Supplementary Appendix

Contents

§1. A neural network model comprised of KCs, DANs and MBONs.

To simulate associative conditioning and extinction processes in *Drosophila melanogaster*, we created a computational model of the *Drosophila* mushroom body's neural circuit dynamics. The model describes the activity and plasticity patterns of Kenyon cells (KCs), mushroom body output neurons (MBONs) and dopamine neurons (DANs) within 3 interconnected learning modules (γ1, α2 and α3) of the mushroom body (MB) (**Fig. 5a**). The KCs sparsely encode the odor stimuli used in our conditioning experiments. The DANs encode the shock punishments received by the fly and modulate the strengths of the synaptic connections between KCs and MBONs, thereby shaping the storage and extinction of memories stored in the MB. The MBONs gather signals from the KCs to control motor behaviors. We described the interactions of these 3 neuron-types by using a set of ordinary differential equations to model the circuit's time-dependent patterns of neural activity and synaptic plasticity. The model thus depicts how associative information is stored and retrieved in

both the short-term (γ1 module) and long-term (α2 and α3 modules) learning compartments.

We used a recurrent neural network to characterize the neural dynamics [Eqs. (1.1), (1.2), (1.3) and (1.4)]. The recurrent circuit architecture of our model is based directly on the physical connections revealed in recent electron microscopy studies of the fly brain connectome[1] (**Fig. 5a**). If the number of synapses between any two neurons in the biological connectome is <10, as determined by electron microscopy, we set the synaptic strength between these two neurons in our model to be zero. This simplification significantly reduced the number of parameters in the model. However, we did not infer non-zero synaptic weight values in the model based on the numbers of synapses found in the biological connectome between neuron pairs, which might have oversimplified the important role of synaptic plasticity in mushroom body circuit dynamics. Instead, we estimated the values of all non-zero synaptic weights by parametric fitting of our experimental neural recording data.

In our experiments, we performed olfactory conditioning using odor pairs with distinct molecular structures, implying that the two odors are likely to be encoded orthogonally. To capture this, the model has 4 KCs, each of which responds to a single odor. The 4 odors included in the model are an attractive CS^+ odor, an attractive CS^- odor, a repulsive CS^+ odor, and a repulsive CS^- odor. Using the subscript *i* to refer to an individual KC, Eq. (1.1) describes the sparse activation of each KC by its preferred odor input,

$$\tau_{KC,i}\frac{d}{dt}x_{KC,i} = f_{a,KC,i}\left(w_{odor,i}(t)\,x_{odor,i}(t)+b_{KC,i}\right)-x_{KC,i} \quad , \tag{1.1}$$

where $x_{KC,i}(t)$ is the spiking rate of the *i*'th KC, $f_{a,KC,i}$ is the activation function of this KC, $\tau_{KC,i}$ is a time-constant characterizing the rate at which the cell's spiking rate converges to its steady state value, $x_{odor,i}(t)$ equals 1 when the cell's preferred odor is presented to the fly and 0 otherwise,

$w_{odor,i}(t)$ is the time-dependent amplitude of neural input signals conveying the presence of odor *i*, and $b_{KC,i}$ is a bias term that sets the cell's baseline spiking rate ($B_{KC,i}$) in the absence of odor *i* and prior to any conditioning, $B_{KC,i} = f_{a,KC,i}\left(b_{KC,i}\right)$. When odor is present continuously at a uniform level, the activity levels of olfactory sensory neurons do not stay uniform but instead adapt over time. The model captures this phenomenon through the time-dependence of $w_{odor,i}(t)$:

$$\frac{d}{dt}w_{odor,i} = -\frac{x_{odor,i}(t)}{\tau_{KC,adapt}}w_{odor,i} + \frac{1-x_{odor,i}(t)}{\tau_{KC,recover}}\left(A_{odor,i} - w_{odor,i}\right) \quad , \tag{1.2}$$

where $A_{odor,i}$ denotes the amplitude of odor *i*, and $\tau_{KC,adapt}$ and $\tau_{KC,recover}$ are time-constants that respectively characterize the rates of adaptation in the presence of odor and following the offset of odor presentation.

DANs are activated by electric shocks, and they also receive feedforward, feedback, and cross-module signals from KCs and MBONs (**Fig. 5a**). The MBONs gather signals from the KCs and also transmit signals between different MB learning units. For the DANs, we used the subscripts 1, 2 and 3 to refer to PPL1-γ1pedc, -α'2α2, and -α3. For the MBONs, these subscripts refer to each DAN's corresponding MBON, namely MBON-γ1pedc>αβ, -α2sc and -α3. Defining $x_{DAN,j}$ as the spike rate of DAN *j*, $x_{MBON,j}$ as the spike rate of MBON *j,* and $f_{a,DAN,j}$ and $f_{a,MBON,j}$ as activation functions, the dynamics of $x_{DAN,j}$ and $x_{MBON,j}$ are governed by

