15 cm long) that provided the test odor in a constant

306 airstream that was clean (500 ml active charcoal filtered) and humidified
307 (distilled water, 90% relative humidity). All measurements were conducted at
308 25 °C. For each stimulation, we delivered an odor pulse for 3 s, mixing it into
309 the continuous flow. To record antennal responses, we used a custom stimulus
310 controller, a modified EAG amplifier (Wen et al., 2017) outputting a signal into
311 a HP34405A Digital Multi Meter (Agilent, USA) and BenchVue software
312 (Keysight, USA) running on a PC.

313 Each bee was exposed to only one level of TP (0, 10, or 100 µg/ml) and
314 tested with one odor type (IPA, OA, or BA). Each bee was tested with the
315 following ascending odor doses: 0 ng (blank control), 100 ng, 1000 ng, 10,000
316 ng. The blank control was 5 µl of pure hexane (0 ng test odorant) and all
317 subsequent doses were also provided in 5 µl of hexane. All test odors were
318 pipetted onto clean filter paper (0.4 cm × 2.0 cm) placed inside a glass Pasteur
319 pipette for delivery via the EAG system (see above). During testing, we
320 provided the test odor for 3 s with an inter-trial interval of 30 s to provide
321 enough recovery time (Wang et al., 2016).

322

323 | 2.6 Statistics

324 Our bioassay choice experiments consisted of three different arrays (0 vs.
325 10 µg/ml, 0 vs. 100 µg/ml, and 10 vs. 100 µg/ml). Each bee only experienced
326 one kind of array, but made 10 trips to that array. Per bee, we therefore
327 calculated the percentage of choices for the lower TP concentration feeder. We
328 then generated a distribution of bee choices per array type and tested if
329 means of the distributions of these choices were significantly different from no
330 preference (50%) using 2-sided Wilcoxon Signed-Rank tests.

331 | **We ran separate analyses for learning (PER) and memory (Discrimination**
332 | **Index).** For memory, we examined each memory time point

333 | **Our sample sizes ranged from 60-117 honey bee workers per treatment**
334 | **(Table S1) and we therefore used Repeated-Measures Mixed Models with a**
335 | **REML algorithm (bee identity is the repeated measure) to allow between group**
336 | **and within group comparisons (Matsumoto et al., 2012). We used sequential**
337 | **model simplification, first running all interactions, and then eliminating them if**
338 | **they were not significant. Tukey Honestly Significant Difference (HSD) tests**
339 | **were used to make corrected pairwise comparisons.**

340 | **For the EAG experiment, we analyzed each alarm pheromone odor**
341 | **separately, using a Repeated-Measures Mixed Models with a REML algorithm**
342 | **and bee identity nested within odor type because each bee was tested with**
343 | **different concentrations of one type of odor. We log-transformed the EAG**
344 | **responses. We used sequential model simplification, first running all**
345 | **interactions, and then eliminating them if they were not significant. Tukey**
346 | **Honestly Significant Difference (HSD) tests were used to make corrected**
347 | **pairwise comparisons. We used JMP Pro v13.0.0 (SAS Institute, USA) for all**
348 | **statistical analyses and show mean $\pm$ 95% CI (confidence interval) in our plots.**

349

350 | **3. Results**

351 | ***3.1 Exp 1. Caffeine and TP within the tea nectar***

352 | **Our collected tea nectar had a natural caffeine concentration of 15.83 ± 0.06**
353 | **$\mu\text{g/ml}$ (0.0792 mM , Fig. 1). Thus, the natural caffeine concentration of tea**
354 | **nectar is similar to the lower concentration of $10 \mu\text{g}$ caffeine/ml that we used.**

355 | **Total tea polyphenols were a mixture of multiple compounds in the following**
356 | **average concentrations: $7.87 \pm 0.38 \mu\text{g/ml}$ (0.0257 mM) galocatechin (GA),**

357 1.13± 0.07 µg/ml (0.0039 mM) epicatechin (EC), 9.18± 0.10 µg/ml (0.02 mM)
358 epigallocatechin gallate (EGCG), and 0.921 ± 0.01 µg/ml (0.0021 mM)
359 epicatechin gallate (ECG) (Fig. 1 and Table 1). We did not detect any epig-
360 allocatechin (EGC): 0 µg/ml (0 mM). This yields a total of 19.1± 0.56 µg/ml of
361 TP compounds in natural tea nectar. We therefore prepared two different
362 concentrations of TP compounds, all in the same proportions found in natural
363 tea nectar, to the effects of lower (10 µg TP/ml) and higher (100 µg TP/ml)
364 concentrations bracketing the natural concentrations.

