Dopamine-based mechanism for transient forgetting

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Active forgetting is an essential component of the memory management system of the brain². Forgetting can be permanent, in which prior memory is lost completely, or transient, in which memory exists in a temporary state of impaired retrieval. Temporary blocks on memory seem to be universal, and can disrupt an individual’s plans, social interactions and ability to make rapid, flexible and appropriate choices. However, the neurobiological mechanisms that cause transient forgetting are unknown. Here we identify a single dopamine neuron in Drosophila that mediates the memory suppression that results in transient forgetting. Artificially activating this neuron did not abolish the expression of long-term memory. Instead, it briefly suppressed memory retrieval, with the memory becoming accessible again over time. The dopamine neuron modulates memory retrieval by stimulating a unique dopamine receptor that is expressed in a restricted physical compartment of the axons of mushroom body neurons. This mechanism for transient forgetting is triggered by the presentation of interfering stimuli immediately before retrieval.

Memory formation, consolidation and retrieval are well-known functions that support memory expression; however, the processes that limit these functions—including forgetting—are less understood. Forgetting has been characterized as either passive or active, and is crucial for memory removal, flexibility and updating¹–³. Memory may be removed completely, resulting in permanent forgetting; or temporarily irretrievable, resulting in transient forgetting.

One form of active forgetting—known as intrinsic forgetting— involves one dopamine neuron (DAN) that innervates the γ2α′1 compartment of the axons of mushroom body neurons (MBNs) and the dendrites of the downstream, compartment-specific mushroom-body output neurons (MBONs)⁴–⁶. This DAN resides in a cluster of 12 DANs in each brain hemisphere that is known as the protocerebral posterior lateral 1 (PPL1) cluster⁴. Current evidence indicates that the ongoing activity of these DANs after aversive olfactory conditioning slowly and chronically erodes labile and nonconsolidated behavioural memory⁵, as well as a corresponding cellular memory trace that forms in the MBONs⁵. This intrinsic forgetting mechanism is shaped by external sensory stimulation and sleep or rest⁴, and is mediated by a signalling cascade in the MBNs that is initiated by the activation of the dopamine receptor DAMB, which leads to the downstream activation of the actin-binding protein Cofilin and the postulated reorganization of the synaptic cytoskeleton⁴–⁶.

By contrast, there is little understanding of the mechanisms that arbitrate transient forgetting. Neuropsychological studies of failures or delays in retrieval in humans have primarily focused on lexical access. Phonological blockers or interfering stimuli produce a tip-of-the-tongue state⁷— the failure to recall the appropriate word or phrase. Tip-of-the-tongue states are resolved when the distracting signals dissipate⁸. Several brain regions have been implicated in tip-of-the-tongue states from functional magnetic resonance imaging studies⁹, but the neurobiological mechanisms that produce a temporary state of impaired retrieval are unknown. Our study offers an entry point into this area of brain function.

External stimuli briefly block retrieval

Wild-type flies that were subjected to aversive olfactory conditioning (an odour coupled with an electric shock) using several, spaced training cycles displayed robust long-term memory (LTM) by 72 h after training (Fig. 1a, b). To determine how exposure to an interfering stimulus might affect expression of LTM, flies were briefly stimulated with airflow, electric shock or blue light before the memory retrieval test. Memory expression was weakened with increasing stimulus strength after experiencing these distractors (Extended Data Fig. 1a–c). These effects were observed only on cycloheximide-sensitive memory (Fig. 1b, c). As cycloheximide blocks protein-synthesis-dependent LTM (PSD-LTM), the difference in performance index between untreated and cycloheximide-treated flies represents the magnitude of PSD-LTM. The expression of PSD-LTM resurfaced at significant levels by 1 h after presentation of airflow, shock or blue light (Fig. 1b, c, Supplementary Information), which indicates that the memory impairments were temporary and produced by transient forgetting. Furthermore, flies presented with these interfering stimuli exhibited shock and odour avoidance that was indistinguishable from that of flies not presented with interfering stimuli, which indicates that the observed effects were not due to anomalous sensorimotor behaviour (Supplementary Information).

Transient forgetting through dopamine

Because DANs of the PPL1 cluster (PPL1 DANs) are involved in intrinsic forgetting, we asked whether they might also be involved in the processes that underlie transient forgetting. Pilot experiments

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demonstrated that strong, prolonged thermogenetic stimulation of all 12 PPL1 DANs per hemisphere (TH-D’>TrpA1 flies; for a full list of fly lines with their genotypes, see Supplementary Information) significantly reduced expression of PSD-LTM (Extended Data Fig. 2a–c) even 24 h before retrieval. We observed the opposite effect upon blocking synaptic output from PPL1DANs (TH-D’>Shibire), which suggested the existence of a memory reserve that remains hidden unless synaptic output from the DAN is suppressed (Extended Data Fig. 2c). We used these assays and a collection of split-gal4 fly lines to spatially restrict TrpA1 expression to subpopulations of the PPL1DANs (Extended Data Fig. 3). The phenotype observed from stimulating the entire cluster was recapitulated after manipulating only a single DAN (one per hemisphere; OS8B>split-gal4), whereas other PPL1-DAN subgroups had no significant effect (Extended Data Fig. 3). Notably, this single DAN (OS8B>TrpA1) innervates the α2α’2 compartment of the MBN axons, which is distinct from the compartment that is involved in intrinsic forgetting.

