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1 Ants act as olfactory bio-detectors of 2 tumour in patient-derived xenograft 3 mice 4

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16

17 **Abstract**

18 Early detection of cancer is critical in medical sciences, as the sooner a cancer is diagnosed, the higher
19 the chances of recovery. Tumour cells are characterized by specific volatile organic compounds (VOCs)
20 that can be used as cancer biomarkers. Through olfactory associative learning, animals can be trained
21 to detect these VOCs. Insects, such as ants, have a refined sense of smell and can be easily and rapidly
22 trained with olfactory conditioning. Using urine from patient-derived xenograft mice as stimulus, we
23 demonstrate that individual ants can learn to discriminate the odour of healthy mice from that of tumour
24 bearing mice, and do so after only three conditioning trials. Chemical analyses confirmed that the
25 presence of the tumour changed the urine odour, supporting the behavioural results. Our study
26 demonstrates that ants reliably detect tumour cues in mice urine and have the potential to act as efficient
27 and inexpensive cancer bio detectors.

28 **Keywords:** *Formica fusca*, olfactory conditioning, triple-negative breast cancer, GC-MS

29

30 **Background**

31 With 10 million deaths, and more than 19 million cases in 2020, cancer is a leading cause of mortality
32 worldwide (1). A way to increase cancer survival rate is to improve diagnosis methods. The sooner the

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33 tumours are detected, the higher are the chances of recovery for the patients. Current early detection
34 methods are often invasive (e.g., colonoscopy) and/or expensive (e.g., magnetic resonance imaging, MRI),
35 therefore relatively few people profit from available detection techniques. For example, in France, the
36 most prevalent cancer for women is breast cancer. However, despite France is a relatively rich country,
37 less than half (48.6 % in 2019) of the population at risk (women between 50 and 74) underwent
38 screening for this cancer.

39 A promising method to increase early cancer detection rate is the use of animal olfaction. Trained dogs
40 can detect tumours in cell samples or body odour samples (2,3). This method is based on the detection
41 of volatile organic compounds (VOCs) that are characteristic of tumours and linked to their altered cell
42 metabolism (4). Dogs are not the only animal species used as bio-detectors of cancer. Mice were trained
43 to discriminate tumour-bearing mice from healthy ones (5). The nematode *Caenorhabditis elegans*
44 showed attractive chemotaxis to some cancer VOCs (6). In fruit flies, by using *in vivo* calcium imaging,
45 a cutting edge and expensive technology, it was shown that olfactory neurons formed specific activation
46 patterns when exposed to the volatiles from a given cancer cell line (7). Using a well-known and simple
47 paradigm for olfactory conditioning, the proboscis extension response (8), honeybees were tested when
48 exposed to cancer odour (9). Insects are promising detection tools as they are relatively easy to handle,
49 do not require expensive rearing facilities, are available in large numbers and can be trained to recognize
50 an odour in very few trials. Amongst insects, ants, and especially *Formica fusca*, demonstrated
51 remarkable learning abilities using ecologically relevant odours. In this species, one training trial is
52 enough to form a genuine long-term memory lasting for days. Furthermore, these ants are highly
53 resistant to memory extinction: after training, they can be tested up to 9 times without reward before
54 their responses start to decline (10). Recently, we tested the olfactory detection abilities of *F. fusca*
55 towards cultured human cancer cell lines. Using ovarian (IGROV-1) and breast cell lines (MCF-7, MCF-
56 10A and MDA-MD-231) as stimuli, we showed that ants can correctly discriminate between a cancerous
57 cell line and a healthy one, and between two cancerous cell lines (11). Cell lines have the advantage of
58 being easily available and to provide reproducible odour samples over time, but they do not represent
59 the exact reality of tumours, which are complex tissues composed of different cell types. The use of
60 tumour tissues or whole organisms is therefore a critical step for testing ants as clinical detection tools.

61 In this study, we used olfactory stimuli (urine samples) from patient-derived xenograft (PDX) mice
62 carrying human tumours. Compared to cell lines that grow on a stable and known environment (culture
63 medium), PDX mice represent a more realistic model, as the cancerous cells composing a tumour are
64 growing in a live organism with all its complexity. Furthermore, human tumours retain their
65 characteristics when grafted on mice (heterogeneity, relative proportion of cells, and genomic
66 architecture) (12). Finally, tumours used in PDX are stable in time and can be duplicated, which allows
67 for a virtually infinite number of drug tests and preclinical investigations, ensuring that the patient at the
68 origin of the grafted tumour receives the optimal treatment (12,13).

