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Current Opinion in Neurobiology

# Systems memory consolidation in *Drosophila*

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From an information processing perspective, memories need to be acquired, encoded, stored, maintained and retrieved. As time passes after training, memories become less easily retrieved, but also become progressively more stable in the face of experimental perturbations. This process is referred to as consolidation. But the term has been used to describe two different biological processes whose relationship is poorly understood [1,2]. The first, which we refer to as biochemical consolidation, involves cell-signaling events within a neuron. The second, which we call systems consolidation, involves ongoing communication between brain regions or cell types. Although systems consolidation was first thought to be at play only in complex brains, a number of recent studies reveal its importance in *Drosophila*. The ease of cell type specific genetic manipulations in flies provides a unique opportunity to forge an integrated mechanistic understanding of biochemical and systems consolidation.

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**Current Opinion in Neurobiology** 2012, **23**:xx–yy

This review comes from a themed issue on **Neurogenetics**

Edited by **Ralph Greenspan** and **Christine Petit**

0959-4388/$ – see front matter, 

http://dx.doi.org/10.1016/j.conb.2012.09.006

## Introduction

Biochemical consolidation involves stabilization of the synaptic plasticity that was driven by behavioral experience. By contrast with short-term memory and plasticity, long-term synaptic plasticity and memory require translation of new proteins that are synthesized in response to the memory inducing stimuli. The best-studied example is the activation of cAMP response element binding protein (CREB), a transcription factor that lies downstream of several signaling cascades including the cAMP pathway [3–5]. Biochemical consolidation involves signaling cascades within a neuron that lead to progressively more stable changes at the synapse over time. By contrast, systems consolidation involves ongoing communication between neurons, which in some cases results in an apparent 'transfer' of information from one brain region to another [1,2].

The combination of approaches available in *Drosophila* provides a unique set of tools to investigate the relationship between biochemical and systems consolidation because individual genes can be manipulated within defined neuronal cell types. This gives a conceptually integrated view of information flow within a neural circuit. We focus on aversive olfactory memory, for which consolidation has been thoroughly investigated [6–10], and for which several recent studies strongly support a systems view.

## Biochemical consolidation

### The mushroom body circuit

The mushroom bodies (MBs), which are a primary olfactory learning center in insects, consist in *Drosophila* of about 2500 Kenyon cells (KC) per brain hemisphere (Figure 1). MBs receive olfactory inputs from the antennal lobe (AL) via a population of several hundred largely cholinergic projection-neurons (PNs). Both electrophysiological recordings and functional imaging demonstrate that odor information is coded in MB as a pattern of sparse activity in the KC populations such that a specific odor leads to activation of only a small subset of the KCs in the MB [7,11].

In addition to odor inputs, KCs also integrate inputs from several types of neuromodulatory neurons, including the unconditioned stimulus (US) reinforcement for olfactory learning [9,10]. In the case of aversive learning, the US reinforcement is carried by a specific subset of dopaminergic (DA) neurons (Figure 2) that respond to electric shock [10,12•,13,14]. DopR, a D1-like dopamine receptor required in MB [15••,16], is thought to mediate the US inputs.

### Biochemical consolidation in MB

Odor-driven calcium influx and G-coupled signaling downstream of DopR are thought to cause synergistic activation of *rutabaga* (*rut*), a calcium/calmodulin sensitive adenylyl cyclase. In this cellular model, which is conceptually convergent with findings from *Aplysia* [17], activation of *rut*-dependent signaling causes short-term plasticity and also can activate CREB, leading to a transcriptional cascade that underlies long-term plasticity and long-term memory [3,4]. Such synaptic plasticity is thought to alter the strength of output or the number of responding KCs during subsequent exposure to the