We considered the possibility that the long-lasting decrement (24 h) in performance due to extended stimulation of the PPL1 DAN that innervates the α2α’2 compartment (hereafter, PPL1-α2α’2) might be an extreme experimentally produced variant of short-lived transient forgetting caused by distractors. We therefore tested whether a milder stimulation presented immediately before retrieval mimicked the effects observed with external stimuli (Fig. 1). Indeed, PSD-LTM was temporarily suppressed by 5 min of TrpA1 stimulation 5 min before retrieval (Fig. 2a, left), and PSD-LTM was temporarily enhanced by briefly blocking synaptic output (OS8B>Shibire) (Fig. 2a, right). Memory performance spontaneously recovered within 1 h of a brief bout of TrpA1 stimulation (Extended Data Fig. 4a). Thus, the behavioral effects observed with brief artificial stimulation of PPL1-α2α’2 mimic the transient forgetting that is produced by presenting interfering stimuli immediately before retrieval, consistent with the model that PPL1-α2α’2 stimulation mediates these effects.

As a 6-h stimulation inhibited memory expression 24 h later, we wondered whether this stimulation—or more extreme variants—could convert transient forgetting to a permanent loss of memory. To test this, we delivered several epochs of TrpA1 stimulation. Notably, the deficit in expression of PSD-LTM was prolonged to more than one week after several spaced TrpA1-stimulation cycles (Extended Data Fig. 4b–d), but returned to normal levels by day 14.

**Transient forgetting recruits DAMB**

The disruption of memory retrieval induced by PPL1-α2α’2 predicts the existence of a dopamine receptor expressed in the mushroom body, to propagate the transient forgetting signal. One candidate is DAMB, a Gα12-coupled receptor12,13 that transduces the DAN γ2α’1–mushroom body signal that is involved in intrinsic forgetting5,6. Thus, we hypothesized that DAMB is the major receptor that mediates DAN-induced transient forgetting.

The expression of PSD-LTM in flies that are mutant for DAMB was markedly elevated at all time points tested up to day 14 (Extended Data Fig. 5a). To identify the key neural site that is responsible for increased PSD-LTM, we drove DAMB (also known as DopIR2) RNA interference (RNAi) expression pan-neuronally and in various types of neurons that are implicated in olfactory memory14 (Extended Data Fig. 5b, c). Because PPL1-α2α’2 forms synaptic contact with the α2 and α’2 compartments of MBNs, we predicted that we would see differences in performance for the gal4 lines that drive RNAi expression in either of the two subtypes of MBN. The enhanced-memory phenotype was recapitulated only when using gal4 lines with restricted expression in the α/β MBNs, and not with the α’/β’ MBN lines (Extended Data Fig. 5c, d). We also reinstated DAMB expression only in α/β MBNs, which led to a full rescue of the phenotype (Extended Data Fig. 5e)—consistent with studies that have implicated the α/β MBNs for storage of PSD-LTM15,16. These findings are consistent with the model that transient forgetting is mediated by the DAMB receptor located in the α2α’2 compartment of α/β MBN axons.

We next determined whether DAMB function is epistatic (downstream) to PPL1-α2α’2 stimulation using an intersectional genetics approach. We used the lexA–lexAop binary system to stimulate PPL1-α2α’2 and the gal4–uas binary system to knock down DAMB in...
the α/β/γ MBNs (Fig. 2b). The Gal4 derivative GeneSwitch allows for spatiotemporal control of gene expression, with its activity regulated by the presence or absence of the progesterone analogue mifepristone (RU486)15. Without RU486, all groups displayed comparable performance; as expected, PPL1-α2α’2 stimulation diminished LTM (Fig. 2b). However, upon RU486 treatment after training (to express DAMB<sub>RNAi</sub>) the flies with reduced DAMB showed increased performance compared to control flies, whereas flies with reduced dDA1 did not. Moreover, the effects of PPL1-α2α’2 stimulation were blocked in the DAMB<sub>RNAi</sub>-expressing flies, consistent with the conclusion that DAMB is epistatic to PPL1-α2α’2 stimulation for PSD-LTM. Memory performance of dDA1<sub>RNAi</sub> flies paralleled the control flies, which suggests that dDA1 has no role in this behavioural plasticity. These experiments identify DAMB as the dopamine receptor that transduces the forgetting signal in the α2 MBN to inhibit the retrieval of PSD-LTM.