$$\tau_{DAN,j}\frac{d}{dt}x_{DAN,j} = f_{a,DAN,j}\left(\begin{array}{l} w_{punish,j}x_{punish}(t) + \sum_i w_{KD,i,j}\left(x_{KC,i} - B_{KC,i}\right)\ldots \\ +\sum_l w_{MD,l,j}\left(x_{MBON,l} - B_{MBON,l}\right) + b_{DAN,j} \end{array}\right) - x_{DAN,j} \tag{1.3}$$

$$\tau_{MBON,j}\frac{d}{dt}x_{MBON,j} = f_{a,MBON,j}\left(\begin{array}{l} \sum_i w_{KM,i,j}\left(x_{KC,i} - B_{KC,i}\right)\ldots \\ +\sum_l w_{MM,l,j}\left(x_{MBON,l} - B_{MBON,l}\right) + b_{MBON,j} \end{array}\right) - x_{MBON,j} \quad , \tag{1.4}$$

where $\tau_{DAN,j}$ and $\tau_{MBON,j}$ are time-constants of spiking adapation, $w_{KD,i,j}$ is the synaptic weight from KC *i* to DAN *j*, $w_{MD,l,j}$ is the synaptic weight from MBON *l* to DAN *j*, $w_{KM,i,j}$ is the synaptic weight from KC *i* to MBON *j*, $w_{MM,l,j}$ is the synaptic weight from MBON *l* to MBON *j*, $B_{KC,i}$, $B_{DAN,j}$ and $B_{MBON,j}$ are baseline spiking rates, and $b_{DAN,j}$ and $b_{MBON,j}$ are bias terms analogous to that in Eq. (1.1). When the fly receives an electric shock punishment, $x_{punish}(t)$ equals 1; otherwise, it equals 0. $w_{punish,j}$ is the weight of punishment signals received by DAN *j*. Because PPL1-α′2α2 did not respond to electric shocks (**Fig. 2a**), this DAN received no punishment signals in the model. Further, since in our experiments the presentation of different odors with similar innate valences led to comparable levels of DAN activation (**Fig. 2i; Extended Data Fig. 3a**), we modeled this result by setting $w_{KD,1,j} = w_{KD,2,j}$ and $w_{KD,3,j} = w_{KD,4,j}$. In other words, the model incorporates the finding that each odor of an attractive odor pair with similar innate valences drives equivalent levels of DAN spiking, as do the two odors of a repulsive odor pair with similar innate valences.

§2. Modeling the plasticity of synapses between KCs and MBONs.

Based on the biological finding that concurrent activation of a DAN and KCs leads to plasticity of the odor-evoked spiking responses by the DAN's corresponding MBON[2,3], in our model the concurrent activation of KC *i* and DAN *j* modifies the weight of the KC *i* to MBON *j* synapse, $w_{KM,i,j}$, according to an anti-Hebbian learning rule (**Fig. 5b**). If the fly receives a punishment following odor presentation (a forward pairing), $w_{KM,i,j}$ decreases, whereas $w_{KM,i,j}$ increases if the punishment occurs before odor presentation (a backward pairing) (**Fig. 5b**). We modeled these effects as follows[4]:

$$\frac{d}{dt} y_{KC,i} = k_{CS}\left(x_{KC,i} - B_{KC,i}\right) - \gamma_{CS} y_{KC,i} \tag{2.1}$$

$$\frac{d}{dt} y_{DAN,j} = k_{US}\left(x_{DAN,j} - B_{DAN,j}\right) - \gamma_{US} y_{DAN,j} \tag{2.2}$$

$$\frac{d}{dt} u_{KM,i,j} = \left[\left(x_{KC,i} - B_{KC,i}\right) y_{DAN,j} - \left(x_{DAN,j} - B_{DAN,j}\right) y_{KC,i}\right] - \frac{u_{KM,i,j}}{\tau_{u,j}} \tag{2.3}$$

$$\tau_{w,j} \frac{d}{dt} w_{KM,i,j} = u_{KM,i,j} - w_{KM,i,j} \quad . \tag{2.4}$$

In Eqs. (2.1) and (2.2), $y_{KC,i}$ and $y_{DAN,j}$ are low-pass filtered versions of the spiking signals conveyed to the MBON by KC *i* and DAN *j*. Unlike past modeling studies[4] but in accord with prior experimental findings[2,3], in our model there are distinct amplitudes (k_{CS} and k_{US}) and time-constants (γ_{CS} and γ_{US}) characterizing the plasticity that arises from forward *vs.* backward pairings. In Eq. (2.3), $\tau_{u,j}$ is a plasticity time-constant for MBON *j*. $w_{KM,i,j}$ is the synaptic weight between KC *i* and MBON *j*. $u_{KM,i,j}$ is an intermediate variable used to calculate $w_{KM,i,j}$ and $\tau_{w,j}$ is the time-constant of the low-pass filter in Eq. (2.4), which ensures that the synaptic weight $w_{KM,i,j}$ is a temporally low-pass filtered version of $u_{KM,i,j}$. This prevents rapid fluctuations of the synaptic weights in the model, which might have occurred if we had directly used $u_{KM,i,j}$ as the synaptic weight between KC *i* and MBON *j*.