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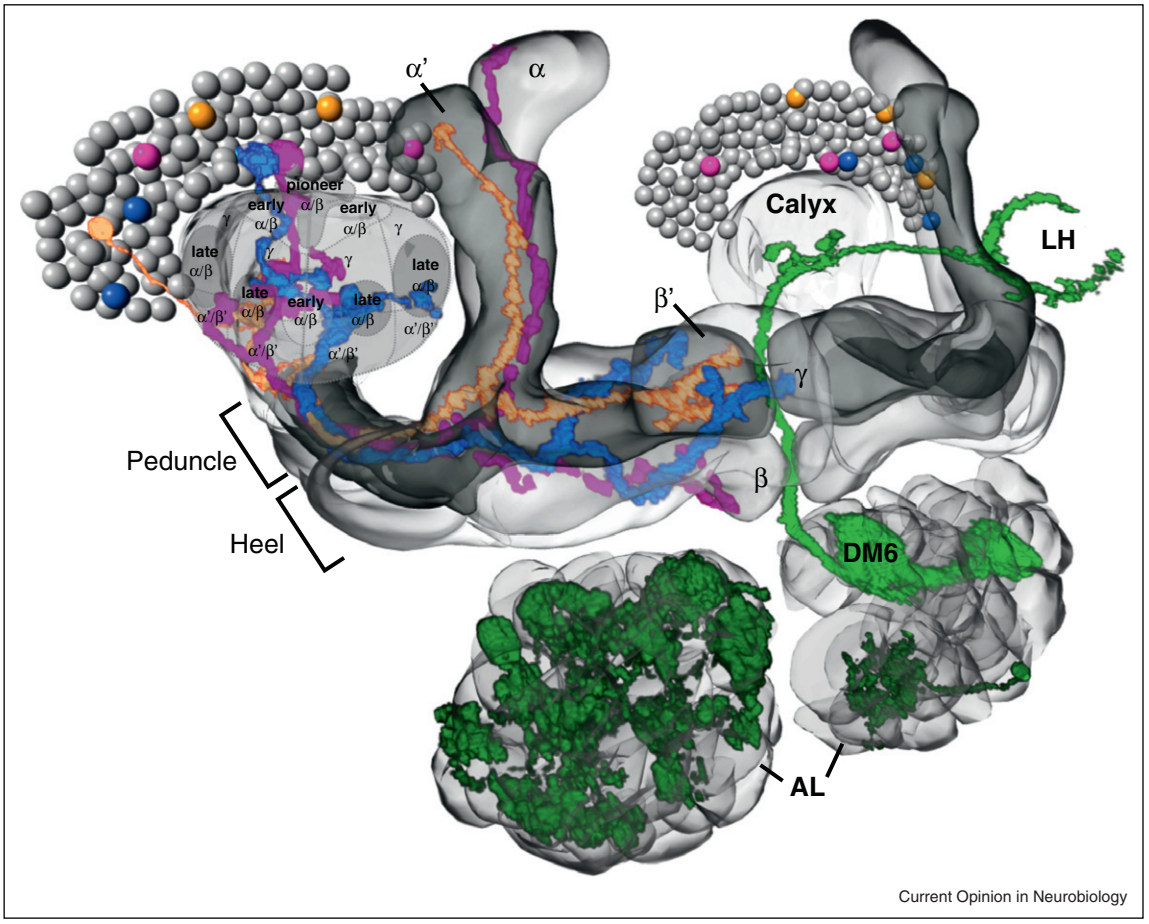
**Figure 1**




Sparse odor coding in the MB. The figure shows frontal-lateral view of ALs and MBs containing representative KCs involved in odor information processing. In *Drosophila* olfactory system, odor sensory neurons synapse with AL PNs within glomerulus, where odor information is modulated by a diversity of AL local neurons. Odor representations in AL glomeruli are then relayed by specific PNs, such as DM6 PNs (light green), to distinct regions in the MB calyx and lateral horn (LH). The MB is composed of approximately 2500 small Kenyon cells (KCs), derived from sequentially born γ (blue), α′/β′ (yellow) and α/β (magenta) neurons from 4 neuroblasts. The KC dendritic fields are spatially segregated into 17 complementary domains according to their neuroblast clonal origins and birth orders. Electrophysiological recordings and calcium imaging show that, unlike PNs, individual KCs have highly odor-specific responses and odors are represented by robust sparse coding in small subset of KCs.

CS+ odor. This change is read out by MB extrinsic neurons that lie downstream. But this biochemical model cannot account for each of the kinetically and mechanistically distinct memory 'phases' that have been documented in flies.