**Fig. 2** | Transient suppression of memory engages a single pair of PPL1 DANs and the dopamine receptor DAMB. a, Schematic depicting the right hemisphere of a fly brain. One DAN (dark grey circle) in the PPL1 cluster synapses onto the MBN α2α’2 compartment (dark grey). Brief stimulation of PPL1-α2α’2 (058B>TrpA1) immediately before retrieval transiently suppressed, whereas blocking synaptic output (058B>Shibire) temporarily enhanced, expression of PSD-LTM. b, Conditioned flies were fed with RU486 to induce RNAi expression in the MBN. Knocking down DAMB, but not dDA1, blocked the PPL1-α2α’2-induced suppression of memory. PPL1-α2α’2<sub>lexA</sub>, MB-GeneSwitch>uas-RNAi, lexAop-TrpA1 flies. Box-and-whisker plots show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 12, two-way ANOVA with Tukey’s test. Exact P values and comparisons are shown in Supplementary Information.

**Persistence of PSD-LTM cellular trace**

Olfactory memory is established through the generation of cellular memory traces, defined as the biochemical and cellular changes that are instilled by memory formation14,18. Cellular memory traces such as these have often been detected by the functional imaging of transgenic supplied neurons such as GaMP, with memory traces reported as increased19,20 or decreased21 responses to the positive conditioned stimulus (CS; odour combined with an electric shock), or as a combination of an increased response to the CS’ and a decreased response to the negative conditioned stimulus (CS; odour without an electric shock)22. The latter memory traces are best represented as the CS’/CS’ differential response. Because activation of PPL1-α2α’2 causes transient forgetting, we hypothesized that cellular memory traces downstream of this signal would persist, with behavioural forgetting being due to a block on retrieval rather than a transient suppression of the cellular memory trace.

We focused on the neuron MBON-α2sc because the synaptic output of the α2α’2 compartment of the mushroom body is funnelled to the dendrites of this neuron24. We first confirmed the importance of MBON-α2sc for retrieval of PSD-LTM by blocking synaptic release before and during retrieval. This block reduced expression of PSD-LTM (Extended Data Fig. 6a). Thus, we chose MBON-α2sc to search for a 72-h cellular trace and then to assay its persistence in response to transient forgetting.

Flies that express GCaMP6m in MBON-α2sc were subjected to spaced training and the MBON-α2sc dendrites were imaged 72 h later (Extended Data Fig. 6b, c). For each trained fly, the responses to the conditioned odours (octanol (OCT) and benzaldehyde (BEN)) were measured: the naive odour responses served as controls. In naive flies, responses to the two odours were relatively similar and showed no detectable differential response. However, there was a significantly increased enhancement in the GCaMP6m differential fluorescence signal in conditioned versus naive flies (Extended Data Fig. 6d–f), which was more robust when OCT was the CS’ than when BEN was the CS’. To confirm that this trace is dependent on protein synthesis, flies were fed with cycloheximide before conditioning. Notably, the training-induced increase in the CS’/CS’ differential response was blunted for the cycloheximide-treated flies (Extended Data Fig. 6g–i), thus identifying a previously unknown PSD-LTM cellular trace at 72 h after conditioning.

We focused on OCT training (OCT as CS’ and BEN as CS”) for the subsequent analysis as it produced the strongest responses. Most importantly, ectopic activation of PPL1-α2α’2 did not suppress the PSD-LTM trace (Fig. 3a–c, Extended Data Fig. 6j). There were no obvious changes in GCaMP6m differential signal between genotypes and across temperature conditions after a 6-h TrpA1 stimulation of PPL1-α2α’2 (Fig. 3b). Furthermore, the calcium traces in MBON-α2sc were not affected immediately after a shorter bout (5 min) of TrpA1 stimulation (Fig. 3c). Thus, our results support the first hypothesis: activation of the transient forgetting signal blocks retrieval, rather than temporarily suppressing a PSD-LTM cellular trace (see ‘Discussion’ for further details).
Stimuli suppress memory through PPL1-α2α’2 and DAMB

We hypothesized that the transient forgetting caused by distracting stimuli before retrieval occurred via the PPL1-α2α’2–DAMB pathway. To test this possibility, we first blocked synaptic output from PPL1-α2α’2 while simultaneously delivering the external stimuli. As inhibiting PPL1-α2α’2 before a memory retrieval test enhanced PSD-LTM (Fig. 2a) (which may be represented by cellular memory traces of greater strength or number), we undertook the 058B+Shibire flies using only three cycles of training so that their LTM performance was similar to the control flies (Fig. 4a). This enabled us to balance the strength of the behavioural response across all groups so that we could then subject them to interfering stimuli of constant strength—obtaining a balanced measure of PSD-LTM performance with and without the blockade of PPL1-α2α’2 activity. The 058B+Shibire flies with the presynaptic block and exposed to airflow or blue light exhibited significantly increased performance relative to control flies exposed to the same external stimuli, and performance that was indistinguishable from control flies that were not exposed to the external stimuli (Fig. 4a). This result indicates that blocking the single pair of DANs repressed the transient forgetting effects of the stimuli.

