nature research

Peer Review File

Manuscript Title: Dopamine-based mechanism for transient forgetting

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

In the manuscript entitled “Dopamine-based mechanism for transient forgetting”, the authors first showed that several external stimuli are capable of transiently disrupting retrieval of long-term memory. In the following experiments, the authors identified a single pair of dopamine neurons to mediate this transient forgetting bidirectionally. It is a novel and exciting finding that deserves to be published in Nature, with the following concerns addressed.

Major concerns:
1). As a major conclusion, authors claim that a single pair of DAns, PPL1-α2α’2, mediates transient forgetting induced by external stimuli including airflow, electric shock or blue light. It would be critical to show that synaptic blockade or inhibition of PPL1-α2α’2 activity leads to suppression of transient forgetting induced by external stimuli examined.

2). In Fig. 5, the authors show that 72-h memory trace represented in MBOn-α2sc neuron was not disrupted by a 6-h TrpA1 stimulation of PPL1 DAn-α2α’2 24 h before retrieval. Based on this observation, the authors conclude that “transient forgetting blocks retrieval rather than temporarily suppressing a PSD-LTM cellular trace”. It would be more comfortable if authors could show that a 5-min TrpA1 stimulation does not affect the calcium traces recorded in MBOn-α2sc immediately after stimulation, instead of waiting for a long period after the 6-h stimulation of PPL1-α2α’2.

3). In Fig.2, the manipulation is within a single pair of PPL1 DAns. Therefore, it might be more accurate to conclude that “Activity of a single (pair of) PPL1 DAn suppresses or enhances retrieval of PSD-LTM.

Minor concerns:
1) In lines 49-51, the sentence is lack of the supporting reference.
2) In line 59, reference 8 is missing.
3) According to the Extended Fig.2, the authors reported that except PPL1-α2α’2 DAn, other PPL1 DAn subgroups can not yield a similar decrement in PSD-LTM expression as the whole PPL1 DAn cluster after ectopic stimulation. However, it seems that no PSD-LTM was formed before ectopic stimulation of PPL1-γ1ped and PPL1-γ2α’1, even in the control flies. Therefore, it is inappropriate to make such a conclusion.

It might be better to remove this figure.
4) In line 139, “knockdown” should be “knock down”.
5) Expression patterns for used Gal4 lines are required in extended data.

Referee #2 (Remarks to the Author):

Forgetting is the important but understudied flip side of learning and memory. Far less is known at a molecular and circuit level about how memories are lost than about how they are formed. The Davis group has pioneered studies of forgetting in Drosophila, and the present study continues this welcome effort.

The focus is on what the authors call "transient forgetting." They show that dopaminergic input to the α2 and α'2 mushroom body compartments, which is mediated by the neuron PPL1-α2α'2, causes a transient block in memory retrieval. Blocking the mushroom body output neuron MBON-α2sc, which has its dendrites in the α2 compartment and shows a memory trace after conditioning, recapitulates the memory retrieval block.

Although I am sympathetic to the line of research as a whole, I have two serious reservations about the present paper.

First, the question of novelty. A previous study (Krashes et al., Cell 2009), which the authors do not acknowledge, showed that dopaminergic input to the γ1 mushroom body compartment transiently suppresses long-term appetitive memory performance in satiated flies. This hunger-dependent gating of memory retrieval by PPL1-γ1pedc (also known as MB-MP1) is conceptually difficult if not impossible to separate from the kind of transient forgetting the authors study. The earlier paper therefore represents a fatal infringement of novelty.

Second, the question of mechanism. The authors show that 6 h stimulation of PPL1-α2α'2 inhibits memory expression even 24 h later (ED Fig. 3), that memory performance depends on MBON-α2sc (ED Fig. 6), that the odor responses of MBON-α2sc are altered after conditioning ("memory trace", Fig. 4) and yet, when they stimulate PPL1-α2α'2 under conditions that cause transient forgetting, nothing happens to the odor responses of MBON-α2sc (Fig. 5)! The locus and mechanism of transient forgetting thus remain to be discovered, contradicting the claim of the title.

Because these two major reservations preclude a serious consideration of the paper in my view, I will not list in detail the more minor shortcomings, of which there are also several.