## Genetically distinct memory phases

Aversive olfactory memory in flies consists of several distinct underlying mechanisms that act both in parallel and in sequence. Even memory performance measured within a few minutes after training involves more than one signaling pathway. This is illustrated by comparing the magnitude of performance defects observed with null alleles in various genes in this pathway. Genetic disruptions of the presumed US inputs, for example mutations in DopR or in G-proteins that lie immediately downstream are sufficient to completely eliminate detectable short-term and long-term memory performance [15••,16,18,19]. These effects are as severe as complete ablation of the MB structure [20]. This dramatic effect with disrupting the US input pathway is starkly contrasted by all other memory and learning mutations that have been identified [6,9,10,21]. Most of these mutations yield quantitative reductions in performance, but do not eliminate performance measured immediately after training. Even null mutations in the core cAMP signaling components such as *rut* yield only partial reductions in performance. The situation gets even more complex when one considers the evolving genetic requirements during consolidation. For instance, memory performance measured between one and 3 hours after training can be dissected into components that are anesthesia resistant (ARM) and anesthesia sensitive (ASM). ARM and ASM are also genetically distinct because mutations in *radish*



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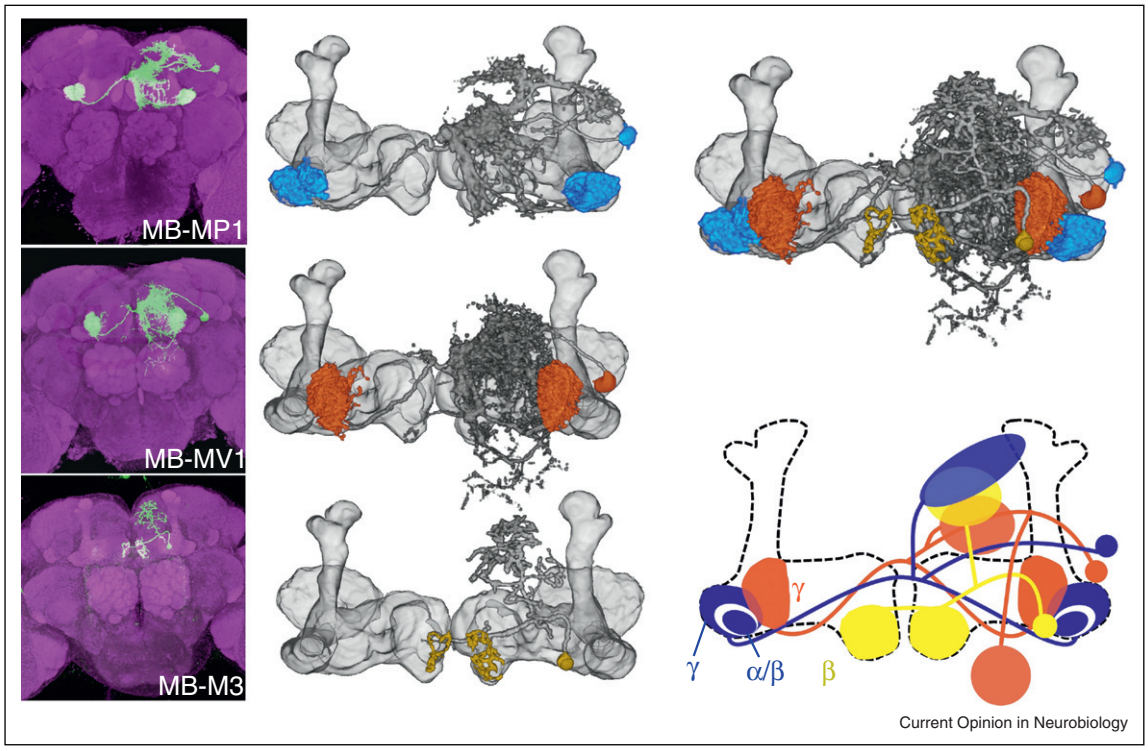
**Figure 2**




Dopamine-MB circuits involving in odor-shock associative memory. Three dopaminergic neurons, MB-MP1 (blue), MB-MV1 (orange), and MB-M3 (yellow), have been shown to relay aversive US signals to MB. MB-MP1 and MB-MV1 neurons are neighbors located within PPL1 cluster. MB-MP1 axons innervate MB heel and MB-MV1 axons innervate middle segment of MB γ lobe, respectively. MB-M3 neurons belonging to PAM cluster project their axonal terminals in the β-lobe tip. Dendrites of all three dopaminergic neurons are widely distributed in the dorsal- frontal-medial protocerebrum.

disrupt the former while mutations in *amnesiac* disrupt the latter [9,10].