We further tested the hypothesis by obstructing the PPL1-α2α’2–DAMB pathway postsynaptically by expressing $DAMB^{RNAi}$ in the α/β MBNs (Fig. 4b). Because $DAMB^{RNAi}$ expression in the α/β MBNs enhanced PSD-LTM (Fig. 2b), we also undertook the $DAMB^{RNAi}$-expressing flies; this yielded PSD-LTM performances comparable to those of the control flies (Extended Data Fig. 5c). The $DAMB^{RNAi}$-expressing flies exposed to airflow, shock or blue light displayed significantly enhanced performance relative to control flies exposed to the same three external stimuli (Fig. 4b), and performance indistinguishable from control flies.
that were not exposed to the stimuli. The data obtained from this post-synaptic insult parallels precisely our observations from disrupting the proposed neural circuit at the presynaptic level. Taken together, these findings confirm that the interfering stimuli trigger the PPL1-α2γ2-ΔMB function normally to suppress expression of PSD-L TM. However, this insult attenuates PSD-L TM expression (Extended Data Fig. 6a), the simplest hypothesis posits that cellular memory traces form with conditioning in the MBN in addition to the cytoplasmic Ca2+-based memory trace that we detect here. This is expected: neurons undergo broad changes in physiology as they adopt new states, so it is plausible that such plastic mechanisms—especially ones that gate synaptic release—are inactivated by DAN activity while leaving the Ca2+-based memory trace intact.

The discovery that loss of function of DAMB leads to enhanced PSD-L TM was surprising, because of a previous study reporting that this insult attenuates PSD-L TM. Our experiments argue strongly that DAN functions normally to suppress expression of PSD-L TM. However, this leads to the question of why a receptor involved in transient forgetting would lead to enhanced PSD-L TM when inactivated. Previous experiments have shown that PPL1-α2γ2-Δ similar to PPL1-γ2α′1 exhibits ongoing activity, leading to a slow release of dopamine onto MBNs. This activity should slowly degrade or suppress existing memory so that when the receptor is inactivated memory expression is enhanced. PPL1-α2γ2-Δ has no important role in the forgetting of labile nonconsolidated memory. Instead, previous studies have identified a different DN (PPL1-γ2α′1) as having a role in this process and the apparent erasure of the downstream cellular memory trace—perhaps an indication of ‘permanent forgetting’. This process is modulated by internal and external factors, and is mediated by key molecules expressed in the MBN that receive PPL1-γ2α′1 input (Extended Data Fig. 7). We found a robust decrement in expression of PSD-L TM after PPL1-γ2α′1 stimulation (Extended Data Fig. 2), which points to the existence of two separate dopamine-based circuits for permanent and transient forgetting. This functional separation may indicate a fundamental principle in the organization of circuits that mediate several forms of forgetting.

However, the DAMB receptor is used for both permanent and transient forgetting. DAMB is widely expressed across the MBN axons but alters synaptic plasticity differently across MBN compartments. It is possible that DAMB signalling may be distinct for the two forms of forgetting. DAMB preferentially couples with Gαq, the knockdown of which inhibits the potent erasure of memory, but its potential role in transient forgetting is unknown. The scaffolding protein Scribble orchestrates the activities of Rac1, Pak3 and Cofilin, all of which are important for the permanent forgetting pathway (Extended Data Fig. 7). However, Scribble knockdown or inhibition of Rac1 does not enhance the PSD-L TM as is the case in DAMB-knockdown flies, which suggests that this scaffolding signalsome does not have a large role in transient forgetting. In summary, the two distinct forms of forgetting—transient and permanent—share a dopaminergic mechanism and a common dopamine receptor, but differ in upstream and downstream neural circuits and in downstream signalling pathways within MBNs.

Online content
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Methods

No statistical methods were used to predetermine sample size. The experiments were randomized with appropriate controls present in each independent experiment. All experiments were self-blinded to the experimenter. For example, when six genotypes or conditions were tested, each genotype or condition was assigned a number (1 through 6) prior to spaced training. This method was maintained throughout testing and initial data analysis. For each independent experiment, the assigned code to the genotype or condition was different from the previous set. After reaching a sample size of 8 or 12, all groups were self-decoded. All data analysed were consistently reproducible. More details regarding research design are in Reporting Summary.

Drosophila husbandry

Fly stocks were raised on regular Drosophila medium at room temperature. Experimental fly crosses were kept at 23 °C unless otherwise stated, and 70% relative humidity on a 12-h light:12-h dark cycle. A full list of fly strains is given in the Supplementary Information. Unless specifically noted in the figures, legends or text, the fly lines used were from the gal4>uas binary system collection.