69 Here, PDX were established using a “triple-negative” human breast tumour. Urine was chosen as body-
70 odour stimuli, as it can easily be collected and stored (14). Ant workers of the species *F. fusca* were
71 used as bio-detector since they are able to learn and detect cancer related odours stemming from

72 standardized cancer cell lines (11). Using a simple conditioning paradigm, we tested whether ants could
73 discriminate the urine of mice carrying a tumour from that of healthy mice. To characterize changes in
74 VOCs composition due to the presence of cancer, urine odour samples were analysed using solid-phase
75 micro-extraction (SPME) and gas chromatography coupled with mass spectrometry (GC-MS).

76

77 **Methods**

78 **(a) Insects and origin of colonies**

79 *Formica fusca* is a relatively common ant species in the Northern hemisphere. Colonies may contain
80 several hundred individuals and are headed by one queen (monogynous) or several queens
81 (polygynous). Three queenright colonies were collected in the forest of Ermenonville (France,
82 49°09'51.5" N, 2°36'49.2" E) and kept under laboratory conditions (25 ± 2 °C, $50 \pm 10\%$ relative humidity,
83 12 h/12 h: day/night) at the Laboratory of Experimental and Comparative Ethology (LEEC, University
84 Sorbonne Paris Nord). Tested ants were foragers (ants that leave the nest to search for food) and were
85 individually marked with a dot of oil-based paint (Mitsubishi Pencil) on the abdomen and / or thorax one
86 day prior to the conditioning. In all cases, each individual ant was used only for one conditioning and
87 test experiment.

88

89 **(b) Mice and odour source**

90 Female immunodeficient mice (Swiss nude mice from Charles River Laboratories) were maintained
91 under specific pathogen-free conditions at the Laboratory of pre-clinical investigation (LIP, Institut Curie,
92 Paris, France). The housing facility was kept at 22°C (± 2 °C) with a relative humidity of 30-70%. The
93 light/dark cycle was 12 h light/12 h dark. Patient-derived xenografts (PDX) from a triple-negative breast
94 cancer (HBCx-11, mesenchymal subtype (15)) were established with consent of the patient. The study
95 was approved by the local ethics committee (Breast Group of *Institut Curie* Hospital). Twelve mice were
96 used in our study: half ($n = 6$) were graft with tumour at 9-week-old into their interscapular fat pad, the
97 other half ($n = 6$) underwent the same chirurgial procedure but without the graft of the tumour (healthy
98 sham group). Mice were kept for seven weeks and were euthanized at the end of the experiment before
99 the PDX had a critical size of 15/15 mm. Tumour size was measured with a manual calliper, and the
100 total volume of a tumour was calculated as $V = a \times b^2 / 2$, a being the largest diameter, b the smallest
101 (Table 1 gives details about the size of each tumour). Care and housing of animals were in accordance
102 with Institutional Animal Care and French Committee–approved criteria (project authorization no.
103 02163.02).

104 Urine of mice was used as olfactory stimuli and was collected seven weeks after the graft. Mice were
105 placed individually in a cage (43 cm x 27 cm, height = 16 cm) without food and water for 3h. The floor
106 of the cage was covered with filter paper, which was collected at the end of the 3h soaked with urine.
107 Samples of 1 cm² of urine imbibed filter paper were inserted individually in an Eppendorf tube and stored
108 at -20 °C.

109

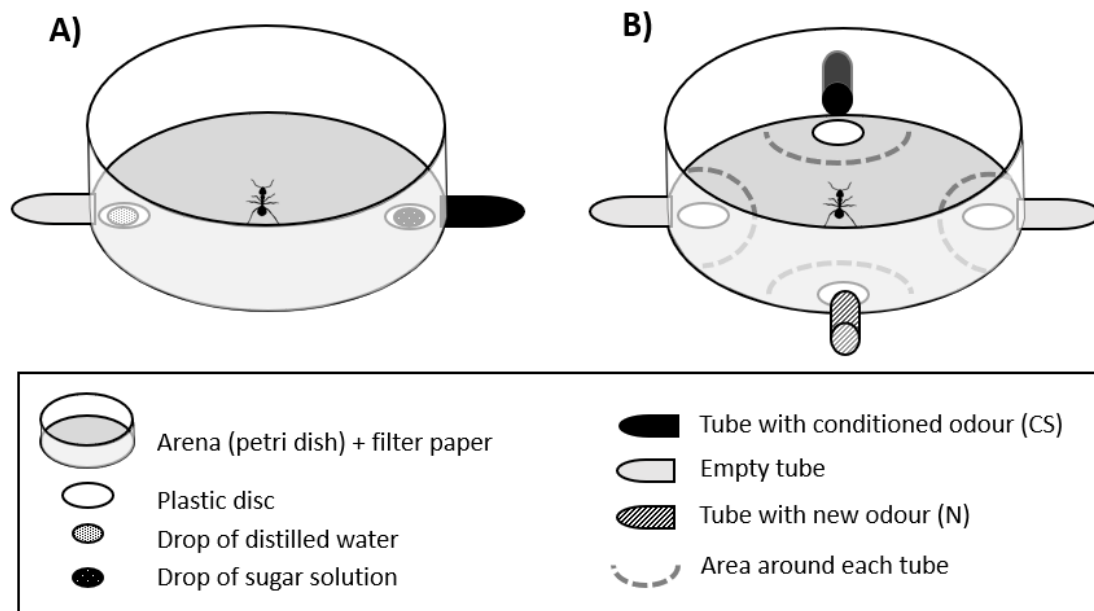
110 **Table 1| Size of the tumour of each mouse 7 weeks after the initial graft.** The estimated volume of
 111 tumour (V) was calculated as $V = a \times b^2 / 2$, with a being the largest side of the tumour and b the smallest
 112 side of the tumour.

