Mizunami et al. Development of behavioral automaticity by extended Pavlovian training in an insect.

Supplementary table S1 and figures S1-S4.

Table S1. Statistical results.

Groups	n	Test	H or W or U	P	Fig.
p/v, before (17 groups)		K-W	H=18.4 (df=19)	0.50	-
a:b, before (12 groups)		K-W	H=15.0 (df=15)	0.45	-
4trials x 1d, p/v; control: before vs after training	22	WCX	W=31	0.0033	1a
devalued: before vs after training	20	WCX	W=69	0.189	1a
After training: control vs devalued		M-W	U=346	0.0031	1a
4trials x 1d, p/v; sucrose US, no water: before vs after training	17	WCX	W=18	0.00038	1b
sucrose US, water: before vs after training	16	WCX	W=10	0.0049	1b
water US, water: before vs after training	19	WCX	W=66	0.41	1b
sucrose US, after training: no water vs water		M-W	U=161	0.38	1b
4trials x 3d, p/v; control: before vs after training	29	WCX	W=60	0.0036	2a
devalued: before vs after training	32	WCX	W=78	0.00013	2a
after training: control vs devalued		M-W	U=573.5	0.12	2a
4trials x 1d, p/v; control: before vs 3d after training	18	WCX	W=11	0.00084	2b
devalued: before vs 3d after training	20	WCX	W=123	0.52	2b
3d after training: control vs devalued		M-W	U=313	0.00032	2b
4trials x 1d, a:b; control: before vs after training	37	WCX	W=277	0.000067	2c
devalued: before vs after training	34	WCX	W=88	0.96	2c
After training: control vs devalued		M-W	U=368.5	0.0055	2c
4trials x 3d, a:b; control: before vs after training	37	WCX	W=142	0.0023	2d
devalued: before vs after training	38	WCX	W=130	0.00084	2d
after training: control vs devalued		M-W	U=785.5	0.38	2d
4trials x 1d, p/v; saline: before vs after training	33	WCX	W=72	0.00023	4a
epinastine: before vs after training	28	WCX	W=169	0.45	4a
after training: saline vs epinastine		M-W	U=619	0.047	4a
4trial x 3d, p/v; saline: before vs after training	26	WCX	W=95	0.026	4b
epinastine: before vs after training	21	WCX	W=117	0.029	4b
after training: saline vs epinastine		M-W	U=313.5	0.39	4b
4trials x 1d,a:b; saline: before vs after training	47	WCX	W=205	0.00022	4c
epinastine: before vs after training	41	WCX	W=351	0.43	4c
after training: saline vs epinastine		M-W	U=1351	0.0024	4c

Groups	n	Test	W or U	P	Fig.
4trials x 3d,a:b; saline: before vs after training	35	WCX	W=145	0.0090	4d
epinastine: before vs after training	28	WCX	W=52	0.00082	4d
after training: saline vs epinastine		M-W	U=525	0.73	4d
4trialx 1d, time to visit peppermint; control: before vs after training	24	WCX	W=40	0.0035	S1a
devalued: before vs after training	24	WCX	W=133.5	0.90	S1a
After training: control vs devalued		M-W	U=90.5	0.00014	S1a
4trials x 1d, time to visit vanilla; control: before vs after training	24	WCX	W=176	0.47	S1b
devalued: before vs after training	24	WCX	W=216.5	0.18	S1b
After training: control vs devalued		M-W	U=213.5	0.25	S1b
4trials x 1d, total time to visit odors; control: before vs after training	24	WCX	W=74	0.11	S1c
devalued: before vs after training	24	WCX	W=209	0.094	S1c
After training: control vs devalued		M-W	U=120.5	0.0017	S1c
no training p/v: before vs after water devaluation	19	WCX	W=116	0.19	S2
12 trials x 1d, p/v; control: before vs after training	22	WCX	W=3	0.0000072	S3a
devalued: before vs after training	25	WCX	W=175	0.48	S3a
after training: control vs devalued		M-W	U=438	0.0010	S3a
6trials x 2d, p/v; control: before vs after training	22	WCX	W=14	0.00016	S3b
devalued: before vs after training	24	WCX	W=62.5	0.023	S3b
after training: control vs devalued		M-W	U=388	0.013	S3b
12trials x 1d, a:b; saline: before vs after training	21	WCX	W=43	0.037	S4a
epinastine: before vs after training	25	WCX	W=136	0.48	S4a
after training: saline vs epinastine		M-W	U=364	0.026	S4a
6trials x 2d, a:b; saline: before vs after training	21	WCX	W=37	0.015	S4b
epinastine: before vs after training	17	WCX	W=48	0.63	S4b
after training: saline vs epinastine		M-W	U=212	0.33	S4b

p/v: peppermint odor was used as CS and vanilla odoe was used as control odor: a:b: either of the apple odor or banana odor was used as CS and the other as the control odor in a counterbalanced manner. K-W: Kruskal-Wallis test. WCX: Wilcoxon's test. M-W: Mann-Whitney test. P values were adjusted by Holm's method in the case of multiple comparisons. Experiments with the K-W test are described in the third paragraph of the Results section.