Although *radish*-dependent ARM is resistant to anesthetic experimental disruption, this memory is not 'consolidated' by most definitions because its formation is independent of CREB-mediated gene expression and decays within 24 hours [22]. Consolidation of long-term memory (LTM) in this paradigm requires repeated training sessions with a rest interval between each session. In contrast to *radish*-dependent ARM which is formed after a single training session as well as after multiple massed trainings without rest interval, the LTM that forms after spaced training is sensitive to pharmacological inhibition of protein synthesis [22], requires CREB-mediated gene transcription [23], and can last for up to one week. Thus memory consolidation involves several different cellular mechanisms acting both sequentially and in parallel. Hypothetically, these could all act within the same set of MB neurons where the initial coincidence detection takes place. But investigation of the underlying neuroanatomical substrates demonstrates that this is not the case.

## Systems consolidation

In *Drosophila*, the ease of restricting genetic manipulations to specific and reproducible neuronal cell-types has provided a unique ability to investigate circuit mechanisms. These findings, which we review next, reveal the presence of two different feedback loops for memory consolidation.

### Distinct functions of different subsets of MB neurons

MBs consist of three morphologically and functionally distinct cell types. All three of the major sub-types of KCs send dendritic projections to the calyx, where the olfactory inputs are received. The axons of the three major KC classes bifurcate into several distinct subsets of lobes (Figure 1). The α/β neurons send two axon branches, one that extends vertically and one horizontally projecting to the α and β lobes respectively. α′/β′ neurons send axons to the vertical α′ and horizontal β′ lobes. And γ lobe neurons have both branches extending horizontally to form the enlarged γ lobe.

Reversible manipulation of neural activity with the 'shibire approach' (which makes use of a temperature sensitive dynamin protein) reveals distinct temporal requirements



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for neuro-transmission within each of these MB cell types. Neurotransmission in α′/β′ neurons is required during acquisition and shortly afterwards, but not during retrieval [24,25]. α′/β′ neurons play a key role in encoding the memory trace and in its early stabilization, but are not the site of storage because transmission is dispensable during retrieval. By contrast, output from the combination of α/β and γ lobes is not needed during acquisition or early storage [26–28]. Retrieval, on the other hand, is disrupted when transmission in α/β neurons is blocked, consistent with the view that information is stored either within or upstream of α/β [24,27,29]. But this idea is at odds with findings from transgenic rescue experiments, which map the odor–shock coincidence detection to γ lobe neurons rather than α/β [15••].

DopR is thought to mediate the US inputs to MB, and null mutations in DopR eliminate memory performance at all time-points after training [15••,16]. DopR function for this form of memory appears to be required only in MB because expression of DopR only in MB can fully rescue the STM [16], ARM and LTM [15••] defects of the mutants. More surprisingly, expression just in the γ lobe neurons is sufficient to fully restore normal memory performance to DopR mutants at each retention interval after training [15••]. Thus the relevant DA signals onto DopR occur solely in the γ lobe. Though α/β output plays a critical role in retrieval [24,27,29], DA inputs to DopR expressed in γ neurons are sufficient to encode memory [15••]. These findings are hard to explain with a simple model in which coincidence detection occurs in γ lobe followed by intracellular signaling leading to biochemical consolidation within these same neurons. Some clues come from manipulations of individual DA inputs to MB.

