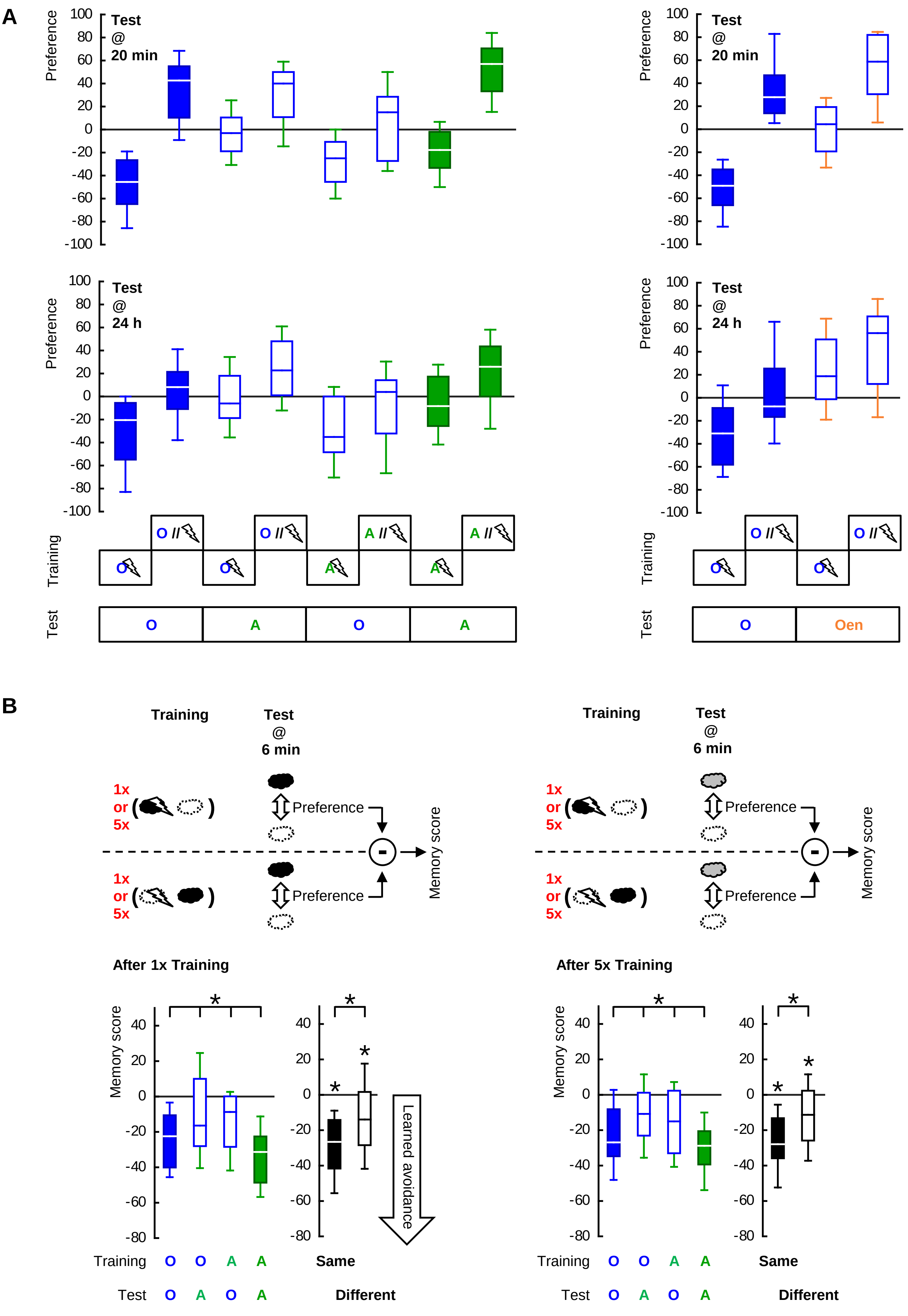
A
Preference
Test
@
20 min
Test
@
24 h
Training
Test
O
A
Oen
B
Training
Test
@
6 min
1x
or
5x
Preference
Memory score
After 1x Training
After 5x Training
Learned avoidance
Same
Different

Figure S1.

