

1 Prediction error drives associative olfactory learning and  
2 conditioned behavior in a spiking model of *Drosophila larva*

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14 **Abstract**

15 Predicting reinforcement from the presence of environmental clues is an essential component  
16 of guiding goal-directed behavior. In insect brains, the mushroom body is central to learning  
17 the necessary associations between sensory signals and reinforcement. We propose a biologically  
18 realistic spiking network model of the *Drosophila larva* olfactory pathway for the association of  
19 odors and reinforcement to bias behavior towards approach or avoidance. We demonstrate that  
20 prediction error coding through the integration of currently present and expected reinforcement  
21 in dopaminergic neurons can serve as a driving force in learning that can, combined with a  
22 synaptic homeostasis mechanism, account for experimentally observed features of acquisition  
23 and loss of associations in the larva that depend on the intensity of odor and reinforcement and  
24 temporal features of their pairing. To allow direct comparisons of our simulations with behav-  
25 ior data [1], we model learning-induced plasticity over the complete time course of behavioral  
26 experiments and simulate the locomotion of individual larvae towards or away from odor sources  
27 in a virtual environment.

## 28 Introduction

29 Goal-directed behavior in dynamic situations benefits from the ability to predict future conditions  
30 in the environment from the occurrence of sensory clues. In insects, the mushroom body (MB) is  
31 the central brain structure for multi-sensory integration, involved in memory formation and recall  
32 [2, 3]. It is at the core of learning and retaining valuable associations between sensory inputs and  
33 reinforcement in the synapses between the MB intrinsic and its output neurons [4–7].

34 One of the underlying mechanisms is associative learning, a process that gradually establishes a  
35 relationship between two previously unrelated elements. In classical conditioning, the conditioned  
36 sensory stimulus (CS) obtains behavioral relevance through its concurrence with the reinforcing  
37 unconditioned stimulus (US), an acquisition process depending dynamically on their spatiotemporal  
38 proximity. The temporal evolution of this process has been formalized in the Rescorla-Wagner (RW)  
39 model (eqn. 1) [8].

$$\begin{aligned}\Delta V &= \alpha \cdot (\lambda_{US} - V(t)), \\ V(t + \Delta t) &= V(t) + \Delta V.\end{aligned}\tag{1}$$

40 Here, a CS obtains predictive power of concurrent or successive US [8], that depends on the  
41 strength of the already acquired association between the CS and US  $V(t)$ , allowing for anticipatory  
42 behavior to the expected US [9, 10]. The acquisition of this association terminates when the US is  
43 fully predicted. Until then, the change in associative strength  $\Delta V$  is proportional to the difference  
44 between the maximum associative strength (or asymptote)  $\lambda_{US}$  and the current associative strength  
45  $V(t)$  (eqn. 1). The maximum associative strength is a property of the US, determined mainly by  
46 the intensity of the reinforcement. While the current associative strength  $V(t)$  is defined by the  
47 shared learning history of CS and US [8]. The concept of prediction error (PE) [11] is a derivative  
48 of the Rescorla-Wagner model [8]. The error signal equals the difference between the current  $\lambda_{US}$   
49 and the predicted value of US  $V(t)$ . Over the course of the memory acquisition/training phase, the  
50 pace of learning, which can be formalized as the slope of the acquisition curve, decreases as the PE  
51 is reduced, minimizing the driving force for changes of the association [8, 11]. This difference is  
52 multiplied with a learning rate parameter ( $\alpha$ ), here combined for the CS and the US (eqn. 1).

53 This continuous optimization of predictions, guided by the PE, could allow animals to efficiently  
54 adapt their goal-directed behavior in dynamic environments. Among the most relevant associations  
55 to be learned are those that enable the prediction of reward or punishment. Dopaminergic neurons  
56 (DANs) have long been known to encode information about reward and punishment. These types of  
57 neurons respond to the presence of rewards and punishments in the environment, both in vertebrates  
58 [12–17], as well as invertebrates [18–21]. The electrical stimulation or optogenetic activation of DANs  
59 induces approach or avoidance both in vertebrates [22–25] and invertebrates [20, 26–32]. In adult  
60 [5, 6, 33, 34] and larval [20, 35, 36] *Drosophila* this approach or avoidance learning is facilitated by

61 the modulation of MB output synapses by DAN activity. Ultimately DANs do not only signal the  
62 presence of rewards or punishments but have also been suggested to encode PE in various vertebrate  
63 species [16, 37–40] and might have a similar function in insects [19, 20, 32, 34, 41–44].

64 We utilize our spiking model of the *Drosophila* larva MB in one brain hemisphere that forms  
65 associations of odors with reinforcement to further test the hypothesis that PE coding within this  
66 circuit takes place in DANs that receive input from the output neurons of the MB or their down-  
67 stream partners [20, 45–47], that might provide feedback to the DANs. Beyond the scope of similar  
68 models [20, 48–51](see Discussion, section: Comparison with other MB models), we demonstrate  
69 that this mechanism can reproduce the experimentally observed findings on the acquisition of asso-  
70 ciations of odors with reinforcement in a time-resolved manner [1]. To facilitate direct qualitative  
71 and quantitative comparisons with animal behavioral data, we couple this model with a realistic  
72 locomotory model of the larva [52] that captures the effects of learned associations on chemotactic  
73 behavior in individual animals.

## 74 Results

### 75 Connectome-based circuit model of the larval olfactory pathway

76 The network architecture of our model (fig. 1 A) is based on the anatomy of the olfactory pathway  
77 in one *Drosophila* larva brain hemisphere [20, 29, 53, 54] (for more details see Methods, section:  
78 Network model). Peripheral processing is carried out by 21 olfactory receptor neurons (ORNs), each  
79 expressing a different olfactory receptor type [53, 55, 56]. ORNs form one-to-one excitatory synaptic  
80 connections with 21 projection neurons (PNs) and 21 local interneurons (LNs) in the antennal lobe  
81 [53]. Each LN connects with all PNs via inhibitory GABAergic synapses, establishing a motif for  
82 lateral inhibition within the antennal lobe. The 72 mature larval Kenyon cells (KCs) [54] are the  
83 excitatory principal cells of the MB. Each KC receives excitatory input from 2-6 randomly selected  
84 PNs [54]. The KCs are subjected to feedback inhibition, provided via the GABAergic anterior paired  
85 lateral (APL) neuron, which receives input from all KCs [29]. Only mature KCs, characterized by  
86 a fully developed dendrite, are included in this model, yielding a complete convergent synaptic KC  
87 >APL connectivity. The output region of the MB is organized in compartments, in which the  
88 KC axons converge with the dendrites of one or few MB output neurons (MBONs) [20, 54]. Our  
89 model assumes two MBONs from two different compartments that are representative of two different  
90 categories of output neurons of the MB that mediate either approach or avoidance [4–6, 33, 35, 36,  
91 57–59] with a single MBON each. Both MBONs receive excitatory input from all of the KCs to  
92 fully capture the information that is normally represented by the complete set of MBONs. Each  
93 compartment is also innervated by a single DAN, signaling either reward or punishment and targeting  
94 the KC>MBON synapses to facilitate learning (for a discussion of all simplifications compared to  
95 the animal brain, see Methods, section: Network model).

## 96 Learning through KC>MBON plasticity

97 We assume that the KC>MBON synapses undergo plasticity, based on strong experimental evidence  
98 in larval [20, 35, 36] and adult flies [5, 6, 34]. This plasticity requires the convergence of the sensory  
99 pathways in the form of KC activation and of the reinforcing pathway, mediated by neuromodu-  
100 latory DAN signaling at the synaptic site. We employ a two-factor learning rule (eqn. 2) at each  
101 KC>MBON synapse (fig. 1 A,B). The first factor is expressed in the pre-synaptic KC activation by  
102 an odor, tagging the synapse eligible for modification. This is modeled via an exponentially decay-  
103 ing eligibility trace  $e_i(t)$ , which is set to a 1 whenever the respective KC elicits a spike (fig. 1 B).  
104 The decay time constant determines the window of opportunity for synaptic change. The pres-  
105 ence of reinforcement (reward or punishment) constitutes the second factor and is signaled by the  
106 reward-mediating DAN<sub>+</sub> or punishment-mediating DAN<sub>-</sub>, respectively. Spiking of the DAN provides  
107 a neuromodulatory reinforcement signal  $R(t)$  to the synaptic site. If a DAN spike coincides with  
108 positive eligibility at the synapse, the respective synaptic weight is reduced. At each synapse  $i$ , the  
109 reduction of synaptic weight  $\Delta w_i$  depends on the learning rate  $a$  (table S1) and is proportional to  
110 the amplitude of the eligibility trace  $e_i(t)$  (fig. 1 B):

$$\Delta w_i = -a \cdot e_i(t) \cdot R(t) \leq 0. \quad (2)$$

111 We introduce a synaptic homeostasis mechanism (eqn. 3) that modulates the effects of plasticity  
112 at each KC>MBON synapse to account for the experimentally observed loss of a learned associ-  
113 ation when reinforcement is omitted [41, 60, 61] and to ensure continued input to both MBONs.  
114 With each MBON spike, the current weight  $w_i$  of each respective KC>MBON synapse is increased,  
115 proportionally to the extent to which the weight differs from its original value  $w_{init}$  (table S1) and  
116 multiplied with a homeostatic regulation factor  $h$  (table S1). This mechanism serves as an imple-  
117 mentation for the loss of the association when the reinforcement is omitted. While reinforcement  
118 is present, the learning curve will either continue to rise or remain at the asymptote if already  
119 saturated. The interaction of the two mechanisms of learning and unlearning at the level of the  
120 individual KC>MBON synapses allows to include the loss of learned associations, when continued  
121 reinforcement is omitted (see Discussion, section: A mechanistic implementation of the RW model)  
122 and also ensures continued input to the MBONs, despite the reduction of input weights over the  
123 course of the learning process (eqn. 2). The homeostatic factor  $h$  hereby serves as an implementation  
124 of a time constant of this exponential process. The interaction of synaptic plasticity and homeostatic  
125 regulation defines the magnitude of the weight at the next simulation timestep  $t + \Delta t$  as

$$w_i(t + \Delta t) = w_i(t) + \Delta w_i + (w_{init} - w_i(t)) \cdot h. \quad (3)$$