Three different DA neurons have been shown to play important roles (Figure 2), and interestingly they each project to distinct sub-regions of the MB lobes [12•,13,30•,31•,32]. MB-MP1 and MB-MV1 neurons reside nearby to each other in the PPL1 cluster of DA neurons. MB-MP1 axons innervate the MB heel region, which provides an opportunity for contacts with both γ and α/β neurons, whereas MB-MV1 neurons send axons to a defined region of the γ lobe. MB-M3 resides in the PAM cluster and sends axons to the β lobe. With all three of these neurons, experiments have been performed in which neurotransmission is blocked, using the shibire approach, or stimulated using a temperature sensitive TrpA1 channel in an attempt to substitute for the aversive US treatment. In each case, there is evidence for a role in delivering relevant information to MB. For the cases of MB-MP1 and MB-MV1, the findings are consistent with the idea that DopR is the receptor because these neurons can contact γ lobe neurons where DopR expression is sufficient. But the effects of MB-M3, which appear to contact the β lobe, cannot easily be interpreted in the context of DopR, which is solely required in γ lobe. Though this issue is not fully understood, there are several interesting possibilities. One hint comes from the fact that MB-M3 neurons do not impact memory measured 2-min after training, but instead impact ASM measured 2-hours later [12•]. In fact, several studies have implicated DA inputs to MB in modulating consolidation and/or forgetting, perhaps via an ongoing synchronized activity [30•,31•]. One possibility is involvement of another DA receptor such as DAMB [31•] in α/β neurons to modulate consolidation or forgetting rather than acquisition.

The requirement for α/β neurons for memory consolidation also is supported by genetic rescue of signaling thought to lie downstream of DopR. Unlike DopR, *rut* expression in γ lobe neurons is only sufficient to rescue the STM defect of *rut* mutants. Rescue of the LTM defects of *rut* mutants requires additional expression in α/β lobes [33]. Taken together, the reversible manipulations of neural activity described above and the cell-type specific genetic manipulations support a model in which activity in α′/β′ neurons drives formation of the initial memory trace in γ lobes followed by signaling in α/β lobes to support memory consolidation. This model also dovetails with a series of functional imaging studies that reveal associative changes in calcium responses in all three subsets of MB neurons, with an early traced observed in α′/β′ neurons and a later trace observed in α/β and γ lobes [25,34,35].

## An information processing loop within MB

The idea that the odor–shock association forms in γ lobes but requires signaling in α/β lobes for consolidation invokes a need for communication between MB neuron cell types. There are three direct functional observations that support this notion. First is the observation that ongoing activity in α′/β′ neurons is required to maintain memory during the first hour or so after training [24]. Second, is the requirement for ongoing activity in both APL [36••,37••] and DPM [6] neurons. APL and DPM are large, electrically coupled neurons with fibers extending throughout all MB lobes (Figure 3), providing connections to thousands of KCs. The APL and DPM neurons are both odor generalists that respond even in untrained animals to all tested odors [36••]. Moreover, the heterotypic gap junctions that couple the APL and DPM neurons are essential [36••]. These data have led to a model in which recurrent activity in an α′/β′ KC–DPM–APL–KC loop is required to stabilize intermediate-term ASM formed in the α/β KCs. ARM formation may also require such an intra-MB persistent activity because ARM requires 5HT released from DPM neurons acting onto d5HT1A receptors in the α/β neurons [38].

## Beyond MB

Cell type specific genetic manipulations of KCs, DA input neurons and the APL–DPM neurons yield a model



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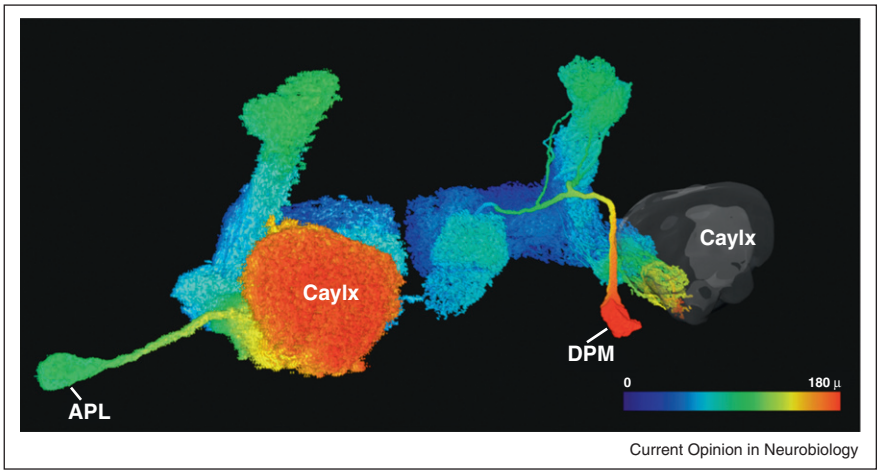
**Figure 3**