Identity of the mouse	Largest side of the tumour (mm)	Smallest side of the tumour (mm)	Estimated volume (mm ³)
#1 – 6 (Sham)			
#11	10	9	405
#12	12	11	726
#13	11	11	665.5
#14	13	11	786.5
#15	9	7	196
#16	11	10	550
Mean size	11	9.83	554.83

113

114 **(c) Experimental protocol**

115 The experimental protocol was similar to the one used in Piqueret et al., (10). In brief, we trained
 116 individual ants to associate an odour (Conditioned Stimulus - CS, urine of mice) with a reward
 117 (Unconditioned Stimulus - US, 30% sugar solution) using a circular arena (Figure 1A). Three
 118 conditioning trials were performed by each ant (Figure 2). The time needed by the ant to find the reward
 119 was the variable measured in each conditioning trial.



120

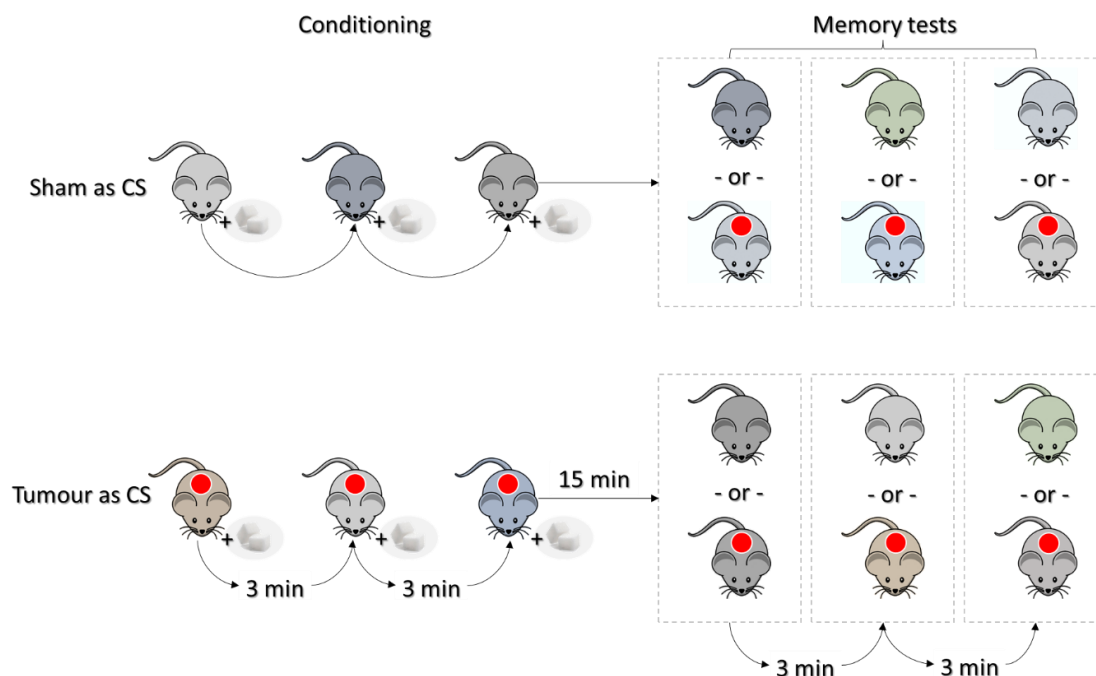
121 **Figure 1. Schema of the experimental device (arenas).**

122 A: Set-up used during the conditioning phase. The time to find the reward was noted.

123 B: Slightly modified set-up used during the memory tests. The time spent by the ant in the vicinity of the
 124 odours (areas, in dashed lines) was recorded during two minutes. Each area measured 35.5 cm². The
 125 orientation of the arena in the experimental room was changed between trials so that ants could not
 126 learn visual cues.