126 It has been shown in behavioral experiments that specific MBONs encode a behavioral tendency  
127 to either approach or avoid a currently perceived stimulus, depending on the acquired stimulus  
128 valence [4–6, 36, 57, 58]. In the naive state of our model, all KC>MBON synapses have the same

129 initial weights  $w_{init}$  (table S1), and hence the spiking activity of both MBONs is highly similar.  
130 Learning alters the KC>MBON synaptic weights and thus skews the previously balanced MBON  
131 output. This acquired imbalance between MBON outputs biases behavior towards the approach or  
132 avoidance of the conditioned odor. To quantify the effect of learning, we compute the behavioral  
133 bias BB (eqn. 4) from the firing rates of both MBONs over  $T = 1s$  as follows:

$$BB = \frac{MBON_+ - MBON_-}{T}. \quad (4)$$

### 134 Implementation of prediction error coding in the KC-MBON-DAN motif

135 In the larva, many DANs and other modulatory neurons receive excitatory or inhibitory input  
136 from different MBONs, either in a direct manner or via one or two interneurons [20]. Based on  
137 this observation, we constructed our hypothetical feedback motif (for similar models see discus-  
138 sion section: Comparison with other MB models). In the model, DANs are activated by external  
139 reward/punishment signals and also receive excitatory and indirect inhibitory feedback from both  
140 MBONs (fig. 1 A). As the initial balance between the two MBON outputs shifts over the course of  
141 the training process, the amount of excitatory and inhibitory feedback that DANs receive continues  
142 to diverge, allowing the DANs to access the model's learning history. Ultimately DAN activation  
143 signals the difference between the current external activation and the expected activation based  
144 on prior learning, implemented as the difference between excitatory and inhibitory MBON>DAN  
145 feedback. Including this feedback leads to learning curves that saturate when the reward is fully  
146 predicted, and the prediction error approaches zero (fig. 2 A,D). This effect disappears, when the  
147 feedback circuit is disabled (fig. 2 A). In this case the behavioral bias quickly reaches the maximum  
148 value of the measure when the MBON<sub>-</sub> elicits no more spikes and can not encode further learning.  
149 Increasing reward intensity, learning rate or odor intensity (see Methods, section: Experimental  
150 protocols) foster a faster acquisition of the association and increases the maximum strength of the  
151 association at the same time (fig. 2 A).

152 Increasing the reward intensity after a 2.5 min (black curve), or 5 min (gray curve) of appetitive  
153 training, results in a steeper slope of the learning curve and also increases the maximum during  
154 training trials of 2.5min duration with increased reward intensity (fig. 2 B). Higher intensity of the  
155 reward results in an average DAN spike rate of 39.14Hz( $std = 1.27$ (standard deviation)) compared  
156 to 33.11Hz( $std = 1.34$ ).

157 Additionally, we tested for loss of the acquired association as the reduction in behavioral memory  
158 expression, over the course of prolonged exposure to the CS without the US, following initial memory  
159 acquisition [8, 62]. We test this in our model experiments by presenting the odor, previously paired  
160 with reward, for an extended period of time, in the absence of reinforcement. During the test  
161 phase and without the presence of reward to trigger synaptic KC>MBON<sub>-</sub> weight reduction, the  
162 extinction mechanism is no longer outweighed by learning and drives each individual weight back  
163 towards  $w_{init}$  (fig. 2 C, upper panel). We also demonstrate the interaction of the learning rule with

164 this mechanism in figure S1, where the learning rate remains constant but the magnitude of the  
165 homeostatic regulation was manipulated to show that both mechanisms need to be in balance.

## 166 **Learned preference and behavior generalize to similar odors**

167 We trained our model by pairing a reward with a single odor for 4min. After the training procedure,  
168 we tested the behavioral bias either for the same or a different odor, following the experimental  
169 approach used in the larva [63]. Mimicking the experimental data, we show that the odor preference  
170 is highest if training odor and testing odor are identical in the case of training with 3-octanol. When  
171 amylacetate is used during training, 3-octanol preference is increased (fig. 3 A). Since 3-octanol  
172 activates a subset of the ORNs activated by amylacetate (fig. 1 D), some of which with higher rates  
173 than in the case of amylacetate, we also tested for generalization using a set of ORN activation  
174 patterns with a controlled degree of overlap (see Methods, section: Sensory input, fig. 1 D) and  
175 show that with decreasing similarity, the generalization effect to a new odor is diminished (fig. 3 A).  
176 Figure 3 B shows the network response to 30sec stimulations with amylacetate and 3-octanol in a  
177 single exemplary model instance. On the level of the ORNs, 3-octanol merely activated a subset  
178 of the amylacetate-activated neurons. The uniqueness of the odor identities is enhanced in the KC  
179 population [64].

## 180 **The model reproduces temporal features in trace conditioning experiments**

181 Including an odor-evoked eligibility trace at the KC>MBON synapses allows the model to maintain  
182 the sensory odor representation for a time window, during which reinforcement will trigger synaptic  
183 change (fig. 1 B). The time window between odor and reward onset (0, 10, 20, 30, 40, 50, 60, 120s)  
184 was varied for trace conditioning experiments with a 30s presentation of odor and reward that was  
185 repeated three times. A small inter-stimulus-interval (ISI) of 10 to 30s leads to an increase in  
186 behavioral bias compared to the complete overlap of odor and reinforcement (fig. 3 C), using the  
187 extended window of opportunity for synaptic change triggered by each KC spike. Long ISIs do not  
188 lead to learning as the eligibility trace declines back to zero during this time (fig. 3 C). These findings  
189 match observations from experiments in larvae [29, 65, 66] with the caveat that the trace in the real  
190 larva brain seems to extend for a slightly longer period of time, compared with our experiments.

## 191 **The model reproduces paired and unpaired associative conditioning experiments**

193 To test if learning, driven by prediction error, can account for learned larval behavior, we replicated  
194 single-trial conditioning experiments performed with larvae [1] in simulation. In these experiments,  
195 animals were trained with the odor amylacetate in a single trial of varying duration (1 – 8 min).  
196 To this end, larvae were placed on a Petri dish coated with an agar-sugar substrate and the odor in

197 two small containers for diffusion in the air (paired training). Either before or following this train-  
198 ing protocol, larvae underwent a single trial without sugar and odor. Afterward, the animals were  
199 transferred to a new dish with two odor containers placed on different sides (one of them contained  
200 amylacetate, and the other one was empty). This paired training was compared with an unpaired  
201 protocol with separate (randomized order) presentations of amylacetate and sugar. Following the  
202 paired training protocol (odor and reward are presented concurrently), the animals showed a ten-  
203 dency to approach the previously rewarded odor, as measured by the difference in the number of  
204 animals on each side at the end of a 3min test phase, divided by the total number of animals. Fol-  
205 lowing the experimental literature, we will refer to this measure as the preference index ([1] eqn. 15).  
206 The animals' preference is relatively consistent across training trials of different duration. Prolonged  
207 paired training did not lead to an increase in preference (fig. 5 A). These experiments did not in-  
208 clude a test for odor preference before training, but untrained larval odor preference of odors used in  
209 learning experiments has been demonstrated elsewhere [67–69]. This paired training was compared  
210 with an unpaired protocol with separate (randomized order) presentations of amylacetate and sugar.  
211 Here the extent to which animals preferred amylacetate over no odor varied with the duration of  
212 the training trial. The longer the duration of the training, the more the preference index decreased  
213 from an initially high value but saturated around 2.5min (fig. 5 A).

