

Parallel processing of olfactory memories in *Drosophila*

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One of the hallmarks of both memory and the underlying synaptic plasticity is that they each rely on short-lived and longer-lived forms. Short-lived memory is thought to rely on modification to existing proteins, whereas long-term memory requires induction of new gene expression. The most common view is that these two processes rely on signaling mechanisms within the same neurons. We recently demonstrated a dissection of the signaling requirements for short and long-lived memory into distinct sets of neurons. Using an aversive olfactory conditioning task in *Drosophila*, we found that cAMP signaling in different neuron cell types is sufficient to support short or long-term memory independently.

A highly conserved feature of memory storage is a process called consolidation, in which an initially labile trace becomes progressively stabilized. Soon after a learning experience, memories are robust in terms of performance levels of an animal, but are easily disrupted by experimental perturbation. Over time, following a behavioral experience, memories can be consolidated into a form that is resistant to experimental manipulation.¹⁻⁴ At the cellular level, the synaptic plasticity that is believed to underlie memory also has been dissected into short, intermediate and long-term forms.^{5,6} Short-term plasticity generally involves trafficking or modification of pre-existing synaptic proteins that rapidly alter synaptic strength, but these changes decay away on the timescale of minutes to hours. In contrast, long-lasting forms of synaptic plasticity, as well as long-term memory, involve recruitment of newly synthesized

proteins both through local translation of existing mRNAs and through an induced cascade of CREB (cyclic-AMP-responsive-element-binding-protein)-dependent gene expression.^{5,7-9} This cellular model has not been fully integrated with findings from neuroanatomical studies.

At the level of neural circuitry, different temporal phases of memory storage have also been experimentally dissected and an emerging theme is that anatomical regions involved in long-term memory can be distinct from those whose function is required immediately after or during a learning task.¹⁰⁻¹⁴ Such anatomical lesion experiments have suggested a circuit level reorganization of memory storage over time after the trace is established.

While both the biochemical/cellular and circuit level views of memory consolidation suggest mechanistic differences between short and long-term memory, the cellular view is consistent with the idea that both short- and long-lived modifications occur sequentially in the same set of neurons. In this model, cellular mechanisms of coincidence detection, for instance through Ca²⁺ responsive adenylyl cyclase or NMDA (N-methyl d-aspartate receptor) receptors,¹⁵⁻¹⁹ set in motion both short-term and more stable forms of synaptic plasticity. The former involves local signaling at the synapse, and the latter involves signaling to the nucleus through CREB-mediated transcription.^{1,6,9,20,21} In contrast with this “biochemical” consolidation, anatomical lesions suggest a dissection of temporal phases of memory into different circuits.¹⁰⁻¹⁴ We recently investigated the relationship between the biochemical/cellular and the neural circuit models of memory consolidation using

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olfactory aversive conditioning in flies as a model.^{22,23}

Olfactory Memory in Mushroom Bodies

In insects, a brain center called the mushroom bodies (MBs), has been shown to play a key role in olfactory memory and learning.²⁴⁻²⁷ A large body of evidence supports a model in which cAMP signaling in MB neurons is sufficient to support olfactory memories. In this widely accepted model, the MBs receive multi-modal inputs including both olfactory information via acetylcholinergic projection neurons and neuromodulatory inputs that likely convey the unconditioned stimuli (US)—dopamine in the case of electric shock aversive reinforcement.²⁸⁻³⁴ The *rutabaga* adenylyl cyclase is widely thought to play a key role within MB as a coincidence detector for the association of these two stimuli because it can be synergistically activated by Ca²⁺ (driven by odors) and by G-coupled receptor signaling (Dopamine receptor in the case of electric shock mediated aversive conditioning).¹⁵ In this model, the activation of *rutabaga*-mediated cAMP signaling causes short-term changes in synaptic strength within the MB neurons, and also can induce a CREB-mediated transcriptional response in the MB neuron nuclei. This CREB-transcriptional cascade then is thought to result in stabilization of learning-driven synaptic changes that were formed earlier.³⁵ But several observations in the literature suggested to us that this model represents an oversimplification. First, *rutabaga* null mutants still exhibit appreciable levels of learning. In fact, the performance levels of *rutabaga* mutants are about 50% that of the wild type.²² This in itself suggests that other forms of plasticity, not dependent on *rutabaga*, also are capable of supporting this type of association. A second observation that did not easily fit within the above model is the requirement for NMDA-receptor function for long-term memory within a subset of neurons in the ellipsoid body (EB), a different neural center from MBs.³⁶ This finding suggested the possibility that information might be transferred out of MBs and into EBs. At a minimum, it suggests a more complex and

dynamic circuit requirement. Finally, differing requirements for signaling within distinct cell-types of the mushroom bodies themselves suggested a more complex model. Most striking is the observation that *rutabaga* signaling within one subset of MB neurons is sufficient for short-term memory.³⁷ In contrast, disruption of normal CREB function in a different subset of MB neurons was shown to inhibit expression of long-term memory.³⁸ Finally, reversible manipulations of neural activity within MBs suggests a dynamic and evolving requirement for neurotransmission in different subsets of these neurons.³⁹⁻⁴³

