Lateralization of short- and long-term visual memories in an insect

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Supplementary materials

(a) Generalised linear mixed models for responses during training

For comparisons between groups, trial number and training type were included as fixed effects, whereas the individual ant was a random factor, using the command `glmer (MaLER ~ Trial + Training + (1|Individual))`. For assessing the responses within a training group, we used the command `glmer (MaLER ~ Trial + (1|Individual))`.

(b) Barnard tests for comparing MaLER responses during testing

Two-tailed tests were applied for comparisons between two paired groups or between two unpaired or CSO groups. For comparisons between a paired group with an unpaired or with CSO, tests were one-tailed.

(c) Bonferroni corrections

Bonferroni corrections were applied for comparisons of the ants' performances during testing with the CSO control ants, with a cut-off of p=0.0071 for the 10 minutes test and p=0.00625 at 1 hour and 24 hours. Further corrections were applied for the comparison of RUPL with UPL and PL at 24 hours, with a cut-off of p=0.025.

(d) Analysis of facing direction during training

To determine whether the direction ants faced (right or left) during training influenced learning, we ran a logistic regression model with the paired groups PR and PL, where trial number, type of training and the direction ants faced were fixed effects and the individual ant was a random effect, using the command `glmer (MaLER ~ Trial + Training + Facing + (1|Individual))`.

Supplementary figures

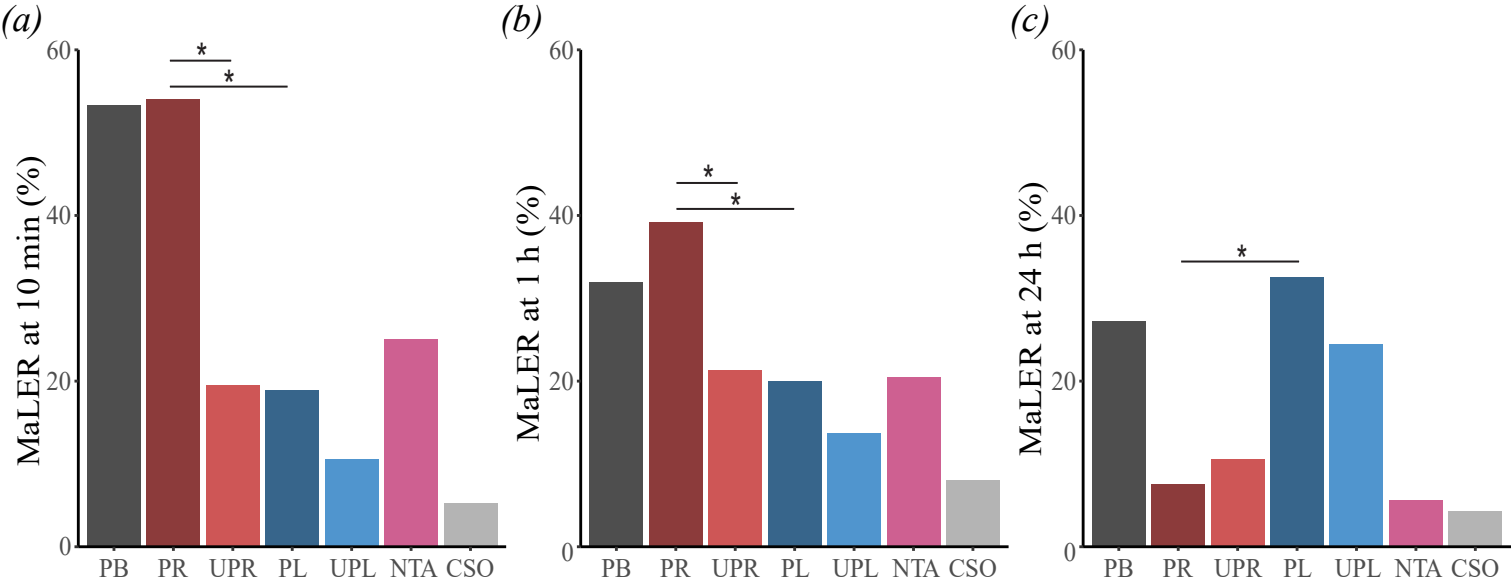
Supplementary figure 1. Sequence of events during a paired training trial with a reinforcement on the right antenna. A) 10 seconds of CS visualization, where the ant is shown the visual cue attached to the syringe. B) Drop of sugar touching the right antenna. C) Ant feeding on sugar during approximately 5 seconds. The ants' eyes, antenna and mouthparts, the pin and wax fixing the ant and the needle with sugar droplet are visible in every frame. The CS is attached to the other end of the needle (not visible here).