Behavioural experiments

The 1–4-d-old flies, of mixed sex, were used for standard olfactory conditioning experiments as previously described30,31. Blotting filter paper was cut to fit the bottom of an empty vial, and 450 μl of vehicle (5% glucose, 3% ethanol solution) or 35 mM cycloheximide dissolved in vehicle were pipetted onto the paper; flies were kept on the vehicle or cycloheximide for 18 h overnight. The flies were transferred into regular food vials 1 h before conditioning, and were acclimated under dim red light, in an environmentally controlled chamber set at 23 °C and 70% humidity. Odour-laced air was produced by bubbling fresh air through odorant dissolved in mineral oil. Groups of about 65 flies were gently tapped into a training tube, where they received 30 s of fresh air, 1 min of odour A paired with electric shock (12 shocks at 90 V lasting 1.25 s each, at 5-s intervals) (CS−), 30 s of fresh air, 1 min of odour B without electric shock (CS+) and 30 s of fresh air. This constituted one cycle. The trained flies were then transferred back onto their respective food vials for a 15-min rest interval, and this protocol was repeated for a total of 5 spaced cycles. After spaced training, flies were housed in a covered box inside a 23 °C incubator at 70% humidity. For memory tests beyond 24 h, flies were flipped into fresh food vials every 2 d. After the specified time (24 h to 14 d), flies were loaded into a T maze and given 1 min to acclimate, and subsequently allowed to choose between arms containing either the CS− or CS+ odour for 2 min. The number of flies were counted and used to calculate a performance index ([CS− – (CS+)]/[CS− + (CS+)]). The final performance indices shown in the figures denotes the averaged performance indices between the two CS+ odours (half performance indices). Odours were counterbalanced and included BEN (0.05–0.10%) and OCT (0.15%) diluted in mineral oil. The concentrations were slightly adjusted according to experimental condition, memory time point assayed and genotype, so that flies displayed comparable half performance indices when tested in the T maze.

The naïve avoidance of the flies to the odours and electric shock were assessed by allowing flies to choose between the odour or fresh air (odour avoidance) or between the copper-grid arms with or without electric shock (shock avoidance) in the T maze for 2 min. Exposing wild-type flies to blue light for 20 min, or manipulating PPL1-α2α'2 activity before a memory retrieval test, did not affect the innate capacity of flies to avoid BEN, OCT or electric shock. The full list of behavioural control experiments with detailed experimental conditions are given in the Supplementary Information.

In the external-stimulus presentation experiments for Figs. 1, 4, spaced-trained flies were transferred into fresh food vials for 30 min and the following protocols were used. For airflow, flies were tapped into clean and empty cylindrical tubes. Pressurized filtered airflow was manually passed through the tubes, spanning 10 cycles of 10-s airflow with 5-s interstimulus rest intervals. Flow meters were used monitor the airflow delivery. Flies were allowed to rest for 1 min before being transferred to the T maze for a memory retrieval test; this timing was also adopted for the electric shock and blue light experiments. For electric shock, conditioned flies were briefly exposed to mild electric shock using clean and fresh training tubes. For blue light, a 34 × 13 × 19-cm box was lined with reflective mirror boards and a computer fan for internal cooling42. Two rows of three 448-nm emitting LED modules (Luxeon SP-01-V4) were attached to the top inside the box, with a projected light intensity of 0.15 mW mm−2. Flies were housed in this box for the time indicated in the figures. Exposure to the blue light for 20 min did not compromise the capacity of the flies to form odour-shock memory (Supplementary Information).

For the temperature-shift experiments involving TrpA1 stimulation or Shibire blockade, flies were tapped into fresh food vials before placing them inside an environmentally controlled chamber at 30 °C and 70% humidity for the length of time indicated in the figures. Subsequent memory tests were performed in a separate chamber at 23 °C and 70% humidity, after some rest (as denoted in the figures). Experiments using longer heat treatments (such as those in Extended Data Figs. 2, 3) required use of an incubator set at 30 °C with 70% humidity.

For the intersectional genetics experiments using MB-GeneSwitch in Fig. 2d, fly food vials were freshly prepared containing a final concentration of 500 μM RU486 dissolved in 1% ethanol solution (+RU486) or food vials with the ethanol alone (−RU486). The drug treatment allowed us to pharmacogenetically induce uas-dDA1RNAi or uas-DAMBRNAi expression. Spaced-trained flies were reared on RU486 food 24 h after conditioning, until 1 h before the retrieval test measuring 72-h memory. Stimulation of PPL1-α2α'2 (R82C10-lexA) was achieved by using the lexAA>lexAop binary system, which enabled us to confirm the memory-suppression phenotype with an independent fly line with restricted expression in the single DAn. The lexAop-TrpA1 stimulation was performed as described with TrpA1 (uas-line version).

