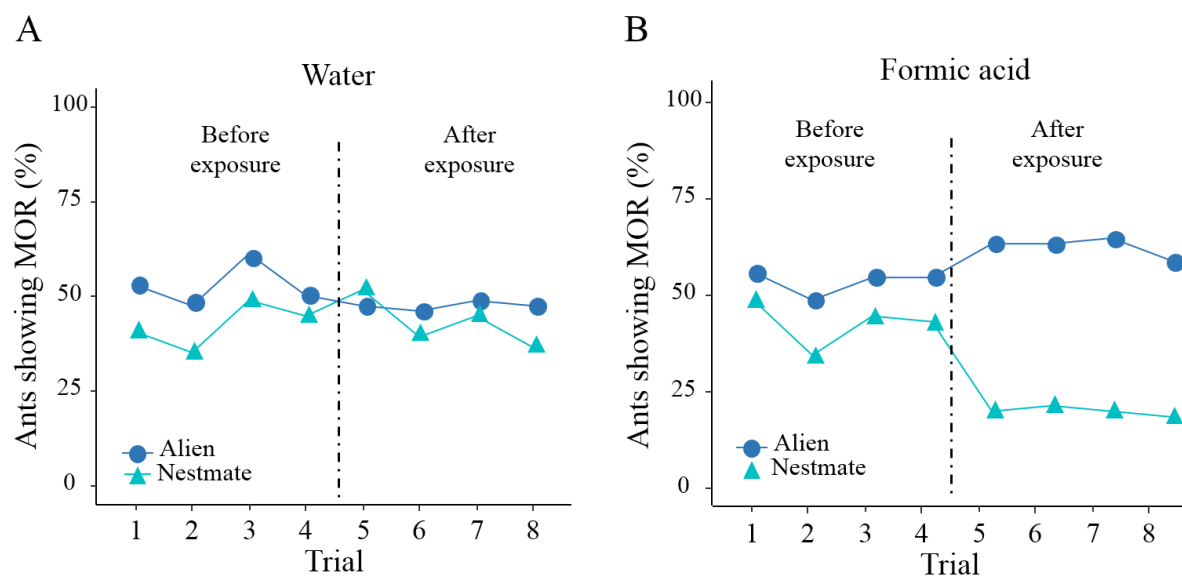
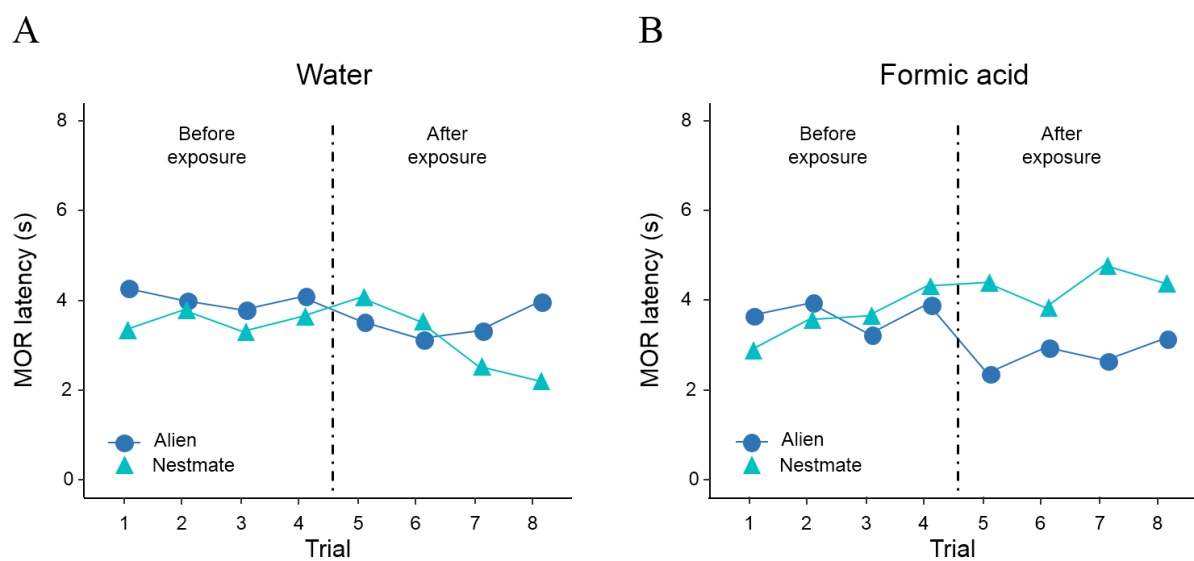


**Fig. S1.** Mean locomotor activity of ants previously exposed to either FA (pale blue) or water (dark blue). Error bars show standard deviation.