Referee #3 (Remarks to the Author):

In this manuscript Davis and colleagues investigated circuit and molecular mechanisms underlying interference-based forgetting. They employed an interfering stimuli-triggered transient forgetting task, a Drosophila model of a retroactive interference in which new experience or learning interferes with the retrieval of previously acquired memory, although they did not explicitly mention this. Interference-based forgetting, such as forgetting for a moment and tip-of-the-tongue state, often occurs in our daily life and there have been many psychological studies thus far. However, underlying mechanisms, either in neural circuit, cellular, or molecular level, are totally unknown. The authors took full advantage of Drosophila molecular genetics and identified a unique neural circuit that mediates the interference-based transient forgetting.

The authors established an interference-based forgetting task where interfering stimuli, airflow, electric shock, or blue light, presented just before retrieval test transiently disrupted retrieval of previously acquired long-term memory. Specific dopamine neuron in PPL1 cluster innervating the α2α'2-MB compartment triggered the transient forgetting. This effect was mediated by DAMB in αβMBn. Activation of this pathway resulted in transient forgetting without suppressing the cellular memory trace in MBOn-α2sc, thus indicating the transient forgetting being due to retrieval failure rather than temporal suppression of memory trace. Most importantly, transient forgetting by the external stimuli was mediated by this circuit, which is distinct from PPL1-DA-MBn-MBOn circuit mediating an active forgetting that the author's group previously identified.

The experiments are well designed and carefully carried out. Overall, the data presented support the authors' conclusions. Abstract, introduction, and result sections are logically structured, and flow of data presentation is natural and easy to understand. Most statistical tests were appropriately carried out except for some cases, which I point out in the comments.

This is a particularly important study and interests not only to the neuroscience community but also to a general audience of scientists, given that the retroactive forgetting occurs frequently in our daily life. While the author's conclusion is supported by their experimental data, I have several points that, if addressed or some least discussed, collectively would improve and strengthen the manuscript.

1. The authors' finding that transient forgetting recruits a distinct neural circuit from that of active forgetting is particularly important and interesting. I am convinced that transient and intrinsic (active) forgetting recruit two distinct PPL1 DAn-MBn-MBOn circuits. However, I wonder whether PPL1-α2α'2 indeed exerts its role on the external stimuli-induced transient forgetting. What the authors showed is the involvement of dopamine receptor DAMB expressed in αβMBn. Would it be possible to examine the effect of PPL1-α2α'2 suppression on the interfering-stimuli-induced transient forgetting, which should strengthen the authors' conclusion. Fly line of 058B > Shibire may be employed for this purpose.

2. Authors demonstrate that interfering stimuli-induced forgetting is transient as PSD-LTM resurfaced 1 h after the stimuli (Fig. 1e, f, g). They compared PI between control and experimental groups at 73 h and found no statistically significant differences (Extended Table). I suggest, in addition, to compare PI between 72h and 73h of experimental groups with two-way repeated measures ANOVA and show

significant differences. This strengthens the observed interference-based forgetting being a transient. The same logic may also apply to Fig. 2a.

3. Fig. 4e: Although differential response of calcium transient to BEN in conditioned animals was not different from naïve animals, BEN worked well as a CS odor in behavioral response. Authors may discuss possible interpretation.

4. Repeated and prolonged activation of PPL1-α2α'2 produced longer-lasting forgetting (Ext. Fig. 3). This suggests an interesting possibility that, in addition to mediating the transient forgetting, the PPL1-α2α'2 has potential to mediate strong or impressive interfering experience-induced forgetting that lasts extremely long period. PPL1-α2α'2 may flexibly regulate the duration of suppressing memory retrieval depending on the extent of external interfering stimuli. This may be discussed in the paragraph that mentioned the ongoing activity of DAn-α2α'2 (line 238).

5. I suggest authors to briefly describe in the text characteristics such as cell-, circuit-, or compartment-specificity of some of the fly lines, specifically 058B (split gal4) and c739 (gal4) that were used to identify the specific neural circuit mediating the transient forgetting. Alternatively, URLs describing the characteristics may help readers understand. It could not be easy for scientists who are unfamiliar with Drosophila molecular genetics to reach necessary information through genotype description, source, and identification in Ext Data Table. It took long time for me to critically understand the characteristics of these fly lines.