For the experiments done in Fig. 4, Extended Data Fig. 5c, we used the TARGET5 system. Fly crosses were reared in an incubator set at 18 °C and in 70% humidity. Flies were then trained in an 18-°C, 70% humidity chamber to minimize leaky RNAi expression. To thermogenetically induce uas-DAMBRNAi expression in the α/β MBN (c739;gal80ts), flies were shifted to a 30-°C, 70% humidity incubator 24 h after spaced training until the time of testing.

In vivo GCaMP imaging

The fly mounting preparation, cuticle dissection, haemolymph saline solution (124 mM NaCl, 3 mM KCl, 20 mM MOPS, 1.5 mM CaCl2, 4 mM MgCl2·6H2O, 5 mM NaHCO3, 1 mM NaH2PO4·H2O, 10 mM trehalose, 7 mM sucrose and 10 mM glucose, pH 7.2) and microscope conditions for in vivo calcium imaging were adopted from a previously published protocol27, with slight modifications. To determine whether the LTM cellular trace in MBON-α2sc was dependent on protein synthesis, female R34B02-lexA>lexAop-GCaMP6m flies were first fed with either 35 mM cycloheximide solution mixed with 1% blue dye, or vehicle alone with the dye. After 18 h, the flies with the most robust blue-coloured abdomen were aspirated (without CO2) and spaced-trained as described in ‘Behavioural experiments’.

A Leica TCSSPS II confocal microscope with single-photon excitation was used for GCaMP6m fluorescence, incorporating a 488-nm argon laser at a resolution of 512 × 512 pixels using a 20× objective (HCX APOL 20.0/0.70NA, Leica). Emitted light was collected using a PMT (S10–550 nm) for GCaMP emission at a frame rate of 2 Hz with the pinhole fully open. To deliver odours to flies under the microscope, a small stream of air (100 ml min−1) was diverted (via solenoids) from flowing through a clean 20-ml glass vial to flow through a 20-ml glass vial containing 1 ml of a 1:1,000 odour: mineral oil solution. The air stream was then
diluted into a faster air stream (1,000 ml min⁻¹) before travelling 95 cm through Teflon tubing (about 2.5-mm diameter) to reach the fly mounted beneath the microscope objective. Flies were exposed to 5 s of one odour, then 45 s of fresh air and then 5 s of a second odour with half of the flies for a given group receiving OCT then BEN, and the other half BEN then OCT. The final responses for a particular odour were averaged across these two odour sequences.

MBON-α2sc GCaMP activity was quantified using ImageJ and MATLAB. A region of interest was first drawn around the MBON-α2sc dendrites, which wrapped around the compartment of the α2 mushroom body axons, as depicted in Extended Data Fig. 6c, d. The MBON-α2sc neural ‘activity’ was calculated across time (t), by normalizing the GCaMP fluorescence (F) signal to the mean signal (F₀) across the 5 s time window before the start of an odour pulse as follows: activity (%ΔF/F₀) (t) = 100 × ((F(t) – F₀)/F₀). The line graphs represent the MBON-α2sc activity across 20 s (~5 s to 15 s of odour onset) and the bar graphs denote the mean MBON-α2sc response within the first 5 s of odour onset. The calculated differential reflects the change between CS+ and CS− odour responses for each fly. Representative images of the odour onsets for the naive, BEN+- or OCT+-treated flies are shown both in window before the start of an odour pulse as follows: activity (%ΔF/F₀) range.

For the PPL1-α2α'+2 stimulation (R82C10-gal4) experiments in Fig. 3b, Extended Data Fig. 6j, the flies were shifted to an incubator set at 30 °C with 70% humidity for 6 h (from hour 42 to hour 48), mimicking the environmental experiments for which we observed strong suppression of behavioural memory. The flies were flipped into fresh food vials and remained in an incubator at 23 °C with 70% humidity until they were imaged for changes in the cellular memory trace detected 72 h after conditioning. For the shorter TrpA1 stimulation experiments in Fig. 3c, we first recorded the odour responses (pre), which represents the 72-h cellular memory trace without PPL1-α2α'+2 stimulation. To activate PPL1-α2α'+2 acutely, we then perfused saline at 30 °C for 5 min, allowed for a 5-min rest period before recording the odour responses (post), thus mimicking our short-stimulation behavioural paradigm. The post response represents the cellular memory trace after the PPL1-α2α'+2 stimulation.

Statistics and reproducibility
All data were compiled and analysed using Excel version 16.43 (20110804) and GraphPad Prism 7, respectively. Different groups of flies, along with appropriate controls per experimental condition, were distributed evenly across each set of experiments. Each experiment was performed three to four independent times. All replication attempts were successful. The graphs displayed in all figures are box-and-whisker plots that show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. The sample sizes per group, P values, and statistical tests used for each experiment are detailed in the Supplementary Information.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability
All relevant data are available from the corresponding author upon request.

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Author contributions
J.M.S. planned and performed all behavioural experiments, data analysis and interpretation, and figure design, and wrote the initial draft of the manuscript. J.A.B. helped to plan experiments, performed all in vivo imaging and contributed to data interpretation and manuscript writing. R.L.D. acquired funding, helped with planning experiments, supervised the overall execution of the project, provided feedback on data interpretation and edited the manuscript along with J.M.S. and J.A.B.

