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Report

# Parallel Processing of Appetitive Short- and Long-Term Memories In *Drosophila*

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## Summary

**It is broadly accepted that long-term memory (LTM) is formed sequentially after learning and short-term memory (STM) formation, but the nature of the relationship between early and late memory traces remains heavily debated [1–5]. To shed light on this issue, we used an olfactory appetitive conditioning in *Drosophila*, wherein starved flies learned to associate an odor with the presence of sugar [6]. We took advantage of the fact that both STM and LTM are generated after a unique conditioning cycle [7, 8] to demonstrate that appetitive LTM is able to form independently of STM. More specifically, we show that (1) STM retrieval involves output from γ neurons of the mushroom body (MB), i.e., the olfactory memory center [9, 10], whereas LTM retrieval involves output from αβ MB neurons; (2) STM information is not transferred from γ neurons to αβ neurons for LTM formation; and (3) the adenylyl cyclase RUT, which is thought to operate as a coincidence detector between the olfactory stimulus and the sugar stimulus [11–14], is required independently in γ neurons to form appetitive STM and in αβ neurons to form LTM. Taken together, these results demonstrate that appetitive short- and long-term memories are formed and processed in parallel.**

## Results and Discussion

Short-term memory (STM) forms right after learning and is based on transient molecular and cellular events lasting from a few minutes to a few hours, whereas long-term memory (LTM) forms later on and involves gene expression and de novo protein synthesis following conditioning. The nature of the links between STM and LTM has long been debated [1–4], but there is consensus that STM and LTM are sequential processes and that LTM formation is built on the short-term trace [5]. However, other studies have led to the conclusion that the mechanisms underpinning STM and LTM in vertebrates are at least partially independent [2, 15, 16].

Studies in insects have highlighted that mushroom bodies (MBs) play a major role in learning and memory [17, 18]. In particular, in *Drosophila*, MBs play a key role in olfactory learning and memory [9, 10]. The MBs in each brain hemisphere of *Drosophila* consist of approximately 2,000 neurons called Kenyon cells that can be classified into three major types: αβ, whose axons branch to form a vertical (α) and a medial (β) lobe, α′β′, which also form a vertical (α′) and a medial (β′) lobe, and γ, which form a single medial lobe in the adult [19].

Several molecular-level studies have demonstrated that the cyclic AMP (cAMP) pathway plays a pivotal role in associative learning [20]. In particular, calcium/calmodulin-dependent adenylyl cyclase (AC) encoded by the *rutabaga* (*rut*) gene [21] is necessary to aversive olfactory conditioning where an odorant is associated to electric shock. RUT AC was proposed to function as a coincidence detector [11, 12, 22–24], integrating both the olfactory information carried by projection neurons to the MB and the electric shock carried by dopaminergic neurons [25, 26]. Interestingly, RUT cAMP signaling is required in γ neurons to form aversive STM [22, 24] and in αβ neurons to form LTM [24], suggesting an independence of these two memory phases. However, several results suggest that aversive STM and LTM are not processed by fully independent neuronal pathways. Thus, a more efficient rescue of *rut* STM or LTM defect is observed when RUT is expressed in both γ and αβ neurons [13, 23, 24, 27], suggesting that RUT is also involved in αβ neurons for aversive STM and in γ neurons for LTM. In addition, blocking αβ neuron synaptic transmission during memory retrieval impairs both aversive STM and LTM [23, 28, 29]. Moreover, the induction of aversive STM and LTM requires different conditioning protocols, because STM is induced by one cycle of conditioning, whereas LTM formation requires spaced conditioning consisting of repeated training sessions separated by 15 min rest periods. These different training protocols may induce different physiological states within the relevant neurons, making it more difficult to interpret whether LTM is or is not built upon STM.

### Output from MB αβ Neurons Is Required for Appetitive LTM Retrieval but Not for STM Retrieval

In *Drosophila*, appetitive STM and LTM are both generated after a single session of odorant-plus-sugar association [7, 8], offering a powerful situation to study the link between the short- and the long-term trace. RUT AC has been hypothesized to be the coincidence detector in olfactory appetitive memory, because *rut* mutants exhibit poor immediate memory [6, 13, 14]. It was shown that RUT AC in MB αβ and γ neurons or in projection neurons is sufficient for appetitive learning and STM [13, 14], but it remains unknown which brain structure involves RUT activity for appetitive LTM.

Consolidation of appetitive STM and LTM requires output from α′β′ neurons for 1 hr after training but not from αβ neurons [30]. The role of γ neurons in appetitive STM or LTM consolidation has not yet been addressed. STM retrieval involves output from αβ and/or γ neurons [13], but the role of α′β′ neurons in STM retrieval remains unknown. LTM retrieval involves output from αβ neurons but not from α′β′ neurons [7], and the role of γ neurons in LTM retrieval has not yet been addressed. Thus a full picture of the role of MB neurons in appetitive memory processing has yet to emerge.