Author Rebuttals to Initial Comments:
Note: Author rebuttals in blue

Referee 1

In the manuscript entitled "Dopamine-based mechanism for transient forgetting", the authors first showed that several external stimuli are capable of transiently disrupting retrieval of long- term memory. In the following experiments, the authors identified a single pair of dopamine neurons to mediate this transient forgetting bidirectionally. It is a novel and exciting finding that deserves to be published in Nature, with the following concerns addressed.

Major concerns:
1). As a major conclusion, authors claim that a single pair of DAns, PPL1-α2α'2, mediates transient forgetting induced by external stimuli including airflow, electric shock or blue light. It would be critical to show that synaptic blockade or inhibition of PPL1-α2α'2 activity leads to suppression of transient forgetting induced by external stimuli examined.

Authors' Response:

Thank you for this critique and we have added the suggested experiment in the revised manuscript (Fig. 6a). Our results demonstrate that inhibiting synaptic output from PPL1-α2α'2 blocked the transient forgetting induced by the external stimuli. This further implicates the PPL1- α2α'2/DAMB

pathway as a key neural circuit mechanism triggered by the distractors/interfering stimuli to transiently suppress memory retrieval.

2). In Fig. 5, the authors show that 72-h memory trace represented in MBOn-α2sc neuron was not disrupted by a 6-h TrpA1 stimulation of PPL1 DAn-α2α'2 24 h before retrieval. Based on this observation, the authors conclude that "transient forgetting blocks retrieval rather than temporarily suppressing a PSD-LTM cellular trace". It would be more comfortable if authors could show that a 5-min TrpA1 stimulation does not affect the calcium traces recorded in MBOn- α2sc immediately after stimulation, instead of waiting for a long period after the 6-h stimulation of PPL1-α2α'2.

Authors' Response:

Thank you for this critique and we have added the suggested experiment in the revised manuscript (Fig. 5d, e). We showed, using the experimental genotype (DAn-α2α'2 > TrpA1), that the cellular memory traces are not affected immediately following a 5 min TrpA1 stimulation. This observation is similar when employing the longer 6 h stimulation. Importantly, these results support our original conclusion that transient forgetting blocks memory retrieval rather than temporarily suppressing the cellular memory trace.

3). In Fig.2, the manipulation is within a single pair of PPL1 DAns. Therefore, it might be more accurate to conclude that "Activity of a single (pair of) PPL1 DAn suppresses or enhances retrieval of PSD-LTM.

Authors' Response:

Thank you for pointing this out and we have appropriately changed the title of the figure (line 583).

Minor concerns:

1) In lines 49-51, the sentence is lack of the supporting reference. – We have added the reference (line 51).

2) In line 59, reference 8 is missing. – We have added the reference (line 59).

3) According to the Extended Fig.2, the authors reported that except PPL1-α2α'2 DAn, other PPL1 DAn subgroups cannot yield a similar decrement in PSD-LTM expression as the whole PPL1 DAn cluster after ectopic stimulation. However, it seems that no PSD-LTM was formed before ectopic stimulation of PPL1-γ1ped and PPL1-γ2α'1, even in the control flies. Therefore, it is inappropriate to make such a conclusion. It might be better to remove this figure. - The control data for PPL1-γ2α'1 (*296B-split-gal4*, grey bars), although more variable than other datasets, do show that PSD-LTM is formed since the PIs for the CXM+ and CXM- are statistically different. However, the reviewer's comment is correct regarding the PPL1-γ1ped (*320C-split-gal4*) data. It is worth noting that our data are also consistent with those of Aso and Rubin (*eLife*, 2016, Fig 2c), who showed that the *320C-split-gal4* fly line labeling PPL1-γ1ped has negligible 4 d memory even after 10 cycles of spaced conditioning. Our opinion is that it is prudent to show all of the relevant data.

4) In line 139, “knockdown” should be “knock down”. - We have made this change (line 152).

5) Expression patterns for used Gal4 lines are required in extended data. - All of the extended data figures had cartoons of the expression patterns for the driver lines or an included descriptive table (Extended Fig. 5) except for Extended Fig. 6. We added a cartoon to this figure to show the expression pattern of *R34B02-lexA*. We have also included a separate column in our Extended Table (1 – Reagents) with links directing to the expression pattern and other pertinent information available for each driver line.