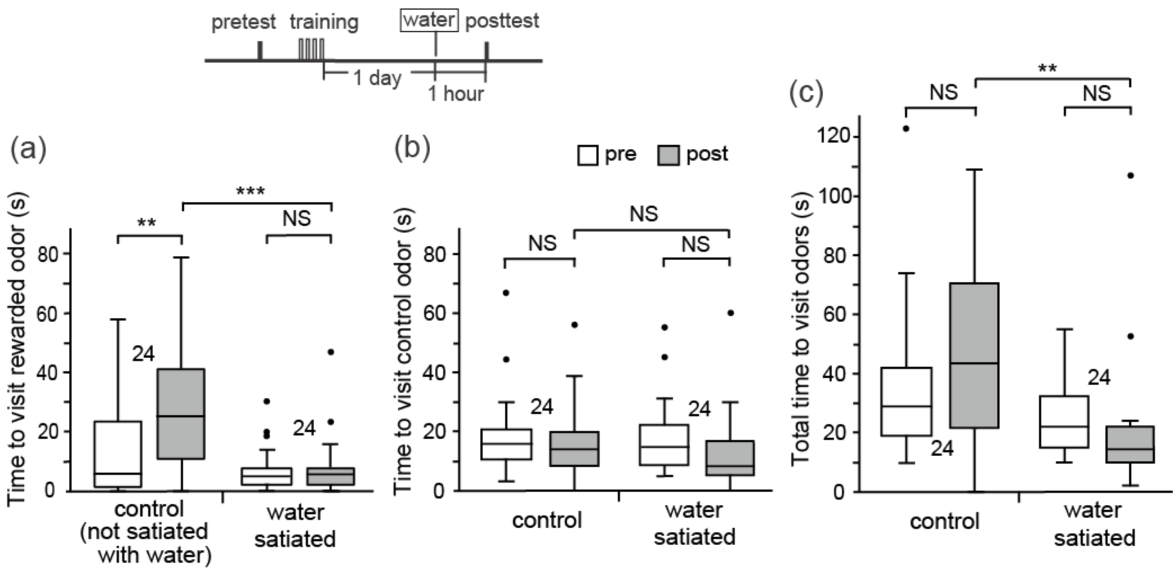

Figure S1. Effects of water satiation on responses to water-associated odors after standard training were evaluated by the time spent visiting odors. The time visiting rewarded (peppermint) odor (*a)* and control (vanilla) odor (*b*), and the total time visiting either odors (*c*) in the control (no water provided) group and water-satiated group shown in Fig. 1a are plotted as box plots. In this analysis, we included data from crickets that visited odor sources for less then 10 sec (two crickets for the control group and four crickets for the satiated group), which were not used for evaluation of odor preferences (see Methods). The results of statistical comparison of visiting time before and after training (WCX test) and between groups (M-W test) are shown as asterisks (＊＊$p<0.01$; ＊＊＊$p<0.001$; NS $p>0.05$, with the p values adjusted by Holm's method). The time visiting rewarded odor was significantly longer after training than that before training in the control group but not in the devalued group (*a*). On the other hand, the time visiting control odor did not significantly differ before and after training in both groups (*b*). The total time to visiting odors did not significantly differ before and after training in both groups, but between-group comparison showed that the total visiting time after training was significantly shorter in the devalued group than that in the control group (*C*). The results confirm that water satiation fully suppresses responses to water-associated odor but responses to control odors are less affected.

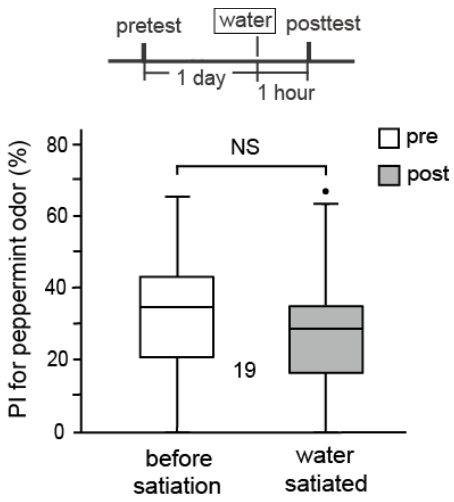

Figure S2. Water satiation does not alter odor preference in untrained crickets. Crickets in one group (n=19) were given odor preference test, and on the next day they were given water until stopped drinking, and one-hour later they were given the test again. Relative preferences between peppermint and vanilla odors, measured as the preference index (PI) for the peppermint odor, are shown as box plots. The odor preference after water satiation did not significantly differ from that before satiation (NS, $p>0.05$, WCX test), indicating that water satiation does not alter odor preference in untrained crickets.