To clarify the role of the different MB neurons in appetitive STM and LTM, we first used the *c739-GAL4* driver and the *UAS-shi$^{ts}$* (*shi*) transgene to block synaptic transmission in

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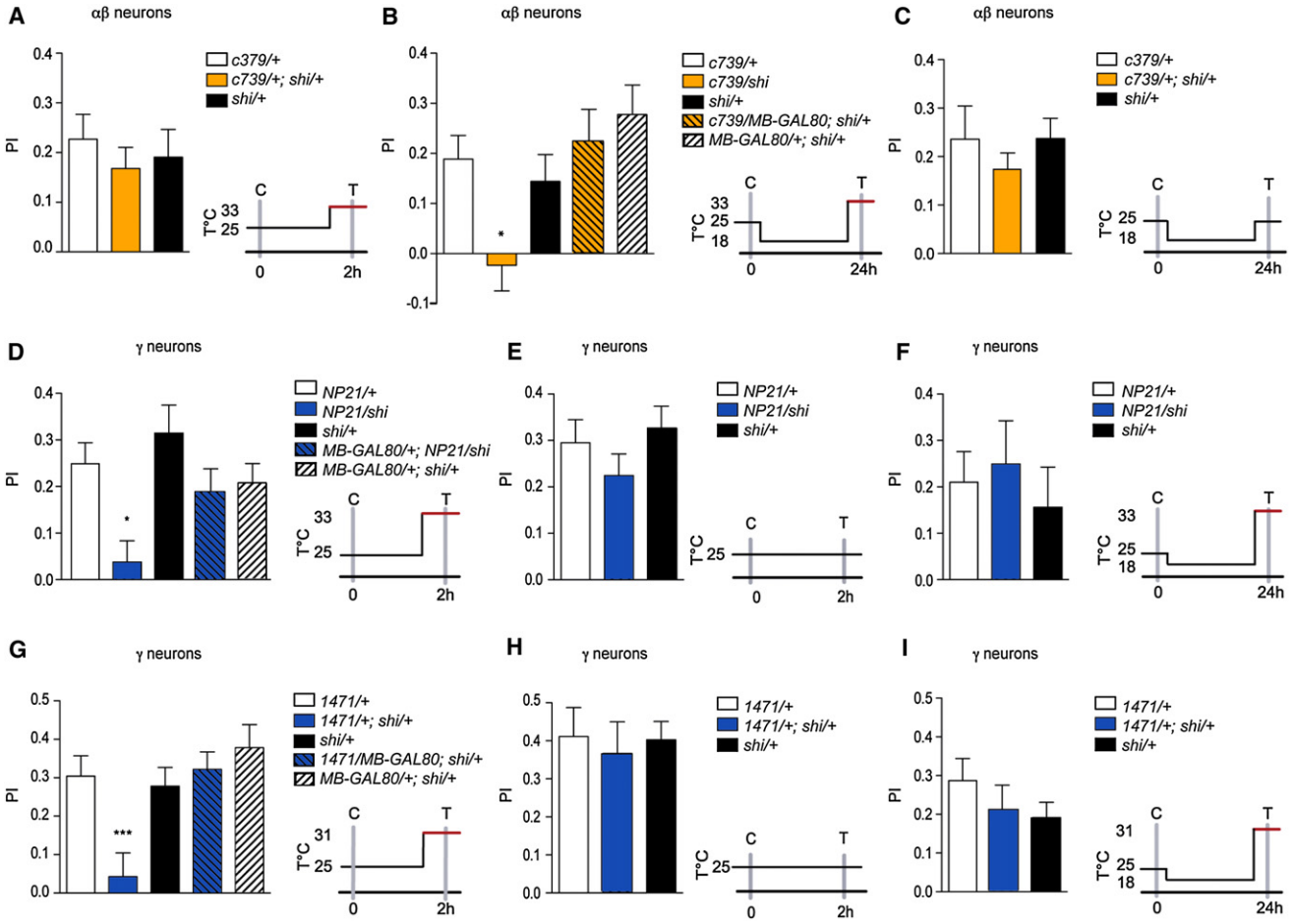


Figure 1. Mushroom Body αβ Neuron Output Is Required for Appetitive Long-Term Memory Retrieval, and MB γ Neuron Output Is Required for Appetitive Short-Term Memory Retrieval

(A) Blocking mushroom body (MB) αβ neuron output during the test does not affect 2 hr memory [$F_{(2,41)} = 0.354$, $p = 0.704$; $n = 14$].
(B) Blocking αβ neuron neurotransmission during the test impairs 24 hr memory [$F_{(4,37)} = 5.325$, $p = 0.002$; $n \geq 6$]. The memory defect is rescued by expressing *MB247-GAL80* combined with *shi* in αβ neurons using *c739-GAL4* driver.
(C) At permissive temperature, long-term memory (LTM) retrieval is not affected [$F_{(2,26)} = 0.554$, $p = 0.582$; $n = 9$].
(D and G) Blocking MB γ neuron output during the test abolished appetitive short-term memory (STM). The 2 hr memory defect is rescued by expressing *MB247-GAL80* transgene. (D) shows [$F_{(4,88)} = 4.312$, $p = 0.003$; $n \geq 16$], and (G) shows [$F_{(4,60)} = 5.819$, $p = 0.0005$; $n \geq 9$].
(E and H) At permissive temperature, 2 hr memory is normal. (E) shows [$F_{(2,36)} = 1.43$, $p = 0.252$; $n \geq 12$], and (H) shows [$F_{(2,26)} = 0.011$, $p = 0.896$; $n = 9$].
(F and I) Blocking γ neuron output during the test does not affect LTM. (F) shows [$F_{(2,26)} = 0.32$, $p = 0.729$; $n = 9$], and (I) shows [$F_{(2,32)} = 0.897$, $p = 0.418$; $n \geq 10$].
The following abbreviations are used: C, conditioning; T, test. Each graph displays mean performance indices ± standard error of the mean (SEM). The red line indicates time when synaptic transmission is blocked.