Referee 2

Forgetting is the important but understudied flip side of learning and memory. Far less is known at a molecular and circuit level about how memories are lost than about how they are formed. The Davis group has pioneered studies of forgetting in Drosophila, and the present study continues this welcome effort.

The focus is on what the authors call "transient forgetting." They show that dopaminergic input to the α2 and α'2 mushroom body compartments, which is mediated by the neuron PPL1-α2α'2, causes a transient block in memory retrieval. Blocking the mushroom body output neuron MBON-α2sc, which has its dendrites in the α2 compartment and shows a memory trace after conditioning, recapitulates the memory retrieval block.

Although I am sympathetic to the line of research as a whole, I have two serious reservations about the present paper.

First, the question of novelty. A previous study (Krashes et al., Cell 2009), which the authors do not acknowledge, showed that dopaminergic input to the γ1 mushroom body compartment transiently suppresses long-term appetitive memory performance in satiated flies. This hunger- dependent gating of memory retrieval by PPL1-γ1pedc (also known as MB-MP1) is conceptually difficult if not impossible to separate from the kind of transient forgetting the authors study. The earlier paper therefore represents a fatal infringement of novelty.

Authors’ Response:

Krashes, Waddell and collaborators (2009) show in one section of their paper, “A neural circuit mechanism integrating motivational state with memory expression in Drosophila,” that stimulating the PPL1-γ1pedc dopamine neurons beginning 15-60 min before and during the retrieval test impairs appetitive memory performance of starved flies (Fig 6B; S6C). However, there are several fundamental mistakes in the reviewer’s criticism that when corrected provide a clear separation between the singular observation of Krashes et al., and the deep focus we present on transient forgetting. First, the word “transient” is incorrectly used to describe the two panels of data shown in Krashes et al. There are no data presented showing that the behavioural impairment recovers spontaneously. This is critical. One cannot claim prior discovery of the processes underlying transient forgetting without these data. Second, their protocol utilized stimulation before and during the retrieval test, whereas we show that brief distracting stimuli or dopamine neuron stimulation prior to, but not during testing,

impairs performance. One cannot eliminate the possibility that the persistent stimulation during retrieval used by Krashes et al., produced a state of behavioural impairment that is distinct from transient forgetting. Third, Krashes et al., tested performance at 3 h after appetitive training whereas we tested performance at 1-14 days after aversive, spaced conditioning. The modes used for conditioning and the times employed for testing after conditioning are very distinct, with the high likelihood of the underlying neurobiology being distinct as well. Caution prohibits attempts to equate results from one to the other. Fourth, the data presented in the two experiments of Krashes et al., paper combined with a large amount of other data are synthesized into the interpretation that the dopamine neurons regulate the motivation of the fly to seek food, as the title indicates. Assuming that the interpretation made by Krashes et al., is correct, the internal state of motivation is very different from transient forgetting caused by brief exposure to external stimuli or dopamine neuron stimulation.

Second, the question of mechanism. The authors show that 6 h stimulation of PPL1-α2α'2 inhibits memory expression even 24 h later (ED Fig. 3), that memory performance depends on MBON-α2sc (ED Fig. 6), that the odor responses of MBON-α2sc are altered after conditioning ("memory trace", Fig. 4) and yet, when they stimulate PPL1-α2α'2 under conditions that cause transient forgetting, nothing happens to the odor responses of MBON-α2sc (Fig. 5)! The locus and mechanism of transient forgetting thus remain to be discovered, contradicting the claim of the title.

Authors' Response:

One part of this criticism is also incorrect. We show that the activation of PPL1 dopamine neurons just prior to a retrieval test inhibits the expression of memory, but that memory expression spontaneously recovers (Fig 2). We also show that a characterized dopamine receptor, DAMB, is required for transient forgetting (Figs 3 and 6). These data by themselves justify the title, "Dopamine-based mechanism for transient forgetting."

All behaviour to our knowledge is mediated by neurons and other cells working within one or more neural circuits. We identify in the manuscript for the first time, some of the neurons and part of the neural circuit that mediates transient forgetting of protein synthesis-dependent memory. Because behaviour is dependent on neural circuits, it is highly unlikely that there exists a "locus" for transient forgetting or for that matter, any other type of behaviour. For instance, olfactory memory formation in Drosophila engages cells in the olfactory circuit to include neurons in the antennal lobe, several different types of mushroom body neurons, the mushroom body output neurons, and certainly neurons that are downstream of the output neurons.