Innervation patterns of two MB modulatory neurons. The anterior paired lateral (APL) neurons innervate the entire MB including calyx. The dorsal paired medial (DPM) neurons innervate all MB lobes and anterior half of the peduncle but not calyx. Posterior view of one APL neuron (left) and one DPM neuron (right) are shown. Spatial distribution is represented by a depth code from anterior (blue) to posterior (red).

in which persistent communication between MB neuron sub-types is required to support maintenance of memory over the first few hours after training. The preponderance of evidence supports the conclusion that the initial CS–US association occurs in γ [15••], but over time comes to rely on signaling within α/β neurons as well [24,27,29,33,34,39]. LTM in particular relies heavily on α/β neurons. Formation of LTM requires *rut* function within α/β neurons [40], LTM retrieval requires neurotransmission both from α/β neurons [29] and a MB α/α′ efferent neuron, MB-V2 [39]. In addition, the formation of LTM is correlated with changes in MB calcium responses to the conditioned odor in both α/β and γ neurons [34,35]. Moreover, this effect in α/β neurons is abolished in each of 26 mutants with defective LTM [41,42]. Despite the above evidence that MB α/β neurons play an important role in LTM, there also is now clear evidence that the MB-APL/DPM-MB feedback loop is only part of the circuit required for consolidation. The main evidence comes from identification of the site of CREB-dependent *de novo* gene expression that underlies LTM formation.

A search for the neural substrate of new gene expression has lead to identification of dorsal–anterior–lateral (DAL) neurons, rather than MB, as a relevant site of CREB mediated transcription [43••]. This conclusion rests on convergent findings from multiple lines of experiment. First, was the use of a temperature-sensitive ribosome-inactivating toxin Ricin$^{cs}$, to acutely inhibit protein synthesis. Surprisingly, LTM was impaired when protein synthesis was inhibited in two DAL neurons, but not when it was inhibited in MB. Second, disruption of CREB-mediated transcription in DAL neurons by acute induction of a dominant negative CREB-blocker is sufficient to disrupt formation of LTM, but not ARM. In contrast, acute expression of the same CREB-blocker transgene in MB does not disrupt LTM. Third, direct visualization of *de novo* protein synthesis with a photo-convertible fluorescent protein reveals a CREB and spaced training dependent up-regulation in DAL of the reporter expression. Again, such induction is not observed in MB.

These findings do not rule out the possibility that some relevant gene expression occurs in MB [43••,44]. If it occurs, however, it falls below detection with all of these methods (in contrast with DAL). Rather, the conclusion is that there is a clear requirement for CREB-mediated transcription outside of MB, and there currently is no evidence for such a requirement within MB. Despite this, the importance of MB as a substrate for LTM is clear from all of the other evidence in the literature.

Indeed the DAL neurons appear to send inputs to a subset of MB neurons because DAL axonal terminals are detected in the K5 subregion of the calyx, which contains dendrites of the pioneer subset of α/β neurons [43••]. DAL dendrites are detected in the dorsal frontal protocerebrum (Figure 4). The fact that DAL neurotransmission is required for LTM retrieval but not for consolidation supports the conclusion that DAL is part of the storage system rather than part of a network that is only involved in consolidation or encoding. Thus it makes sense that DAL neurons are ‘upstream’ of MB, which is the place where odor coding is thought to take place. Although the inputs to DAL are less clear, DAL is downstream of MB in a functional sense because CREB mediated function is presumably driven by the signaling