αβ neurons during memory retrieval. The dominant temperature-sensitive $SHI^{TS}$ protein blocks dynamin-dependent membrane recycling and synaptic vesicle release at restrictive temperature (33°C). This effect is reversible when the temperature is shifted back to 25°C [31]. It was previously reported that *c739/+; shi/+* flies have normal olfactory acuity [7]. We first checked that *c739/+; shi/+* flies present a normal sugar response (see Table S1 available online) at restrictive temperature. We then found that blocking synaptic transmission from MB αβ neurons did not affect appetitive STM retrieval (Figure 1A). This was a surprising observation, because it was previously shown that output from MB αβ neurons is required for appetitive LTM retrieval [7], which we confirmed here (Figure 1B). To rule out the possibility that the LTM retrieval defect might be due to *c739*-driven expression of $shi^{ts}$ outside of the MB, we used the *MB247-GAL80*$^{+}$ construct (*MB-GAL80*) to inhibit GAL4 activity in αβ neurons. As expected, *c739/MB-GAL80; shi/+* flies showed normal LTM performance when tested at restrictive temperature (Figure 1B). Furthermore, appetitive LTM performance of *c739/+; shi/+* flies at permissive temperature was normal (Figure 1C). Hence, these data indicate that MB αβ neuron output is required for appetitive LTM retrieval but not for STM retrieval.

### Output from MB γ Neurons Is Required for Appetitive STM Retrieval but Not for LTM Retrieval

It was previously shown that output from MB αβ and γ neurons is required for STM retrieval [13]. Because our data established that output from αβ neurons is not required for this process, we analyzed the role of γ neurons in STM retrieval. We expressed $shi^{ts}$ in γ neurons using the *NP21-GAL4* driver [32]. We first checked that *NP21/shi* flies present normal sugar response and olfactory acuity (Table S1) at restrictive temperature. Blocking γ neuron output during the memory test significantly

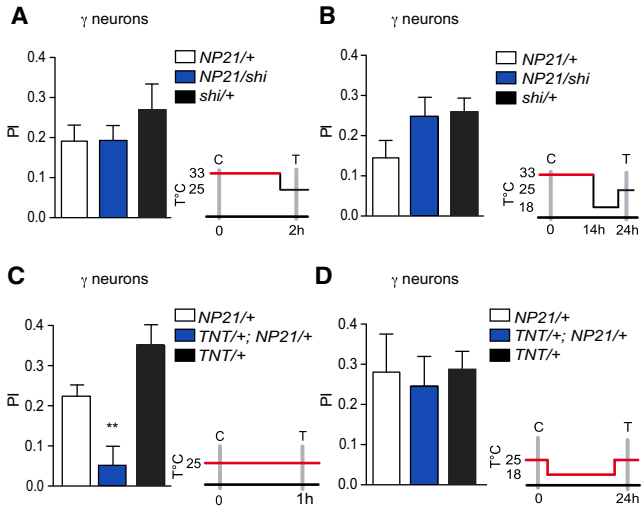


Figure 2. LTM Formation Does Not Require γ Neuron Output

(A) Blocking γ neurons during training and consolidation does not affect STM formation [$F_{(2,56)} = 0.853$, $p = 0.43$; $n \geq 18$].

(B) Blocking γ neurons during training and consolidation does not affect LTM formation [$F_{(2,42)} = 2.042$, $p = 0.143$; $n \geq 14$].

(C) Constitutively blocking synaptic transmission from γ neurons impairs STM [$F_{(2,38)} = 12.28$, $p < 0.0001$; $n = 13$].

(D) Constitutively blocking γ neuron output does not affect LTM [$F_{(2,31)} = 0.253$, $p = 0.778$; $n \geq 10$].

Each graph displays mean performance indices ± SEM. The red line indicates time when synaptic transmission is blocked.

impaired appetitive STM (Figure 1D). Inhibition of NP21 activity in the MB by MB-GAL80 (Figure S1) rescued the STM defect of *NP21/shi* flies (Figure 1D). Furthermore, the STM performance of *NP21/shi* flies was indistinguishable from controls at permissive temperature (Figure 1E). Interestingly, blocking γ neuron output during the test did not affect LTM retrieval (Figure 1F). The specific role of γ neurons in appetitive STM retrieval was confirmed with another γ neuron driver, *1471-GAL4* [29] (Figures 1G–1I; Table S1). Taken together, the results indicate that MB γ neuron output is indispensable for appetitive STM retrieval but dispensable for LTM retrieval.