A. The preference values underlying the memory scores in Figs. 1B (left) and 1C (right) are presented.

B. Same as in Fig. 1A, but either one training trial or five training trials without substantial pause in between were used and the test took place 6 min after the end of the last trial. Flies were trained with 3-octanol (O) or n-amylacetate (A) and tested with the trained odor (conditions O-O and A-A) or the respective other odor (conditions O-A and A-O). After single-trial training, memory scores significantly differed across the conditions O-O, O-A, A-O and A-A (KW-test: H= 13.68, d.f.= 3, *P*= 0.0034, N= 24, 24, 24, 23). Pooling the scores across the O-O and A-A as well as O-A and A-O conditions, which pair-wise did not differ (U-test O-O *versus* A-A: U= 208.00, P= 0.1509; U-test O-A *versus* A-O: U= 268.00, *P*= 0.6876), we obtained two groups where the training and test odors were the Same or Different. In the Different group scores were weaker than in the Same group (U-test: U= 662.00, *P*= 0.0005). Significant learned avoidance was however detectable in each case (OSS-tests: *P*< 0.0001 and *P*= 0.0055 for Same and Different groups, respectively). After five-trial training, memory scores significantly differed across the conditions O-O, O-A, A-O and A-A (KW-test: H= 13.63, d.f.= 3, P= 0.0035, N= 24, 24, 24, 24). Pooling the scores across the O-O and A-A as well as O-A and A-O conditions, which pair-wise did not differ (U-test O-O *versus* A-A: U= 249.00, P= 0.4273; U-test O-A *versus* A-O: U= 253.50, *P*= 0.4833), we obtained two groups where the training and test odors were the Same or Different. In the Different group scores were weaker than in the Same group (U-test: U= 677.00, *P*= 0.0005). Significant learned avoidance was however detectable in each case (OSS-tests: *P*< 0.0001 and *P*= 0.0055 for Same and Different groups, respectively). Thus, regardless of whether single or five training trials were used learned avoidance was partially specific to the trained odor, and partially generalized to a novel odor. Box plots as well as * and NS are as explained in Fig. 1.

Journal of Experimental Biology • Supplementary information

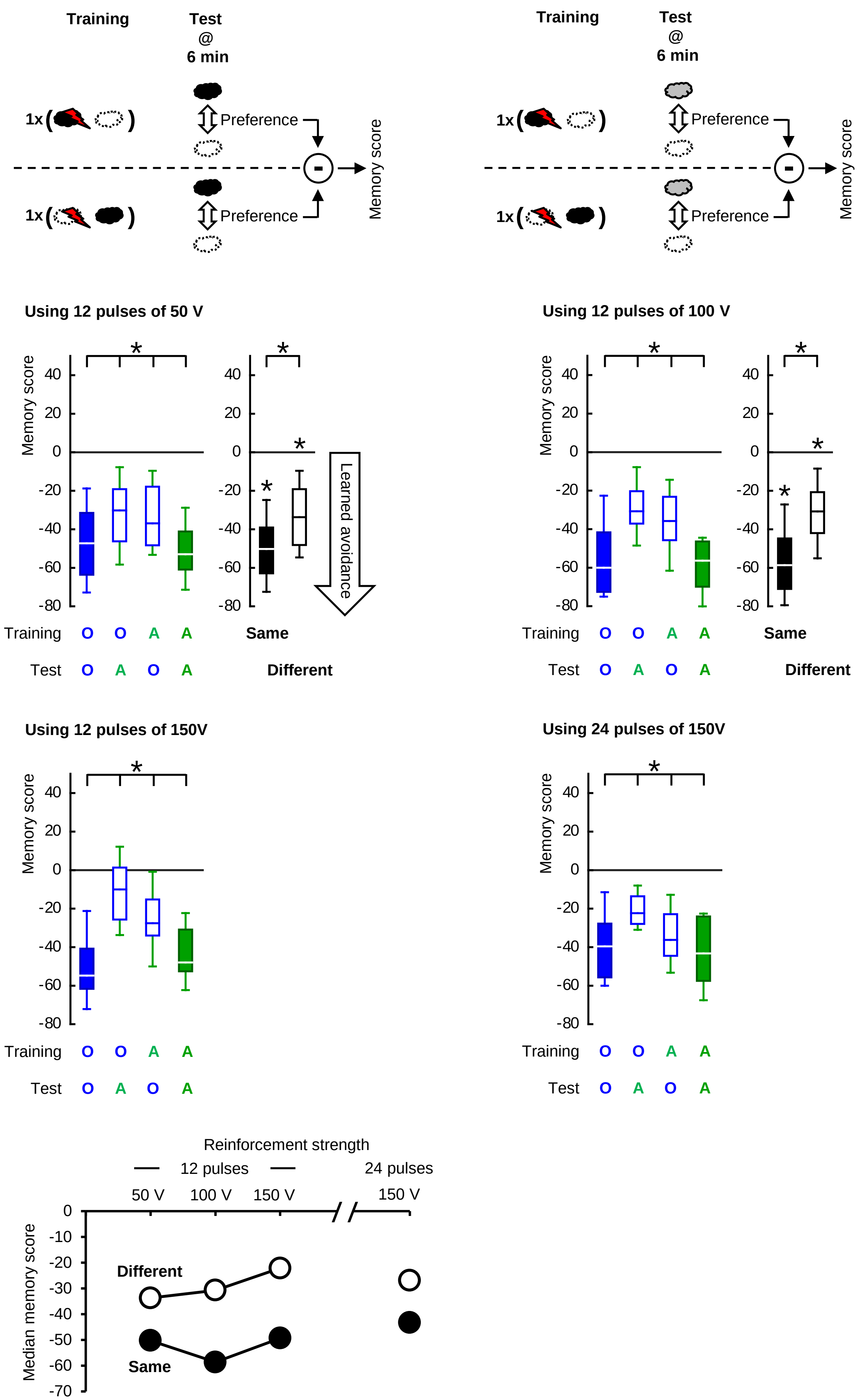
Training
Test
@
6 min
1x
Preference
Memory score
Using 12 pulses of 50 V
Using 12 pulses of 100 V
Using 12 pulses of 150V
Using 24 pulses of 150V
Memory score
Training
Test
O
A
Same
Different
Learned avoidance
Reinforcement strength
12 pulses
24 pulses
50 V
100 V
150 V
Median memory score
Different
Same