Certainly, one can state that mushroom body neurons, for instance, are prominent in the process of olfactory memory formation, but one should not describe them as "the locus" given the involvement of other cell types.

Referee 3

In this manuscript Davis and colleagues investigated circuit and molecular mechanisms underlying

interference-based forgetting. They employed an interfering stimuli-triggered transient forgetting task, a Drosophila model of a retroactive interference in which new experience or learning interferes with the retrieval of previously acquired memory, although they did not explicitly mention this. Interference-based forgetting, such as forgetting for a moment and tip-of-the-tongue state, often occurs in our daily life and there have been many psychological studies thus far. However, underlying mechanisms, either in neural circuit, cellular, or molecular level, are totally unknown. The authors took full advantage of Drosophila molecular genetics and identified a unique neural circuit that mediates the interference-based transient forgetting.

The authors established an interference-based forgetting task where interfering stimuli, airflow, electric shock, or blue light, presented just before retrieval test transiently disrupted retrieval of previously acquired long-term memory. Specific dopamine neuron in PPL1 cluster innervating the α2α'2-MB compartment triggered the transient forgetting. This effect was mediated by DAMB in αβMBn. Activation of this pathway resulted in transient forgetting without suppressing the cellular memory trace in MBOn-α2sc, thus indicating the transient forgetting being due to retrieval failure rather than temporal suppression of memory trace. Most importantly, transient forgetting by the external stimuli was mediated by this circuit, which is distinct from PPL1-DA- MBn-MBOn circuit mediating an active forgetting that the author's group previously identified.

The experiments are well designed and carefully carried out. Overall, the data presented support the authors' conclusions. Abstract, introduction, and result sections are logically structured, and flow of data presentation is natural and easy to understand. Most statistical tests were appropriately carried out except for some cases, which I point out in the comments.

This is a particularly important study and interests not only to the neuroscience community but also to a general audience of scientists, given that the retroactive forgetting occurs frequently in our daily life. While the author's conclusion is supported by their experimental data, I have several points that, if addressed or some least discussed, collectively would improve and strengthen the manuscript.

1. The authors' finding that transient forgetting recruits a distinct neural circuit from that of active forgetting is particularly important and interesting. I am convinced that transient and intrinsic (active) forgetting recruit two distinct PPL1 DAn-MBn-MBOn circuits. However, I wonder whether PPL1-α2α'2 indeed exerts its role on the external stimuli-induced transient forgetting. What the authors showed is the involvement of dopamine receptor DAMB expressed in αβMBn. Would it be possible to examine the effect of PPL1-α2α'2 suppression on the interfering-stimuli- induced transient forgetting, which should strengthen the authors' conclusion. Fly line of 058B > Shibire may be employed for this purpose.

Authors' Response:

Thank you for this critique and we have added the suggested experiment in the revised manuscript (Fig. 6a). Our results demonstrate that inhibiting synaptic output from PPL1-α2α'2 blocked the transient forgetting induced by the external stimuli. This further implicates the PPL1- α2α'2/DAMB pathway as a key neural circuit mechanism triggered by the distractors/interfering stimuli to transiently suppress memory retrieval.

2. Authors demonstrate that interfering stimuli-induced forgetting is transient as PSD-LTM resurfaced 1 h after the stimuli (Fig. 1e, f, g). They compared PI between control and experimental groups at 73 h and found no statistically significant differences (Extended Table). I

suggest, in addition, to compare PI between 72h and 73h of experimental groups with two-way repeated measures ANOVA and show significant differences. This strengthens the observed interference-based forgetting being a transient. The same logic may also apply to Fig. 2a.

Authors' Response:

We appreciate this feedback and have added the suggested statistical comparisons in the Extended Table (sheet 5). In addition to the two-way repeated measures ANOVA, we also performed the two-tailed t-test with Welch's correction. The comparisons showed a significant difference between the 72 h and 73 h memory – transient forgetting – for all external stimuli using the t-test, but only the electric shock and blue light stimuli using the two-way ANOVA. Based on the latter comparison, this is likely due to the strength of the stimuli – with the shock and blue light having a more potent effect on suppressing memory compared to the airflow. We also applied this logic for the TrpA1 data set (Fig. 2a) and found a similar significant difference when using either statistical comparisons.