Competing interests
The authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to R.L.D.
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Extended Data Fig. 1 | Dose-dependent suppression of LTM.

a–c, Conditioned wild-type (Canton S) flies were exposed to distracting stimuli of increasing potency: airflow (a), electric shock (b) or blue light (c), terminating 1 min before a 72 h memory retrieval test. Box-and-whisker plots show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 8 (a–c), one-way ANOVA with Dunnett’s test. Exact P values and comparisons are shown in Supplementary Information.
Extended Data Fig. 2 | PPL1 DAN bidirectionally modulate PSD-LTM expressed at 72 h. 

**a.** Schematic illustrating the PPL1 DAN cluster (TH-D′-gal4) that innervates five subcompartments of the mushroom body neuropil.

**b,c.** Seventy-two-hour PSD-LTM without (b) or with (c) a manipulation of PPL1 DAN activity. Stimulation (TH-D′>TrpA1) decreased, whereas synaptic blockade (TH-D′>Shibire) enhanced, the expression of PSD-LTM. Box-and-whisker plots show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 9 (b, c), two-way ANOVA with Tukey’s test.

Exact P values and comparisons are shown in Supplementary Information.
Extended Data Fig. 3 | Mapping the phenotype of impaired expression of PSD-LTM to a single DAN. PPL1 DAN screen using split-gal4 lines and uas-TrpA1 to stimulate discrete DAN subpopulations. Stimulating PPL1-α2α′2 significantly decreased the expression of PSD-LTM when tested at 72 h. Other neurons from the PPL1 cluster did not impair expression of PSD-LTM. Box-and-whisker plots show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 12, two-way ANOVA with Tukey’s test. Exact P values and comparisons are shown in Supplementary Information.
Extended Data Fig. 4 | Multiple epochs of TrpA1 stimulation extend the suppression of memory expression. a, Memory rapidly recovered after a brief bout of TrpA1 stimulation. b, Retention of PSD-LTM across 14 d, after spaced conditioning without PPL-α2α′2 stimulation. c, Expression of PSD-LTM was significantly dampened at 3 d after a single 6-h bout of TrpA1 stimulation, but resurfaced at 6 d. d, Three 6-h-spaced TrpA1 stimulations prolonged the memory expression deficit to 10 d, but expression of PSD-LTM resurfaced at normal levels at 14 d. Box-and-whisker plots show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 8 (a), one-way ANOVA with Dunnett’s post hoc test; n = 6 (b), n = 12 (c, d), two-way ANOVA with Tukey’s test. Exact P values and comparisons are shown in Supplementary Information.
Extended Data Fig. 5 | Enhanced PSD-LTM of DAMB maps to α/β MBN. a, Loss of DAMB (loss-of-function allele) elevated expression of PSD-LTM up to 14 d. Wild type, Canton-S. b, Pan-neuronal knockdown of DAMB increased 24-h PSD-LTM. The RNAi lines target nonoverlapping sites of DAMB that affect all transcript variants. Coding exons, green; noncoding exons, blue; introns, black line. nSyb-gal4>uas-RNAi, uas-dicer2. RNAi no. 1: KK line. RNAi no. 2: GD line. RNAi no. 3: TRiP line. c, DAMB knockdown in α/β MBN enhanced 24-h LTM. Gal4>uas-RNAi(KK), uas-dicer2. d, DAMB knockdown in the α/β MBN elevated PSD-LTM up to 14 d after spaced conditioning. C739-gal4>uas-RNAi(KK), uas-dicer2. e, Functional reinstatement of DAMB restored PSD-LTM to normal levels. Wild type, Canton-S. f, DAMB RNAi knockdown and normalizing expression of PSD-LTM by differential spaced conditioning. C739-gal4, gal80ts>uas-RNAi, uas-dicer2. Box-and-whisker plots show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 9 (c), unpaired two-tailed Welch’s t-test; n = 12 (a, b, d–f), two-way ANOVA with Tukey’s test. Exact P-values and comparisons are shown in Supplementary Information.
Extended Data Fig. 6 | Robust 72-h-old PSD-LTM plasticity is formed in MBON-α2sc. a, Blocking synaptic output (R34B02-lexA>lexAop-Shibire) from MBON-α2sc impaired retrieval of PSD-LTM. b, Schedule for training and imaging. c, MBON-α2sc dendrites imaged as the region of interest. d, Representative pseudocoloured images (scale bar, 10 μm) showing responses to octanol (OCT) or benzaldehyde (BEN) for naive, OCT+ (OCT as CS+), or BEN+ (BEN as CS+) flies. e, f, Response traces of group data and quantification for OCT+ (e) and BEN+ (f) conditioned flies relative to naive. Left, activity as a function of time with odour stimulation. Right, average response magnitude within the first 5 s of odor onset (duration of odor delivery). Calculations of the activity versus mean responses are provided in the Methods. The differential reflects the difference in odour response between CS+ and CS−. Multiple spaced cycles generated increased calcium transients compared to naive flies, with OCT+ generating a more-potent differential than that of BEN+. g, Feeding schedule for cycloheximide before spaced conditioning and representative pseudocoloured images (scale bar, 10 μm) showing the effects of cycloheximide on odour responses. h, i, Cycloheximide blunted the OCT+ (h) or BEN+ (i) training-induced calcium transients, indicating that the differential represents a PSD-LTM trace. j, Training schedule and representative pseudocoloured images (scale bar, 10 μm) without (23 °C) or with (30 °C) a 6-h TrpA1 stimulation. Response traces and quantification are in Fig. 3b. Box-and-whisker plots show the range of individual data points, with the interquartile spread as the box and the median as the line bisecting each box. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 8 (a), two-way ANOVA with Tukey’s test; n = 20 (e, naive), n = 21 (e, OCT+), n = 20 (f, naive), n = 20 (e, BEN+). n = 20 (h, i), unpaired two-tailed Mann-Whitney test (e, f, h, i). Imaging experiments (d–j) were performed three independent times with proper controls present within each set. All activity traces, mean responses and representative images shown were reproducible. Exact P values and comparisons are shown in Supplementary Information.
Extended Data Fig. 7 | Working model comparing permanent and transient forgetting. Two forms of forgetting include permanent (red) and transient (orange) forgetting. Left, Permanent forgetting involves a PPL1 DAN that synapses onto the MBN-γ2α′1 compartment (red). The slow ongoing DAN activity after learning is transduced by the Gq-coupled DAMB receptor. This forgetting signal mobilizes the Scribble scaffolding complex and recruits Rac1, Pak3 and Cofilin to erode labile nonconsolidated memory. The cellular memory traces formed and stored in the following neuron (MBON-γ2α′1) are also eroded. This process can be exacerbated by enhanced sensory stimulation (+) or repressed by sleep or rest (−). Right, Transient forgetting incorporates a different PPL1 DAN (to that in permanent forgetting) that synapses onto the MBN-α2α′2 compartment (orange). This forgetting signal, transduced by DAMB, temporarily impairs the expression of consolidated PSD-LTM. The cellular memory traces stored in MBON-α2sc are not abolished after activating the forgetting pathway. This process can be triggered by interfering or distracting stimuli (+) to transiently block the retrieval of PSD-LTM.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: n/a