To characterize long-term memory consolidation processes, we let the time-constants of the synaptic weights of MBON-α2sc and -α3 ($\tau_{u,2}$ and $\tau_{u,3}$) change with time. Namely, within the first 3 hrs after conditioning, $\tau_{u,2}$ and $\tau_{u,3}$ were set to a relatively low level; after 3 hrs, $\tau_{u,2}$ and $\tau_{u,3}$ were switched to a larger value. The reason for changing the values of $\tau_{u,2}$ and $\tau_{u,3}$ was that

long-term memory consolidation involves protein synthesis, which typically takes several hours[5].

In multiple of this paper's figures and extended data figures, we plotted changes in neural spiking rates from baseline levels prior to conditioning:

$$\Delta x_{KC,i} = x_{KC,i} - B_{KC,i} \tag{2.5}$$

$$\Delta x_{DAN,j} = x_{DAN,j} - B_{DAN,j} \tag{2.6}$$

$$\Delta x_{MBON,l} = x_{MBON,l} - B_{MBON,l} \quad . \tag{2.7}$$

§3. Simplifications and simulations of the neural network model.

We simulated the differential equations (1.1–1.4) and (2.1–2.4) using the MATLAB (Mathworks Inc.) function ode15s(), which solves differential equations numerically. This approach was time-consuming and required ~14 s to provide results for a single, fixed set of parameter values. To accelerate the simulations and analyze the key features of the neural network across many different sets of parameter values, we simplified the model by applying several approximations.

First, we approximated the activation functions of KCs and DANs as linear functions. In our experiments, we did not use extremely high concentration odorants, which might have saturated the flies' odorant receptors (**Extended Data Fig. 3a**). Thus, we approximated KC activation functions as linear, $f_{a,KC,i}(x) = x$. Using this approximation and combining Eq. (1.1) with Eq. (2.5) yields:

$$\tau_{KC,i} \frac{d}{dt} \Delta x_{KC,i} = w_{odor,i}(t) x_{odor,i}(t) - \Delta x_{KC,i} \quad . \tag{3.1}$$

In our imaging studies of DANs, we observed that they linearly integrate the valences of jointly presented stimuli, such that the spiking rate changes triggered by an odor and shock presented

jointly approximately equal the sum of the spiking rate changes triggered by the individual stimuli (**Fig. 4e–h**). Thus, DANs operate approximately in the linear range of their activation function, and Eq. (1.3) can be simplified as:

$$\tau_{DAN,j}\frac{d}{dt}\Delta x_{DAN,j} = w_{punish,j}x_{punish}(t) + \sum_i w_{KD,i,j}\Delta x_{KC,i} + \sum_l w_{MD,l,j}\Delta x_{MBON,l} - \Delta x_{DAN,j} \quad . \quad (3.2)$$

Unlike our results from DANs, we observed in our experiments that the spiking rates of MBONs can attain upper and lower bounds (**Fig. 4a–d**). Therefore, we did not approximate the activation functions of MBONs as linear. Instead, we used a piecewise linear function for the activation function of MBON *j*:

$$f_{a,MBON,j}(x) = \begin{cases} 0 & x < 0 \\ x & 0 \le x < x_{M,j} \\ x_{M,j} & x \ge x_{M,j} \end{cases} \quad , \quad (3.3)$$

where $x_{M,j}$ is the maximum spiking rate of MBON *j*. Based on our experimental data, we chose $x_{M,1} = 71.6\ \text{s}^{-1}$, $x_{M,2} = 17.9\ \text{s}^{-1}$, $x_{M,3} = 31.2\ \text{s}^{-1}$, $B_{MBON,1} = 35.2\ \text{s}^{-1}$, $B_{MBON,2} = 9.0\ \text{s}^{-1}$, and $B_{MBON,3} = 11.25\ \text{s}^{-1}$ (**Extended Data Figs. 4,6**). Plainly, the baseline spiking rates of MBONs do not reach the upper and lower limits. Thus, we concluded that $b_{MBON,j} = B_{MBON,j}$. Combining Eqs. (1.4) and (2.7) yields Eq. (3.4), which describes the spiking rate changes:

$$\tau_{MBON,j}\frac{d}{dt}\Delta x_{MBON,j} = f_{a,MBON,j}\left(\begin{array}{l}\sum_i w_{KM,i,j}\Delta x_{KC,i} \\ +\sum_l w_{MM,l,j}\Delta x_{MBON,l} + B_{MBON,j}\end{array}\right) - B_{MBON,j} - \Delta x_{MBON,j} \quad . \quad (3.4)$$

The time-constants $\tau_{KC,i}$, $\tau_{DAN,j}$ and $\tau_{MBON,j}$ are sufficiently brief (~10 ms) that the dynamics of Eqs. (3.1) and (3.2) quickly reach steady-state values when the input values of $x_{odor,i}(t)$ and

