Fly 4:2, 163-166; April/May/June 2010; © 2010 Landes Bioscience

Parallel processing of olfactory memories in Drosophila

Allison Blum[1,3] and Joshua T. Dubnau[2,*]

[1]Watson School of Biological Sciences; [2]Cold Spring Harbor Lab; Cold Spring Harbor, NY USA

[3]Current address: NYU Medical Center; Skirball Institute; USA;

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.

Key words: Drosophila, memory, learning, mushroom bodies, cAMP, *rutabaga*, short-term memory, long-term memory

Submitted: 12/24/09

Revised: 01/25/10

Accepted: 02/08/10

Previously published online: www.landesbioscience.com/journals/fly/article/11445

*Correspondence to: Joshua T. Dubnau; Email: dubnau@cshl.edu

Commentary to: Blum AL, Li W, Cressy M, Dubnau J. Short- and long-term memory in Drosophila require cAMP signaling in distinct neuron types. Curr Biol 2009; 19:1341–50; PMID: 19646879; DOI: 10.1016/j.cub.2009.07.016.

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 have 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 Ca^{++} 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

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 Ca^{++} (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

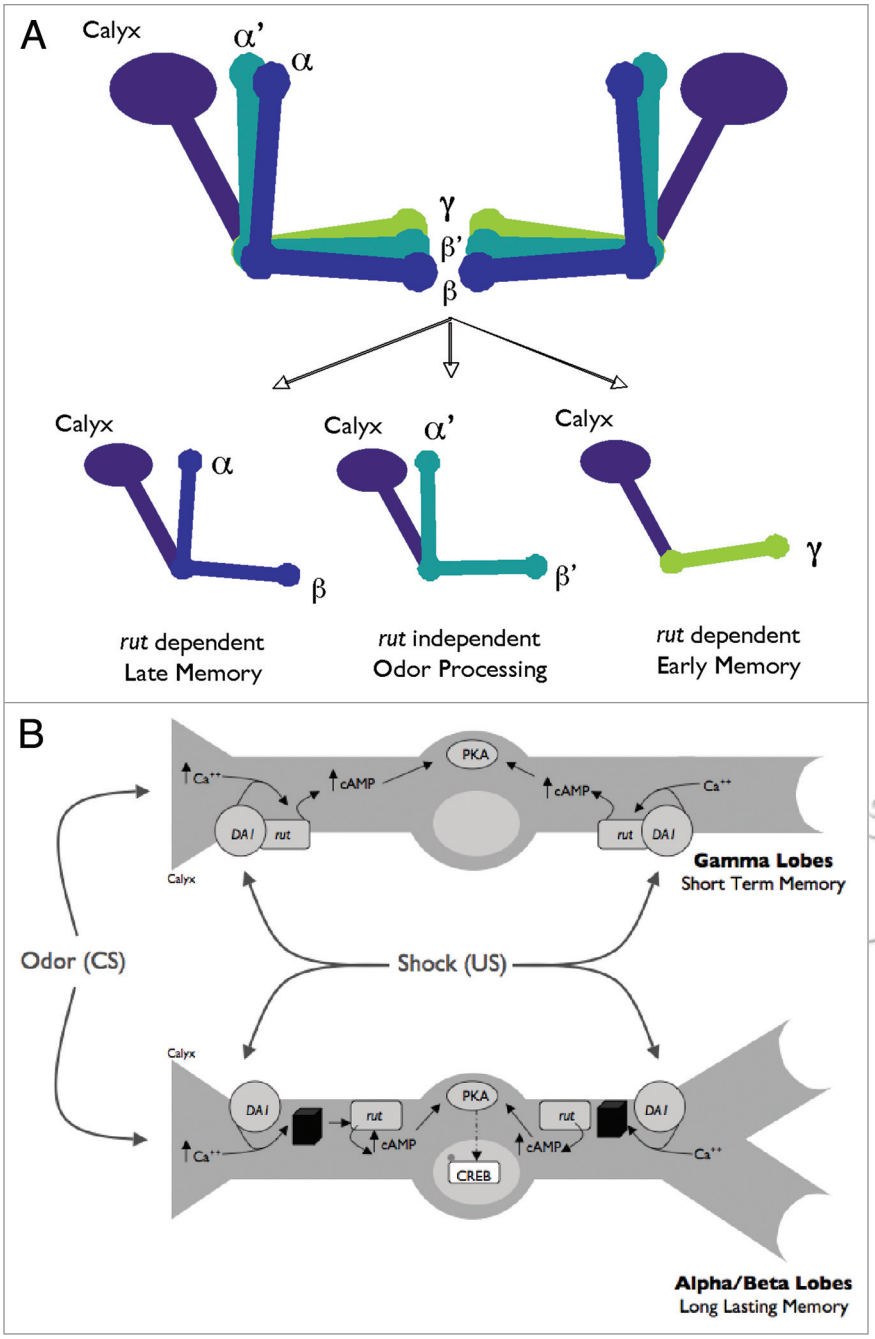

Figure 1. Parallel processing of short and long term memories in Drosophila MB. (A) MBs in Drosophila consist of approximately 2,500 neurons per brain hemisphere.[45,46] The MB neurons send dendrites to a field called the Calyx. MB axons form a bundle that then bifurcates into 5 lobed structures. The 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 demonstrated[22] 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.

References

1. Alberini CM. Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? Trends Neurosci 2005; 28:51-6.