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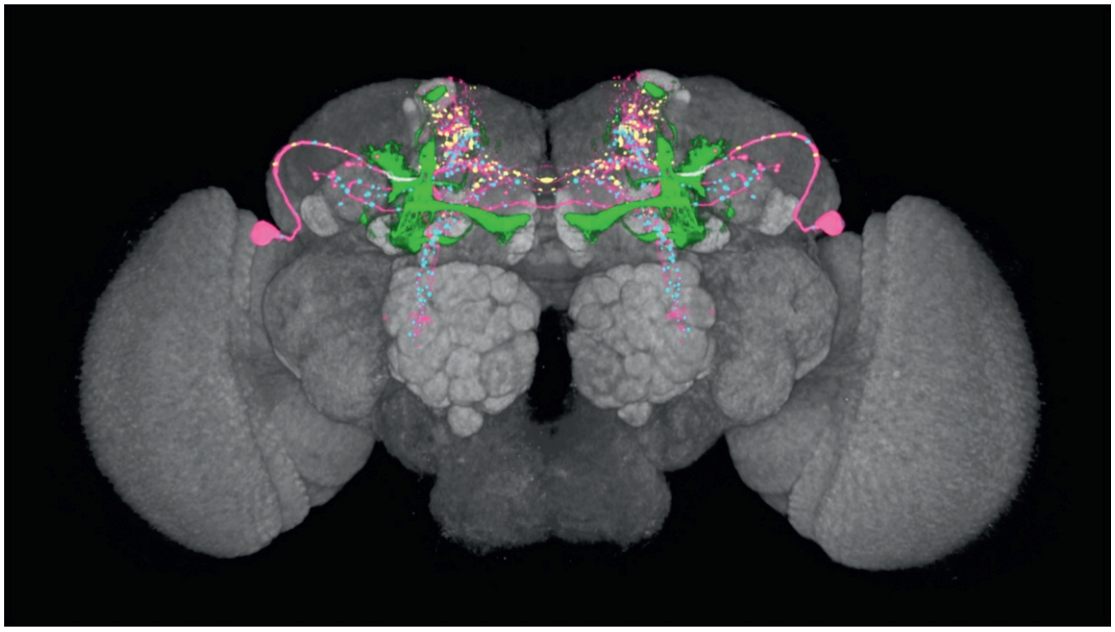
**Figure 4**




Polarity and connectivity of DAL neurons. New protein synthesis for LTM occurs in the two dorsal–anterior–lateral (DAL) neurons (magenta) with axonal terminals (light blue) linking to pioneer α/β neurons (green) at the K5 dendritic field (white) in the calyx. The DAL neuron may receive inputs from dorsal frontal protocerebrum where it has numerous dendrites (yellow). The brain is counterstained with anti-disc large immunostaining (grey).

that occurs in MB. Together, these data suggest an MB–DAL–MB feedback loop for LTM. The anatomical link from MB to DAL remains to be established, but it is tempting to speculate the involvement of ellipsoid body R4 m neurons, which also are required for LTM retrieval [45].

## Towards an integrated model

Biochemical and systems consolidation need to be conceptually integrated. In the case of aversive olfactory memory in flies, there is strong evidence that the initial CS–US association occurs in the γ KCs, and later comes to rely more heavily on signaling in α/β KCs. This process appears to involve at least two feedback loops. The first, which is required for maintenance and consolidation of ASM and ARM over the first several hours, involves a persistent activity in MB α′/β′ neurons as well as in the APL–DPM neurons that are electrically coupled and positioned to communicate between all of the different classes of MB intrinsic neurons. The second feedback loop is required for CREB-dependent LTM and involves DAL neurons, which are functionally downstream of MB and anatomically upstream of the pioneer α/β MB neurons. It will be important to investigate the functional relationship between these two feedback loops for maintenance of labile memory versus formation of consolidated memory. Several recent studies also provide evidence for both biochemical and circuit mechanisms governing forgetting [31,46] and the maintenance of a sensory buffer during trace conditioning [47]. Thus it will be of interest to determine whether or not persistent activity via these feedback mechanisms also impacts these features of memory processing. Finally, it is important to note that a simple model that relies on synaptic plasticity in the efficacy of two DAL neurons is clearly insufficient to store or retrieve memory of a complex sensory stimulus such as an odor. Rather, plasticity in synaptic function must occur within the context of an ensemble of neurons to alter information flow through a neural circuit that changes behavioral outcomes.

## Acknowledgements

We are grateful to Tsung-Pin Pai, Chun-Chao Chen, Jie-Kai Wu and Wanhe Li for assistance and helpful discussions on the manuscript. We regret that – because of space constraints – not all papers contributing to the advancement in the field could be cited. JD receives funding support from DART LLC and from NIH (TR01 5R01NS067690-03). ASC receives funding support from DART LLC and Taiwan's National Science Council.

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