$x_{punish}(t)$ change. This allows us to simplify Eqs. (3.1), (3.2), and (3.4) as follows:

$$\Delta x_{KC,i} = w_{odor,i}(t)\, x_{odor,i}(t) \tag{3.5}$$

$$\Delta x_{DAN,j} = w_{punish,j} x_{punish}(t) + \sum_i w_{KD,i,j} \Delta x_{KC,i} + \sum_l w_{MD,l,j} \Delta x_{MBON,l} \tag{3.6}$$

$$\Delta x_{MBON,j} = f_{a,MBON,j}\left(\sum_i w_{KM,i,j} \Delta x_{KC,i} + \sum_l w_{MM,l,j} \Delta x_{MBON,l} + B_{MBON,j} \right) - B_{MBON,j} \quad . \tag{3.7}$$

During resting bouts, $x_{odor,i}(t) = 0$ and $x_{punish}(t) = 0$. From Eqs. (3.5), (3.6) and (3.7), we found that $\Delta x_{KC,i}$, $\Delta x_{DAN,j}$ and $\Delta x_{MBON,j}$ are all zero during resting bouts. During training, testing and imaging bouts, at least one of $x_{odor,i}(t)$ and $x_{punish}(t)$ is non-zero. Then, $\Delta x_{KC,i}$, $\Delta x_{DAN,j}$ and $\Delta x_{MBON,j}$ may be non-zero. Considering that the durations of training, testing and imaging bouts are much less than the resting intervals between them (**Fig. 3a,d; Fig. 4l; Fig. 5i**), we focused on the discrete time points t_k corresponding to each training, testing, and imaging bout, and we used the time-averaged mean neural spiking rates to represent the neurons' activities. With this approach, Eqs. (3.5), (3.6), and (3.7) become:

$$\overline{\Delta x_{KC,i}}(t_k) = \overline{w_{odor,i}}(t_k)\, x_{odor,i}(t_k) \tag{3.8}$$

$$\overline{\Delta x_{DAN,j}}(t_k) = w_{punish,j} x_{punish}(t_k) + \sum_i w_{KD,i,j} \overline{\Delta x_{KC,i}}(t_k) + \sum_l w_{MD,l,j} \overline{\Delta x_{MBON,l}}(t_k) \tag{3.9}$$

$$\overline{\Delta x_{MBON,j}}(t_k) = f_{a,MBON,j}\left(\begin{array}{l} \sum_i \overline{w_{KM,i,j}}(t_k)\, \overline{\Delta x_{KC,i}}(t_k) \\ + \sum_l w_{MM,l,j}\, \overline{\Delta x_{MBON,l}}(t_k) + B_{MBON,j} \end{array} \right) - B_{MBON,j} \quad , \tag{3.10}$$

where $\overline{\Delta x_{KC,i}}(t_k)$, $\overline{\Delta x_{DAN,j}}(t_k)$, and $\overline{\Delta x_{MBON,j}}(t_k)$ are the average neural spiking rates during the k^{th} training, testing, or imaging bout. $\overline{w_{odor,i}}(t_k)$ is the average weight of odor i on KC i at the k^{th}

training, testing, or imaging bout. $\overline{w_{KM,i,j}}(t_k)$ is the average weight from KC *i* to MBON *j* at the k^{th} training, testing, or imaging bout.

In the 3 equations above, $w_{odor,i}$ and $w_{KM,i,j}$ change with time, whereas the other synaptic weights remain constant. Given that the durations of training, testing and imaging bouts are much less than the durations of the resting intervals between them (**Fig. 3a,d; Fig. 4l; Fig. 5i**), we attained equations from which we could calculate the average $w_{odor,i}$ of Eq. (1.2):

$$w_{odor,i}(t_1, start) = A_{odor,i} \tag{3.11}$$

$$w_{odor,i}(t_k, end) = \begin{cases} w_{odor,i}(t_k, start)\, e^{-t_{on,k}/\tau_{KC,adapt}} & x_{odor,i}(t_k) = 1 \\ A_{odor,i} - \left(A_{odor,i} - w_{odor,i}(t_k, start)\right) e^{-t_{on,k}/\tau_{KC,recover}} & x_{odor,i}(t_k) = 0 \end{cases} \tag{3.12}$$

$$w_{odor,i}(t_{k+1}, start) = A_{odor,i} - \left(A_{odor,i} - w_{odor,i}(t_k, end)\right) e^{-t_{off,k}/\tau_{KC,recover}} \tag{3.13}$$

$$\overline{w_{odor,i}}(t_k) \approx \left[w_{odor,i}(t_k, start) + w_{odor,i}(t_k, end)\right]/2 \quad , \tag{3.14}$$

where $w_{odor,i}(t_k, start)$ and $w_{odor,i}(t_k, end)$ are the weight of odor *i* activating KC *i* at the starting and ending time-points of the k^{th} training, testing or imaging bout. $t_{on,k}$ is the duration of the k^{th} training, testing, or imaging bout. $t_{off,k}$ is the duration of the resting bout after the k^{th} training, testing, or imaging bout. Because $A_{odor,i}$ can be combined with other amplitude terms like $w_{KM,i,j}$ and $w_{KD,i,j}$, we set $A_{odor,i}$ = 1. We set $\tau_{KC,adapt}$ = 20 s based on prior research on olfactory receptor neurons in *Drosophila*[6] and optimized the value of $\tau_{KC,recover}$ through our fits to experimental data.