2. Dubnau J, Tully T. Gene discovery in Drosophila: new insights for learning and memory. Annu Rev Neurosci 1998; 21:407-44.
3. Hawkins RD, Kandel ER, Bailey CH. Molecular mechanisms of memory storage in Aplysia. Biol Bull 2006; 210:174-91.
4. Rose SP. God's organism? The chick as a model system for memory studies. Learn Mem 2000; 7:1-17.
5. Pittenger C, Kandel E. A genetic switch for long-term memory. C R Acad Sci III 1998; 321:91-6.
6. Carew TJ, Sutton MA. Molecular stepping stones in memory consolidation. Nat Neurosci 2001; 4:769-71.
7. Yin JC, Tully T. CREB and the formation of long-term memory. Curr Opin Neurobiol 1996; 6:264-8.
8. Alberini CM. Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 2009; 89:121-45.
9. Frank DA, Greenberg ME. CREB: a mediator of long-term memory from mollusks to mammals. Cell 1994; 79:5-8.
10. Patterson TA, Rose SP. Memory in the chick: multiple cues, distinct brain locations. Behav Neurosci 1992; 106:465-70.
11. Frankland PW, et al. Stability of recent and remote contextual fear memory. Learn Mem 2006; 13:451-7.
12. Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions 1957. J Neuropsychiatry Clin Neurosci 2000; 12:103-13.
13. Milner B. Some cognitive effects of frontal-lobe lesions in man. Philos Trans R Soc Lond B Biol Sci 1982; 298:211-26.
14. Gilbert DB, Patterson TA, Rose SP. Dissociation of brain sites necessary for registration and storage of memory for a one-trial passive avoidance task in the chick. Behav Neurosci 1991; 105:553-61.
15. Tomchik SMaD, RL. Dynamics of Learning-Related cAMP Signaling and Stimulus Integration in the Drosophila Olfactory Pathway. Neuron 2009; 64:510-21.
16. Ferguson GD, Storm DR. Why calcium-stimulated adenylyl cyclases? Physiology (Bethesda) 2004; 19:271-6.
17. Tsien JZ. Linking Hebb's coincidence-detection to memory formation. Curr Opin Neurobiol 2000; 10:266-73.

