Electronic Supplementary Information

Ants learn fast and do not forget: olfactory associative learning, memory and extinction in *Formica fusca*

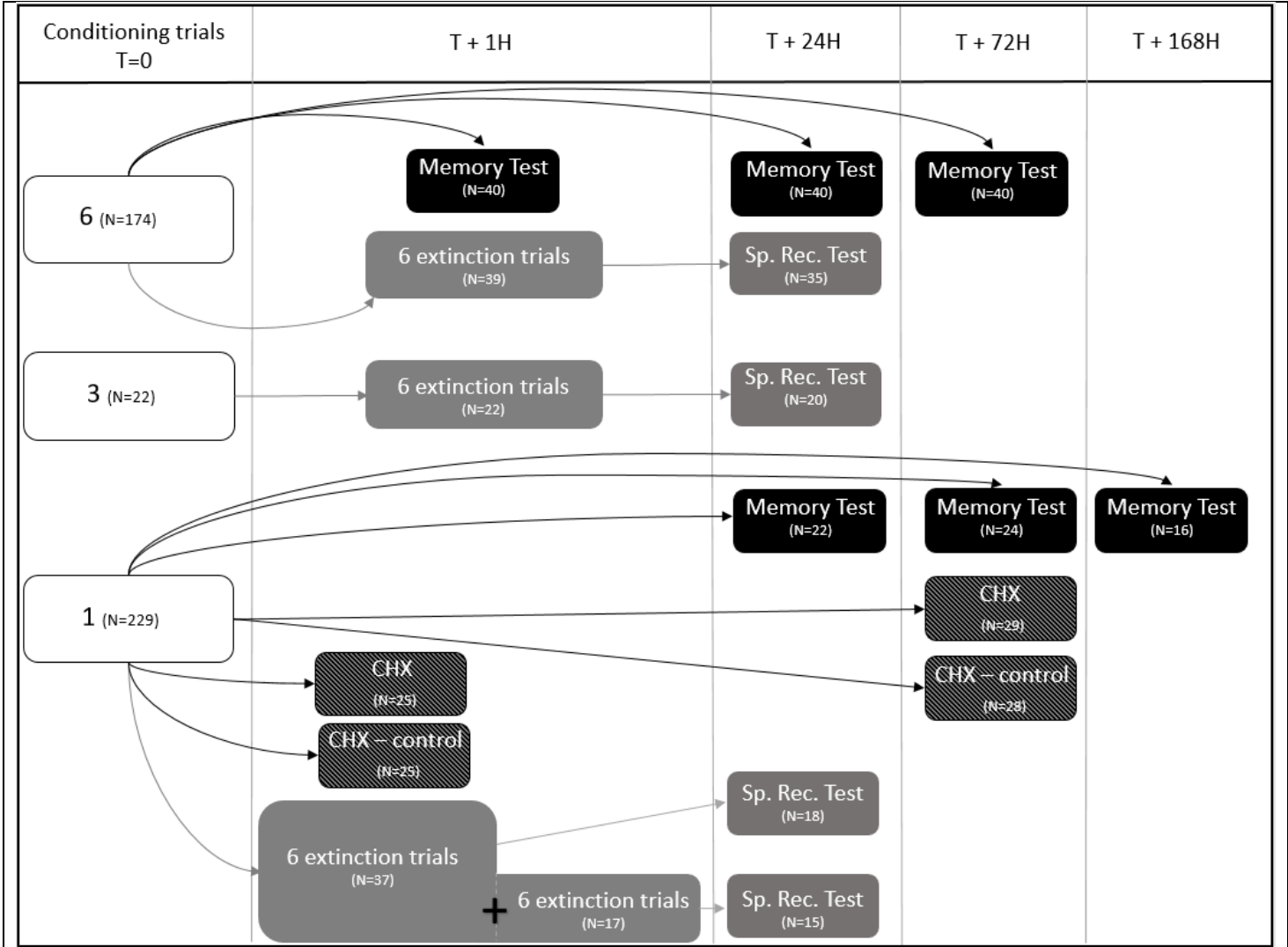Baptiste Piqueret, Jean-Christophe Sandoz, Patrizia d'Ettorre