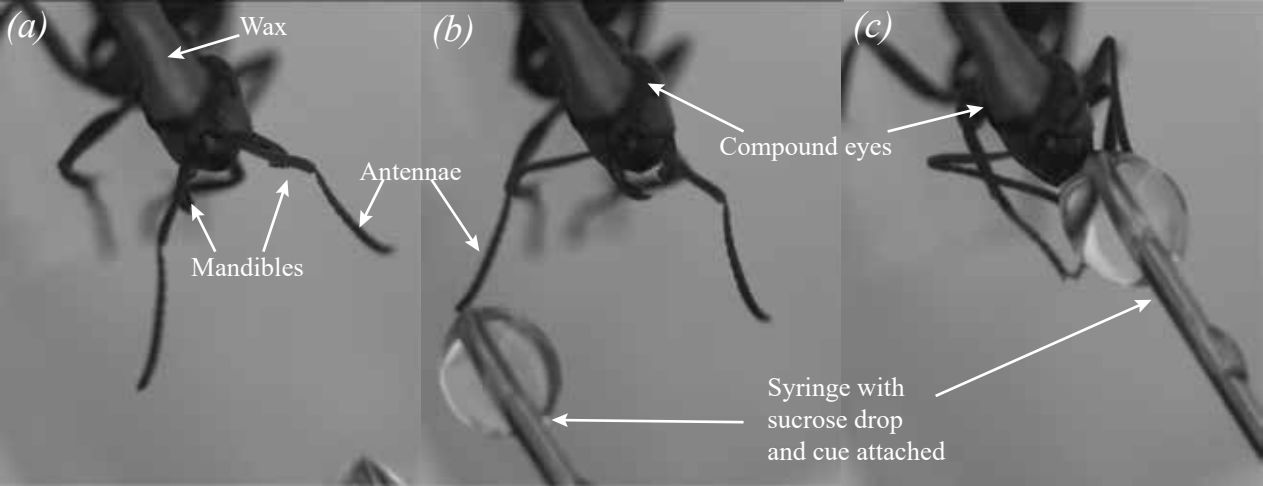
Supplementary figure 2. Wood ants recall of a visual memory after 24 hours is less precise than during training. A) Percentage of MaLER responses during training, for all groups reinforced on the left antenna (PL: dark blue; UPL: medium blue; RUPL: light blue, N=45) and ants that did not contact with the sugar reward (CSO: grey). Ants subjected to a paired training show a significant increase of responses with training trial, but ants with either type of unpaired training or with a CSO type of training don't. B) MaLER performance of all left reinforcement groups are low after 1 hour with no distinction with the CSO baseline. C) 24 hours after training, MaLER responses are elevated for all ants trained with a reinforcement on the left antenna. These are not significantly different than each other but PL is the only group that responded significantly more than the CSO after Bonferroni corrections were applied. Asterisks indicate significant differences in MaLER percentages during testing, between training types.