We opted out of including these extra comparisons in the figures. We believe that the current figures will be more digestible and suitable to the general reader. However, we have included the statements in the text indicating such comparisons (lines 78-84, 109-111).

3. Fig. 4e: Although differential response of calcium transient to BEN in conditioned animals was not different from naïve animals, BEN worked well as a CS odor in behavioral response. Authors may discuss possible interpretation.

Authors' Response:

Thank you for this critique and we have added a paragraph to this point in the Discussion (lines 252-257). BEN as the CS+ is capable of forming spaced training-induced calcium differentials (- CXM group, Extended Fig. 6). But, in our hands, it does not appear to be as consistent in producing robust calcium differentials compared to when OCT is used as the CS+. Therefore, we only used OCT+ for the later functional imaging experiments to probe whether DAn stimulation affects the cellular traces or not. In addition, the behavioural memory is measured as PIs (performance index) being the average of both OCT+ and BEN+ with perhaps OCT+ being more salient.

4. Repeated and prolonged activation of PPL1-α2α'2 produced longer-lasting forgetting (Ext. Fig. 3). This suggests an interesting possibility that, in addition to mediating the transient forgetting, the PPL1-α2α'2 has potential to mediate strong or impressive interfering experience- induced forgetting that lasts extremely long period. PPL1-α2α'2 may flexibly regulate the duration of suppressing memory retrieval depending on the extent of external interfering stimuli. This may be discussed in the paragraph that mentioned the ongoing activity of DAn-α2α'2 (line 238).

Authors' Response:

We appreciate this observation and have added a paragraph in the Discussion that provides a testable hypothesis (lines 276-283). We do agree that this adds another intriguing layer to the modulatory role of PPL1-α2α'2 on memory retrieval.

5. I suggest authors to briefly describe in the text characteristics such as cell-, circuit-, or compartment-specificity of some of the fly lines, specifically 058B (split gal4) and c739 (gal4) that were used to identify the specific neural circuit mediating the transient forgetting. Alternatively, URLs describing the characteristics may help readers understand. It could not be easy for scientists who are unfamiliar with Drosophila molecular genetics to reachnecessary information through genotype description, source, and identification in Ext Data Table. It took long time for me to critically understand the characteristics of these fly lines.

Authors' Response:

We apologize for this. We have added text descriptions throughout the manuscript to aid in the readers' understanding of the spatial expression patterns. We also include cartoons on many of the figures that depict the expression patterns. Furthermore, we have added a separate column in our Extended Table (1 – Reagents) with links directing to the expression pattern and other pertinent information available for each driver line.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The revised manuscript is greatly improved. Most of my concerns are well addressed, except for the first one. It is an important conclusion that a single pair of DAns, PPL1-α2α'2, mediates transient forgetting induced by external stimuli (air flow, electric shock or blue light). Thus, it is critical to show that inhibition of this pair of neurons can resist the disruption by external stimuli.
To some extent, the revised data (Fig. 6a) support that inhibiting synaptic output from PPL1-α2α'2 blocks the transient forgetting induced by the external stimuli. However, it would be more convicing if the training cycle (Fig. 6a) for the experimental group (058B > Shibire) (3-cycle) is of same as controls (058B > + and Shibire > +) (5-cycle).
As shown in Figure 1, the disruption of PSD-LTM retrieval by external stimuli is found under 5-clcle spaced training. It is more rigorous to test whether PPL1-α2α'2 inhibition blocks the transient forgetting induced by the external stimuli under the same condition (5-cycle). It is also acceptable if experimental group (058B > Shibire) and its respective control groups (058B > + and Shibire > +) are trained in the same cycle, either 5 or 3-cycle.
The authors mentioned, in lines 210-212, that "since inhibiting PPL1-α2α'2 before a memory retrieval

test enhanced PSD-LTM, we undertrained 058B > Shibire flies using only three cycles of training so that their LTM performance was similar to the control flies." It would also be acceptable if authors provide insights into why enhanced PSD-LTM may affect the suggested experiments (with the same training cycle in experiemental and control groups).

Referee #2 (Remarks to the Author):

I have read the authors' response and the revised manuscript and continue to have strong reservations.

1. If the authors wish to draw a conceptual distinction between earlier work on dopaminergic suppression of memory retrieval and their current work on dopaminergic suppression of memory retrieval, they should make the basis for this distinction explicit in the paper.