127

128 To test if ants had learned the CS, we performed memory tests without reward using an arena similar
129 to the one used for the conditioning (Figure 1B). In these memory tests, two odorant stimuli were
130 presented at the same time, on each side of the arena. One was the CS, while the other one was a new
131 stimulus (N). When urine from a mouse with tumour was used as CS, we used urine from a sham-mouse
132 as N, and vice versa. Two unscented stimuli were also present (empty Eppendorf tube) to control for
133 the potential learning of visual cues. Circular areas were drawn around each stimulus, which allowed us
134 to record as variable the time spent by the ant in the vicinity of each odorant. Each memory test lasted
135 2 min and the behaviour of the ant was scored using a behavioural transcription tool (software Ethoc v.
136 1.2, CRCA, Toulouse, France). All experiments were video recorded with a camera (Canon, Legria
137 HFR806) placed above the experimental arena. Ants underwent three consecutive unrewarded memory
138 tests at 15, 20, and 25 min after the end of the last conditioning trial (Figure 2). We used different stimuli
139 (urine from different mice) in the conditioning trials and in the memory test. Therefore, ants could not
140 rely on the possible individual odour of a given mice between the training and the tests.



141

142 **Figure 2. Schema of the procedure ants underwent during the behavioural experiments.**

143 Individual ants were trained to associate the odour (urine) of mice to the reward (sugar solution) three
144 times during the conditioning procedure, and later tested with odours from other mice during the memory
145 tests. Ants were trained either with urine of mice from the *sham* group (sham as CS, above), or from the
146 *tumour* group (tumour as CS, below). The red circles indicate that mice have a tumour. During the
147 memory tests, ants had to choose between urine of individuals from the two groups (*tumour vs sham*).
148 Each ant encountered urine samples from 9 different mice during the full procedure. They never
149 encountered the same stimulus more than once to exclude the possible use of individual identity cues.
150

151 The same stimuli were used for a group of six ants, which is one advantage of using urine stimuli (14).
152 However, we tested whether significant differences in terms of memory abilities were present when ants
153 were tested with *fresh* samples (urine never used in a test) or *reused* one (used for at least another

154 test). Ants tested with *reused* samples did not perform differently from ants tested with *fresh* samples
155 (*stimulus* × *sequence*: $F = 1.20$, $d.f = 2$, $p \geq 0.1$).

156

157 **(d) Chemical analysis**

158 Odour emitted by the urine of mice used in the behavioural experiments were characterized using
159 chemical analysis. A filter paper (1 cm²) soaked with urine was inserted in a 15 mL glass vial and placed
160 at 37 °C using a water bath. A SPME fibre (50/30 DVB/CAR/PDMS, Supelco) was introduced through
161 the PTFE/silicone 1.5 mm cap of the vial for 50 min (11,16). After that, the fibre was immediately inserted
162 into an Agilent Technologies 7890A gas-chromatograph, equipped with a HP5MS GC column (30 m ×
163 0.25 mm × 0.25 µm, Agilent Technologies, Les Ulis Cedex, France). The carrier gas was helium (1
164 mL.min⁻¹), and the injection was split less (250 °C). The oven temperature was programmed at 40 °C
165 for 5 min, then increased to 220 °C at 7 °C.min⁻¹, and to 300 °C at 15 °C.min⁻¹ and was held for 3 min.
166 The GC was coupled with a 5975 C mass-spectrometer (Agilent Technologies). Mass spectra were
167 recorded with electron impact ionization at 70 eV. Peak areas were integrated with MSD ChemStation
168 software version E.02.01.1177 (Agilent Technologies). Peaks were identified by comparing their ion
169 spectrum to the NIST library (NIST v2.2, 2014) and to standards injected with the same temperature
170 program (benzyl alcohol, acetophenone, nonanal, dodecane, decanal, all from Sigma Aldrich, Saint-
171 Louis, MO, USA).

172

173 **(e) Statistics**

174 **(a) Behaviour analysis**

175 Data were analysed using R software (v. 4.0.0, (17)). Significance was fixed at $\alpha = 5\%$. All data were
176 analysed using linear mixed models (LMMs, *lmer* function from the package *lme4*, (18)). Normality of
177 dataset was obtained either with log function (for the conditioning dataset) or with square root
178 transformation (for the memory tests dataset). The identity of the ants and the colony were included as
179 nested random factors.