### LTM Formation Does Not Require Information Transfer from MB γ Neurons

Appetitive STM and LTM retrieval each mobilize specific subsets of MB neurons, namely γ neurons for STM and αβ neurons for LTM. This might be due to the fact that STM and LTM are actually mutually independent, being formed and stored in spatially distinct compartments. Alternatively, γ and αβ neurons might be sequentially recruited: in this scenario, STM would form in γ neurons and information would be further transferred from γ to αβ neurons during the consolidation phase to build LTM. Under this latter assumption, blocking output from γ neurons during the LTM consolidation phase should lead to an LTM defect. To discriminate between the two hypotheses, we blocked γ neuron neurotransmission during training and consolidation and then measured LTM performance. First, we observed that blocking γ neuron output during training and consolidation did not affect STM (Figure 2A). Then, to test the putative role of γ neurons in LTM formation, we trained *NP21/shi* flies at restrictive temperature and maintained them at this temperature for 14 hr during the memory consolidation phase (the flies were kept at 33°C for 14 hr and not for the full 24 hr consolidation period because they started to die after 14 hr (data not shown); given that appetitive LTM is being detectable 6 hr after training [7], it is likely that LTM consolidation takes place during the 14 hr time-period of γ neuron neurotransmission blockade). *NP21/shi* flies showed a normal 24 hr memory in this condition (Figure 2B), suggesting that γ neuron output is dispensable during appetitive LTM acquisition and consolidation.

To further prove that LTM could be formed independently of STM, we constitutively blocked neurotransmission from γ neurons using *UAS-TNT* (*TNT*) construct encoding the tetanus toxin [33]. *TNT/+; NP21/+* flies are viable and present normal sugar response and olfactory acuity (Table S1). Interestingly, a continuous blockade of γ neurons abolished STM (Figure 2C) but left LTM unaffected (Figure 2D). Thus, *TNT/+; NP21/+* flies trained with a single protocol showed no appetitive STM but a normal LTM at 24 hr. These results indicate that appetitive LTM formation is independent of STM and does not require synaptic communication between γ and αβ neurons.

### RUT Adenylyl Cyclase Is Required in γ Neurons for STM Formation and in αβ Neurons for LTM Formation

RUT AC has been hypothesized to be the coincidence detector in olfactory appetitive memory [6, 13, 14]. *rut* appetitive STM defect can be rescued by expressing RUT in αβ and γ neurons [13, 14], but it had not yet been addressed whether RUT is specifically involved in γ or αβ neurons. Because circuit blocking experiments suggested that STM and LTM operate independently and involve different subsets of MB neurons, we investigated whether *rut* STM and LTM defects could be rescued independently by expressing RUT in γ and αβ neurons, respectively. We used *NP21* and *c739* transactivators to express *UAS-rut* in $rut^{2080}$ mutant flies. Expressing RUT in γ neurons fully rescued the *rut* STM defect (Figure 3A), whereas expressing RUT in αβ neurons failed to rescue the *rut* STM defect (Figure 3B). Conversely, expressing RUT in γ neurons failed to rescue the *rut* LTM defect (Figure 3C), whereas expressing RUT in αβ neurons fully rescued the *rut* LTM defect (Figure 3D). These results indicate that RUT AC is specifically required in γ neurons to form STM and in αβ neurons to form LTM, which further argues that appetitive STM and LTM are formed independently of each other.

### Appetitive Immediate Memory Is Formed in MB γ Neurons

Our data suggest that appetitive STM and LTM are processed independently in γ and αβ neurons, respectively. Accordingly, immediate appetitive memory processing should involve γ neurons. To test this hypothesis, we constitutively blocked neurotransmission from γ neurons. As expected, *TNT/+; NP21/+* flies displayed a 3 min memory defect (Figure 4A). The involvement of γ neurons was further confirmed as *1471/+; shi/+* flies displayed a 3 min memory defect at restrictive temperature (Figure 4B) but not at permissive temperature (Figure 4C). Strikingly, blocking neurotransmission from αβ neurons did not affect immediate memory (Figure 4D). These results are in agreement with our previous observations, suggesting that γ neurons support appetitive STM and αβ neurons support appetitive LTM. It has been shown that appetitive immediate memory is abolished by expressing $SHI^{TS}$ in αβ and γ neurons under the MB247 driver [13]. The partial inhibition observed with NP21 and 1471 GAL4 drivers might be due to the fact that MB247 shows a very strong expression in γ neurons [26], unlike 1471 [26] or NP21 [32]. To further prove that the immediate appetitive memory forms in γ neurons, we

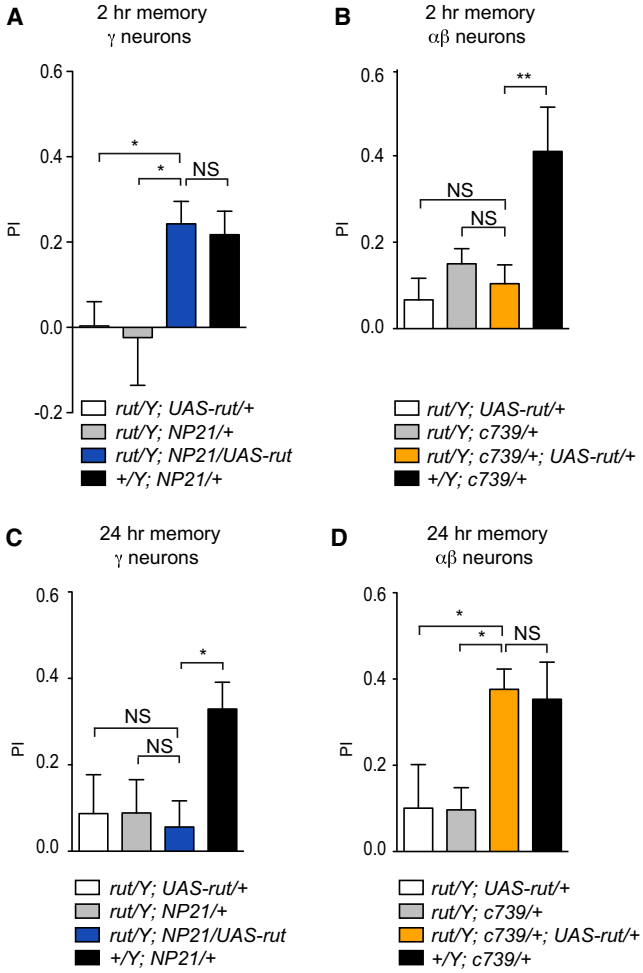


Figure 3. RUT Adenylyl Cyclase Is Recruited in γ Neurons for Normal Appetitive STM and in αβ Neurons for Normal LTM