**Fig. S2.** Effect of FA and water exposure on MOR accuracy. Percentage of ants showing mandible opening response (MOR) when stimulated with eight presentation trials (four with nestmate odours and four with non-nestmate odours) before and after FA ( $n = 69$ ) or water ( $n = 73$ ) exposure. **(A)** Water neither affected MOR accuracy to nestmate odours nor to non-nestmate odours (GLMM, *Odour stimulus* \* *Exposure*:  $p = 0.27$ ). **(B)** FA made ants slightly more responsive to non-nestmate odours (GLMM, *Tukey post-hoc test*:  $p = 0.09$ ) and significantly less responsive to nestmate odours (GLMM, *Tukey post-hoc test*:  $p < 0.0001$ ).



**Fig. S3.** Effect of FA and water on MOR latency. Mandible opening response (MOR) latency showed by ants when stimulated with eight presentation trials (four with nestmate odours and four with non-nestmate odours) before and after FA ( $n = 69$ ) or water ( $n = 73$ ) exposure. **(A)** Water affected neither the latency of the aggressive response (MOR) to nestmate odours nor to non-nestmate odours (GLMM, *Odour stimulus* \* *Exposure*:  $p = 0.31$ ). **(B)** Ants exposed to FA had a shorter MOR latencies towards alien CHCs (LMM, *Tukey post-hoc test*:  $p < 0.0001$ ) but did not change the MOR latency to nestmate odours (GLMM, *Tukey post-hoc test*:  $p = 0.76$ ).

**Table S1.** (A) Anova output of the GLMM analysis of the Water group' accuracy performance in the test 1 (before water exposure). (B) Anova output of the GLMM analysis of the FA group' accuracy performance in the test 1 (before pheromone exposure).

| <b>(A)</b> glmer (resp ~ stimulus + trial + (1   ID) + (1   Col), family = binomial (link = "logit"),<br>data = ASSAY1W, na.action = na.omit, control = glmerControl (optimizer = 'bobyqa'))  |          |    |          |
|---|----------|----|----------|
| Dep var: <i>resp</i>  | $\chi^2$ | Df | p-values |
| <i>Intercept</i>  | 0.1169   | 1  | 0.68286  |
| <i>Stimulus</i>   | 5.6301   | 1  | 0.01765* |
| <i>Trial</i>  | 0.8292   | 1  | 0.36251  |
| <b>(B)</b> glmer (resp ~ stimulus + trial + (1   ID) + (1   Col), family = binomial (link = "logit"),<br>data = ASSAY1FA, na.action = na.omit, control = glmerControl (optimizer = 'bobyqa')) |          |    |          |
| Dep var: <i>resp</i>  | $\chi^2$ | Df | p-values |
| <i>Intercept</i>  | 0.3393   | 1  | 0.56022  |
| <i>Stimulus</i>   | 5.9364   | 1  | 0.01483* |
| <i>Trial</i>  | 0.0287   | 1  | 0.86556  |

**Table S2.** (A) Anova output of the GLMM analysis of the FA group' accuracy performance before and after pheromone exposure. (B) Anova output of the GLMM analysis of the Water group' accuracy performance before and after water exposure.

| <b>(A)</b> glmer (resp ~ Exposure + Stimulus + Exposure:Stimulus + (1   ID) + (1   Col), family = binomial (link = "logit"), data = LongdataFA, na.action = na.omit) |           |         |          |
|--|-----------|---------|----------|
| Dep var: <i>resp</i>   | $\chi^2$  | Df      | p-values |
| <i>Intercept</i>   | 6.0444    | 1       | 0.01395  |
| <i>Exposure</i>  | 5.3857    | 1       | 0.02030  |
| <i>Stimulus</i>  | 99.6940   | 1       | < 0.0001 |
| <i>Exposure*Stimulus</i>   | 36.3515   | 1       | < 0.0001 |
| contrast   | estimates | z-ratio | p-values |
| <i>after A - before A</i>  | 0.433     | 2.321   | 0.0933   |
| <i>after A - after N</i>   | 2.138     | 9.985   | < 0.0001 |
| <i>after A - before N</i>  | 0.877     | 4.658   | < 0.0001 |
| <i>before A - after N</i>  | 1.705     | 8.126   | < 0.0001 |
| <i>before A - before N</i>   | 0.444     | 2.399   | 0.0772   |
| <i>after N - before N</i>  | -1.260    | -6.048  | < 0.0001 |
| <b>(B)</b> glmer (resp ~ Exposure + Stimulus + Exposure:Stimulus + (1   ID) + (1   Col), family = binomial (link = "logit"), data = LongdataW, na.action = na.omit)  |           |         |          |
| Dep var: <i>resp</i>   | $\chi^2$  | Df      | p-values |
| <i>Intercept</i>   | 1.1472    | 1       | 0.2841   |
| <i>Exposure</i>  | 1.9195    | 1       | 0.1659   |
| <i>Stimulus</i>  | 0.6138    | 1       | 0.4334   |
| <i>Exposure *Stimulus</i>  | 1.2108    | 1       | 0.2712   |