180

181 *a. Acquisition*

182 We analysed the effect of the number of conditioning trials (factor *trials*) on the dependent variable *time*
183 (continuous variable, the time to find the reward). We analysed the effect of the *conditioning odorant*
184 (factor with two levels, tumour bearing mice or sham mice). We also looked at the interaction
185 *conditioning odorant* × *trials* to detect possible differences in ants' responses depending on the odorant
186 used.

187

188 *b. Memory tests*

189 First, we checked whether ants spent more time near the tubes with odours than near the control
190 unscented tubes by analysing the effect of the independent variable *presence of odour* (factor with two
191 levels, yes or no) on the dependent variable *time* (continuous variable, the time spent near the odorant
192 vials or the unscented ones). Then, in all experiments, we analysed the effect of the independent
193 variable *stimulus* (factor with two levels, CS or N) on the dependent variable *time* (continuous variable,
194 the time spent in the vicinity of a stimulus) during the 3 memory tests. Finally, we analysed the memory
195 tests separately for each stimulus origin (*tumour* or *sham* stimulus).

196

197 **2. Chemical analysis**

198 The areas of 49 regularly occurring peaks were standardized by calculating the $\ln(P_i/g(P))$ (19), where
199 P_i is the area of a peak and $g(P)$ is the geometric mean of all the peak areas of the individual. On these
200 variables, we run a Principal Component Analysis (PCA) (Table S3) and compared the scores of the
201 first two principal components (PC1, explaining 45.57% of the total variance, and PC2 that account for
202 29.90%) between groups (urine of tumour mice vs sham mice) using Wilcoxon's rank sum test.

203 We then calculated the chemical distance of each tumour sample from the centroid of the sham group
204 and we tested whether this distance could be correlated to the size of the tumour.

205

206 **Results**

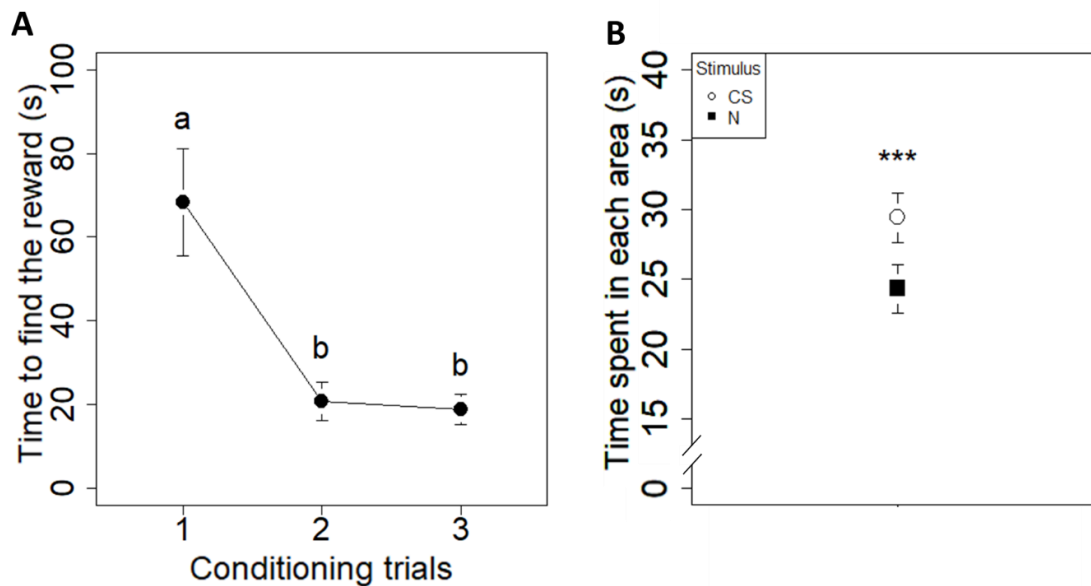
207 **(a) Ants can associate the odour mice urine to a food reward**

208 We conditioned half of the ants with the urine of tumour-bearing mice and the other half with urine of
209 healthy, sham treated mice. During the acquisition phase, the ants learned the two different stimuli (urine
210 from tumour mice and from sham mice) in a similar way, as shown by the absence of any significant
211 *conditioning odorant* \times *trial* interaction ($p > 0.1$). The time spent by the ants to find the reward decreased
212 significantly across the three training trials ($p < 0.001$, Table S1). This suggests that ants learned that
213 the urine odour (CS) predicted the sucrose reward (Figure 3A). Memory tests without reward were then
214 performed to assess if ants could recognize the learned urine type (tumour or healthy).