365

366 3.2 Exp 2. Bioassay of forager choices for TP

367 Bees significantly preferred the TP feeder when given a choice between 0
368 and 10 µg/ml TP (62.9% of choices for TP, 2-tailed Wilcoxon Signed-Rank test,
369 $W=1163$, $P<0.0001$) and between 0 and 100 µg/ml TP (63.3% of choices for the
370 TP feeder, 2-tailed Wilcoxon Signed-Rank test, $W=1249$, $P<0.0001$. Fig. 2).
371 However, when given a choice between 10 vs 100 µg/ml TP, foragers had no
372 significant preference for either feeder (2-tailed Wilcoxon Signed-Rank test,
373 $W=321$, $P=0.12$). Bees therefore preferred 10-100 µg/ml TP over the control.

374

375 3.3 Exp 3. Learning and memory

376 Effect of caffeine on learning

377 Bees learned (significant trial effect: $F_{5,4855}=363.54$, $P<0.0001$) and caffeine
378 weakly improved learning (dose effect: $F_{2,969}=4.44$, $P=0.012$). The 100 µg/ml
379 dose (each bee was fed 10 µl of this concentration) resulted in significantly
380 higher learning than the control dose (Tukey HSD test, $P<0.05$. Fig.3A). There
381 was no significant effect of treatment wait time (either 2 h or 1 d after
382 treatment, $F_{1,969}=0.07$, $P=0.79$). However, there were significant effects of the

383 interaction's treatment wait time x trial ($F_{5,4855}=25.16$, $P<0.0001$) and trial x
384 dose ($F_{10,4855}=2.60$, $P=0.004$). Caffeine did not increase learning in any
385 individual trial (Tukey HSD test, $P>0.05$). ~~In trial 2, bees fed caffeine 1 d before~~
386 ~~training had better learning than those fed caffeine 2 h before (Tukey HSD test, $P<0.05$).~~
387 ~~In trial 6, however, bees fed caffeine 2 h before training had better learning than those~~
388 ~~fed caffeine 1 d before (Tukey HSD test, $P<0.05$; Fig. 3A).~~ No other interactions were
389 significant, and colony accounted for <1% of model variance.

390

391 *Effect of caffeine on memory*

392 We note that nonanal may have potentially greater salience than hexane (Wright and
393 Smith, 2004) for bees. However, an analysis of responses to the CS alone yielded similar
394 results to the analysis of the DI. There was a significant effect of memory trial on
395 memory retention, which declined over time ($F_{3,2657}=7.97$, $P<0.0001$, Fig. 3A).
396 There were significant effects of treatment wait time ($F_{1,1175}=12.23$, $P=0.0005$)
397 and dose ($F_{2,1366}=37.80$, $P<0.0001$). The interaction trial x dose ($F_{6,2685}=2.35$,
398 $P=0.029$) was significant. The treatment wait time x dose was also significant
399 ($F_{2,1170}=8.45$, $P=0.0002$), and caffeine improved memory retention (dose effect
400 per bee): 2 h wait time (100 $\mu\text{g/ml}$ better than the control dose) and 1 d (100
401 and 10 $\mu\text{g/ml}$ better than control, Tukey HSD test, $P<0.05$). Colony accounted
402 for <1% of model variance (Fig. 3A).

403

404 *Effect of TP on learning*

405 As expected, bees learned in the TP trials (trial effect: $F_{5,4925}=1016.86$,
406 $P<0.0001$). However, there was no significant effect of TP dose ($F_{2,981}=1.78$,
407 $P=0.17$, Fig. 3B). ~~There was a significant effect of treatment wait time ($F_{1,981}=5.91$,~~
408 ~~$P=0.015$) and the interaction treatment wait time x trial ($F_{5,4925}=42.06$, $P<0.0001$) in trial~~

409 | 2 (1 d better), in trial 5 (2 h better), and in trial 6 (2 h better. Fig. 3B). Colony
410 | accounted for <1% of model variance.

411

412 | *Effect of TP on memory*

413 | Memory significantly declined over 7 d ($F_{3,2690}=13.37$, $P<0.0001$, Fig. 3B), but
414 | TP increased memory retention (dose effect: $F_{2,11761}=10.70$, $P<0.0001$. Fig. 3B).
415 | A dose of 100µgTP/ml (fed as 10 µl per bee) increased memory as compared to
416 | the control dose at the 5 h trial and the 24 h trial. In contrast, 10 µg TP/ml only
417 | increased memory to the control dose at the 5 h trial (Tukey HSD test, $P<0.05$).
418 | The interaction treatment wait time x trial was significant ($F_{3,2692}=3.47$,
419 | $P=0.016$), but there were no significant differences between the effects of
420 | treatment wait time on memory at any tested time point (Tukey HSD test,
421 | $P>0.05$). Colony accounted for <1% of model variance.