We used the time-averages of the KCs' and DANs' spiking rate changes to calculate the synaptic weight changes between KCs and MBONs. Since the durations of training, testing, and

imaging bouts are much less than the time-constant $\tau_{u,j}$, we approximated $u_{KM,i,j}$ as follows:

$$u_{KM,i,j}\left(t_k, end\right) \approx \int_0^{t_{on,k}} \left(\Delta x_{KC,i} y_{DAN,j} - \Delta x_{DAN,j} y_{KC,i}\right) dt + u_{KM,i,j}\left(t_k, start\right) \tag{3.15}$$

$$u_{KM,i,j}\left(t_{k+1}, start\right) \approx u_{KM,i,j}\left(t_k, end\right) e^{-t_{off,k}/\tau_{u,j}} \quad , \tag{3.16}$$

where $u_{KM,i,j}\left(t_k, start\right)$ and $u_{KM,i,j}\left(t_k, end\right)$ are the values of $u_{KM,i,j}\left(t\right)$ at the starting and ending time-points of the k^{th} training, testing or imaging bout.

Because the time-constant $\tau_{w,j}$ is much greater than $t_{on,k}$ but much smaller than $t_{off,k}$, $w_{KM,i,j}$ does not significantly change during the k^{th} training, testing, or imaging bout but instead converges to $u_{KM,i,j}$ at the end of the subsequent resting bout:

$$\overline{w_{KM,i,j}}\left(t_k\right) \approx u_{KM,i,j}\left(t_k, start\right) \quad . \tag{3.17}$$

By combining Eqs. (3.15), (3.16), and (3.17), we derived the recurrence formula Eq. (3.18):

$$\overline{w_{KM,i,j}}\left(t_{k+1}\right) \approx \left(\int_0^{t_{on,k}} \left(\Delta x_{KC,i} y_{DAN,j} - \Delta x_{DAN,j} y_{KC,i}\right) dt + \overline{w_{KM,i,j}}\left(t_k\right) \right) e^{-t_{off,k}/\tau_{u,j}} \quad . \tag{3.18}$$

The initial value of this formula is the synaptic weight from KC *i* to MBON *j*. Before our experiments, the fly does not have a bias or preference between the CS^+ and CS^- odors. Therefore, we set the initial values of the synaptic weights from all KCs to MBON *j* to be the same:

$$\overline{w_{KM,i,j}}\left(t_1\right) = w_{KM,initial,j} \quad . \tag{3.19}$$

To calculate the integral term of the Eq. (3.18), we derived the analytic solution of Eqs. (2.1) and (2.2), and defined a functional ΔW:

$$y_{CS}\left(t\right) = k_{CS} e^{-\gamma_{CS} t} \int_0^t e^{\gamma_{CS} t_1} x_{CS}\left(t_1\right) dt_1 \tag{3.20}$$

$$y_{US}(t) = k_{US} e^{-\gamma_{US} t} \int_0^t e^{\gamma_{US} t_1} x_{US}(t_1)\, dt_1 \tag{3.21}$$

$$\begin{aligned} &\Delta W\left(\Delta x_{CS}(t), \Delta x_{US}(t)\right) = \int_0^{t_{on}} \left(\Delta x_{CS}(t)\, y_{US}(t) - \Delta x_{US}(t)\, y_{CS}(t)\right) dt \\ &= \int_0^{t_{on}} \left(\Delta x_{CS}(t)\, k_{US} e^{-\gamma_{US} t} \int_0^t e^{\gamma_{US} t_1} x_{US}(t_1)\, dt_1 - \Delta x_{US}(t)\, k_{CS} e^{-\gamma_{CS} t} \int_0^t e^{\gamma_{CS} t_1} x_{CS}(t_1)\, dt_1\right) dt \end{aligned} . \tag{3.22}$$