(A) *NP21*-driven RUT expression in γ neurons fully restores *rut*$^{2080}$ STM defect [$F_{(3,46)}$ = 3.95, p = 0.014; n ≥ 8].
(B) *c739*-driven RUT expression in αβ neurons does not restore STM *rut*$^{2080}$ defect [$F_{(3,48)}$ = 4.99, p = 0.0045; n ≥ 6].
(C) RUT expression in γ neurons does not restore *rut*$^{2080}$ LTM defect [$F_{(3,36)}$ = 3.54, p = 0.025; n ≥ 6].
(D) RUT expression in αβ neurons fully restores *rut*$^{2080}$ LTM defect [$F_{(3,33)}$ = 4.80, p = 0.0075; n ≥ 6].
Each graph displays mean performance indices ± SEM.

investigated whether *rut* defect could be rescued by expressing RUT in γ neurons. Indeed, RUT expression under the NP21 driver restored *rut* immediate memory defect (Figure 4E). On the contrary, expressing RUT in αβ neurons failed to rescue the *rut* immediate memory defect (Figure 4F).

Appetitive conditioning offers a powerful situation for studying the link between STM and LTM, because both are formed after a single training cycle [7, 8]. It remained unknown whether the same MB neurons process both appetitive STM and LTM formation or whether these two memory phases are underpinned by specialized pathways. Our study leads to a new understanding of the role of αβ and γ neurons in appetitive STM and LTM. Using distinct GAL4 drivers to specifically express SHI$^{TS}$ or the tetanus toxin in either αβ or γ neurons, we showed that appetitive STM and LTM involve γ and αβ neurons, respectively. We found that (1) immediate memory and STM processing involves RUT AC specifically in γ neurons, whereas LTM formation involves RUT in αβ neurons; (2) MB γ neuron output is required to retrieve immediate memory and STM but not LTM, and conversely, αβ neuron output is required to retrieve LTM but neither immediate memory nor STM; (3) γ neuron output is dispensable during memory consolidation, and therefore short-term information is not transferred from γ to αβ neurons to form LTM. Blocking γ neurons using tetanus toxin resulted in a striking phenotype, because flies completely deprived of appetitive STM exhibited normal LTM at 24 hr. In conclusion, the present study provides strong evidence that in *Drosophila*, appetitive STM and LTM are two parallel and independent processes, involving different subsets of neurons within the MB (Figure 4G).

The dynamics of the appetitive memory phase involve other neural circuits than just αβ and γ neurons. Blocking output from α′β′ neurons for 1 hr after training affects both STM and LTM. Similarly, blocking output from dorsal paired medial (DPM) neurons, which project onto the MB lobes, for 1 hr after appetitive conditioning affects both STM and LTM [30, 34]. And it was recently shown that blocking GABAergic anterior paired lateral (APL) neurons, which project onto the MB lobes and dendrites, for 2 hr after appetitive conditioning affects STM but not LTM [35]. It has been proposed that α′β′-DPM neurons form a recurrent loop that stabilizes STM and LTM [30] and that APL activity regulates this loop for STM-related processes [35]. Because α′β′ neurons are not required for either LTM [30] or STM retrieval (Figure S2), our results are in agreement with this scheme, where independent STM and LTM traces in γ and αβ neurons are maintained by output from α′β′ neurons and MB-extrinsic neurons.

This model of STM and LTM independence is supported by several studies in other species. In *Aplysia*, synaptic connection between tail sensory neurons and motor neurons exhibits short- and long-term synaptic facilitation following learning [36]. It was shown that the induction of short-term synaptic plasticity is not necessary for the induction of long-term plasticity [37]. Studies in vertebrates indicate that STM and LTM involve different biochemical pathways [2, 15, 16, 38, 39] or distinct connected brain areas [4, 40–42]. The study presented here goes one step further, because it identified neuronal structures that independently process STM and LTM, providing a unique opportunity to analyze biochemical and cellular processes specifically associated with STM and LTM.

## Experimental Procedures

### Fly Stocks

Fly stocks were raised on standard food at 18°C and 60% relative humidity under a 12:12 hr light:dark cycle. The wild-type *Drosophila melanogaster* strain used was the Canton-Special (CS) strain. All mutations were used in a CS background. To block MB synaptic transmission, we used flies carrying a single insertion of the *UAS-shi*$^{ts1}$ transgene on the third chromosome [31] or of the *UAS-TNT* transgene on the second chromosome [33]. To express transgenes in αβ neurons, we used the *c739-GAL4* driver (on the second chromosome). To express transgenes in γ neurons, we used either *NP21-GAL4* [32] (on the third chromosome) or *1471-GAL4* (on the second chromosome) [29]. To express transgenes in α′β′ neurons, we used *c305a-GAL4* [30]. The *MB247-GAL80*$^{+}$; *UAS-shi*$^{ts}$ stock (from H. Tanimoto) was used for rescue experiments. We used the *rut*$^{2080}$ mutant allele to study the implication of the cAMP pathway in appetitive STM and LTM [43]. Behavioral rescue experiments were conducted by crossing *UAS-rut*; *rut*$^{2080}$ females with *c739-GAL4* or *NP21-GAL4* males. Because *rut* is an X-linked gene, only the data resulting from male progeny were taken into account in these experiments.