We recently cleared up some of this confusion by examining the requirements for *rutabaga*-dependent signaling within each of the major MB neuron cell types both for short and long-term memory.²² The findings support the surprising hypothesis that flies rely on two parallel subsets of MB neurons to store short- and long-term memory respectively.

Parallel Memories in Parallel Groups of Mushroom Body Neurons

MBs of the fly consist of approximately 2,500 neurons on each side of the brain.⁴⁴⁻⁴⁷ These neurons have a striking organization—their cell bodies are packed into a dorsal posterior region, their dendrites occupy a common field called the calyx and their axons form a bundle called the peduncle, which then bifurcates to form five lobed structures that contain the axon terminals (Fig. 1A). It has been known for some time that MB neurons are made up of three major cell types whose axon branches are restricted to subsets of these five lobes.⁴⁴ The α/β neurons have two branches, one of which projects medially into the β lobe and the other vertically into the α lobe.^{46,47} The α'/β' neurons similarly have two branches that occupy the vertical α' and the horizontal β' lobes. The γ neurons have an un-branched axon that occupies the horizontally oriented γ lobe. Previous work had established that expression of *rutabaga* in just the γ lobe neurons is sufficient to restore nearly normal levels of performance to an otherwise *rutabaga* null animal.³⁷ We recently used the same approach to restrict expression of

rutabaga to each of the three major classes of MB neurons α/β , α'/β' and γ neurons.²² In each case we measured memory at different time-points after a standard aversive Pavlovian olfactory task.

Consistent with previous reports, we found that expression in γ lobe neurons is sufficient to restore short-term memory to nearly normal levels. Expression in either α/β or α'/β' neurons did not restore short-term memory to the *rutabaga* mutants. The results with long-term memory were more surprising. In this case, we used a repetitive spaced training protocol to induce CREB-dependent long-term memory and then measured memory retention 24 hours later. Interestingly, expression in γ lobe neurons provided no restoration of long term memory performance to the *rutabaga* mutants, even though this pattern of expression is sufficient to fully restore learning to mutant animals. However, expression in α/β neurons was sufficient to significantly restore long-term memory, which is particularly surprising because this expression does not improve short-term memory. This reciprocal outcome with γ versus α/β lobe *rutabaga* expression supports the hypothesis that *rutabaga* functions in both places, but for different temporal stages of memory. Our findings are consistent with the established model in which *rutabaga* functions during the learning experience as a coincidence detector in γ lobe neurons.¹⁵ In addition, we propose that a second form of plasticity is induced in α/β neurons. Induction of this second plasticity mechanism is likely *rutabaga* independent, which would explain the residual performance observed in *rutabaga* mutants. In our model, *rutabaga* function in α/β neurons is needed to consolidate this second memory 'trace' via induction of CREB-dependent signaling (Fig. 1B). This notion is consistent with the observation that expression of a CREB-repressor within the α/β neurons can inhibit long-term memory.³⁸

The observation that different temporal stages of memory can rely on distinct brain regions has been well documented with a variety of species and tasks. Our recent findings in *Drosophila* take advantage of the ability to genetically manipulate not only specific brain regions, but also individual cell types within a region. This