214 We aimed to replicate these behavioral experiments on two levels. Firstly, we focused on the  
215 direct model output that reflects the strength of the acquired association between amylacetate  
216 and reward (behavioral bias, eqn. 4) and later also simulated behavior based on these biases. We  
217 simulated both the paired and unpaired training protocol (fig. 4 B). While the unpaired training  
218 yielded almost no behavioral bias, the models that underwent the paired training show an increased  
219 behavioral bias, that depended on the duration of the training and saturated for longer training  
220 duration (fig. 4 B). The simulation results reported in figure 4 B were obtained using odor-naive  
221 models that exhibited no odor preference, prior to training. To account for the experimental finding  
222 that real larvae often do have an odor preference even without any training [67–69], we readjusted  
223 our experiments to include a pre-training period of 10 minutes to start the conditioning experiments  
224 with the amylacetate-reward association already established. This adaptation of the protocol leads to  
225 results (fig. 4 C) that match the results obtained in real behavioral experiments (fig. 5 A). The paired  
226 condition in figure 4 C shows that once the behavioral bias is saturated (fig. 2 A), continued pairing  
227 maintains the association, without further increasing it. Unpaired training on the other hand, causes  
228 the behavioral bias to decrease and saturate at a lower level. For a discussion of different potential  
229 causes of a reinforcement expectation prior to training, please refer to the discussion (Comparison of  
230 modeling results to experimental findings). Figure 2 A demonstrates that disabling MBON>DAN  
231 feedback leads to a learning curve that does not saturate but instead increases with a steep slope  
232 until it reaches the maximum value for the behavioral bias eqn. 4) with a MBON. rate of 0. To verify  
233 if this PE feedback mechanism is responsible for the difference between maintenance and loss of the  
234 association in figure 4 C, we repeated the same experiment with disabled MBON>DAN feedback.

235 The behavioral bias overall is much higher, compared to the intact network (fig. 4B). The maximum  
236 is reached before the test phase of even the shortest 1min training experiment, with no MBON-  
237 spikes elicited.

238 Secondly, since the effect of training in lab experiments is quantified behaviorally via spatially  
239 defined, group-level metrics (preference index and performance index (eqn. 15,eqn. 16), [1]), we  
240 performed behavioral simulations of the testing phase with groups of virtual larvae for both the  
241 paired and unpaired condition [1], allowing a straightforward comparison with the animal experi-  
242 ments (fig. 5A). To this end, we utilized a realistic model for the simulation of larval locomotion  
243 and chemotactic behavior [52] that uses the behavioral bias at the output of the MB model as  
244 a constant gain factor to modulate the locomotory behavior of individual larvae towards or away  
245 from a spatially placed odor source in a virtual arena (see Methods, section: Realistic modeling of  
246 larval locomotion). The resulting preference indices, acquired across groups of independently sim-  
247 ulated larvae (fig. 5C), can directly be compared to the experimentally obtained preference indices  
248 (fig. 5A). We also compare performance indices from our simulated experiments (fig. 5D) with those  
249 from the lab experiments (fig. 5B) and find that the model can replicate these when accounting for  
250 the odor preference at the beginning of the experiment.

## 251 Discussion

252 Seeking rewards and avoiding punishments by predicting change in the environment is a major  
253 motivator of animal behavior. Sensory clues can acquire the necessary predictive power to guide  
254 behavior through classical conditioning, an associative learning process potentially driven by re-  
255 ward/punishment PE [8, 11], as observed in vertebrates [16, 37–40]. To test the biological plausi-  
256 bility of the proposed PE coding motif in the larval MB and test its capacity to explain behavioral  
257 data we implemented a spiking network model of the olfactory pathway, coupled with a simulation  
258 of locomotory behavior [52]. We demonstrate that our model of PE coding results in saturating  
259 group-level and individual learning curves, where the slope and maximum of the learning curve are  
260 determined by the intensity of both the reward and the odor signal. Learning is also influenced by  
261 the timing of odor and reinforcement and can be extinguished if reinforcement is omitted during the  
262 presentation of the sensory clue. After verifying that this circuit motif enables learning as predicted  
263 by the PE theory, we show that it can also explain time-resolved larval behavior in conditioning  
264 experiments.

## 265 A mechanistic implementation of the RW model

266 A number of predictions can be derived from the phenomenological RW model [8] and tested in our  
267 mechanistic model thereof. We found that regardless of odor/reward intensity or the model’s learn-  
268 ing rate, the strength of the odor-reward association (quantified as the behavioral bias) saturates  
269 over time (fig. 2A), as the strength of the already acquired association  $V(t)$  approaches the maxi-



270 mum value supported by the given reinforcement input ( $\lambda_{US}$ ) (eqn. 1). Consequently, our model's  
271 acquisition curve saturates at a higher value when the intensity of the reinforcement is increased  
272 (fig. 2 A,B), as predicted by the RW model, in which a stronger US should result in a higher value  
273 of  $\lambda_{US}$  [8]. In our model, a higher reinforcement intensity relates to a higher input rate into the  
274 respective DAN (see Methods, section: Sensory input) which translates into more frequent DAN  
275 spikes within a given window of 1 second, used to compute the behavioral bias (eqn. 4). This defines  
276 the asymptote of the learning curve. According to the RW model, increasing either the intensity of  
277 the odor or the learning rate  $\alpha$  [8] should lead to faster acquisition of the association. In our model,  
278 the learning rate directly influences the increment of each respective synaptic weight  $\Delta w^i$ , while an  
279 increase of the odor intensity allows for a more frequent execution of the weight update routine, by  
280 influencing the eligibility trace (eqn. 2).

281 The RW model predicts that the omission of reward should result in the loss of the learned  
282 association (eqn. 1, [8]). From the equation itself, we can not infer if this loss is due to extinction  
283 or forgetting. Extinction, characterized by the possibility of recovery of the association, after its  
284 temporary loss [70], has been demonstrated in adult [71, 72] but not larval *Drosophila*. To retain  
285 the association for recovery, extinction relies on the formation of parallel memory traces for the  
286 acquisition and the loss of the association [41, 60]. The mechanism implemented in our model is  
287 overwriting the association, since the homeostatic mechanism drives the synaptic weights toward  
288 their initial value, thereby deleting the learned association with no chance of recovery, but only in  
289 the presence of olfactory input, eliciting MBON spikes. The resulting behavior during the extinction  
290 phase of the experiments presents itself in a similar way, while the underlying mechanism is different.

## 291 **Comparison of modeling results to experimental findings**

292 A variety of experiments have demonstrated group-level acquisition curves that saturate over mul-  
293 tiple training trials or with increasing duration of a single trial in olfactory conditioning [1, 51,  
294 73–75]. To replicate larval behavior in reward learning experiments [1] with varying duration of the  
295 learning phase (fig. 5 A,B) we trained our model with an odor and reward in a paired vs. unpaired  
296 fashion (fig. 4 B). Real larvae show a strong odor preference even after a very short training and  
297 no significant increase in their preference when trained in a paired manner for longer periods of  
298 time [1, 67]. Instead, the animals trained in an unpaired protocol start out with a similarly high  
299 odor preference, which then decreases over time [1, 67]. This behavior is very counter-intuitive since  
300 the coincidence of odor and reward should yield an association of those two stimuli and thus an  
301 increased behaviorally expressed preference for the CS [8]. To resolve this contradiction, we include  
302 the observation that animals might not be naive to the training odor prior to the beginning of the  
303 experiments in the model. In that case, the animals would enter into the experiment with an already  
304 established reward prediction that would be violated during unpaired training. Three scenarios lend  
305 themselves as plausible causes of this effect: Firstly, accidental conditioning over the course of their  
306 lifespan during which they are raised on a food substrate while being exposed to air that carries

307 many different odorants. Alternatively, or in fact, additionally, the animals might exhibit an innate  
308 preference for many odors [76–78]. Finally, the presence of the reward during reward-only phases  
309 might lead to an association of the experimental context with that reward (previously discussed  
310 by Saumweber et al. [67]). The resulting reward expectation (solely based on the always present  
311 context), unmet during the odor-only phases could lead to a prediction error signal. All three candi-  
312 date explanations would yield a similar projection for the unpaired experimental protocol: A reward  
313 expectation acquired prior to the actual experiment would cause a violation of that expectation  
314 during odor-only trials of the unpaired experiments. In all three cases, the animal’s preferences  
315 might also generalize to a broader array of odors, leading to an overall preference for some odors, as  
316 observed experimentally. To test this hypothesis we pre-trained our model before simulating condi-  
317 tioning experiments (fig. 4C) and observed that this allows us to reproduce the animal experiments  
318 (fig. 5 A,B). Including odor preference at the beginning of the experiment ensures the model not only  
319 behaves in accordance with the RW model [8], but also fits the animal experimental results [1]. A  
320 possible alternative explanation could be a sensory habituation process to the odor that might cause  
321 odor preferences to decrease over time, resulting in the observed patterns for unpaired learning. In  
322 the paired condition this effect might be abolished by the continued presentation of odor and reward  
323 together [79].