The integral term in Eq. (3.18) equals $\Delta W\left(\Delta x_{KC,i}(t), \Delta x_{DAN,j}(t)\right)$. It can be proved that the ΔW defined by Eq. (3.22) is a linear functional of the two input functions $\Delta x_{CS}(t)$ and $\Delta x_{US}(t)$. Eq. (3.6) indicates that the spiking rate of each DAN is a linear combination of the punishment-related input and the spiking rates of KC and MBON inputs to the DAN. Therefore, the changes in the synaptic weights between KCs and MBONs are linear combinations of the contributions from the punishment and the spiking rates of KCs and MBONs:

$$\begin{aligned} &\Delta W\left(\Delta x_{KC,i}(t), \Delta x_{DAN,j}(t)\right) \\ &= \Delta W\left(\Delta x_{KC,i}(t), w_{punish,j} x_{punish}(t) + \sum_i w_{KD,i,j} \Delta x_{KC,i}(t) + \sum_l w_{MD,l,j} \Delta x_{MBON,l}(t)\right) \\ &= w_{punish,j} \Delta W\left(\Delta x_{KC,i}(t), x_{punish}(t)\right) + \sum_i w_{KD,i,j} \Delta W\left(\Delta x_{KC,i}(t), \Delta x_{KC,i}(t)\right) \\ &+ \sum_l w_{MD,l,j} \Delta W\left(\Delta x_{KC,i}(t), \Delta x_{MBON,l}(t)\right) \end{aligned} . \tag{3.23}$$

To calculate the terms $\Delta W\left(\Delta x_{KC,i}(t), x_{punish}(t)\right)$, $\Delta W\left(\Delta x_{KC,i}(t), \Delta x_{KC,i}(t)\right)$, and $\Delta W\left(\Delta x_{KC,i}(t), \Delta x_{MBON,l}(t)\right)$ from the mean spiking rates of KCs, DANs and MBONs, we input two square wave functions (Eqs. (3.24) and (3.25)) into Eq. (3.22). Given the linear dependence noted above of ΔW on its inputs, we defined the anti-Hebbian amplitude function $A_{AH}(\Delta t)$ by Eq. (3.26).

$$\Delta x_{CS}(t) = \begin{cases} \overline{\Delta x_{CS}} & 0 < t \le \tau \\ 0 & otherwise \end{cases} \tag{3.24}$$

$$\Delta x_{US}(t) = \begin{cases} \overline{\Delta x_{US}} & \Delta t < t \le \Delta t + \tau \\ 0 & otherwise \end{cases} \tag{3.25}$$

$$A_{AH}(\Delta t) = \frac{\Delta W\left(\Delta x_{CS}(t), \Delta x_{US}(t)\right)}{\overline{\Delta x_{CS}}\,\overline{\Delta x_{US}}}$$

$$= \begin{cases} \frac{k_{US}}{\gamma_{US}^2}\left(1-e^{-\gamma_{US}\tau}\right)^2 e^{-\gamma_{US}(-\Delta t-\tau)} & \Delta t \le -\tau \\ \left(\frac{k_{US}}{\gamma_{US}} - \frac{k_{CS}}{\gamma_{CS}}\right)(\Delta t+\tau) + \frac{k_{US}}{\gamma_{US}^2}\left(e^{-\gamma_{US}(\tau-\Delta t)} - 2e^{-\gamma_{US}(-\Delta t)} + 1\right) - \frac{k_{CS}}{\gamma_{CS}^2}\left(e^{-\gamma_{CS}(\Delta t+\tau)} - 1\right) & -\tau < \Delta t \le 0 \\ \left(\frac{k_{US}}{\gamma_{US}} - \frac{k_{CS}}{\gamma_{CS}}\right)(\tau-\Delta t) + \frac{k_{US}}{\gamma_{US}^2}\left(e^{-\gamma_{US}(\tau-\Delta t)} - 1\right) - \frac{k_{CS}}{\gamma_{CS}^2}\left(e^{-\gamma_{CS}(\Delta t+\tau)} - 2e^{-\gamma_{CS}\Delta t} + 1\right) & 0 < \Delta t \le \tau \\ -\frac{k_{CS}}{\gamma_{CS}^2}\left(1-e^{-\gamma_{CS}\tau}\right)^2 e^{-\gamma_{CS}(\Delta t-\tau)} & \Delta t > \tau \end{cases} \tag{3.26}$$

where $\overline{\Delta x_{CS}}$ and $\overline{\Delta x_{US}}$ are time averages of the input functions, Δt is the time difference between CS and US, and τ is the duration of the CS and US. (In our experiments, the CS and US lasted for an equal duration in the training bouts). The anti-Hebbian learning rule implies that the time difference between the CS and US, Δt , influences the changes in synaptic weights between KCs and MBONs. The amplitude function $A_{AH}(\Delta t)$ captures this property (**Fig. 5b**).

In the training bout, the electric shock punishment is delivered 5 s later than the odorant. Thus, the amplitude of the punishment term should be $A_{AH}\left(\Delta t_{punish}\right)$ where Δt_{punish} = 5 s. From Eq. (3.7), we saw that the firing of the MBONs is synchronized with that of the KCs. Thus, the amplitude of the MBON terms should be $A_{AH}(0)$, the same as that of the KC terms. We thereby achieve Eq. (3.27) using Eqs. (3.18) and (3.23),

$$\overline{w_{KM,i,j}}(t_{k+1}) \approx \left(\overline{\Delta x_{KC,i}}(t_k) \left[\begin{array}{l} A_{AH}(\Delta t_{punish}) w_{punish,j} x_{punish}(t_k) \\ +A_{AH}(0) \left(\begin{array}{l} \sum_i w_{KD,i,j} \overline{\Delta x_{KC,i}}(t_k) \\ +\sum_l w_{MD,l,j} \overline{\Delta x_{MBON,l}}(t_k) \end{array} \right) \end{array} \right] + \overline{w_{KM,i,j}}(t_k) \right) e^{-t_{off}/\tau_{u,j}} . \quad (3.27)$$