422

423 | *3.4 Exp 4. TP effect on EAG response to alarm pheromone components*

424 | For each alarm odor compound, bees fed 10 or 100 µg TP/ml generally had
425 | increased EAG responses as compared to bees fed the control treatment of 0
426 | µg TP/ml (Fig. 4). Interestingly, this increase in EAG responsiveness occurred
427 | even in the absence of any test odor (0 ng of test compound hexane alone),
428 | suggesting that TP induces a general increase in antennal responsiveness. We
429 | therefore highlight exceptions to this trend below.

430 | For IPA, there were significant effects of TP concentration ($F_{2,77}=26.01$,
431 | $P<0.0001$), odor concentration ($F_{3,153}=491.31$, $P<0.0001$), and the interaction
432 | TP concentration x odor concentration ($F_{6,153}=4.59$, $P=0.0003$). Colony
433 | accounted for <1% of model variance. When presented with 10000 ng of IPA,

434 control bees and bees fed 100 µg/ml TPA did not have significantly different
435 EAG responses.

436 For OA, there were also significant effects of TP concentration ($F_{2,91}=35.70$,
437 $P<0.0001$), odor concentration ($F_{3,153}=711.43$, $P<0.0001$), and the interaction
438 TP concentration x odor concentration ($F_{6,153}=7.46$, $P<0.0001$). Colony
439 accounted for <1% of model variance. When presented with 1000 ng of OA,
440 control bees and bees fed 10 µg/ml TPA did not have significantly different EAG
441 responses.

442 For BA, TP concentration ($F_{2,82}=39.07$, $P<0.0001$), odor concentration
443 ($F_{3,153}=1019.33$, $P<0.0001$), and the interaction TP concentration x odor
444 concentration ($F_{6,153}=4.40$, $P=0.0004$) were also all significant. Colony
445 accounted for <1% of model variance.

446

447 4. Discussion

448 We provide the first evidence that bees prefer nectar with tea polyphenols
449 (TP) over control nectar at natural and elevated TP concentrations. In addition,
450 TP can affect bee olfactory cognitive ability and olfactory sensitivity. Caffeine,
451 but not TP, improved learning. Since bees can be exposed to these compounds
452 and immediately begin to learn or experience a longer delay before learning,
453 we tested the effects of exposure 2 h or 1 d before learning. The effects of
454 exposure time delay before learning were complex and varied depending upon
455 the learning trial and compound (caffeine or TP). However, both caffeine and
456 TP significantly improved memory retention and, in general, more recent
457 treatment (2 h) resulted in better retention than treatment 1 d before. TP also
458 elevated antennal responsiveness to tested odors.

459 Nonanal may have potentially greater salience than hexane (Wright and Smith, 2004)
460 for bees. However, an analysis of responses to the CS alone yield similar results to the
461 more standard analysis of responses to the DI.

462 **Our chemical analyses of natural tea nectar revealed an average caffeine**
463 **concentration of 0.079 mM, within the range reported by Wright et al. (2013)**
464 **for *Coffea* and *Citrus* (0.003 mM-0.253 mM). In tea nectar, we found that**
465 **caffeine (0.0792 mM) was >8-fold more concentrated than TP (0.0096 mM), a**
466 **result that agrees with prior studies showing that tea polyphenols are**
467 **concentrated in the young leaves of *C. sinensis*, but occur in lower**
468 **concentrations in its nectar (Sharma et al., 1986). These data also support**
469 **prior research demonstrating that caffeine and TP concentrations differ**
470 **depending upon the part of the tea plant analyzed (Morikawa et al., 2013; Lin**
471 **et al., 2003). We identified similar TP compounds in nectar and tea leaves,**
472 **except for EGC, which is one of the most abundant TP components in young tea**
473 **leaves (Graham, 1992). We found an average of 19.1 µg/ml of total TP**
474 **compounds in tea nectar, a concentration between our two test concentrations**
475 **of 10 µg TP/ml and 100 µg TP/ml.**

476 **Interestingly, foragers preferred nectar with TP at concentrations at (10 µg/**
477 **ml) and above (100 µg/ml) what is found in nature (Figure Fig. 2). Singaravelan et**
478 **al. (2005) showed that a low caffeine concentration in nectar (25 ppm in their natural**
479 **caffeine range test) can create a pollinator feeding preference. Similarly, Wright et al.**
480 **(2013) showed that *A. mellifera* foragers preferred to consume nectar with low**
481 **concentrations of caffeine, but also exhibited preferences for some higher**
482 **concentrations. The reasons for these preferences remain unclear, but Kucharski**
483 **and Maleszka (2005) reported that caffeine can alter honey bee gene**
484 **expression patterns in the brain, and caffeine is an adenosine receptor**

485 antagonist and improved responses of mushroom body neurons involved in
486 olfactory learning and memory (Wright et al., 2013). In our study, honey bee
487 memory improved overall when bees fed more recently on caffeine and TP
488 (within 2 h as compared to 1 d before the first learning trial). TP and caffeine
489 improved memory retention, and caffeine weakly improved learning. The tea
490 plant may therefore benefit from these pollinator effects.