215 **(b) Ants can differentiate tumour-bearing mice from healthy ones after a rapid learning** 216 **phase**

217 Ants spent more time near the odorant tubes than near the unscented ones ($p < 0.001$), indicating that
218 ants were not conditioned to the presence of a tube (visual cue), but to the odour. When focusing on the
219 time spent near the tubes with odours, ants conditioned with the urine of tumour mice as CS and ants
220 conditioned with the urine of healthy mice as CS behave similarly, as shown by the absence of significant
221 *stimulus* \times *conditioning odorant* interaction ($p > 0.1$). During the memory tests, ants spent more time
222 near the CS odour than near the N odour independently of the odour used during conditioning ($p <$
223 0.001 , Figure 3B). Analysing the two different subsets of data separately gives similar results (ants
224 conditioned with tumour odour: $p = 0.001$; ants conditioned with healthy odour: $p = 0.006$, Table S2).

225 These results indicate that ants are able to detect whether mice were sick (presence of a tumour) or
226 healthy based solely on the odour of their urine.



227
228 **Figure 3. Ants' performances during acquisition and memory tests in an appetitive conditioning**
229 **experiment with urine of mice as odour stimulus.**

230 A: Ants were conditioned to associate the odour of mice urine (N = 70) either from the tumour group (N
231 = 35) or from the sham group (N = 35) with a reward (sugar solution 30%). The graph shows the time
232 needed by the ants to find the reward in the circular arena (Figure 1). Three conditioning trials were
233 done with an inter-trial interval of three minutes. Significant differences between conditioning trials are
234 indicated with different letters.

235 B: time spent by individual ants in the vicinity of each stimulus during the memory tests (circle:
236 conditioned stimulus, CS area; square: novel odour, N area). Circles and squares represent the mean
237 while error bars show confidence intervals (95%). Significant differences between stimuli are noted with
238 asterisks (***: $p \leq 0.001$).
239

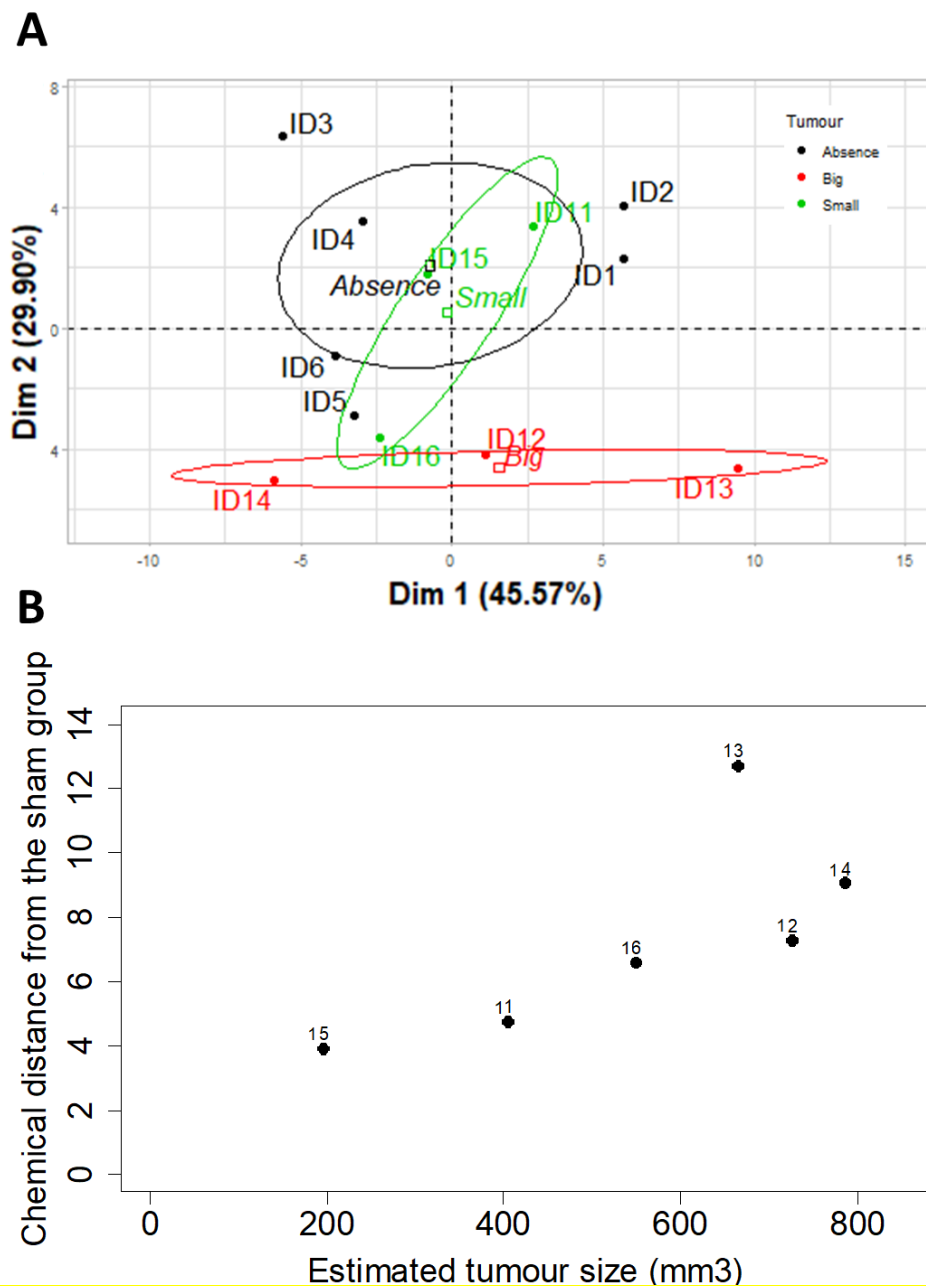
240 (c) Discrimination based on urine VOCs