Figure S2.

Same as in Fig. S1B, but one training trial with 12 pulses of 50, 100 or 150 V or 24 pulses of 150V electric shock, delivered within 1 min was used. Flies were trained with 3-octanol (O) or n-amylacetate (A) and tested with the trained odor (conditions O-O and A-A) or the respective other odor (conditions O-A and A-O). After training with 12 pulses of 50 V shock, memory scores significantly differed across the conditions O-O, O-A, A-O and A-A (KW-test: H= 21.30, d.f.= 3, *P*= 0.0001, N= 26, 34, 27, 38). Pooling the scores across the O-O and A-A as well as O-A and A-O conditions, which pair-wise did not differ (U-test O-O *versus* A-A: U= 448.00, *P*= 0.5340; U-test O-A *versus* A-O: U= 435.00, *P*= 0.7329), we obtained two groups where the training and test odors were the Same or Different. In the Different group, scores were weaker than in the Same group (U-test: U= 1034.00, *P*< 0.0001). Significant learned avoidance was however detectable in each case (OSS-tests: *P*< 0.0001 each). After training with 12 pulses of 100 V shock, memory scores significantly differed across the conditions O-O, O-A, A-O and A-A (KW-test: H= 26.87, d.f.= 3, *P*< 0.0001, N= 21, 26, 14, 15). Pooling the scores across the O-O and A-A as well as O-A and A-O conditions, which pair-wise did not differ (U-test O-O *versus* A-A: U= 151.00, *P*= 0.8473; U-test O-A *versus* A-O: U= 154.00, *P*= 0.4355), we obtained two groups where the training and test odors were the Same or Different. In the Different group, scores were weaker than in the Same group (U-test: U= 231.00, *P*< 0.0001). Significant learned avoidance was however detectable in each case (OSS-tests: *P*< 0.0001 each). After training with 12 pulses of 150 V shock, memory scores significantly differed across the conditions O-O, O-A, A-O and A-A (KW-test: H= 28.75, d.f.= 3, *P*< 0.0001, N= 15, 18, 16, 13). In this case, we did not pool the scores to obtain Same and Different groups as the O-A and A-O conditions showed a tendency towards differing, although this was not formally significant. Instead, we compared the conditions O-O to O-A and A-A to A-O, revealing significant differences in each case (U-test O-O *versus* O-A: U= 18.00, *P*< 0.0001; U-test A-A *versus* A-O: U= 45.00, *P*= 0.0103). All of the four conditions, except O-A gave significant scores (OSS-tests: *P*< 0.0001, *P*= 0.0963, *P*= 0.0005, *P*= 0.0002 for O-O, O-A, A-O and A-A, respectively). After training with 24 pulses of 150 V shock, memory scores significantly differed across the conditions O-O, O-A, A-O and A-A (KW-test: H= 11.80, d.f.= 3, *P*= 0.0081, N= 16, 14, 16, 15). In this case, too, we did not pool the scores to obtain Same and Different groups as the O-A and A-O conditions led to significantly different scores (U-test O-A *versus* A-O: U= 55.00, *P*= 0.0188). We instead compared the conditions O-O to O-A and A-A to A-O, revealing a significant difference in the former case (U-test O-O *versus* O-A: U= 43.00, *P*= 0.0044; U-test A-A *versus* A-O: U= 95.00, *P*= 0.3328). All four conditions gave significant scores (OSS-tests: *P*< 0.0001, *P*= 0.0001, *P*< 0.0001, *P*= 0.001 for O-O, O-A, A-O and A-A, respectively). To summarize, we found no obvious and consistent effect of the reinforcement strength on the specificity of learned avoidance, which seemed to be partial in all cases. This is supported by the semi-schematic graph, featuring the median memory scores of Same *versus* Different groups, obtained by pooling across the conditions O-O and A-A as well as O-A and A-O, respectively. Box plots as well as * and NS are as explained in Fig. 1.

Journal of Experimental Biology • Supplementary information