491 TP consumption generally increased EAG responsiveness, even in the
492 absence of test odors. This was not true in all cases (Figure Fig. 4), but the
493 trend is sufficiently strong to suggest that additional studies are required.
494 Does this heightened EAG responsiveness translates into an ability to
495 discriminate odors or is there simply a heightened basal activity level that does
496 not enhance overall responses to odors? If the former is correct, there are
497 implications for plant fitness. Honey bees will avoid floral resources marked
498 with alarm pheromone (Wen et al., 2017). If bees that have fed upon TP in
499 nectar have heightened sensitivity to alarm odors, this could translate result in
500 an increased spatial area in which bees avoid to alarm pheromones ~~into an increased-~~
501 ~~active space (a more sensitive detection threshold for the active space Q/K ratio),~~
502 reducing honey bee visitation of tea inflorescences upon which foragers had
503 previously released sting alarm pheromone. Such reduced floral visitation is
504 known to decrease plant fitness by decreasing pollination and seed set
505 (Romero et al., 2011).

506 However, *C. sinensis* could also gain from the forager attraction for nectar
507 with TP. The push and pull of these different forces on the co-evolution
508 between *C. sinensis* and its pollinators would be useful to explore in general,
509 particularly since these compounds likely occur in nectar as a side effect of
510 their anti-herbivore effects in general plant tissue. TP compounds may also

511 | occur as defensive compounds in other plant species, a point for further
512 | investigation. Many different pollinators and their plants face similar issues
513 | with the anti-herbivory compounds that plants have evolved. The evolutionary
514 | and theoretical consequences of such spandrels, phenotypic traits such as
515 | defensive nectar compounds that are byproducts with respect to pollinators
516 | rather than the result of adaptive selection to harm or influence pollinators,
517 | should be better understood.

518 |

519 | **5. Data availability**

520 | Upon publication, all data will be publicly available at Zenodo.org.

521 |

522 | **6. Author's contributions**

523 | ZG, KT, and JCN conceived of and contributed to the design of the
524 | experiments. ZG, YW, and SD performed the experiments. ZG, KT, and JCN
525 | contributed reagents, materials, or analysis tools. ZG, KT, and JCN wrote the
526 | paper.

527 |

528 | **Declaration of Competing Interest**

529 | The authors declare that they have no known competing financial interests
530 | or personal relationships that could have appeared to influence the work
531 | reported in this paper.

532 |

533 | **Acknowledgements**

534 | We thank the CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna
535 | Tropical Botanical Garden, Chinese Academy of Sciences. This research was
536 | funded by the China Postdoctoral Science Foundation. Additional funding was

537 | provided by the CAS 135 program (no. 2017XTBG-T01), the China National
538 | Research Fund (31770420), and the Center for Plant Ecology, Core Botanical
539 | Gardens, Chinese Academy of Science, and Science, and the Chinese Academy of
540 | Sciences President's International Fellowship Initiative (no. 2016VBA035).

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543 | **References**

544 | Bitterman, M., Menzel, R., Andrea, F., Sabine, S., 1983. Classical conditioning
545 | of proboscis extension in honeybees (*Apis mellifera*). J. Comp. Psychol. 97,
546 | 107-119.

547 | Blum, M.S., Fales, H.M., Tucker, K.W., Collins, A.M., 1978. Chemistry of the
548 | sting apparatus of the worker honeybee. J. Apic. Res. 17, 218-221.

549 | Couvillon, M.J., Toufaily, A.H., Butterfield, T.M., Schrell, F., Ratnieks, F.L.W.,
550 | Schürch, R., 2015. Caffeinated forage tricks honeybees into increasing
551 | foraging and recruitment behaviors. Curr. Biol. 25, 2815-2818.

552 | Gegear, R.J., Manson, J.S., Thomson, J.D., 2007. Ecological context influences
553 | pollinator deterrence by alkaloids in floral nectar. Ecol. Lett. 10, 375-382.

554 | Giurfa, M., Sandoz, J.C., 2012. Invertebrate learning and memory: fifty years of
555 | olfactory conditioning of the proboscis extension response in honeybees.
556 | Learn. Mem. 19, 54-66.

557 | Gong, Z., Wang, C., Nieh, J.C., Tan, K., 2016. Inhibiting DNA methylation alters
558 | olfactory extinction but not acquisition learning in *Apis cerana* and *Apis*
559 | *mellifera*. J. Ins. Physiol. 90, 43-48.