2. Olfactory associative memories are read out by MBONs in a mushroom body compartment specific manner. The authors show that dopaminergic input to the α2 compartment blocks memory retrieval in their behavioral paradigm, but the neuron responsible for memory retrieval from this compartment (MBON-α2sc) responds equally whether odors are remembered or forgotten. This is an internal contradiction because any upstream changes, for example in the mushroom body neurons to which they map the dopamine receptor requirement for transient forgetting, would also show up as altered MBON odor responses during or following dopaminergic stimulation. The fact that they see no change means that they do not understand how the system works. This fact cannot be brushed aside with a vague appeal to diffuse, unidentified changes elsewhere in the circuit.

Referee #3 (Remarks to the Author):

The authors have adequately addressed all my concerns. The manuscript is now acceptable for publication in Nature.

I suggest revising the title as they clearly show the involvement of dopaminergic pathway in interfering stimulus-triggered forgetting by adding Fig. 6a. For example, "Dopamine-based mechanism for interfering stimulus-triggered transient forgetting", which reflects exactly the essence of the authors' findings.

Kaoru Inokuchi, PhD

Author Rebuttals to First Revision:

Referee 1

The revised manuscript is greatly improved. Most of my concerns are well addressed, except for the first one. It is an important conclusion that a single pair of DANs, PPL1-α2α'2, mediates transient forgetting induced by external stimuli (air flow, electric shock or blue light). Thus, it is critical to show that inhibition of this pair of neurons can resist the disruption by external stimuli.

To some extent, the revised data (Fig. 6a) support that inhibiting synaptic output from PPL1- α2α'2 blocks the transient forgetting induced by the external stimuli. However, it would be more convincing if the training cycle (Fig. 6a) for the experimental group (058B > Shibire) (3-cycle) is of same as controls (058B > + and Shibire > +) (5-cycle). As shown in Figure 1, the disruption of PSD-LTM retrieval by external stimuli is found under 5-cycle spaced training. It is more rigorous to test whether PPL1-α2α'2 inhibition blocks the transient forgetting induced by the external stimuli under the same condition (5-cycle). It is also acceptable if experimental group (058B > Shibire) and its respective control groups (058B > + and Shibire > +) are trained in the same cycle, either 5 or 3-cycle.

The authors mentioned, in lines 210-212, that "since inhibiting PPL1-α2α'2 before a memory retrieval test enhanced PSD-LTM, we undertrained 058B > Shibire flies using only three cycles of training so that their LTM performance was similar to the control flies." It would also be acceptable if authors provide insights into why enhanced PSD-LTM may affect the suggested experiments (with the same training cycle in experimental and control groups).

Authors' Response:

This is an interesting issue. In this experiment, we chose to use 3X training for the experimental group (058B > Shibire flies) and 5X training for the control groups since the brief inhibition of the transient forgetting circuit (PPL1-α2α'2) just prior to retrieval enhances PSD-LTM performance compared to control groups. Thus, we felt at the time that it was best to normalize the performance of the experimental and control groups by providing such differential conditioning since the experiment employs the same strength of the interfering stimuli across all groups beingtested.

Consider that 5X over 3X conditioning probably instills more robust molecular/cellular memory traces, or perhaps additional molecular/cellular memory traces in the brain of the fly, and these lead to the endpoint – enhanced PSD-LTM behavioral performance. Consider also that the brief blockade of the forgetting circuit just prior to retrieval probably also does something that is similar: increase in ways that are currently unknown how PSD-LTM is represented in the brain. If we employed 5X conditioning across all groups, then the experimental group would enjoy this enhanced representation of PSD-LTM over the control groups, yet both control and experimental groups would be subjected to the same strength of interfering stimuli. This would create a disparity and given the unknowns as to exactly how PSD-LTM is represented in the brain across molecules, cells and circuits, we felt that the best way to control for this was to normalize the final behavioral response with differential conditioning.

We really appreciate the reviewer's comment, because it forced us to think more deeply about the issue. But we ended up still feeling that the experiment was performed in the most rigorous way possible. We have followed the reviewer's alternative suggestion and provided an expanded explanation for our choice in using differential conditioning under the assumption that the brain's *representation of PSD-LTM would be more equivalent in the control and experimental groups to* avoid the experimental confound. This expanded explanation is found in the text (lines 210 – 216).