324 Thus far we have tested our model in experiments where the CS and US presentation were fully  
325 overlapping (paired conditions). We now consider different onset times, with the onset of the CS  
326 always preceding the onset of the US (fig. 3,C). For these experiments we used a shorter duration of  
327 30s for both CS and US presentation, repeated over three acquisition trials to mimic experimental  
328 conditions in larval experiments [29, 66] that used optogenetic activation of DANs as a proxy for  
329 sugar reward. Similar to their experiments we show that the behavioral bias clearly depends on the  
330 temporal delay between CS and US (fig. 3,C). Complete temporal overlap of CS and US (ISI=0)  
331 does not seem to expend the full potential of learning the association, instead partial overlap yields  
332 stronger associations due to the extended window of opportunity for synaptic change triggered by  
333 the odor’s eligibility trace. In our model, the eligibility trace  $e(t)$  represents a molecular process that  
334 maintains the odor signal locally in the KC>MBON synapses (eqn. 2). Zeng et al. [80] demonstrate  
335 that feedback from the serotonergic dorsal paired medial neuron onto the KCs directly influences  
336 the length of the KC eligibility trace, making it a candidate mechanism for associative learning with  
337 a delayed US. Appetitive and aversive trace conditioning experiments have been conducted with  
338 larvae [29, 65, 66] and adult flies and other insects [74, 81–83]. In all of these experiments where  
339 the CS is presented before the US demonstrate that longer inter-stimulus intervals abolish learning  
340 of the CS-US association when no KC odor representation persists during the reinforcement period.  
341 In the cases of shorter intervals, the experimental data is not entirely conclusive. Either the odor  
342 preference was higher for partial or no overlap, compared with complete overlap [29, 83] or highest  
343 for complete overlap [51, 66, 74].

344 We also looked at the extent of reinforcement generalization to novel odors. Experiments have

345 shown that associations between an odor and reinforcement generalize, to a varying extent, to other  
346 odors, as shown in experiments [63, 84]. Previous modeling experiments have also shown that  
347 reinforcement generalization depends on odor similarity in adult insects [48, 85–87]. In our larval  
348 model, we also demonstrate both generalization to other odors, as well as a loss in strength, compared  
349 to the training odor (fig. 3A). We also show that the extent of the generalization depends on the  
350 similarity of the training and test odor, as measured by the overlap of the input patterns (fig. 1D).  
351 The larval pathway with its relatively small coding space [53, 55] might be especially prone to such  
352 poor discriminative abilities.

## 353 0.1 Model predictions for behavioral experiments

354 Our approach targets two hypotheses: Firstly, symmetrical inhibitory and excitatory feedback from  
355 MBONs to DANs should yield a circuit capable of saturating learning curves as predicted by the  
356 RW model [8], due to PE [11] driving the learning process, which has also been suggested by  
357 previous models [20, 48–51]. Secondly, saturating learning curves, driven by PE should translate  
358 into (simulated) animal behavior, when comparing different training duration and intensities of  
359 reinforcement. We were able to test these hypotheses in model experiments, on the level of MB  
360 readout (behavioral bias, eqn. 4, fig. 2, 4)) and through the comparison of animal and simulated  
361 behavior of artificial larvae (fig. 5). While the simulation results fit nicely with the real larval behavior  
362 in an experiment with a varied training duration ([1], fig. 5), ultimately, the role of MBON>DAN  
363 feedback needs to be tested in behavioral animal experiments, directly manipulating this feedback.  
364 Some specific predictions that could be tested in such experiments are:

- 365 • Learning curves of individual animals should saturate over time when KC>MBON feedback  
366 is intact.
- 367 • When the MBON>DAN feedback is removed after some training, the learning curve should  
368 increase with a steeper slope and might not saturate.
- 369 • Increasing or decreasing the intensity of the odor or the reinforcement should lead to saturation  
370 on a higher or lower level, respectively.
- 371 • The removal of the KC>MBON feedback should weaken or abolish the saturation of the  
372 learning curve over time.

373 Based on our modeling results, we support the idea that the error computation between the  
374 prediction and reality of reinforcement is done in the DANs and relies on MBON>DAN feedback.  
375 Our hypotheses for experiments are based on this assumption. Nevertheless, some saturation, that  
376 is not based on PE, might still occur, even if MBON input to DANs is removed. The entire MB  
377 circuitry consists of many more elements than our model implementation and would presumably  
378 have additional mechanisms to ensure homeostatic balance and continued MBON input, potentially  
379 leading to some weaker form of saturation in the learning pro.

## 380 Comparison with other MB models

381 Models of the learning in the MB, based on plasticity at the MB output synapses, without PE coding,  
382 have been around for some time, both for *Drosophila* [85, 87–89] and other insects [86, 90]. In all  
383 of these models, plasticity is mediated by the activity of modulatory neurons (e.g., dopaminergic),  
384 coinciding with either KC [86, 87] or coordinated KC and MBON activity [85, 88, 89]. These models  
385 can perform associative learning of a stimulus, paired with reinforcement [85–89], as well as more  
386 complicated forms of learning such as second order conditioning [89] and matching to sample [88] or  
387 reinforcement generalization tasks, the extent of which depends on the stimulus similarity [85, 86].  
388 Additionally, some models were successfully tested in patterning tasks [85, 86], where combinations  
389 of stimuli are reinforced, while their individual components are not or vice versa. Models in which  
390 synaptic plasticity is driven not solely by the activity of modulatory neurons, but by a prediction  
391 error signal lend themselves to studying the evolution of learning over time (either over several  
392 trials, or in a continuous manner), and its dependency on the learning history. We hypothesize that  
393 such mechanisms for PE coding in the MB involve the modulatory DANs [19, 20, 32, 34, 41–44]  
394 and are based on MBON feedback to the DANs, serving as a manifestation of previous learning.  
395 Recently a number of modeling approaches have targeted the idea of PE coding in DANs in the adult  
396 *Drosophila* [48–51] as well as in the larval MB [20]. In these models, some form of MBON>DAN  
397 feedback is implemented, allowing these models to fulfill some of the predictions of the PE theory [8,  
398 11]. One of the most fundamental predictions is the saturation of the learning curve across time, as  
399 the prediction error decreases, demonstrated in a trial-based manner in some of those models [48–51]  
400 as well as the loss of an acquired association [20, 48–50]. Some of the previously published models  
401 include mechanisms for either permanent loss of the association in memory or extinction (parallel  
402 associations in memory). Within the MB circuitry, the formation of a parallel extinction memory  
403 involves an additional DAN of opposite valence [20, 48–50], whereas complete loss is implemented  
404 as a process of changing the KC>MBON weights in the opposite direction of the learning process  
405 [51, 89], as done in our model. Additionally, some of these models capture temporal dynamics  
406 of learning experiments to some extent by utilizing eligibility traces in the KC>MBON synapses  
407 [20, 50, 51], to our understanding, none have tested these predictions in continuous experiments  
408 with spiking dynamics. Therefore, beyond the scope of these contributions, we implemented PE  
409 coding mechanistically in a fully spiking network equipped with synaptic eligibility that we train  
410 and test in continuous experiments to allow for the assessment of dynamic change in the model’s  
411 odor preference. In combination with a time-continuous behavioral simulation [52] during memory  
412 retention tests, this allowed for straightforward comparison with larval experiments.

413 Prediction error coding is not the only mechanism discussed in the literature to explain such  
414 phenomena in learning. Gkaniyas et al. tested a PE-based learning rule against a different dopamine-  
415 based learning rule that does not require the presence of the CS as a reference point for expected  
416 reinforcement [87] in a more complex circuit model consisting of a number of interconnected micro-  
417 circuits. They show that both methods can produce a saturating learning curve across trials. Their

418 alternative learning rule, embedded in a multi-compartment structure of the MB can also explain  
419 extinction, blocking, and second order conditioning, by relying on interactions between different  
420 MBONs and DANs that encode different memory processes.

## 421 Outlook

422 Some experimentally observed effects in insect learning can not be captured by the RW model [8]  
423 and are thus not targeted by our model implementation. Among them are CS and US pre-exposure  
424 effects [91–94] that might be explained by changes either in attention to the CS or habituation to  
425 the CS or the US, caused by prolonged exposure prior to training, rather than changes in associative  
426 strength (for a review see [95]). Also interesting, but not directly predicted by the RW model [8]  
427 is the experimental observation of second order conditioning in adult *Drosophila* [96–99], where a  
428 second CS2 is paired with the CS, after this CS has acquired an association with the US. Through the  
429 CS2-CS pairing without the US, the CS2 acquires predictive power of the US. Different mechanisms  
430 have been proposed to be involved in causing this effect [98, 100]. Among them is an excitatory  
431 synaptic KC>DAN connection, strengthened during first order conditioning, that would allow the  
432 KC odor representation to activate the DAN as a substitute for reinforcement during the CS2-CS  
433 pairing. Exploring this phenomenon using network models could yield valuable insights into the  
434 *Drosophila* circuit, as well as aid in our general understanding of PE coding. Insect experiments  
435 have provided mixed evidence for other phenomena that can be predicted from the RW model, such  
436 as blocking [101–104] and hints at conditioned inhibition [105–107] that would be interesting to  
437 investigate. Furthermore, expanding the model to include different MB output compartments would  
438 offer a perspective to explore parallel associations regarding the same stimulus [41]. This could  
439 enable temporary loss of the learned association, while simultaneously retaining parallel memory  
440 for recovery (extinction vs. forgetting). Ultimately more possible directions arise from the major  
441 benefit of using a spiking model, which offers the potential to conduct experiments at high temporal  
442 resolution, instead of in a trial-based manner [20, 48–50]. In a future closed-loop approach that  
443 connects our continual learning MB model with the locomotory model in the full temporal resolution,  
444 we intend to simulate a behaving agent to investigate the temporal dynamics of adaptive behavior  
445 in analogy to the tracking experiments of real larva [73, 108–111].