Data analysis: ImageJ and MATLAB 2010b were used to analyse raw in vivo imaging files obtained from the confocal microscope. GraphPad Prism 7 was used to analyse all behavioural and imaging data. Detailed statistical analyses and comparisons are described in Extended Data Table.

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Sample size
For behavioural experiments, sample size is between 8 and 12 which is consistent as others in the field. For in vivo imaging experiments, sample size was between 20 and 30. This sample size is sufficient to reach significance with the non-parametric tests we use to analyse the in vivo imaging data. The sample size per experiment is detailed in Extended Data Table.

Data exclusions
No data was excluded.

Replication
All experiments were performed across multiple days - biological replicates. All attempts at replication were successful.

Randomization
All control and experimental lines were present and randomly organised for each independent experiment.

Blinding
All experiments were self-blinded to the experimenter. For example, when six genotypes/conditions were tested, each genotype/condition was assigned a number (i.e. 1 through 6) prior to spaced olfactory training; this method was maintained throughout testing and initial data analysis. For each independent experiment, the assigned code to the genotype/condition was different from the previous set. After reaching a sample size of 8 or 12, all groups were self-decoded. All data analysed were consistently reproducible.

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☐ ChiP-seq
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Animals and other organisms

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Laboratory animals

Drosophila melanogaster, both sexes were used for behavioural experiments, while females were used for in vivo imaging experiments. The fly lines listed below are described in Extended Data Table.

Canton-S
wCS10 (+)
TrpA1 (2nd chrom, uas)
Shibire (uas)
TH-D’ (gal4)
320C (split gal4)
296B (split gal4)
058B (split gal4)
630B (split gal4)
304B (split gal4)
nSyb (gal4)
TH (gal4)
GH146 (gal4)
OK107 (gal4)
R13F02 (gal4)
MB247 (gal4)
1471 (gal4)
R11D09 (gal4)
c739 (gal4)
R28H05 (gal4)
c305a (gal4)
R35B12 (gal4)
dicer2 (uas)
controlRNAi#1 (uas) - KK
controlRNAi#2 (uas) - GD
### Wild animals
The study did not involve wild animals.

### Field-collected samples
The study did not involve samples collected from the field.

### Ethics oversight
Ethics oversight is not required for Drosophila investigations.

Note that full information on the approval of the study protocol must also be provided in the manuscript.