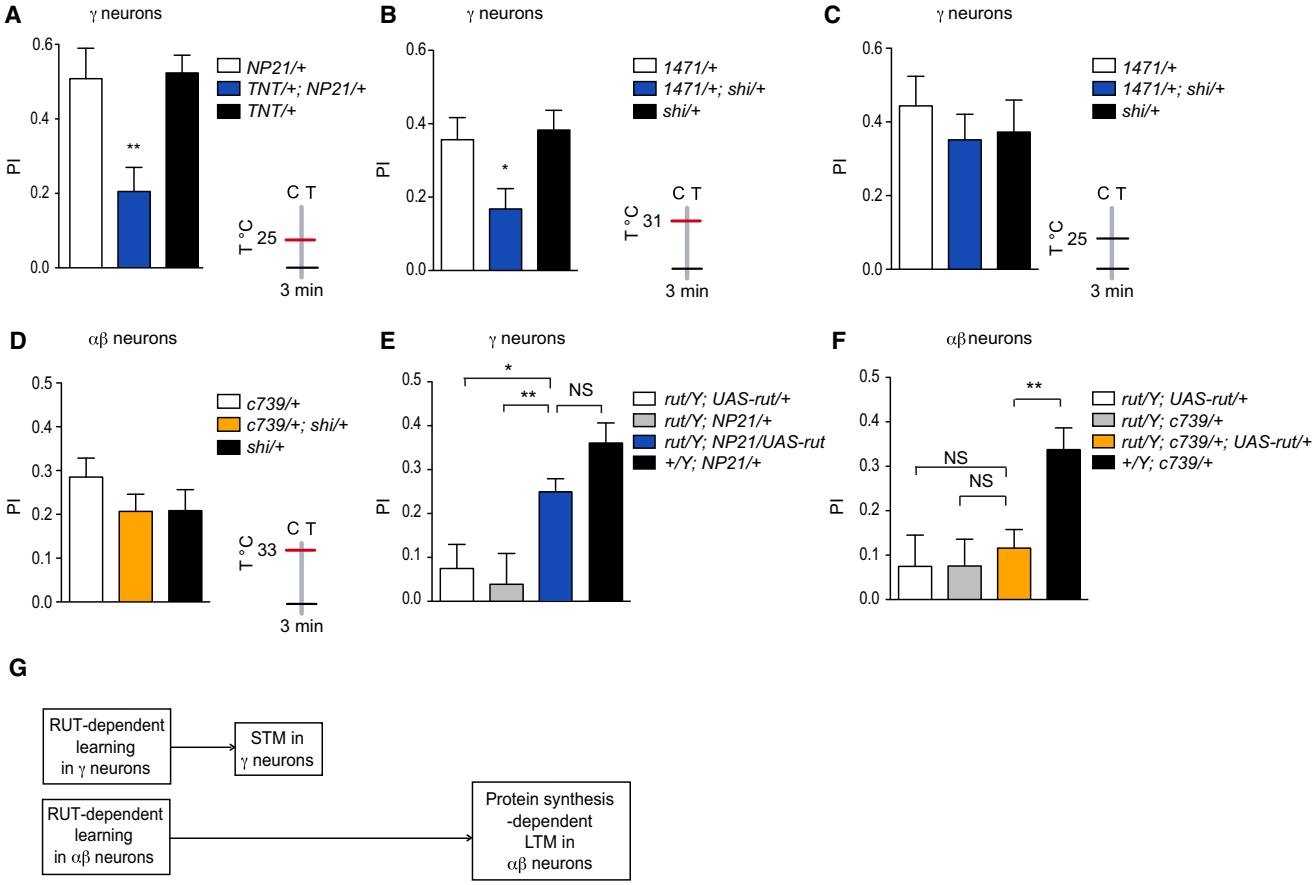


Figure 4. Immediate Appetitive Memory Processing Involves γ but Not αβ Neurons

(A) Blocking constitutively MB γ neuron output affects immediate memory [$F_{(2,23)}$ = 7.39, p = 0.0037; n = 8].
(B) Blocking MB γ neuron output affects immediate memory [$F_{(2,22)}$ = 4.349, p = 0.023; n = 10].
(C) At permissive temperature, immediate memory is normal [$F_{(2,22)}$ = 0.35, p = 0.707; n ≥ 7].
(D) Blocking output of αβ neurons does not impair immediate memory [$F_{(2,36)}$ = 1.02, p = 0.372; n ≥ 12].
(E) *NP21*-driven RUT expression in γ neurons restores $rut^{2080}$ immediate memory [$F_{(2,52)}$ = 8.41, p = 0.0001; n ≥ 12].
(F) *c739*-driven RUT expression in αβ neurons does not restore $rut^{2080}$ defect [$F_{(2,52)}$ = 5.045, p = 0.0041; n ≥ 12].
(G) Representation of appetitive memory phase dynamic: STM and LTM processes are fully independent of each other. STM is RUT dependent and is formed in γ neurons, whereas LTM is RUT dependent but is formed in αβ neurons.
The following abbreviations are used: C, conditioning; T, test. Each graph displays mean performance indices ± SEM. The red line indicates time when synaptic transmission is blocked.