**Table S3. (A)** Anova output of the GLMM analysis of the FA group' latency of the MOR before and after pheromone exposure. **(B)** Anova output of the GLMM analysis of the Water group' latency of the MOR before and after water exposure.

| <b>(A)</b> glmer (resp ~ Exposure + Stimulus + trial + Exposure:Stimulus + (1 ID), family = poisson, data = Longdata1FA, na.action = na.omit) |           |         |          |
|---|-----------|---------|----------|
| Dep var: <i>resp</i>  | $\chi^2$  | Df      | p-values |
| <i>Intercept</i>  | 3610.95   | 1       | < 0.0001 |
| <i>Exposure</i>   | 563.99    | 1       | < 0.0001 |
| <i>Stimulus</i>   | 749.95    | 1       | < 0.0001 |
| <i>Trial</i>  | 146.34    | 1       | < 0.0001 |
| <i>Exposure *Stimulus</i>   | 555.39    | 1       | < 0.0001 |
| contrast  | estimates | z-ratio | p-values |
| <i>after A - before A</i>   | -0.5348   | -23.749 | < 0.0001 |
| <i>after A - after N</i>  | -0.4612   | -27.385 | < 0.0001 |
| <i>after A - before N</i>   | -0.4854   | -21.204 | < 0.0001 |
| <i>before A - after N</i>   | 0.0737    | 3.000   | 0.0144   |
| <i>before A - before N</i>  | 0.0494    | 3.599   | 0.0018   |
| <i>after N - before N</i>   | -0.0242   | -0.975  | 0.7637   |
| <b>(B)</b> glmer (resp ~ Exposure + Stimulus + trial + Exposure:Stimulus + (1 ID), family = poisson, data = Longdata1W, na.action = na.omit)  |           |         |          |
| Dep var: <i>resp</i>  | $\chi^2$  | Df      | p-values |
| <i>Intercept</i>  | 6874.0159 | 1       | <0.0001  |
| <i>Exposure</i>   | 0.1724    | 1       | 0.6780   |
| <i>Stimulus</i>   | 63.7745   | 1       | <0.0001  |
| <i>Trial</i>  | 86.0800   | 1       | <0.0001  |
| <i>Exposure *Stimulus</i>   | 1.0147    | 1       | 0.3138   |

## Supplementary Materials and Methods

### *Locomotor activity assay*

Medium size forager ants were gently collected from the foraging arena of their colony. Ants were immediately exposed either to 25  $\mu$ L of formic acid (FA) (12 % in water) or 25  $\mu$ L of the solvent alone (pure water). To this end each ant was individually confined in a small glass vial (3 ml) glued in the middle of a 50 ml plastic flasks containing a filter paper soaked either with FA or water. The outer part of the glass vial was coated with Fluon® so that the ant was free to move inside the vial but without any possibility to escape and enter in direct contact with the pheromone/water. Immediately after the insertion of the ant and the pheromone/water into the device, the 50 ml flask was closed and placed under a hood. The exposure lasted 15 mins. After exposure, each ant was moved into a Fluon-coated ring (h = 3 cm,  $\varnothing$  = 2.2 cm) positioned in the middle of a testing arena (h = 5.3 cm,  $\varnothing$  = 8 cm) with Fluon-coated walls. After a 30-minute resting time (the same delay used in the MOR experiments), the small cylinder was removed, and the ant was free to explore the arena for additional 30 minutes. The floor of the arena was covered by a clean filter paper with 2 orthogonal drawn lines passing for the centre and delimiting four quadrants. All trials were videotaped. A total of 24 ants from two different colonies were tested (12 ants exposed to FA and 12 ants exposed to water). The number of times each ant crossed the lines was recorded and used as a proxy of locomotor activity. The results show that FA did not impair nor modulate the locomotor activity rate of ants (Two-Sample t test,  $p = 0.64$ ).