## 446 Methods

### 447 Network model

448 All neurons are modeled as leaky integrate-and-fire neurons with conductance-based synapses. They  
449 elicit a spike, whenever the threshold  $V_T$  is crossed (parameters provided in table S1). Each neuronal  
450 membrane potential  $v_i$  is reset to the resting potential  $V_r$  whenever a spike occurs, followed by an  
451 absolute refractory period of 2 ms, during which the neuron does not integrate any inputs. Any

452 neuron from a given population ( $v^O, v^P, v^L, v^K, v^A, v^M, v^D$ ) is governed by the respective equation  
 453 for ORNs, PNs, LNs, KCs, APL, MBONs and DANs (eqs. (5) to (11), fig. 1A). Depending on  
 454 the neuron type, in addition to a leak conductance  $g_L$ , the equations consist of excitatory  $g_e$  and  
 455 inhibitory synaptic input  $g_i$ . In the case of the DANs, one excitatory  $g_e^{M\mp D}(E_E - v_i^D)$  and inhibitory  
 456  $g_i^{M\pm D}(E_I - v_i^D)$  input represent the two types of MBON feedback for the reward and punishment  
 457 encoding DAN, respectively. An additional spike-triggered adaptation conductance was implemented  
 458 for ORNs, KCs, MBONs, and DANs (eqn. 12, [64]), in accordance with our current knowledge of the  
 459 adaptive nature of ORNs in the larva [112] and the adult fly [113, 114]. Adaptation in KCs has so  
 460 far only been demonstrated in other insects [115, 116]. In the model of these neurons, the adaptation  
 461 conductance  $g_{Ia}$  is increased with every spike and decays over time with  $\tau_{Ia}$ . The mechanism of  
 462 synaptic plasticity is described in the results section (Learning through KC>MBON plasticity).

$$C_m \frac{d}{dt} v_i^O = g_L^O (E_L^O - v_i^O) + g_e^{InputO} (E_E - v_i^O) - g_{Ia} (E_{Ia} - v_i^O) \quad (5)$$

$$C_m \frac{d}{dt} v_i^L = g_L^L (E_L^L - v_i^L) + g_e^{OL} (E_E - v_i^L) \quad (6)$$

$$C_m \frac{d}{dt} v_i^P = g_L^P (E_L^P - v_i^P) + g_e^{OP} (E_E - v_i^P) - g_i^{LP} (E_I - v_i^P) \quad (7)$$

$$C_m \frac{d}{dt} v_i^K = g_L^K (E_L^K - v_i^K) - g_i^{APLK} (E_I - v_i^K) + g_e^{PK} (E_E - v_i^K) - g_{Ia} (E_{Ia} - v_i^K) \quad (8)$$

$$C_m \frac{d}{dt} v_i^A = g_L^A (E_L^A - v_i^A) + g_e^{KAPL} (E_E - v_i^A) \quad (9)$$

$$C_m \frac{d}{dt} v_i^M = g_L^M (E_L^M - v_i^M) + g_e^{KM} (E_E - v_i^M) \quad (10)$$

$$C_m \frac{d}{dt} v_i^D = g_L^D (E_L^D - v_i^D) - g_i^{M\pm D} (E_I - v_i^D) + g_e^{M\mp D} (E_E - v_i^D) + g_e^{InputD} (E_E - v_i^D) \quad (11)$$

$$\frac{d}{dt} g_{Ia} = -\frac{g_{Ia}}{\tau_{Ia}}. \quad (12)$$

463 All code for the model implementation is accessible via

464 <https://github.com/nawrotlab/PEcodingDosophilaMB>

465 We based our circuit model on the larval connectome both in terms of connectivity as well as  
 466 numbers of neurons in each population [20, 53, 54] and introduced simplifications to support the  
 467 mechanic investigation of the MBON>DAN feedback circuit and its role in PE coding and excluded  
 468 a number of connections that have been demonstrated in the larva. Due to limited availability of

469 anatomical, functional, and behavioral data most of our circuit implementation is based on the first  
470 instar larva [20, 53, 54], while the information on the APL connectivity within the circuit originates  
471 from studies on the third instar larva [29]. Behavioral experiments used for comparison with our  
472 simulation results were also performed with third instar larvae [1, 29, 66]. We demonstrate that our  
473 model based on the less developed circuit in the first instar larva is sufficient to reproduce animal  
474 behavior as observed in the older animals. From the anatomy of the first instar larva we excluded  
475 DAN>KC [54] and DAN>MBON synapses [54] that may play an additional role in learning-induced  
476 plasticity at KC>MBON synapses [54], the details of which are not fully known. Instead, we induce  
477 plasticity purely via the simulation of a neuromodulatory effect of the DANs onto the KC>MBON  
478 synapses ([54]). We also neglect recurrent interactions among KC themselves [54]. Many of these  
479 interactions affect KC that encode different sensory modalities, which are not included in our purely  
480 olfactory model. Furthermore, we simplified the connectivity between LNs and PNs [53] and between  
481 PNs and KCs to 2 – 6 PN inputs per KC, which excludes the set of KCs in the larva that receives  
482 exclusive input from only one PN [54]. This modification supported model robustness with respect  
483 to odor encoding within the small set of 72 KCs. Finally, from the population of  $\approx 25$  larval MBONs  
484 we only modeled two and correspondingly adapted KC>MBON synapses to provide both MBONs  
485 with input from all KCs.

## 486 Sparse odor representation

487 We implemented four mechanisms supporting population- and temporal sparseness in the MB odor  
488 representation [64]. Population sparseness is defined as the activation of only a small subset of  
489 neurons by any given input [117]. In this circuit population sparseness is enhanced through lateral  
490 inhibition (via LNs), inhibitory APL feedback, and the divergent connectivity from PNs to a larger  
491 number of KCs [64]. Temporal sparseness indicates that an individual neuron responds with only a  
492 few spikes to a specific stimulus configuration [118–120], which supports encoding dynamic changes  
493 in the sensory environment [121, 122]. In our model temporal sparseness is facilitated by spike  
494 frequency adaptation, an adaptive process to prolonged stimulus exposure, in ORNs and KCs and  
495 by inhibitory feedback via the APL[64].

## 496 Sensory input

497 In the olfactory pathway of larval *Drosophila* any odor activates up to  $\approx 1/3$  of ORNs, depending on  
498 its concentration [112, 123]. We implemented receptor input with stochastic point processes to ORNs  
499 via synapses to mimic the noise in a transduction process at the receptors. Each of the 21 receptor  
500 inputs is modeled according to a gamma process (shape parameter  $k=3$ ). The spontaneous firing  
501 rate of larval ORNs has been measured in the range of 0.2 – 7.9 Hz, depending strongly on odor and  
502 receptor type [123, 124]. ORNs in our model exhibit an average spontaneous firing rate of 8.92Hz  
503 (std=0.2). We constructed realistic olfactory input across the ORN population for acetylacetate and

504 3-octanol by estimating ORN spike frequency from the calcium signals measured in the receptor  
505 neurons [112] (dilution of  $10^{-4}$  [112]), ensuring the spike rates would not exceed the rates reported  
506 by [123]. They showed that using an even stronger odor concentration (dilution  $10^{-2}$ ) ORN never  
507 exceeded a frequency of 200Hz. Due to the lower concentration used for amylacetate and 3-octanol  
508 (fig. 1 D) [112] in our experiments and because Kreher et al, 2005 measured only the first 0.5s  
509 after odor onset when the effects of spike frequency in ORNs are the weakest (leading to higher  
510 spike rates) we decided to use a maximum of 150Hz in odor activated ORNs. After generating the  
511 gamma process realizations we clipped multiple spikes occurring in each time step of the simulation  
512 discarding all but the first spike in each time step. Similar to the odor input, the presence of either  
513 reward or punishment in the experimental context was implemented as input to the  $DAN_+/DAN_-$ .  
514 Regular gamma spike trains ( $k = 10$ ) were generated and clipped for the odor input.

515 To assess the effects of odor similarity on generalization we in addition created four artificial  
516 odors (A,B,C,D) (fig. 1 D) and quantified the pair-wise distances in ORN coding space using the  
517 cosine distance (eqn. 13), where vectors a and b each represent the input spike rate of two odors.

$$D_{cos} = 1 - \frac{\sum_{i=1}^n a_i \cdot b_i}{\sqrt{\sum_{i=1}^n a_i^2} \cdot \sqrt{\sum_{i=1}^n b_i^2}}. \quad (13)$$

518 The cosine distance between odors A and B equals 0.21, 0.77 between odors A and C, and 0.99  
519 between odors A and D. The comparison of amylacetate and 3-octanol yields a distance of 0.16.

## 520 Experimental protocols

521 The experiments reported here belong to one of three categories. The first was performed to provide  
522 insight into the model and the effects of specific circuit functions on synaptic plasticity, and prediction  
523 error coding. To this end, we used amylacetate as the primary odor input. We varied the intensity  
524 of the reward via the frequency of gamma spike train, provided as input into the  $DAN_+$  (either  
525 500Hz or 550Hz, resulting in an average output spike rate of 33.11/39.14Hz), and the learning rate  
526  $\alpha$  (0.6nS or 0.8nS). Additionally, MBON>DAN feedback was either enabled or disabled (fig. 1 A).