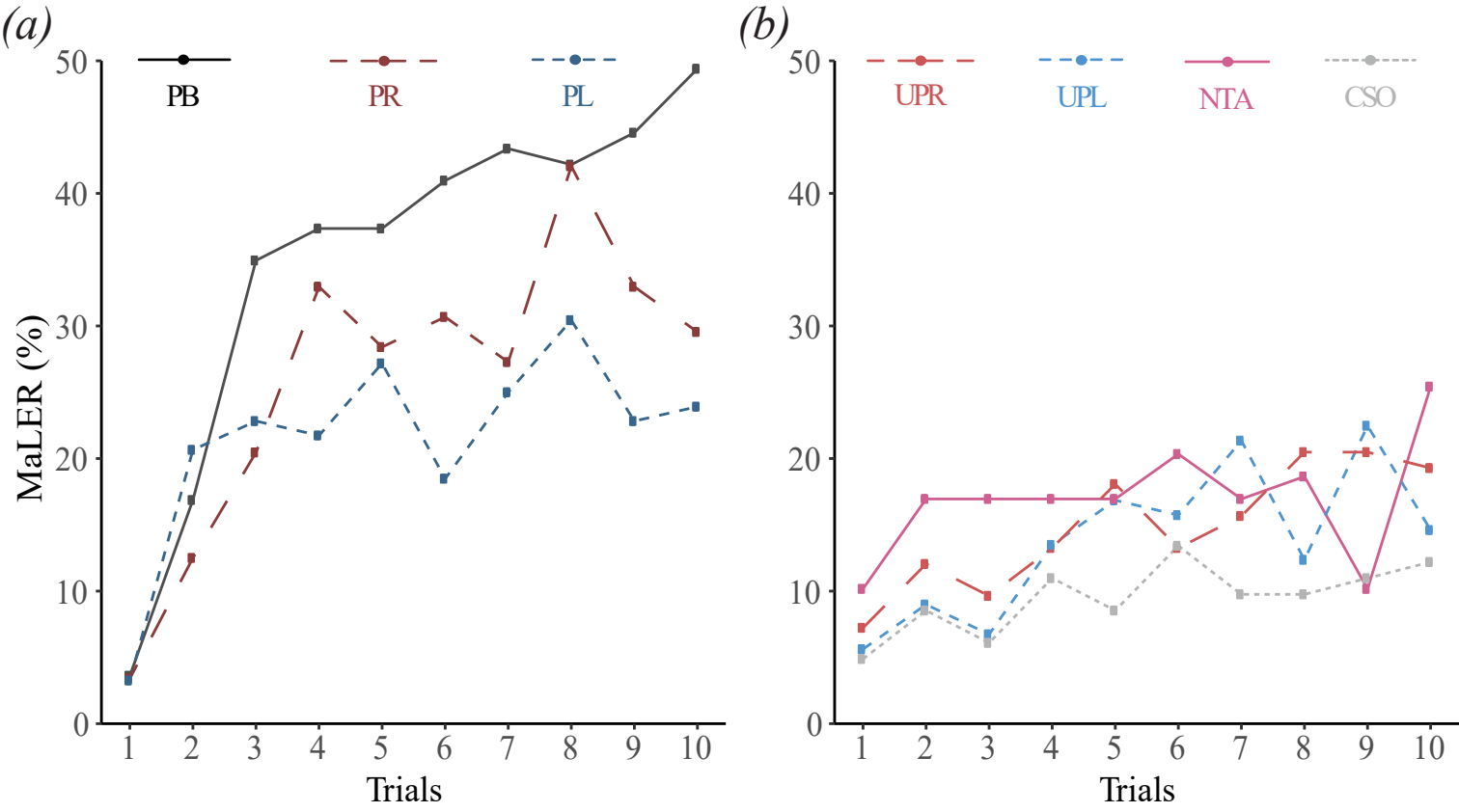
Supplementary tables

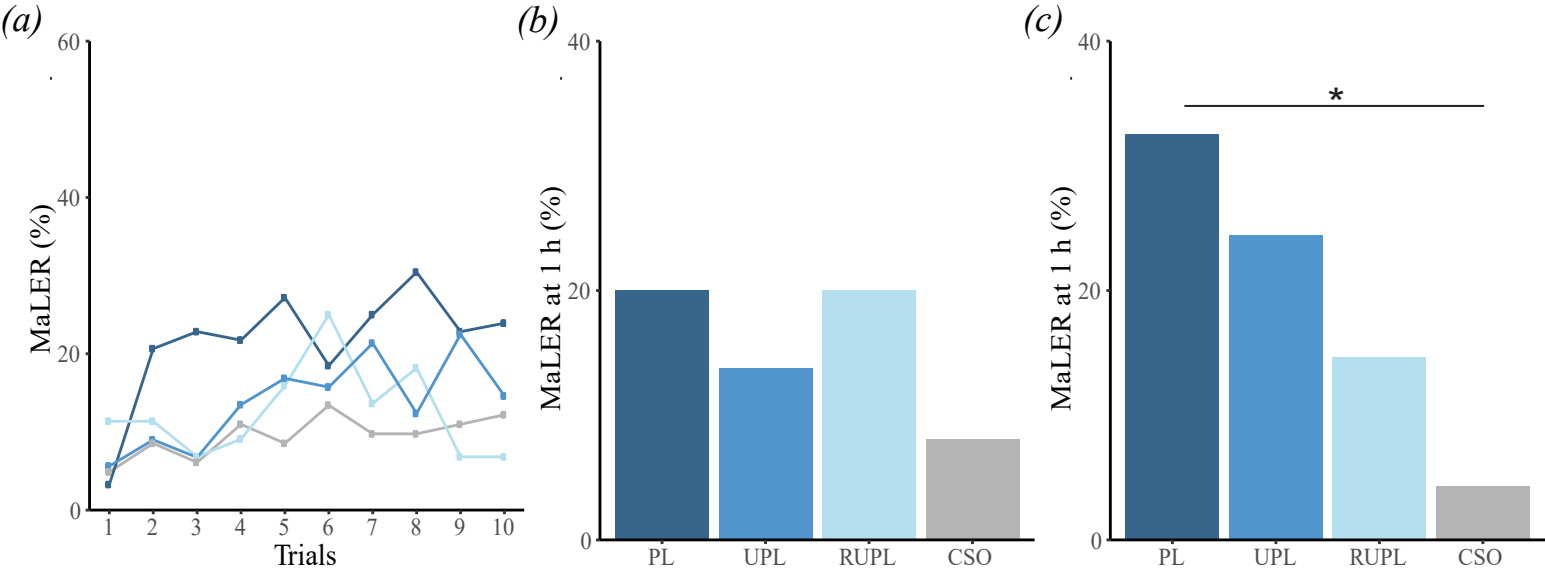
Supplementary table 1. Comparison of the frequency of ants performing MaLER when presented with the visual CS at 10 minutes, 1 hour or 24 hours after training between each group and the CSO control. The cut-off P-value after Bonferroni corrections is P=0.0071 for the 10 minutes test and P=0.00625 for 1 hour and 24 hour tests. The number of ants (N), degrees of freedom (d.f.) and P-value are shown (*P<cut-off).

Test	Treatment compared to CSO	N	d.f.	P
10 min	PB	34	33	0.0008 *
	PR	56	55	0.0003 *
	PL	56	55	0.15
	UPR	55	54	0.17
	UPL	57	55	0.61
	NTA	39	37	0.06
	USO	38	36	0.68
1 hour	PB	109	108	0.0008 *
	PR	113	112	3.6 e-05 *