Referee 2

I have read the authors' response and the revised manuscript and continue to have strong reservations.

1. If the authors wish to draw a conceptual distinction between earlier work on dopaminergic suppression of memory retrieval and their current work on dopaminergic suppression of memory retrieval, they should make the basis for this distinction explicit in the paper.

Authors' Response:

We believe that the reviewer is bolstering once again the paper by Krashes et al. We pointed out in the prior rebuttal the fundamental flaws in the reviewer's comments and will not reiterate them here except for pointing out the most obvious: the distinction that the reviewer desires can be found in the words used in the title of our manuscript, "transient forgetting." As a second, more general point, forgetting can occur for many different reasons, from external sensory stimuli as shown in our current manuscript, perhaps from internal "motivational state" as claimed by Krashes et al, and even as the result from head trauma, in which retrograde memories are inaccessible to retrieval but resurface over time. These are and will continue to be distinguished by the stimulus that causes forgetting along with the underlying brain mechanisms. Thus, there is simply no reason to connect transient forgetting caused by interfering sensory stimuli, with transient/permanent(?) memory due to internal states, or with transient forgetting due to retrograde amnesia, etc., etc., etc.

2. Olfactory associative memories are read out by MBONs in a mushroom body compartment specific manner. The authors show that dopaminergic input to the α2 compartment blocks memory retrieval in their behavioral paradigm, but the neuron responsible for memory retrieval from this compartment (MBON-α2sc) responds equally whether odors are remembered or forgotten. This is an internal contradiction because any upstream changes, for example in the mushroom body neurons to which they map the dopamine receptor requirement for transient forgetting, would also show up as altered MBON odor responses during or following dopaminergic stimulation. The fact that they see no change means that they do not understand how the system works. This fact cannot be brushed aside with a vague appeal to diffuse, unidentified changes elsewhere in the circuit.

Authors' Response:

The experiment we performed was to assay the persistence of a cellular memory trace that forms with PSD-LTM training in downstream neurons, in response to stimulating the dopamine neuron that

mediates transient forgetting. And there were two possible outcomes. First, the memory trace could have disappeared to help mediate the transient behavioral forgetting only to resurface spontaneously at a later time. The envisioned hypothesis would have the trace resurface in order to restore the behavioral responses. Alternatively, the cellular memory trace could persist after the transient forgetting stimulus. We made the latter observation, which indicates that some other process overrides the action of the cellular memory trace in effecting the normal behavioral responses. This is an observation. It is not an internal contradiction as the reviewer claims.

It would be extremely silly for us to claim that we understand completely how the transient forgetting system works. It is similarly unreasonable that the reviewer expects this level of understanding from our pioneering study that defines, for the first time, a dopaminergic circuit impinging on a memory circuit that causes transient forgetting in response to interfering stimuli. We, along with many other students of learning and memory, view the process of memory formation as comprised of at least four separate operations: acquisition of memory, consolidation of memory, retrieval of memory, and the forgetting of memory. Each of these operations requires many decades of work to obtain even a rudimentary understanding.
Although there will be differences of opinion, many neuroscientists would trace the pioneering work on brain mechanisms for acquisition to the discovery and study of LTP by Bliss and Lomo in 1973. Mechanistic studies of acquisition are still being pursued nearly 50 years later. One might trace the pioneering mechanistic work on consolidation of PSD-LTM to the work of Flexner and Flexner, and Agranoff and colleagues performed in the 1960s, from their demonstration that inhibitors of protein synthesis block consolidation. Mechanisms of consolidation and reconsolidation remain under study to this day.

Referee 3

The authors have adequately addressed all my concerns. The manuscript is now acceptable for publication in Nature.

I suggest revising the title as they clearly show the involvement of dopaminergic pathway in interfering stimulus-triggered forgetting by adding Fig. 6a. For example, “Dopamine-based mechanism for interfering stimulus-triggered transient forgetting”, which reflects exactly the essence of the authors’ findings.

Kaoru Inokuchi, PhD

Authors’ Response:

Thank you for your valuable feedback, Dr. Kaoru Inokuchi.

Reviewer Reports on the Second Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have addressed my concerns.

Author Rebuttals to Second Revision:

N/A