527 Experiments belonging to the second category were designed to replicate larva lab experiments  
528 to allow for a direct comparison with our model results. With these comparisons, we aim to validate  
529 the model and show to what extent our assumptions about the circuit functions allow us to recreate  
530 experimental data (fig. 5). Replicating lab experiments also provide more insights into the circuit  
531 mechanisms and offers alternative interpretations of the phenomena observed in data from animal  
532 experiments. Our implementations of the lab experiments were set up following the general procedure  
533 described in the Maggot Learning Manual [125]. Regardless of the specific protocol used in different  
534 experiments, larvae are placed into Petri dishes in groups of 30 animals. They are allowed to move  
535 around freely on the substrate that contains reinforcing substances, such as sugar or bitter tastants.  
536 During the entire time, they are subjected to specific odorants, emitted from two small containers  
537 in the dish to create permanent and uniformly distributed odor exposure within the dish. In the



538 analogy of the experimental setting, in our simulated experiments, each model instance is trained  
539 individually through the concurrent presentation of olfactory stimulation and reward. One-minute  
540 intervals with only baseline ORN stimulation were included between training trials to simulate the  
541 time needed in the lab experiments for transferring larvae between Petri dishes. Unless otherwise  
542 specified and test phases refer to 3 min, during which only odors are presented. All simulations were  
543 implemented in the network simulator Brian2 [126].

## 544 Realistic modeling of larval locomotion

545 Behavior during the testing phase of the olfactory learning experiment is simulated via the freely  
546 available python-based simulation platform Larvaworld (<https://github.com/nawrotlab/larvaworld>,  
547 [52]). A group of 30 virtual larvae is placed with random initial orientation around the center of  
548 a 100 mm diameter Petri dish and left to freely behave for 3 minutes. The previously conditioned  
549 odor is placed at one side of the dish, 10 mm from the arena's boundary. Each larva features a  
550 bi-segmental body, supervised by a layered control architecture [52]. The basic layer of the control  
551 architecture is a locomotory model, capable of realistic autonomous exploration behavior. It consists  
552 of two coupled oscillators, one of which represents the crawling apparatus that generates forward  
553 velocity oscillations, resembling consecutive peristaltic strides [52]. The other oscillator generates  
554 alternating left and right lateral bending, manifested as oscillations of angular velocity [127]. The  
555 crawling and the bending oscillators are coupled via phase-locked suppression of lateral bending to  
556 capture the bend dependency on the stride-cycle phase during crawling (weathervaning). Finally,  
557 intermittent crawling is achieved by a superimposed intermittency module that generates alternating  
558 epochs of crawling and stationary pauses, with more headcasts for orientation during the latter [52].

559 Modulation of behavior due to sensory stimulation is introduced at the second, reactive layer of  
560 the control architecture. An odor signal can transiently alter both, the amplitude and frequency  
561 of the lateral bending oscillator, which biases free exploration towards approach or avoidance along  
562 an olfactory chemical gradient. This modulation of behavior is directly influenced via top-down  
563 signaling from the third, adaptive layer of the control architecture. In our approach, the spiking  
564 MB model populates the adaptive layer and its learning-dependent output, defined as the behavioral  
565 bias  $BB$  (i.e. the difference in MBON firing rates, eqn. 4), provides the top-down signal [36]. We  
566 formalize the gain of behavioral modulation as

$$G = g \cdot BB. \quad (14)$$

567 which is directly proportional to the behavioral bias and the additional proportionality factor  
568  $g = 0.5$ .

569 A set of  $10 * 30$  trained MB model instances is used to generate 10 groups of 30 simulated larvae.  
570 The preference index and the performance index [1] for these simulations are illustrated in figure 5.

571 Preference indices (Pref) are computed individually for the paired and the unpaired experiments

572 [1], based on the number of animals on each side (odor vs. empty) of the Petri dish at the end of  
573 the test phase.

$$Pref = \frac{count_{odor} - count_{no\ odor}}{count_{odor} + count_{no\ odor}}. \quad (15)$$

574 The Performance indices (PI) are computed from the preference indices of the paired and unpaired  
575 experiments [1].

$$PI = \frac{Pref_{paired} - Pref_{unpaired}}{2}. \quad (16)$$

## 576 **Acknowledgements**

577 This project is funded in parts by the German Research Foundation (DFG) within the Research  
578 Unit ‘Structure, Plasticity and Behavioral Function of the Drosophila mushroom body’ (DFG-FOR-  
579 2705, grant no. 403329959, <https://www.unigoettingen.de/en/601524.html> to BG and MN) and by  
580 the Federal Ministry of Education and Research (BMBF, grant no. 01GQ2103A, ‘DrosoExpect’ to  
581 BG and MN). AMJ received additional support from the Research Training Group ‘Neural Circuit  
582 Analysis’ (DFG-RTG 1960, grant no. 233886668). We thank three anonymous reviewers for their  
583 valuable comments that helped us improve this manuscript.

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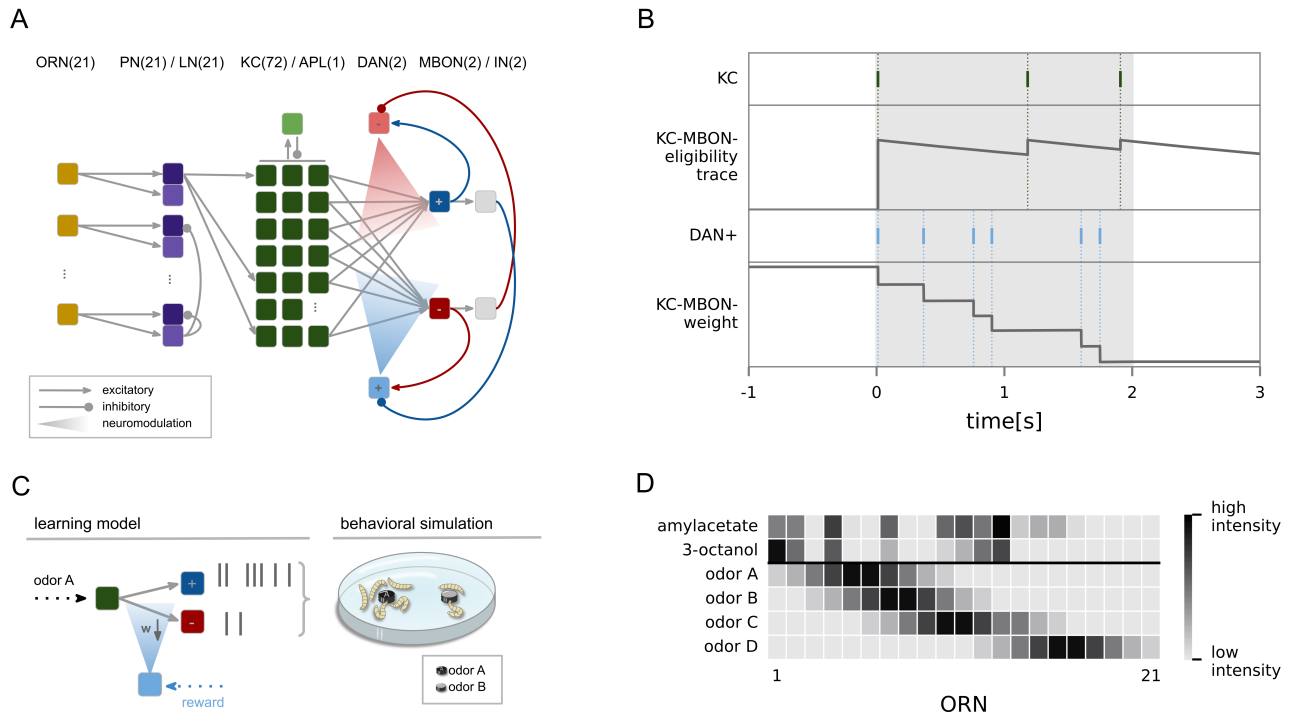
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**Figure 1: Network mechanisms.** (A) Network model of the *Drosophila* larva olfactory pathway including all neurons and connections implemented. One-to-one feed-forward connections between 21 olfactory receptor neurons (ORN) and 21 projection neurons (PN)/local interneurons (LN) and from 2-6 PN to each of the 72 Kenyon cells (KC). Lateral inhibition from each LN innervates all PNs and recurrent feedback inhibition from the anterior paired lateral (APL) neuron is provided onto all KCs. The MB output region is organized in two distinct compartments. The upper compartment holds the approach encoding MBON<sub>+</sub> and is innervated by the punishment mediating DAN<sub>-</sub>, the lower compartment holds the avoidance mediating MBON<sub>-</sub> and is innervated by the reward mediating DAN<sub>+</sub>. Each DAN can exert a neuromodulatory effect on the plastic KC>MBON synapses within its compartment. MBONs provide excitatory and inhibitory (via gray interneurons) feedback to the DANs. (B) Sketch of synaptic weight change at a single KC>MBON synapse with respect to the synaptic eligibility trace elicited by KC spikes and the occurrence of reward-triggered spikes in DAN<sub>+</sub>. Amylacetate is paired with a reward for 2s (gray shaded area). (C) To generate simulated larval behavior in the petri dish during the test phase of the learning experiments, we utilized our locomotory model [52], based on the behavioral bias (eqn. 4) acquired by the MB model during the training phase. The behavioral bias is used directly as input to the locomotory model. (D) All odors (see Methods, section: Sensory input) were used in the experiments. Naturalistic odor patterns for amylacetate and 3-octanol as well as four artificial patterns (odorA, odorB, odorC, odorD) with varying distances (see Methods, section: Sensory input) from odorA. Each odor activates a different set of input neurons with a different spike rate, as indicated by the color bar.