Figure S1: Overview of the conducted experiments. The left column shows the number of conditioning trials ants were subjected to (white box). Three different experiments were performed. Independent groups of ants underwent a memory test (black boxes), an extinction protocol (grey boxes) or were treated with CHX before performing a memory test (black striped boxes). Tests were conducted 1 h, 24 h, 72 h or 168 h after the end of conditioning. For the ants that underwent an extinction protocol, a test of spontaneous recovery (Grey boxes: Sp. Rec. Test) was performed 24h after the last extinction trial. N= number of ants.
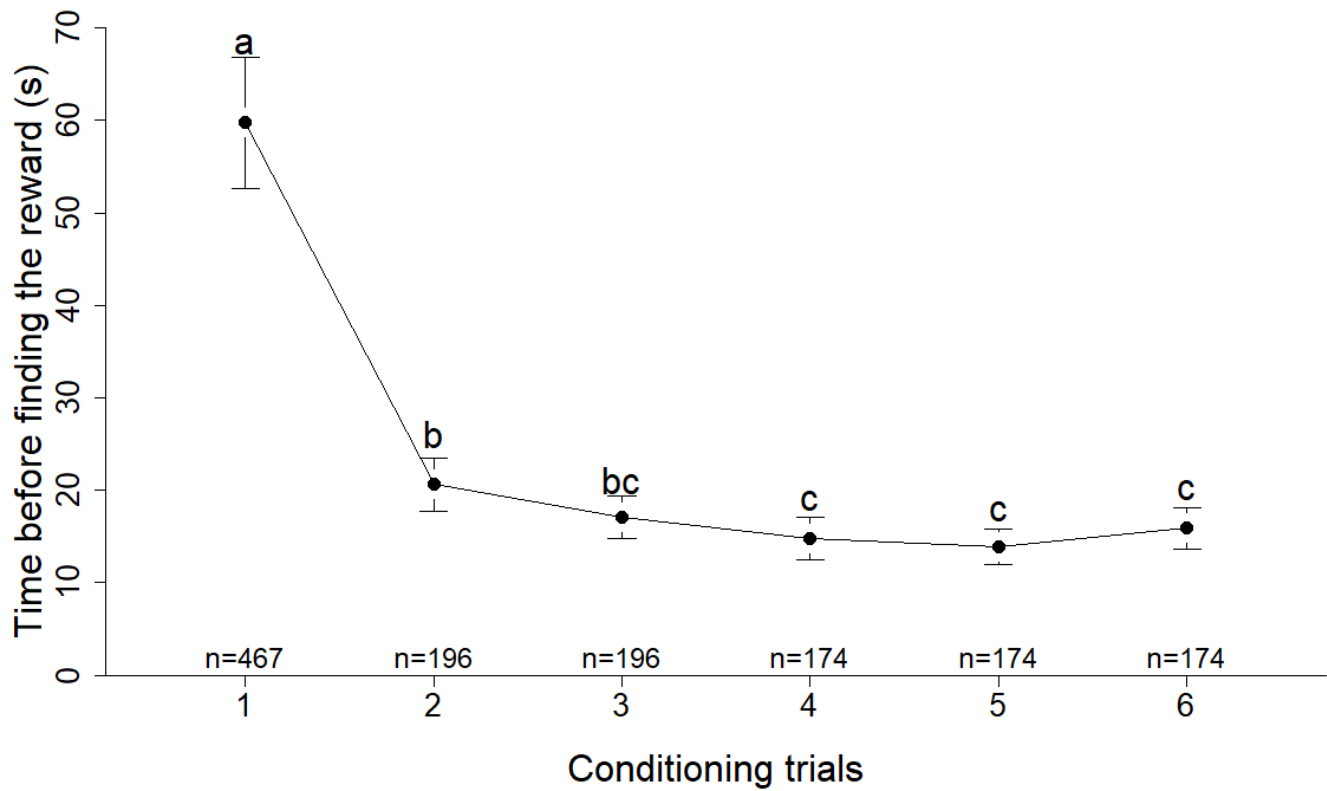
Figure S2: Time (mean + 95% confidence interval) spent by the ants to find the reward along the conditioning trials (N = 174 for six trials, N = 22 for three trials, N = 271 for one trial). Letters indicate difference between trials after Holm-Bonferroni correction. In particular, the first trial was different from all the other trials, in which the time to find the reward was significantly shorter (post-hoc, $p < 0.001$ for all comparison, after Holm-Bonferroni correction). The second and third trials were not significantly different ($p > 0.05$); however, the second trial was different from all the other trials ($p < 0.05$ in all cases). There was no difference from the third trial to the sixth ($p > 0.05$ in all cases).

Table S1| Result of the different model used during the analyses of the ant conditioning (Fig S3): Time was always the dependent variable. Significant effects are given in bold.

Focus on the factor(s)	Df	F value	p value
Conditioning odorant × trials × Conditioning groups	2	0.9271	0.396
Conditioning odorant × trials	5	0.9101	0.473
Trials	5	72.4502	**<0.001**
Trials (1 vs 2)			**<0.001**
Trials (1 vs 3)			**<0.001**
Trials (1 vs 4)			**<0.001**
Trials (1 vs 5)			**<0.001**
Trials (1 vs 6)			**<0.001**
Trials (2 vs 3)			0.287
Trials (2 vs 4)			**0.007**
Trials (2 vs 5)			**<0.001**
Trials (2 vs 6)			**0.048**
Trials (3 vs 4)			0.685
Trials (3 vs 5)			0.270
Trials (3 vs 6)			1.000
Trials (4 vs 5)			1.000
Trials (4 vs 6)			1.000
Trials (5 vs 6)			0.680

Table S2 | Results of the model used during the analyses of the memory tests (Fig 3): Time was the dependent variable; *p*-value are adjusted with Bonferronni-Holm correction when needed. Significant effects are given in bold. Tendency effects are underlined.