### Training Protocol

Eighty to 100 flies (1–2 days old) were transferred to fresh medium during 24 hr and then kept for 21 hr at 25°C in starvation bottles. The conditioning apparatus and protocol are described in [8]. Groups of 30–40 flies of a given genotype were conditioned by exposure to one odor paired with sugar reward (a 1.5 M sucrose solution in mineral water) and subsequent exposure to a second odor in absence of sucrose. A training session consisted of an initial 90 s period of nonodorized airflow, 60 s of one of the odors, 52 s of nonodorized airflow, 60 s of the other odor, and 52 s of nonodorized airflow. Odors were produced using 3-octanol (>95% purity; Fluka 74878, Sigma-Aldrich) at 3.60 × $10^{-4}$ M and 4-methylcyclohexanol (99% purity; Fluka 66360) at 3.25 × $10^{-4}$ M diluted in paraffin oil.

### Test of Memory Performance

During the test, flies were exposed to both odors simultaneously in a T-maze during 1 min. The performance index (PI) was calculated as the number of flies avoiding the conditioned odor minus the number of flies avoiding the unconditioned odor divided by the total number of flies in the experiment. A single PI value is the average of the scores from two groups of flies of identical genotype trained with either octanol or methylcyclohexanol as CS+.

### Temperature-Shift Protocols

For blocking synaptic transmission during training and consolidation, flies were placed at restrictive temperature 30 min before training. When test temperature was different from consolidation temperature, the flies were transferred to the test temperature 30 min before the test. A 33°C temperature was used with *c739/+; shi/+* and *NP21/shi* flies to guarantee fully efficient neurotransmission blockade. Because *1471/+; shi/+* flies display a locomotor defect at 33°C but not at 31°C [29, 44], experiments with this genotype were performed at 31°C. Time courses of the temperature shifts employed in each experiment are shown alongside the graph of memory performance.

### Sugar Response Tests

Tests were performed on starved flies in a T-maze apparatus as described in [8] but without airflow. Flies were trapped in either arm after 1 min. The sugar arm was placed alternatively on the right or left. Sugar response was calculated as above and then used as a score. The sugar response tests were performed at restrictive temperature for *GAL4/shi* flies and at 25°C for *TNT/+; NP21/+* flies.

### Olfactory Acuity

Tests were performed on starved flies in a T-maze apparatus at 33°C for *NP21/shi* genotype and at 25°C for *TNT/+; NP21/+* genotype, as described previously [8]. One odor was tested for 1 min against its solvent (paraffin oil). The response index was calculated as above and then used as a score. The odor was delivered alternately through the right or left arm of the maze. The

response index theoretically ranged from −1 (total repulsion) to 1 (total attraction).

### Statistical Analyses

Comparisons between multiple groups were performed by one-way analysis of variance on each data set followed by pairwise planned comparisons between relevant groups with a Student-Newman-Keuls test. Asterisks denote the smallest significant difference between the relevant group and its controls with the post hoc pairwise comparisons, except for Figure 4, where asterisks denote significant differences with the post hoc pairwise comparisons (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; NS, not significant). Each graph displays mean performance indices ± standard error of the mean.

### Supplemental Information

Supplemental Information includes two figures and one table and can be found with this article online at doi:10.1016/j.cub.2011.08.032.

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# Supplemental Information

# Parallel Processing of Appetitive Short- and Long-Term Memories In *Drosophila*

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## Supplemental Inventory

## Supplemental Figures and Tables

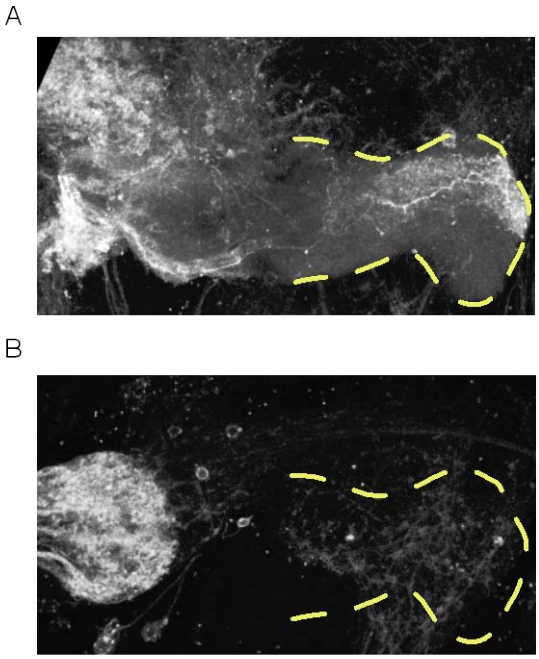


**Figure S1. NP21-Driven GFP Expression in MB γ Neurons, Related to Figure 1**

(A) 2 x *UAS-mCD8-GFP*/+; *NP21-GAL4*/+.