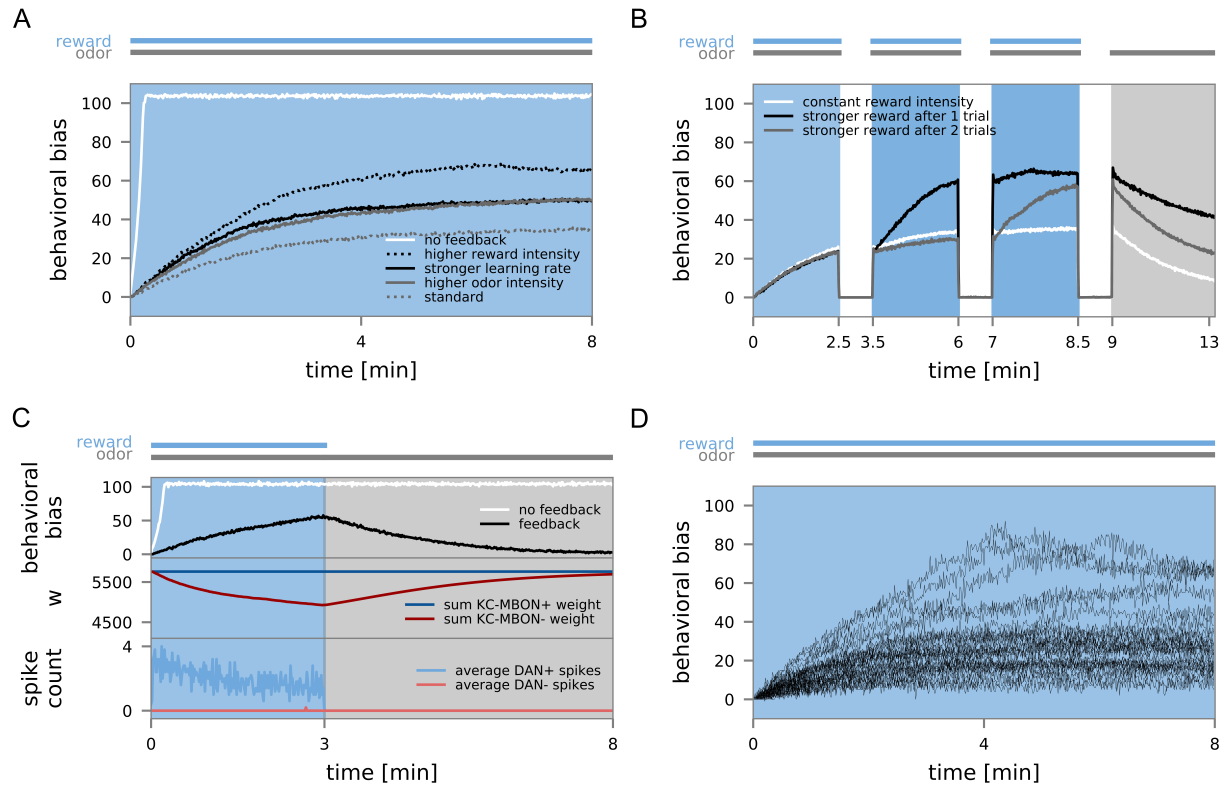


Figure 2: **Learning with prediction errors.** (A)  $N = 30$  model instances were trained with the odor amylocetate (CS) and reward (US, blue background). MBON>DAN feedback, the reward/odor intensity, and the learning rate were manipulated in separate experiments. The odor preference (behavioral bias, eqn. 4) was measured continuously in windows of 1 sec and averaged over all model instances. (B)  $N = 30$  model instances were trained during three trials with amylocetate and reward (blue background). Reward intensity was either constant across the three training trials (white curve), or enhanced during the third (gray) or the second and third trials (black). The training was followed by a 3 min test phase with odor only (gray background). (C)  $N = 30$  model instances were trained with amylocetate and reward (blue background) and then underwent an extended test phase (gray background). (D) Individual acquisition curves for  $N = 30$  model instances (standard experiment fig. 2 A).

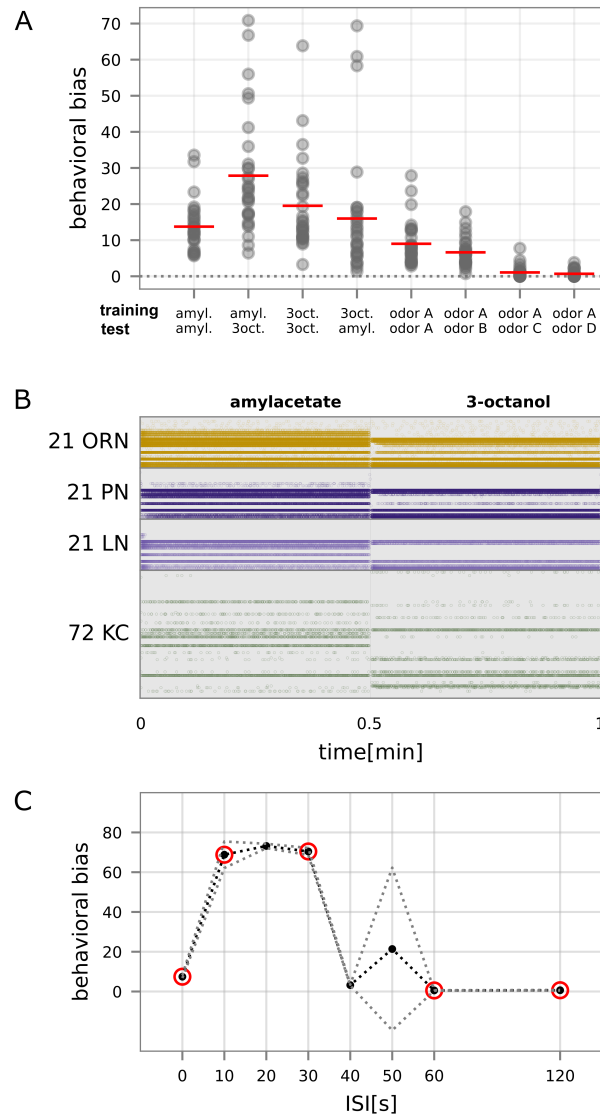


Figure 3: **Reward generalization and trace conditioning.** (A) The behavioral bias generalizes to odors that differ from the training odor after a 4min training (3min test phase). We conducted simulation experiments with different combinations of training and testing odor, each for 10 groups (gray circles represent the mean of a single group) of  $N = 30$  larvae, and red lines indicate the mean between groups. The behavioral bias is highest when the training and the testing odor are the same. (B) Spiking activity in the network during the presentation of amylacetate (left) and 3-octanol (right) in a single naive model instance. (C) Simulated trace conditioning experiments with odor (amylacetate) and reward. Inter-stimulus interval (ISI) indicates the time between odor and reward onset. The black line displays the mean, gray lines the std over  $N = 10$  groups of 30 model instances each. Conditions circled in red correspond to the conditions also used in animal experiments [29, 66]



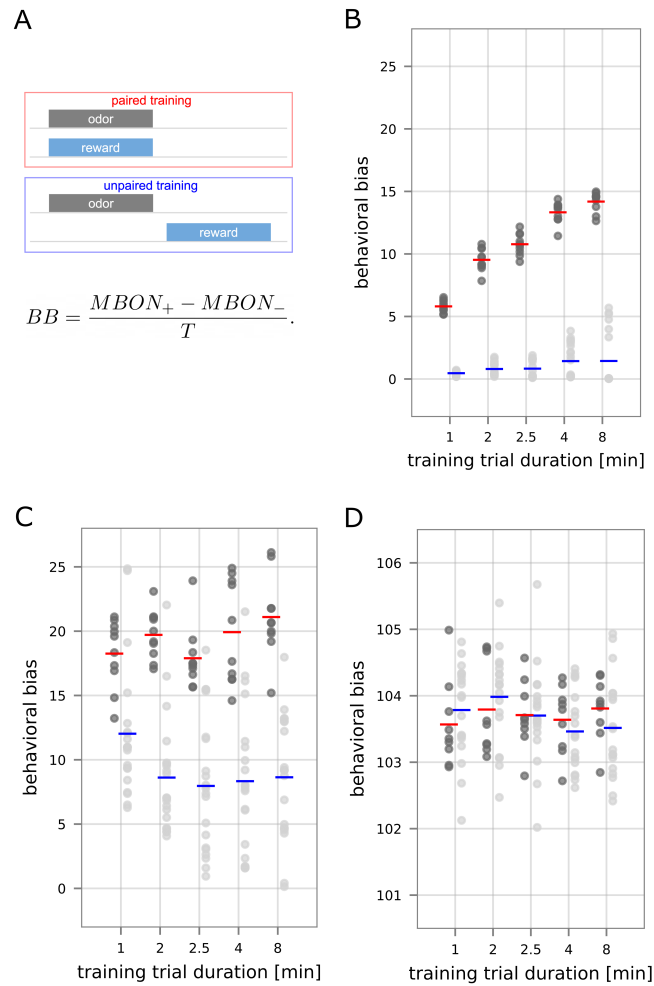


Figure 4: **Paired and unpaired learning in the MB model.** (A) Schematic overview of the paired vs. unpaired training protocol. (B) The model's behavioral bias for training with amylnacetate and reward for  $N = 10$  paired (dark gray, mean in red) and  $N = 10$  unpaired (light gray, mean in blue) experiments with groups of 30 modeled larvae each. In the unpaired condition, half of the groups were trained with the odor preceding the reward, for the other half, the reward preceded the odor. (C) Model behavioral bias for amylnacetate for  $N = 30$  paired and  $N = 30$  unpaired experiments with randomized order of odor and reward. Prior to the conditioning experiment the model instances underwent a 10min pre-training period, during which odor and reward were paired. (D) Model behavioral bias for amylnacetate for  $N = 30$  paired and  $N = 30$  unpaired experiments with randomized order of odor and reward. The MBON>DAN feedback was disabled. Prior to the conditioning experiment the model instances underwent a 10min pre-training period.