In our experiments, we used a fixed Δt_{punish}. Thus, the parameters, k_{CS}, k_{US}, γ_{CS}, and γ_{US} in Eqs. (2.1) and (2.2) are converted into two parameters, $A_{AH}(\Delta t_{punish})$ and $A_{AH}(0)$. We directly optimized these two parameters in our simulation. With these approximations, we obtained a recurrent set of equations [Eqs. (3.8–3.14) and (3.27)]. The time needed to simulate one set of parameters with this set of equations was ~0.02 s, which was ~700-fold faster than simulating the set of differential equations Eqs. (1.1–1.4) and (2.1–2.4).

§4. Estimating the parameter values of the model

We fit the experimental data using the simplified model to estimate values for the set of model parameters, denoted as $\boldsymbol{\theta}$. We assumed that the measured neural spiking rates in experimental condition *i* were drawn from independent normal distributions, $N(\mu_i(\boldsymbol{\theta}), \sigma_i^2)$. Thus, the log-likelihood of the experimental data given a set of parameter values, $\boldsymbol{\theta}$, is:

$$\ln \mathrm{L}(\boldsymbol{\theta}) = -\sum_i \left(\ln \sigma_i + \frac{1}{2} \ln 2\pi \right) - \frac{1}{2} \sum_i \left(\frac{x_i - \mu_i(\boldsymbol{\theta})}{\sigma_i} \right)^2 \quad , \quad (4.1)$$

where, x_i and σ_i are the mean and SEM values of the spiking rate in experimental condition *i* and $\mu_i(\boldsymbol{\theta})$ is the spiking rate in condition *i* as computed from the model with parameter set $\boldsymbol{\theta}$. Maximum likelihood estimation of the model's parameters is equivalent to minimizing the weighted sum of squared errors (*WSSE*):

$$WSSE = \sum_i \left(x_i - \mu_i(\boldsymbol{\theta})\right)^2 / \sigma_i^2 \quad . \tag{4.2}$$

To find the parameter set minimizing *WSSE* in Eq. (4.2), we used an algorithm that combined the MATLAB (Mathworks) genetic algorithm function ga() and the gradient descent algorithm function fmincon(). Although the gradient descent algorithm optimized the parameter set faster than the genetic algorithm, it sometimes caused the parameter set to be trapped in a local minimum of the *WSSE*. Therefore, we first used the genetic algorithm to find a rough estimate of the parameter set, and then we used gradient descent to further fine-tune the parameter set (see **Supplementary Table 1**). The function fmincon() provides the matrix of the second derivatives of *WSSE* (*i.e.* the Hessian matrix of *WSSE*, H_{WSSE}) for the optimized parameter set (Eq. (4.3)):

$$H_{WSSE} = \nabla_{\boldsymbol{\theta}}^2 \sum_i \left(x_i - \mu_i(\boldsymbol{\theta})\right)^2 / \sigma_i^2 \Big|_{\boldsymbol{\theta}=\hat{\boldsymbol{\theta}}} \quad , \tag{4.3}$$

where $\hat{\boldsymbol{\theta}}$ is the set of optimized parameter values and the symbol $\nabla_{\boldsymbol{\theta}}^2$ denotes second derivatives.

Based on Eqs. (4.1) and (4.3), $H_{WSSE}/2$ equals the observed Fisher information provided by the optimized parameter set about the underlying, real biological parameter values. Using the Fisher information approach, the Hessian matrix can be used to estimate the confidence intervals of the parameter set:

$$CI_{\theta_i} = \hat{\theta}_i \pm 1.96\sqrt{\left(\left(H_{WSSE}/2\right)^{-1}\right)_{ii}}, \tag{4.4}$$

where $\hat{\theta}_i$ is the optimized value of the i^{th} parameter and $\left(\left(H_{WSSE}/2\right)^{-1}\right)_{ii}$ is the element in the i^{th} row and the i^{th} column of the covariance matrix $\left(H_{WSSE}/2\right)^{-1}$. The optimized values and confidence intervals of the parameter set are shown in **Supplementary Table 1**. When we used our model to

predict the neural spiking rates, we not only calculated the spiking rates with the maximum probability but also estimated the confidence interval of the predicted values (**Fig. 5; Extended Data Fig. 9**). The spiking rates with the maximum probability were calculated by inputting the optimized value of the parameter set into our model. To estimate the confidence interval of the simulated results, we sampled 10000 sets of parameters from the normal distribution with covariance matrix $\left(H_{WSSE}/2\right)^{-1}$ and ran the simulation with each of them. We used the 16^{th}-84^{th} percentiles of the distribution of the model-predicted values to determine the confidence intervals.