(B) 2 x *UAS-mCD8-GFP*/*MB247-GAL80*; *NP21-GAL4*/+. Combining *MB247-GAL80* with 2 x *UAS-mCD8-GFP*; *NP21-GAL4* eliminates the specific mushroom body γ neuron expression highlighted by the yellow dotted line, but expression elsewhere remains largely intact. The strongly GFP positive round structure on the right hand of (B) is somehow masked by the mushroom body peduncle in (A).

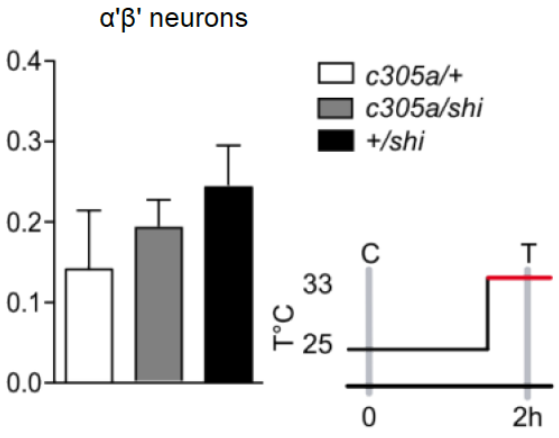


**Figure S2. MB α′β′ Neurons Output Is Not Required for STM Retrieval, Related to Figure 4**

Flies were trained, stored at permissive temperature, then tested for 2 h memory at restrictive temperature. C, conditioning ; T, test. The red line indicates time when synaptic transmission is blocked. Each graph displays mean performance indices ± SEM. There was no statistical différence between the three genotypes [$F_{(3,34)}$ = 0,919, $p$ = 0,409 ; $n \geq 11$].

| Genotypes | Temperature | Sugar Response | Olfactory Acuity | |
|---|---|---|---|---|
| | | | Octanol | Methyl-cyclohexanol |
| *NP21/+* | 33°C | 0,56 +/- 0,06 | 0,63 +/- 0,09 | 0,58 +/- 0,06 |
| *NP21/shi* | 33°C | 0,33 +/- 0,04 | 0,60 +/- 0,07 | 0,47 +/- 0,05 |
| *shi/+* | 33°C | 0,31 +/- 0,06 | 0,66 +/- 0,09 | 0,76 +/- 0,07 |
| *c739/+* | 33°C | 0,43 +/- 0,08 | | |
| *c739/+; shi/+* | 33°C | 0,26 +/- 0,04 | | |
| *shi/+* | 33°C | 0,22 +/- 0,06 | | |
| *1471/+* | 31°C | 0,50 +/- 0,08 | | |
| *1471/+; shi/+* | 31°C | 0,49 +/- 0,06 | | |
| *shi/+* | 31°C | 0,38 +/- 0,06 | | |
| *NP21/+* | 25°C | 0,34 +/- 0,06 | 0,58 +/- 0,09 | 0,60 +/- 0,07 |
| *TNT/+; NP21/+* | 25°C | 0,30 +/- 0,06 | 0,47 +/- 0,05 | 0,49 +/- 0,05 |
| *TNT/+* | 25°C | 0,30 +/- 0,09 | 0,68 +/- 0,05 | 0,57 +/- 0,04 |

**Table S1. Blocking Output from γ or αβ Neurons Affects Neither Sugar Response Nor Olfactory Acuity, Related to Figures 1 and 2**

For sugar response test: ANOVA between *NP21/+*, *NP21/shi* and *shi/+* is significant, but there is no statistical difference between *NP21/shi* and *shi/+* [$F_{(2,.33)} = 7.72$, $p = 0.002$; $n = 11$]. ANOVA between *c739/+*, *c739/+; shi/+* and *shi/+* [$F_{(2.36)} = 3.1$, $p = 0.058$; $n = 12$]. ANOVA between *1471/+*, *1471/+; shi/+* and *shi/+* [$F_{(2.47)} = 1.21$, $p = 0.31$; $n = 16$]. ANOVA between *NP21/+*, *TNT/+; NP21/+*and *TNT/+* [$F_{(2.32)} = 0.094$, $p = 0.91$; $n = 11$]. $rut^{2080}$ was previously reported to present a normal sugar response [14].

For olfactory acuity test: ANOVA between *NP21/+*, *NP21/shi* and *shi/+* (airflow *versus* Octanol) [$F_{(2.65)}= 2.34$, $p = 0.105$; $n = 22$]. ANOVA between *NP21/+*, *NP21/shi* and *shi/+* (airflow versus Methylcyclohexanol) [$F_{(2.65)} = 1.18$, $p = 0.31$; $n = 22$]. ANOVA between *NP21/+*, *TNT/+; NP21/+* and *TNT/+* (airflow *versus* Octanol) [$F_{(2.41)} = 0.13$, $p = 0.87$; $n = 14$]. ANOVA between *NP21/+*, *TNT/+; NP21/+* and *TNT/+* is significant, but there is no statistical difference between *NP21/shi* and *NP21/+* (airflow versus Methylcyclohexanol) [$F_{(2.41)} = 6.01$, $p = 0.005$; $n = 14$]. It has been previously reported that *1471/+; shi/+* [23], *c739/+; shi/+* [7], $rut^{2080}$ and *rut; UAS-rut* flies show normal olfactory acuity [14]. Numbers are mean performance indices ± SEM.