241 To investigate the cues used by ants to differentiate the tumour mice from the healthy ones, we analyzed
242 the urine composition with Solid-Phase Micro-Extraction (SPME) coupled with Gas Chromatography
243 and Mass Spectrometry (GC-MS). The two groups of mice (tumour-bearing and healthy) can be
244 discriminated using PCA. Six principal components (PCs) with eigenvalues > 1 , explaining together
245 more than 90% of the total variance (Table S3) were used for discrimination. The first two PCs accounted
246 for 75.46% of the total variance (Table S3). The two groups (*Tumour* and *Sham* group) are statistically
247 different for the scores of the PC2 ($p < 0.05$), but not for PC1 ($p > 0.1$). Indeed, in the plot of the first two
248 PCs the confidence ellipses are partially overlapping each other (Figure S1). In particular, the samples
249 from two *tumour* individuals (#11 and #15) are similar to those from the sham group. We thus
250 investigated whether this could be linked to the size of the tumour.

251 In fact, mice #11 and #15 were the individuals with the smallest tumours (respectively 405mm^3 & 196
252 mm^3 , Table 1), well below the mean tumour size (554.83mm^3). We then reanalysed the data considering
253 a group of mice with *small* tumour (below the mean size), and a group with *big* tumours (above the mean

254 size, Table 1). With these new categories, the samples of mice with the big tumours are very well
255 separated from those of the sham individuals (Figure 4A, PC2: $p < 0.01$).

256 Using the first 6 PCs ($> 90\%$ of the total variance, Table S3), we calculated the chemical distance
257 (Euclidean distance) of each sample of tumour bearing mice from the mean coordinates (centroid) of
258 the samples of sham group (Figure 4B). We found a strong link between the size of the tumour and
259 chemical distance from the sham group: the larger the size of tumour, the greater the distance from the
260 sham group ($r = 0.73$, $p < 0.1$), although the p-value shows only a tendency given to the sample size
261 ($n=6$). Identification of the detected peaks can be seen in Table S4.



263 **Figure 4: Chemical differences induced by the tumour presence in mice.**

264 **A:** Visualization of the categorization of the urine samples using the first two Principal Components (PC),
265 explaining 68.82% of the total variance. Ellipses represent the confidence interval (0.95). The individuals
266 with large tumour (red) are very well separated from the sham individuals (black). The ones with small
267 tumour (green) are between the two other groups.

268 **B:** Relationship between tumour size of mice and chemical distance of their urine samples from those
269 of the sham group. Estimated tumour sizes (x-axis) are plotted against the chemical distance of each
270 tumour sample from the centroid of the sham group (y-axis). Data are highly correlated (Pearson
271 correlation test, $r_p = 0.73$, $p < 0.1$).

272

273 **Discussion**

274 We demonstrate that ants are able to learn to associate a complex blend of odours with a reward, after
275 only three conditioning trials. This simple and fast conditioning protocol allowed *Formica fusca* ants to
276 discriminate tumour-bearing PDX mice from tumour-free PDX mice in unrewarded tests. This
277 discrimination is based on the odour of the urine of mice altered by the presence of a tumour. Using
278 chemical analysis, we also demonstrate that the larger a tumour is, the more different the odour of the
279 bearing individual is from that of the control.