560 | Gong, Z., Tan, K., Nieh, J.C., 2018. First demonstration of olfactory learning and
561 | long term memory in honey bee queens. J. Exp. Biol. 221, jeb177303.

562 | **Graham, H.N., 1992. Green tea composition, consumption, and polyphenol**
563 | **chemistry. Prev. Med. 21, 334-350.**

564 | **Harborne, J.B., 1993. Introduction to ecological biochemistry (4th edn),**
565 | **Academic Press.**

566 | **Irwin, R.E., Cook, D., Richardson, L.L., Manson, J.S., Gardner, D.R.I., 2014.**
567 | **Secondary compounds in floral rewards of toxic rangeland plants: impacts**
568 | **on pollinators. J. Agric. Food Chem. 62, 7335-7344.**

569 | **Jacobsen, D.J., Raguso, R.A., 2018. Lingering effects of herbivory and plant**
570 | **defenses on pollinators. Curr. Biol. 28, R1164-R1169.**

571 | **Jones, P.L., Agrawal, A.A., 2016. Consequences of toxic secondary compounds**
572 | **in nectar for mutualist bees and antagonist butterflies. Ecology. 97, 2570-**
573 | **2579.**

574 | **Koeniger, N., Weiss, J., Maschwitz, U., 1979. Alarm pheromones of the sting in**
575 | **the genus *Apis*. J. Insec. Physiol. 25, 467-476.**

576 | **Kucharski, R., Maleszka, R., 2005 Microarray and real-time PCR analyses of**
577 | **gene expression in the honeybee brain following caffeine treatment. J.**
578 | **Molec. Neurosci. 27, 269-276.**

579 | **Lin, Y.S., Wu, S.S., Lin, J.K., 2003. Determination of tea polyphenols and**
580 | **caffeine in tea flowers (*Camellia sinensis*) and their hydroxyl radical**
581 | **scavenging and nitric oxide suppressing effects. J. Agric. Food Chem. 51,**
582 | **975-980.**

583 | **Matsumoto, Y., Sandoz, J.C., Giurfa, M., 2012. Revisiting olfactory classical**
584 | **conditioning of the proboscis extension response in honey bees: A step**
585 | **toward standardized procedures. J. Neurosci. Meth. 211, 159-167.**

586 | **Menzel, R., 1999. Memory dynamics in the honeybee. J. Comp. Physiol. A. 185,**
587 | **323-340.**

588 | **Menzel, R., 2001. Searching for the memory trace in a mini-brain, the**
589 | **honeybee. *Learn. Mem.* 8, 53-62.**

590 | **Morikawa, T., Ninomiya, K., Miyake, S., Miki, Y., Okamoto, M., Yoshikawa, M.,**
591 | **Muraoka, O., 2013. Flavonol glycosides with lipid accumulation inhibitory**
592 | **activity and simultaneous quantitative analysis of 15 polyphenols and**
593 | **caffeine in the flower buds of *Camellia sinensis* from different regions by**
594 | **LCMS. *Food Chem.* 140, 353-360.**

595 | **Mustard, J.A., Dews, L., Brugato, A., Dey, K., Wright, G.A., 2012. Consumption**
596 | **of an acute dose of caffeine reduces acquisition but not memory in the**
597 | **honey bee. *Behav. Brain Res.* 232, 217-224.**

598 | **Romero, G.Q., Antiqueira P.A.P., Koricheva, J., 2011. A meta-analysis of**
599 | **predation risk effects on pollinator behaviour, *PLoS one* 6, e20689.**

600 | **Sharma, O.P., Raj, D., Garg, R., 1986. Toxicity of nectar of tea (*Camellia thea***
601 | **L.) to honeybees. *J. Apic. Res.* 25, 106-108.**

602 | **Si, A., Zhang, S.W., Maleszka, R., 2005. Effects of caffeine on olfactory and**
603 | **visual learning in the honey bee (*Apis mellifera*). *Pharm. Biochem. Behav.***
604 | **82, 664-672.**

605 | **Singaravelan, N., Nee'man, G., Inbar, M., Izhakil., 2005. Feeding responses of**
606 | **free-flying honeybees to secondary compounds mimicking floral nectars. *J.***
607 | ***Chem. Eco.* 31: 2791-2804.**

608 | **Stevenson, P.C., Nicolson, S.W., Wright, G.A., 2017. Plant secondary**
609 | **metabolites in nectar: impacts on pollinators and ecological functions. *Func.***
610 | ***Ecol.* 31, 65-75.**

611 | **Sullivan, R.J., Hagen, E.H., Hammerstein, P., 2008. Revealing the paradox of**
612 | **drug reward in human evolution. *Pro. Roy. Soc. B.* 275, 1231.**

