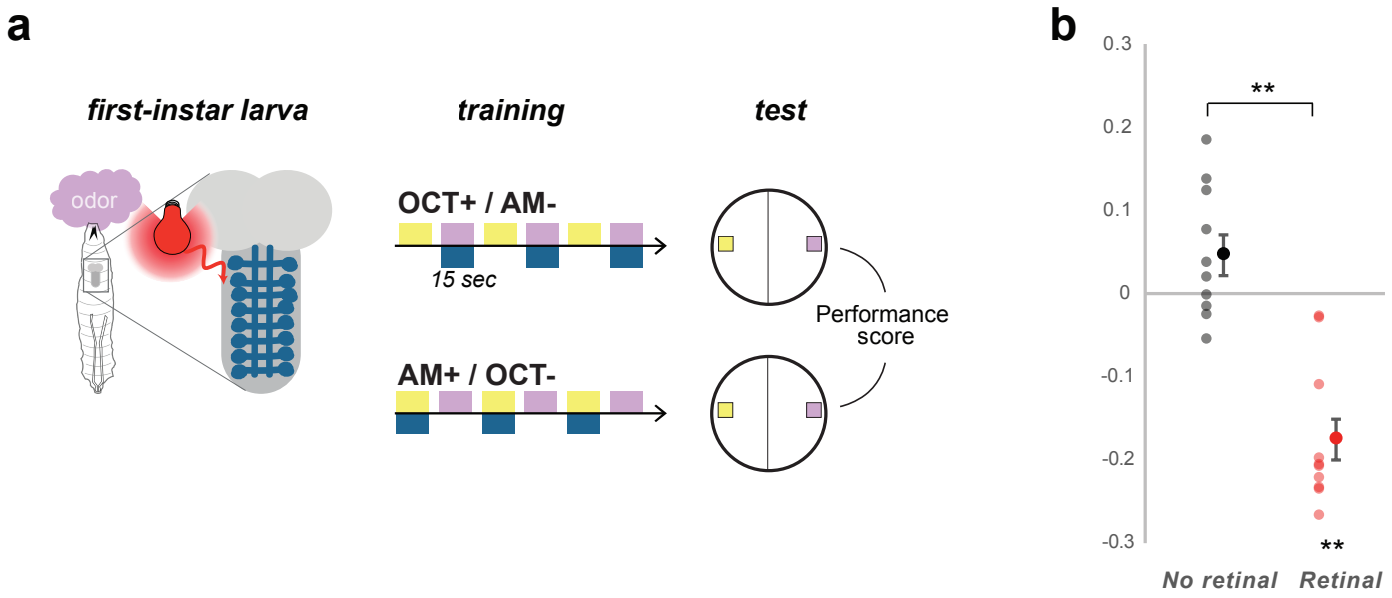

Figure 5 - Figure Supplement 1: Learning performances in first-instar larvae.
Most learning paradigms are done in third-instar larvae, although first-instar larvae are also capable of robust appetitive associative-learning using optogenetic stimuli [13]. Here we demonstrated once more that the first instar larvae are capable of aversive associative learning using the same optogenetic US as in Fig. 5. **a**. We trained pairs of groups of 30 first-instar larvae to associate either 3-octanol (OCT+) or n-amyl acetate (AM+) with optogenetic activation of the aversive Basins neurons (drawn in dark blue) for 15 sec long pairing whereas the other odor was presented alone for 15 sec. The larvae were then tested for their choice between the two odors, which was estimated by $\text{Pref}_{AM} = (\text{N}_{AM} - \text{N}_{OCT}) / (\text{N}_{AM} + \text{N}_{OCT})$, and a Performance Score was computed by subtracting the Pref_{AM} after OCT+/AM- training to the Pref_{AM} obtained in the group that received AM+/OCT- training. A negative score indicates aversive memory, whereas a zero score indicates no memory. **b**. The first-instar larvae of the same genotype as in Fig.5 form an aversive short-term memory if they were raised in the presence of trans-retinal in the food (necessary for a functional channelrhodopsin and thus for the aversive neurons to be depolarized during training), but not if they were raised without retinal. N=10, **: $p<0.01$, Wilcoxon test.