	PL	117	116	0.03
	UPR	109	107	0.05
	UPL	113	111	0.37
	RUPL	109	107	0.08
	NTA	101	100	0.04
	USO	92	91	0.83
24 hours	PB	69	68	0.0063
	PR	87	86	0.31
	PL	82	81	0.0003 *
	UPR	85	83	0.32
	UPL	88	86	0.0063
	RUPL	88	86	0.099
	NTA	83	81	0.43
	USO	70	68	0.99


(a)
(b)
(c)
MaLER at 10 min (%)
MaLER at 1 h (%)
MaLER at 24 h (%)
0
20
40
60
*
PB
PR
UPR
PL
UPL
NTA
CSO


(a)
Wax
Antennae
Mandibles
(b)
Compound eyes
(c)
Syringe with
sucrose drop
and cue attached


(a)
PB
PR
PL
MaLER (%)
0
10
20
30
40
50
1
2
3
4
5
6
7
8
9
10
Trials
(b)
UPR
UPL
NTA
CSO
0
10
20
30
40
50
1
2
3
4
5
6
7
8
9
10
Trials


(a)
MaLER (%)
60
40
20
0
1
2
3
4
5
6
7
8
9
10
Trials
(b)
MaLER at 1 h (%)
40
20
0
PL
UPL
RUPL
CSO
(c)
MaLER at 1 h (%)
40
20
0
*
PL
UPL
RUPL
CSO