§5. Supplementary Table 1 | Definitions and symbols of all model parameters.

Category	Name	Symbol	Equation	Value	Lower bound	Upper bound
Anti-Hebbian amplitudes	Anti-Hebbian amplitude of KCs and MBONs	$A_{AH}\left(0\right)$	(3.27)	-1.78E+01	-2.60E+01	-9.63E+00
	Anti-Hebbian amplitude on PPL1-γ1pedc	$A_{AH}\left(\Delta t_{punish}\right)w_{punish,1}$	(3.27)	-1.46E+02	-1.96E+02	-1.09E+02
	Anti-Hebbian amplitude on PPL1-α'2α2	$A_{AH}\left(\Delta t_{punish}\right)w_{punish,2}$	(3.27)	0.00E+00	0.00E+00	0.00E+00
	Anti-Hebbian amplitude on PPL1-α3	$A_{AH}\left(\Delta t_{punish}\right)w_{punish,3}$	(3.27)	-5.46E+01	-7.32E+01	-4.07E+01
Synaptic weights	KC to PPL1-γ1pedc (Attractive odor)	$w_{KD,1,1}, w_{KD,2,1}$	(3.9), (3.27)	-1.40E+00	-2.83E+00	3.65E-02
	KC to PPL1-α'2α2 (Attractive odor)	$w_{KD,1,2}, w_{KD,2,2}$	(3.9), (3.27)	-5.62E-01	-2.04E+00	9.14E-01
	KC to PPL1-α3 (Attractive odor)	$w_{KD,1,3}, w_{KD,2,3}$	(3.9), (3.27)	2.67E+00	4.18E-01	4.93E+00
	KC to PPL1-γ1pedc (Repulsive odor)	$w_{KD,3,1}, w_{KD,4,1}$	(3.9), (3.27)	2.67E+00	5.83E-01	4.76E+00
	KC to PPL1-α'2α2 (Repulsive odor)	$w_{KD,3,2}, w_{KD,4,2}$	(3.9), (3.27)	6.59E+00	4.60E+00	8.57E+00
	KC to PPL1-α3 (Repulsive odor)	$w_{KD,3,3}, w_{KD,4,3}$	(3.9), (3.27)	1.18E+01	8.88E+00	1.47E+01
	Initial value of KC to MBON-γ1pedc>α/β	$w_{KM,initial,1}$	(3.19)	2.42E+01	2.10E+01	2.73E+01
	Initial value of KC to MBON-α'2α2	$w_{KM,initial,2}$	(3.19)	1.32E+01	5.20E+00	2.12E+01
	Initial value of KC to MBON-α3	$w_{KM,initial,3}$	(3.19)	1.62E+01	1.36E+01	1.89E+01
	MBON-γ1pedc>α/β to PPL1-γ1pedc	$w_{MD,1,1}$	(3.9), (3.27)	-3.56E-02	-1.70E-01	-7.44E-03
	MBON-γ1pedc>α/β to PPL1-α'2α2	$w_{MD,1,2}$	(3.9), (3.27)	-2.02E-01	-2.89E-01	-1.41E-01
	MBON-γ1pedc>α/β to PPL1-α3	$w_{MD,1,3}$	(3.9), (3.27)	-3.08E-01	-4.34E-01	-2.18E-01
	MBON-γ1pedc>α/β to MBON-α2sc	$w_{MM,1,2}$	(3.10)	-2.21E-01	-8.96E-01	-5.45E-02
	MBON-γ1pedc>α/β to MBON-α3	$w_{MM,1,3}$	(3.10)	-8.83E-17	-2.90E-01	0.00E+00
	MBON-α2sc to PPL1-α'2α2	$w_{MD,2,2}$	(3.9), (3.27)	1.24E-01	3.87E-02	3.99E-01
	MBON-α2sc to PPL1-α3	$w_{MD,2,3}$	(3.9), (3.27)	1.11E-18	0.00E+00	2.41E-01
	MBON-α3 to PPL1-α3	$w_{MD,3,3}$	(3.9), (3.27)	4.51E-21	0.00E+00	7.01E-02
Time-constants	τMBON-STM (KC to MBON-γ1pedc>α/β)	$\tau_{u,1}$	(3.27)	1.49E+03	1.08E+03	2.05E+03
	τMBON-STM (KC to MBON-α'2α2 and KC to MBON-α3)	$\tau_{u,2}, \tau_{u,3}$ ($t \leq 3$ hrs)	(3.27)	6.65E+03	5.48E+03	8.08E+03
	τMBON-LTM (KC to MBON-α'2α2 and KC to MBON-α3)	$\tau_{u,2}, \tau_{u,3}$ ($t > 3$ hrs)	(3.27)	3.53E+05	5.64E+04	2.20E+06
	τKC-adaptation	$\tau_{KC,recover}$	(3.12), (3.13)	6.47E+02	4.45E+02	9.40E+02

§6. References.

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