280 *F. fusca* ants are fast learners and show impressive memory abilities after only three associations
281 between an odour and a reward. Such learning abilities were already described in this species (10), but
282 also in other ant species, like *Lasius niger* (20), *Camponotus spp.* (21–23) and *Linepithema humile*
283 (24,25). Odours used in these studies were pure compounds (e.g. hexanal) or odour blends (e.g. food
284 flavouring), which were always ecologically relevant for ants, as they were based on floral or fruit odours.
285 In our previous study focused on learning of cancer-related odours by ants (11), *F. fusca* ants were able
286 to associate the odour of cultured human cancer cells (IGROV-1, MCF-7, MCF-10A, or MDA-MD-231)
287 with a reward. Obviously, cancer cells are not ecologically relevant for ants, but many animals are known
288 to be able to learn olfactory stimuli that are not present in their natural environment, such as drugs or
289 explosives, given the right incentive (26). In our previous study on cancer odours, we showed that ants
290 are able to discriminate a cancerous cell line from a healthy one, and a cancerous cell line from a
291 different cancerous cell line.

292 PDX mice established with human breast tumours are a step forward in clinical cancer detection and
293 represent an even more complex task for bio-detection as *i*) the odour used as stimulus is not directly
294 extracted from the cancer cells, *ii*) tumours are not composed of a single cell type and are therefore
295 heterogeneous, and *iii*) cancer cell odours might be overshadowed by the body odours of individuals or
296 altered when passing from the local micro-environment of the tumour tissue to the body fluids used as
297 conditioning stimulus (e.g. blood, exhaled breath, faeces, sweat, or urine). Despite all this complexity,
298 ants reliably discriminated tumour-free urine samples (*sham* group) from tumour-carrying ones (tumour
299 group) independently of the odour used during conditioning.

300 Our chemical analysis based on the volatiles emitted by urine, showed that odour samples from the
301 tumour group are chemically different from those of the sham group, and that this difference is correlated
302 to the size of the tumour (the larger the tumour, the more dissimilar the odour of the mouse is from the

303 control). These results are in accordance with the behaviour results, in which ants discriminated the
304 tumour-carrying and tumour-free mice based on olfaction.

305 In this study, we used urine from mice with a well-developed tumour (few days before the ethical
306 sacrifice) as odour stimuli. We noted that the level of alteration of the mice odour was linked to the
307 development of the tumour. Based on our chemical analysis, we found that some tumour-bearing
308 individuals were similar to the sham ones. This does not mean that ants were not able to discriminate
309 the small tumour individuals from the sham ones, as ants are able to detect compounds at a very low
310 concentrations (27). During our behaviour tests, we randomly assigned each ant to the odour of three
311 different mice during the conditioning, and six other mice during the memory tests (three from the
312 conditioned group, three from the other group). In this way, ants could not rely on the possible individual
313 odour of a given mouse. However, this experimental plan was not designed to quantify the impact of a
314 weak stimulus (a small tumour) on the ant performances. It would be interesting to test the olfactory
315 abilities of ants using only small and similar sized tumours, in order to find their detection threshold.
316 From a clinical point of view, the optimal conditioning protocol would have the shorter delay possible
317 between the screening and the result. Using cancer related odour (11), we managed to pin down the
318 conditioning time of ants to 30 minutes (three conditioning trials). *F. fusca* ants have the ability to learn
319 a simple odour stimulus after a single presentation (10), and we aim at testing this ability using complex
320 cancer-related odours. Finally, would it be possible to train not a single ant at a time, but hundreds of
321 individuals at the same time? Social insects (such as ants) live in colonies, and communication is
322 essential for them (28). It was demonstrated in another social insect species that one trained honeybee
323 could transfer a learned association to a naïve bee when their antennae were in contact (29). Studying
324 how information are shared amongst nestmates could provide an exponentially faster conditioning
325 methods if cancer related odours are shown to be successfully shared within the colony.

326 In this first study combining olfactory abilities of ants and the use of body fluids from human tumours in
327 PDX mice, we found that ants can be used as bio-detectors, as they reliably discriminate healthy
328 individuals from tumour-bearing ones, and vice versa. Ants could thus become a fast, efficient,
329 inexpensive, and non-invasive tool for detection of human tumours.

330

331 **Author' Contributions:**

332 B.P.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software,
333 Visualization, Validation, Writing – original draft, Writing – review & editing. E.Mo.: Resources, Writing
334 – review & editing. P.D: Methodology. C.L: Investigation, Formal analysis, Validation. E.Ma:
335 Resources, Writing – review & editing. J-.C.S.: Conceptualization, Formal analysis, Funding
336 acquisition, Project administration, Supervision, Validation, Writing – review & editing. P.d.E:
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341 The authors declare no competing interests.

342

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