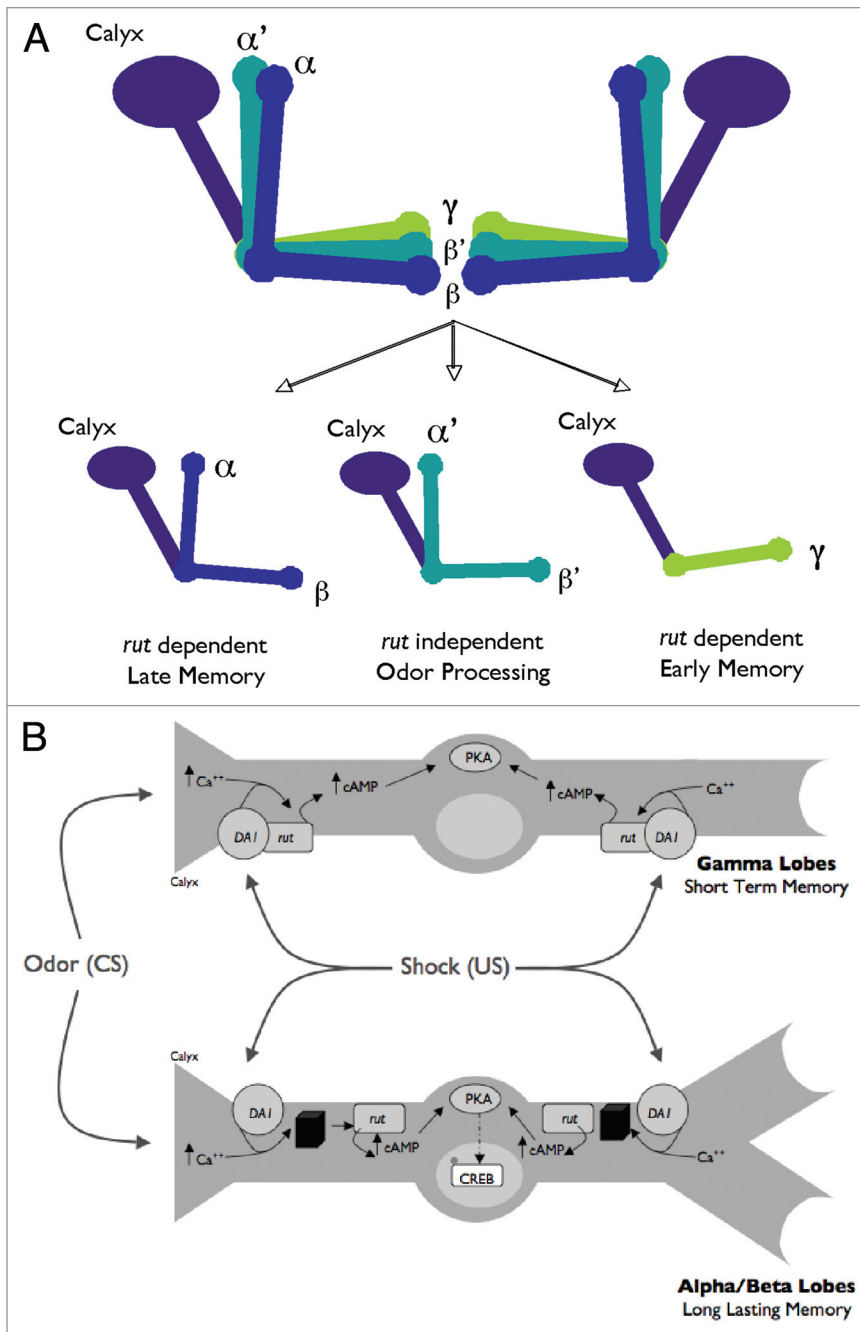


Figure 1. Parallel processing of short and long term memories in *Drosophila* MB. (A) MBs in *Drosophila* consist of approximately 2,500 neurons per brain hemisphere.^{45,46} The MB neurons send dendrites to a field called the Calyx. MB axons form a bundle that then bifurcates into 5 lobed structures. The 5 lobes are made up of 3 main cell types.^{44,46} α'/β' neurons and α/β neurons each contain two main branches that occupy the α'/β' or α/β lobes respectively. γ lobe neurons have an unbranched axon that constitutes the γ lobe. We recently demonstrated²² that expression of the *rutabaga* adenylyl cyclase in the γ lobe neurons is sufficient to support short-term, but not long-term memory. In contrast, long-term but not short-term memory can be supported with *rutabaga* expression in α/β neurons. In contrast with α/β and γ neurons, α'/β' does not appear to require *rutabaga*-dependent plasticity. Instead, these neurons likely play some role in odor encoding during training that is necessary to lay down a memory in the other MB neurons.⁴⁰ (B) These findings suggest a model in which two parallel associations are formed during training. In γ neurons, odor signaling (CS) causes increases in Ca⁺⁺ levels, while shock (US) is relayed through the dopamine receptor (DA1). These two signals activate *rutabaga* which serves as a coincidence detector between these two stimuli. This association forms rapidly and decays rapidly. In contrast, a second association in α/β neurons is *rutabaga*-independent with odor and shock information converging on an unknown protein (black box). This association can then be converted to a long-term memory by a process that requires *rutabaga* function and downstream signaling through CREB in α/β neurons.²²

approach reveals a level of sub-specialization of neural circuit function to support distinct features of memory formation and storage. In particular, the findings support the hypothesis that the cellular underpinnings of short and long-term memory can occur in different sets of neurons.

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