613 **Tan, K., Chen, W., Dong, S., Liu, X., Wang, Y., Nieh, J.C., 2015. A neonicotinoid**
614 **impairs olfactory learning in Asian honey bees (*Apis cerana*) exposed as**
615 **larvae or as adults. *Scientific Rep.* 5, 10989.**

616 **Vincent, D., Ellis, J.D., Neumann, P., 2013. The *Ceoloss_beebook* volume I:**
617 **Standard methods for *Apis mellifera* research. *International Bee Research***
618 ***Association IBRA.* V52.**

619 **Wang, Z., Wen, P., Qu Y., Dong, S, Li, J., Tan, K., Nieh, J.C., 2016. Bees**
620 **eavesdrop upon informative and persistent signal compounds in alarm**
621 **pheromones. *Scientific Rep.* 6, 25693.**

622 **Wen, P., Cheng, Y., Wang, Z., Tan K., Nieh, J.C., 2017. The sex pheromone of a**
623 **globally invasive honey bee predator, the Asian eusocial hornet, *Vespa***
624 ***velutina*. *Scientific Rep.* 7, 12956.**

625 **Wen, P ., Cheng Y.N., Qu, Y.F., Zhang, H.X., Li, J.J., Heather, B., Tan, K., Nieh, J.,**
626 **2017. Foragers of sympatric Asian honey bee species intercept competitor**
627 **signals by avoiding benzyl acetate from *Apis cerana* alarm pheromone.**
628 ***Scientific Rep.* 7, 6721.**

629 **Wright, G.A., Smith, B.H., 2004, Different thresholds for detection and**
630 **discrimination of odors in the honey bee (*Apis mellifera*), *Chem. Senses*, 29,**
631 **127-135.**

632 **Wright, G.A., Baker, D.D., Palmer, M.J., Stabler, D., Mustard, J.A., Power, E.F.,**
633 **Borland, A. M., Stevenson, P.C., 2013. Caffeine in floral nectar enhances a**
634 **pollinator's memory of reward. *Science* 339, 1202-1204.**

635 **Yang, G.H., 2005. Harm of introducing the western honey bee *Apis mellifera* L.**
636 **to the Chinese *Apis cerana* F. and its ecological impact. *Acta Entomologica***
637 ***Sinica.* 48,401-406.**

638 **Zhang, J., Wang, Z., Wen, P., Qu, Y., Tan, K., Nieh, J.C., 2018. The reluctant**
639 **visitor: a terpenoid in toxic nectar can reduce olfactory learning and**
640 **memory in Asian honey bees." J. Exp. Biol.221, jeb168344.**

641 **Zhou, J.H., Yao, L.H., Li, H., Chen, L.Z., Wu, L.M., Zhao, J., 2013. Floral**
642 **classification of honey using liquid chromatography-diode array detection-**
643 **trade mass spectrometry and chemometric analysis. Food Chem. 145,941-**
644 **949.**

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653 **Table 1. The concentration of caffeine and tea polyphenols in nectar collected**
654 **from *C. sinensis* inflorescences, in the TP standards solution used for**
655 **compound verification and in the TP solutions fed to bees (mean±95% CI). We**
656 **tested for the presence of EGC in tea nectar because this compound has**
657 **previously been reported in TP extract from other parts of the plant. However,**
658 **we detected no EGC in tea nectar. We also shown the concentrations of tea**
659 **polyphenol compounds in the synthetic TP solution fed to bees. This TP**
660 **synthetic solution contained no caffeine because we wished to separately test**
661 **the effects of TP apart from caffeine.**
662

	<u>Component</u>	<u>Concentration (µg/ml)</u>	<u>Molarity (mM)</u>
<u><i>C. sinensis</i> nectar</u>	<u>Caffeine (CA)</u>	<u>15.83± 0.06</u>	<u>0.0792</u>
	<u>Galocatechin (GA)</u>	<u>7.87± 0.38</u>	<u>0.0257</u>
	<u>Epicatechin (EC)</u>	<u>1.13± 0.07</u>	<u>0.0039</u>
	<u>Epigallocatechin gallate (EGCG)</u>	<u>9.18± 0.10</u>	<u>0.02</u>
	<u>Epicatechin gallate (ECG)</u>	<u>0.921± 0.01</u>	<u>0.0021</u>
	<u>Epigallocatechin (EGC)</u>	<u>0</u>	<u>0</u>
	<u>Galocatechin (GA)</u>	<u>7.82</u>	<u>0.0255</u>
<u>TP solution fed to bees</u>	<u>Epicatechin (EC)</u>	<u>1.17</u>	<u>0.004</u>
	<u>Epigallocatechin gallate (EGCG)</u>	<u>9.24</u>	<u>0.0201</u>
	<u>Epicatechin gallate (ECG)</u>	<u>0.94</u>	<u>0.0021</u>
	<u>Epigallocatechin (EGC)</u>	<u>0</u>	<u>0</u>
	Component	Concentration (µg/ml)	Molarity (mM)
<u><i>C. sinensis</i> nectar</u>	<u>Caffeine (CA)</u>	<u>15.83± 0.06</u>	<u>0.0792</u>
	<u>Galocatechin (GA)</u>	<u>7.87± 0.38</u>	<u>0.0257</u>
	<u>Epicatechin (EC)</u>	<u>1.13± 0.07</u>	<u>0.0039</u>
	<u>Epigallocatechin gallate (EGCG)</u>	<u>9.18± 0.10</u>	<u>0.0200</u>
	<u>Epicatechin gallate (ECG)</u>	<u>0.921± 0.01</u>	<u>0.0021</u>
	<u>Epigallocatechin (EGC)</u>	<u>0</u>	<u>0</u>
	<u>Galocatechin (GA)</u>	<u>7.82</u>	<u>0.0255</u>
<u>TP standards solution</u>	<u>Epicatechin (EC)</u>	<u>1.17</u>	<u>0.0040</u>
	<u>Epigallocatechin gallate (EGCG)</u>	<u>9.24</u>	<u>0.0201</u>
	<u>Epigallocatechin (EGC)</u>	<u>0</u>	<u>0</u>