Interaction or factor analysed	Post-hoc	Df	F value	p value
elapsed time × conditioning groups × stimulus		4	1.7707	0.133
conditioning groups × stimulus		1	0.5172	0.472
stimulus × elapsed time		3	7.8924	**<0.001**
stimulus × elapsed time	1h/24h	1		0.716
stimulus × elapsed time	1h/72h	1		**0.022**
stimulus × elapsed time	1h/168h	1		**<0.001**
stimulus × elapsed time	24h/72	1		**0.012**
stimulus × elapsed time	24h/168h	1		**<0.001**
stimulus × elapsed time	72h/168h	1		<u>0.068</u>
Stimulus	1h	1	62.997	**<0.001**
Stimulus	24h	1	65.597	**<0.001**
Stimulus	72h	1	13.141	**<0.001**
Stimulus	168h	1	0.488	0.495

Table S3 | Results of the model used during the analyses of the effect of the cycloheximide (CHX, given 3 hours before the conditioning trial) on memory retention (Fig 4): Time was the dependent variable. Significant effects are given in bold. Tendency effects are underlined.

Interaction or factor analysed	Post-hoc	Was CHX given?	Df	F value	p value
Treatment × Stimulus				0.783	0.378
Stimulus	1h	No	1	4.167	**0.046**
Stimulus	1h	Yes	1	8.627	**0.005**
Treatment × Stimulus				2.907	<u>0.091</u>
Stimulus	72h	No	1	6.039	**0.017**
Stimulus	72h	Yes	1	0.025	0.872

Table S4 | Results of the model used during the analyses of the effect of the cycloheximide (CHX, given 72 hours before the conditioning trial) on memory retention: Time was the dependent variable. Significant effects are given in bold.

Interaction or factor analysed	Post-hoc	Df	F value	p value
Treatment × Stimulus		1	2.204	0.143
Stimulus	CHX	1	24.468	**<0.001**
Stimulus	Control	1	12.590	**0.001**

Table S5 | Results of the model used during the analyses of the extinction procedure (Fig 5): Time was the dependent variable. Significant effects are given in bold. Tendency effects are underlined.

Interaction or factor analysed	Post-hoc	Df	F value	p value
stimulus × conditioning group		2	35.621	**<0.001**
Six conditioning trials				
stimulus × extinction trials		5	1.397	0.224
Stimulus	Trial 1	1	106.180	**<0.001**
Stimulus	Trial 2	1	127.940	**<0.001**
Stimulus	Trial 3	1	102.750	**<0.001**
Stimulus	Trial 4	1	142.700	**<0.001**
Stimulus	Trial 5	1	74.979	**<0.001**
Stimulus	Trial 6	1	50.542	**<0.001**
Stimulus	Trial Recuperation	1	66.753	**<0.001**
stimulus × extinction trials	Trial 6/ Recuperation	1	0.104	0.746
Three conditioning trials				
stimulus × extinction trials		5	2.140	<u>0.061</u>
Stimulus	Trial 1	1	29.489	**<0.001**
Stimulus	Trial 2	1	71.702	**<0.001**
Stimulus	Trial 3	1	15.145	**<0.001**
Stimulus	Trial 4	1	35.551	**<0.001**
Stimulus	Trial 5	1	28.973	**<0.001**
Stimulus	Trial 6	1	49.574	**<0.001**
Stimulus	Trial Recovery	1	19.645	**<0.001**
stimulus × extinction trials	Trial 6/Recovery	1	0.008	0.926
One conditioning trial				
Stimulus × extinction trials		11	1.825	**0.046**
Stimulus	Trial 1	1	16.689	**<0.001**
Stimulus	Trial 2	1	23.354	**<0.001**
Stimulus	Trial 3	1	28.386	**<0.001**
Stimulus	Trial 4	1	27.947	**<0.001**
Stimulus	Trial 5	1	12.617	**<0.001**
Stimulus	Trial 6	1	22.053	**<0.001**
Stimulus	Trial 7	1	3.687	<u>0.063</u>
Stimulus	Trial 8	1	8.742	**0.005**
Stimulus	Trial 9	1	0.883	0.354
Stimulus	Trial 10	1	0.001	0.975
Stimulus	Trial 11	1	0.874	0.356
Stimulus	Trial 12	1	4.139	<u>0.051</u>
Stimulus	Trial Recovery 6	1	22.597	**<0.001**
Stimulus	Trial Recovery 12	1	0.262	0.612
Stimulus × extinction trials	Trial 6/ Recovery 6	1	0.177	0.675
Stimulus × extinction trials	Trial 12/ Recovery 12	1	0.749	0.390