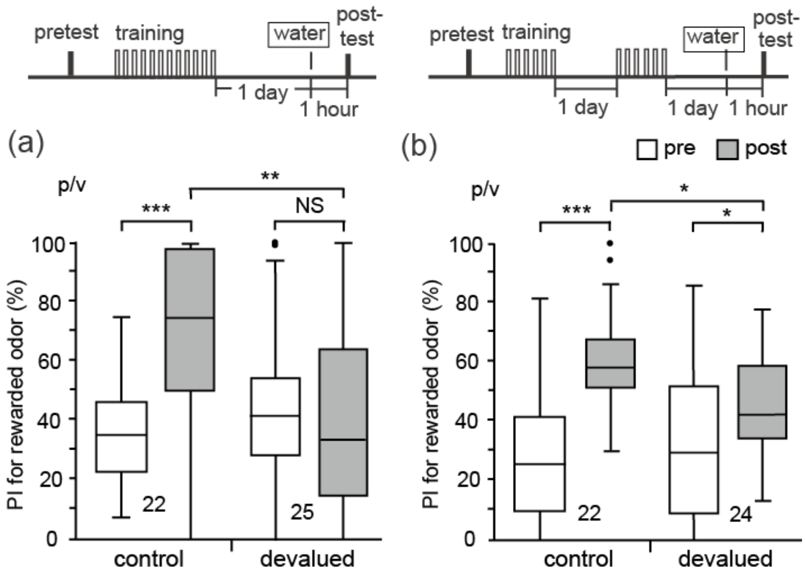

Figure S3. Evaluation of the amount of training necessary to make execution of the CR insensitive to reward devaluation. (a,b) Two groups were each subjected to a pre-test and then 12 trials x 1 day training (a), and another two groups of animals were each subjected to a pretest and then 6 trials x 2 days training (b). On the next day, crickets in one group were given water prior to the posttest and crickets in the other group were not given water. Relative preferences for the rewarded odor before (white box) and 1 day after training (gray box) are shown as box plots. The results of statistical comparison before and after conditioning (WCX test) and between groups (M-W test) are shown as asterisks (＊$p<0.05$; ＊＊$p<0.01$; ＊＊＊$p<0.001$; NS $p>0.05$, with the p values adjusted by Holm's method).

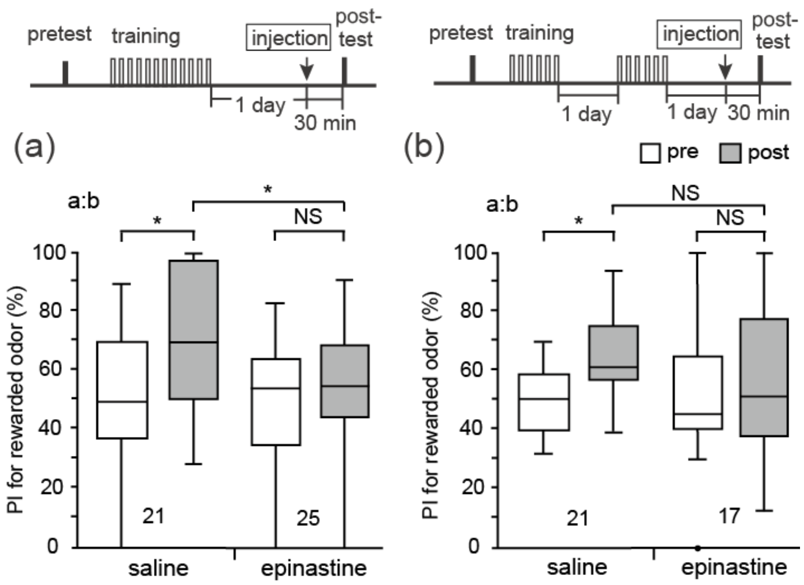

Figure S4. Evaluation of the amount of training necessary to make execution of the CR insensitive to epinastine. (*a,b*) Effects of epinastine on the CR were tested in the 12 trials x 1 day training group (*a*) and in the 6 trials x 2 days training group (*b*). Either apple odor or banana odor was used as CS and the other was used as the control odor. Relative preferences for the rewarded odor (PI) before (white box) and 1 day after training (gray box) are shown as box plots. The results of statistical comparison before and after conditioning (WCX test) and between groups (M-W test) are shown as asterisks (* $p<0.05$; NS $p>0.05$, with the p values adjusted by Holm's method).