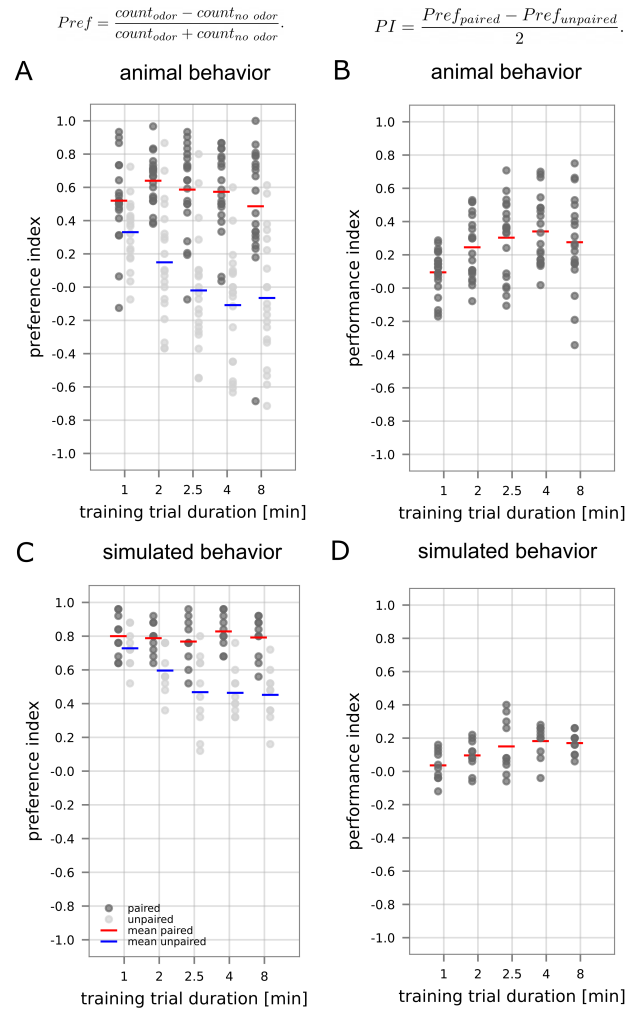


Figure 5: **Replicating behavioral experiments with paired and unpaired training.** (A) Experimental preference indices for amylocetate for 20 groups of 30 real animals each for paired and unpaired experiments with randomized order of odor and reward [1]. (B) Experimental performance indices for amylocetate computed between preference in paired and unpaired real animal experiments [1]. (C) The simulated behavior is based on the protocol in A. Simulated preference indices for amylocetate for  $N = 10$  paired and  $N = 10$  unpaired experiments with varied order of odor and reward. (D) Simulated performance indices for amylocetate computed between preference in paired and unpaired simulation experiments.

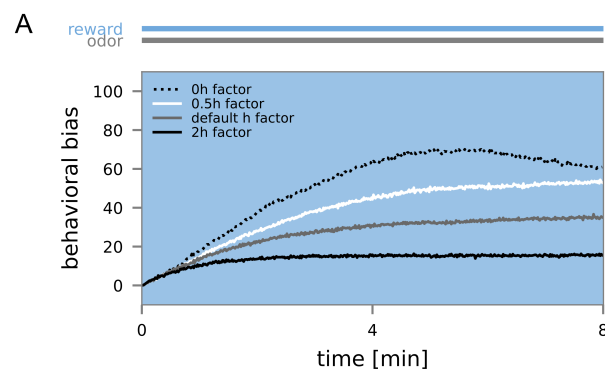


Figure S1: **The effect of the homeostatic mechanism on the learning curve.** (A)  $N = 30$  model instances were trained with the odor amylnacetate (CS) and reward (US, blue background). The odor preference (behavioral bias) was measured continuously in windows of 1 sec and averaged over all model instances. The learning rate was the same in all three experiments, while the magnitude of the homeostatic regulation  $h$  (eqn. 3, table S1) was either at its default value, at 0, or at half or twice the magnitude of the default value.

### Neuron Parameters

Capacitance ORN	$C_m^O$	100pF
Capacitance PN	$C_m^P$	30pF
Capacitance LN	$C_m^L$	50pF
Capacitance KC	$C_m^K$	30pF
Capacitance APL	$C_m^A$	200pF
Capacitance MBON	$C_m^M$	100pF
Capacitance DAN	$C_m^D$	100pF
Leak Conductance ORN	$g_L^O$	5nS
Leak Conductance PN	$g_L^P$	2.5nS
Leak Conductance LN	$g_L^L$	2.5nS
Leak Conductance KC	$g_L^K$	5nS
Leak Conductance APL	$g_L^A$	5nS
Leak Conductance MBON	$g_L^M$	5nS
Leak Conductance DAN	$g_L^D$	5nS
Leak Potential ORN	$E_L^O$	-60mV
Leak Potential PN	$E_L^P$	-59mV
Leak Potential LN	$E_L^L$	-59mV
Leak Potential KC	$E_L^K$	-55mV
Leak Potential APL	$E_L^A$	-60mV
Leak Potential MBON	$E_L^M$	-60mV
Leak Potential DAN	$E_L^D$	-60mV
Threshold Potential ORN	$V_T^O$	-35mV
Threshold Potential PN	$V_T^P$	-30mV
Threshold Potential LN	$V_T^L$	-30mV
Threshold Potential KC	$V_T^K$	-35mV
Threshold Potential APL	$V_T^A$	-30mV
Threshold Potential MBON	$V_T^M$	-30mV
Threshold Potential DAN	$V_T^D$	-30mV
Resting Potential ORN	$V_r^O$	-60mV
Resting Potential PN	$V_r^P$	-59mV
Resting Potential LN	$V_r^L$	-59mV
Resting Potential KC	$V_r^K$	-55mV
Resting Potential APL	$V_r^A$	-60mV
Resting Potential MBON	$V_r^M$	-60mV
Resting Potential DAN	$V_r^D$	-60mV
Refractory Time	$\tau_{ref}$	2ms

<b>Synaptic Parameters</b>		
Excitatory Potential	$E_E$	0mV
Inhibitory Potential	$E_I$	-75mV
Excitatory Time Constant	$\tau_e$	5ms
Inhibitory Time Constant	$\tau_i$	10ms
<b>Plasticity Parameters</b>		
Eligibility Trace Time Constant	$\tau_{eligibility}$	5s
Learning Rate	$\alpha$	0.3nS
<b>Synaptic Weights</b>		
Weight Input-ORN	wORNinputORN	3nS
Weight ORN-PN	wORNPN	10nS
Weight ORN-LN	wORNLN	4nS
Weight LN-PN	wLNPN	1nS
Weight PN-KC	wPNKC	1nS
Weight KC-APL	wKCAPL	20nS
Weight APL-KC	wAPLKC	50nS
Weight KC-MBON	wKCMBON	80nS
Weight Input-DAN	wDANinputDAN	2.5nS
Excitatory Weight MBON-DAN	wMBONDANex	4nS
Excitatory Weight MBON-local interneuron	$w_{MBONMBON_{LN}}$	35nS
Inhibitory Weight local interneuron-DAN	wMBONDANin	70nS
Normalization Factor KC-MBON	$normalization_{factor}$	0.0001
<b>Adaptation Parameters</b>		
Adaptation Time Constant	$\tau_{Ia}$	1000ms
Adaptation Reversal Potential	$E_{Ia}$	-90mV
Increase of Spike Frequency Adaptation Conductance ORN	$ORN_{SFA}$	0.1nS
Increase of Spike Frequency Adaptation Conductance KC	$KC_{SFA}$	0.02nS
Increase of Spike Frequency Adaptation Conductance MBON	$MBON_{SFA}$	0.1nS
Increase of Spike Frequency Adaptation Conductance DAN	$DAN_{SFA}$	0.1nS
<b>Simulation Parameters</b>		
Time Step	$dt$	0.1ms

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Table S1: Network parameters.