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	Epicatechin-gallate (ECG)	0.94	0.0021
TP (10 µg/ml)- solution	Gallocatechin (GA)	4.08	0.0133
	Epicatechin (EC)	0.61	0.0021
	Epigallocatechin-gallate (EGCG)	4.82	0.0105
	Epicatechin-gallate (ECG)	0.49	0.0011
	Gallocatechin (GA)	40.79	0.1330
TP (100 µg/ml)- solution	Epicatechin (EC)	6.10	0.0209
	Epigallocatechin-gallate (EGCG)	48.20	0.1049
	Epicatechin-gallate (ECG)	4.90	0.0110

Fig. 1. Chromatograms showing the relative abundance of caffeine (CA) and total tea polyphenol compounds in *C. sinensis* nectar with reference to TP standards. Abbreviations represent gallic acid (GA), epigallocatechin (EGC), catechin (C), caffeine (CA), epicatechin (EC), epigallocatechin gallate (EGCG), 1,4,6-tri-O-galloyl- β -D-glucose (GG), and epicatechin gallate (ECG).

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Fig. 2. Results of the TP paired-choice bioassay. The mean proportion of choices (out of 10 per bee) for the feeder with the higher TP concentration is shown (P -values from a 2-tailed Wilcoxon Signed-Rank test). Error bars indicate 95% confidence intervals. The dashed line shows the null hypothesis expectation of no preference. Bees preferred 10 and 100 $\mu\text{g/ml}$ TP over the control but had no preference between 10 and 100 $\mu\text{g/ml}$ TP.

(A)

2 h after treatment

1 d after treatment

(B)