18. Mons N, Guillou JL, Decorte L, Jaffard R. Spatial learning induces differential changes in calcium/calmodulin-stimulated (ACI) and calcium-insensitive (ACII) adenylyl cyclases in the mouse hippocampus. Neurobiol Learn Mem 2003; 79:226-35.
19. Xia Z, Storm DR. Calmodulin-regulated adenylyl cyclases and neuromodulation. Curr Opin Neurobiol 1997; 7:391-6.
20. Stough S, Shobe JL, Carew TJ. Intermediate-term processes in memory formation. Curr Opin Neurobiol 2006; 16:672-8.
21. Martin KC, et al. Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. Cell 1997; 91:927-38.
22. Blum AL, Li W, Cressy M, Dubnau J. Short- and long-term memory in Drosophila require cAMP signaling in distinct neuron types. Curr Biol 2009; 19:1341-50.
23. van Swinderen B. Fly memory: a mushroom body story in parts. Curr Biol 2009; 19:855-7.
24. Gerber B, Tanimoto H, Heisenberg M. An engram found? Evaluating the evidence from fruit flies. Curr Opin Neurobiol 2004; 14:737-44.
25. McGuire SE, Deshazer M, Davis RL. Thirty years of olfactory learning and memory research in *Drosophila melanogaster*. Prog Neurobiol 2005; 76:328-47.
26. Keene AC, Waddell S. Drosophila olfactory memory: single genes to complex neural circuits. Nat Rev Neurosci 2007; 8:341-54.
27. Margulies C, Tully T, Dubnau J. Deconstructing memory in Drosophila. Curr Biol 2005; 15:700-13.
28. Krashes MJ, et al. A neural circuit mechanism integrating motivational state with memory expression in Drosophila. Cell 2009; 139:416-27.
29. Claridge-Chang A, et al. Writing memories with light-addressable reinforcement circuitry. Cell 2009; 139:405-15.
30. Selcho M, Pauls D, Han KA, Stocker RF, Thum AS. The role of dopamine in Drosophila larval classical olfactory conditioning. PLoS One 2009; 4:5897.
31. Honjo K, Furukubo-Tokunaga K. Distinctive neuronal networks and biochemical pathways for appetitive and aversive memory in Drosophila larvae. J Neurosci 2009; 29:852-62.
32. Keene AC, Waddell S. Drosophila memory: dopamine signals punishment? Curr Biol 2005; 15:932-4.
33. Schwaerzel M, et al. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in Drosophila. J Neurosci 2003; 23:10495-502.
34. Kim YC, Lee HG, Han KA. D1 dopamine receptor dDA1 is required in the mushroom body neurons for aversive and appetitive learning in Drosophila. J Neurosci 2007; 27:7640-7.
35. Yin JC, et al. Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell 1994; 79:49-58.
36. Wu CL, et al. Specific requirement of NMDA receptors for long-term memory consolidation in Drosophila ellipsoid body. Nat Neurosci 2007; 10:1578-86.
37. Zars T, Fischer M, Schulz R, Heisenberg M. Localization of a short-term memory in Drosophila. Science 2000; 288:672-5.
38. Yu D, Akalal DB, Davis RL. Drosophila alpha/beta mushroom body neurons form a branch-specific, long-term cellular memory trace after spaced olfactory conditioning. Neuron 2006; 52:845-55.
39. Wang Y, Mamiya A, Chiang AS, Zhong Y. Imaging of an early memory trace in the Drosophila mushroom body. J Neurosci 2008; 28:4368-76.
40. Krashes MJ, Keene AC, Leung B, Armstrong JD, Waddell S. Sequential use of mushroom body neuron subsets during drosophila odor memory processing. Neuron 2007; 53:103-15.
41. Schwaerzel M, Heisenberg M, Zars T. Extinction antagonizes olfactory memory at the subcellular level. Neuron 2002; 35:951-60.
42. McGuire SE, Le PT, Davis RL. The role of Drosophila mushroom body signaling in olfactory memory. Science 2001; 293:1330-3.
43. Dubnau J, Grady L, Kitamoto T, Tully T. Disruption of neurotransmission in Drosophila mushroom body blocks retrieval but not acquisition of memory. Nature 2001; 411:476-80.
44. Lee T, Lee A, Luo L. Development of the Drosophila mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development 1999; 126:4065-76.
45. Heisenberg M. Mushroom body memoir: from maps to models. Nat Rev Neurosci 2003; 4:266-75.
46. Ito K, Awano W, Suzuki K, Hiromi Y, Yamamoto D. The Drosophila mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells. Development 1997; 124:761-71.
47. Ito K, Awasaki T. Clonal unit architecture of the adult fly brain. Adv Exp Med Biol 2008; 628:137-58.