2 h after treatment

1 d after treatment

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695 **Fig. 3. Effect of caffeine and TP on bee learning and memory when tested 2 h**
 696 **or 1 d after feeding on the treatment. (A) Bees trained 2 h ($n_0 \mu\text{g/ml}=78$, n_{10}**
 697 **$\mu\text{g/ml}=87$, $n_{100 \mu\text{g/ml}}=75$) after feeding on caffeine had improved learning**
 698 **($P=0.012$), but not if they were trained 1 d ($n_0 \mu\text{g/ml}=72$, $n_{10 \mu\text{g/ml}}=87$, $n_{100 \mu\text{g/ml}}=78$)**
 699 **after feeding on caffeine. Horizontal lines indicate the corresponding trials with**
 700 **significantly higher learning between the treatment wait times: for example, the 2nd-**
 701 **learning trial (arrowhead) for the 1 d wait time had higher learning than the same-**
 702 **learning trial for the 2 h wait time (Tukey HSD test, $*P<0.05$). The plots below pool**
 703 **the data from all memory trials and show that there were significant effects of**
 704 **caffeine at both treatment wait times (Tukey HSD test, $*P<0.05$). (B) TP did not**
 705 **improve learning 2 h after feeding ($n_0 \mu\text{g/ml}=78$, $n_{10 \mu\text{g/ml}}=117$, $n_{100 \mu\text{g/ml}}=87$) or 1 d**
 706 **after feeding ($n_0 \mu\text{g/ml}=75$, $n_{10 \mu\text{g/ml}}=93$, $n_{100 \mu\text{g/ml}}=60$), but there were significant**
 707 **differences between trials 2, 5, and 6 (see horizontal lines and arrowheads) between the**
 708 **treatment wait times. TP improved memory (Tukey HSD test, $*P<0.05$). All plots**
 709 **show mean $\pm$ 95% confidence intervals.**
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Fig. 4. Honey bee electroantennogram (EAG) responses (absolute values shown) to major alarm pheromone components after consuming TP (0, 10 or 100 µg/ml) 2 h before testing. We tested EAG responses to isopentyl acetate (IPA. $n_{0\mu\text{g/ml-}0\text{ng}}=22$, $n_{0\mu\text{g/ml-}100\text{ng}}=22$, $n_{0\mu\text{g/ml-}1000\text{ng}}=22$, $n_{0\mu\text{g/ml-}10000\text{ng}}=22$; $n_{10\mu\text{g/ml-}0\text{ng}}=24$, $n_{10\mu\text{g/ml-}100\text{ng}}=24$, $n_{10\mu\text{g/ml-}1000\text{ng}}=24$, $n_{10\mu\text{g/ml-}10000\text{ng}}=24$; $n_{100\mu\text{g/ml-}0\text{ng}}=23$, $n_{100\mu\text{g/ml-}100\text{ng}}=23$, $n_{100\mu\text{g/ml-}1000\text{ng}}=23$, $n_{100\mu\text{g/ml-}10000\text{ng}}=23$), octyl acetate (OA. $n_{0\mu\text{g/ml-}0\text{ng}}=23$, $n_{0\mu\text{g/ml-}100\text{ng}}=23$, $n_{0\mu\text{g/ml-}1000\text{ng}}=23$, $n_{0\mu\text{g/ml-}10000\text{ng}}=23$; $n_{10\mu\text{g/ml-}0\text{ng}}=24$, $n_{10\mu\text{g/ml-}100\text{ng}}=23$, $n_{10\mu\text{g/ml-}1000\text{ng}}=23$, $n_{10\mu\text{g/ml-}10000\text{ng}}=23$; $n_{100\mu\text{g/ml-}0\text{ng}}=23$, $n_{100\mu\text{g/ml-}100\text{ng}}=23$, $n_{100\mu\text{g/ml-}1000\text{ng}}=23$, $n_{100\mu\text{g/ml-}10000\text{ng}}=23$), and benzyl acetate (BA. $n_{0\mu\text{g/ml-}0\text{ng}}=22$, $n_{0\mu\text{g/ml-}100\text{ng}}=22$, $n_{0\mu\text{g/ml-}1000\text{ng}}=22$, $n_{0\mu\text{g/ml-}10000\text{ng}}=22$; $n_{10\mu\text{g/ml-}0\text{ng}}=23$, $n_{10\mu\text{g/ml-}100\text{ng}}=23$, $n_{10\mu\text{g/ml-}1000\text{ng}}=23$, $n_{10\mu\text{g/ml-}10000\text{ng}}=23$; $n_{100\mu\text{g/ml-}0\text{ng}}=23$, $n_{100\mu\text{g/ml-}100\text{ng}}=24$, $n_{100\mu\text{g/ml-}1000\text{ng}}=24$, $n_{100\mu\text{g/ml-}10000\text{ng}}=24$). TP improved antennal responsiveness (Tukey HSD test, $*P<0.05$). All plots show mean $\pm$ 95% confidence intervals.

SUPPLEMENTAL MATERIAL

Table S1. Sample sizes for learning and memory experiment. The colonies used are named C1, C2, and C3.

Treatment	Colony	2 h delay			1 day delay		
		0 µg/ml	10 µg/ml	100 µg/ml	0 µg/ml	10 µg/ml	100 µg/ml
Caffeine	C1	26	29	25	24	29	26
	C2	26	29	25	24	29	26
	C3	26	29	25	24	29	26
TP	C1	26	39	29	25	31	20
	C2	26	39	29	25	31	20
	C3	26	39	29	25	31	20

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Table S2. Sample sizes for EAG response experiments. The colonies used are named C1, C2, and C3.

Compound	Odor concentration	Colony	TP concentration (µg/ml)		
			0	10	100
IPA	100 ng/ml	C1	7	8	8
		C2	7	8	8
		C3	8	8	7
	1000 ng/ml	C1	7	8	8
		C2	7	8	8
		C3	8	8	7
	10000 ng/ml	C1	7	8	8
		C2	7	8	8
		C3	8	8	7
OA	100 ng/ml	C1	8	8	8
		C2	7	7	7
		C3	8	8	8
	1000 ng/ml	C1	8	8	8
		C2	7	7	7
		C3	8	8	8
	10000 ng/ml	C1	8	8	8
		C2	7	7	7
		C3	8	8	8
BA	100 ng/ml	C1	7	8	8
		C2	7	8	8
		C3	8	7	8
	1000 ng/ml	C1	7	8	8
		C2	7	8	8
		C3	8	7	8
	10000 ng/ml	C1	7	8	8
		C2	7	8	8
		C3	8	7	8