Fly Memory: A Mushroom Body Story in Parts

Recent studies on the compartmentalization of fly mushroom bodies show that learning and memory in *Drosophila* are not as simple as might be expected for an organism with such a tiny brain.

Bruno van Swinderen

Aristotle was notorious for categorizing the world into parts, such as the "three kinds of virtue" or the "four species of movement". Researchers investigating brain functions such as learning and memory often seem to follow this didactic yet effective approach. Thus, we have different phases of memory — short-term, middle-term and long-term — and we try to map these onto different structures in the brain. A study by Blum *et al.* [1], published recently in *Current Biology*, has done this for the fruit fly *Drosophila melanogaster*. Since its beginnings three decades ago, research on learning and memory in *Drosophila* has faithfully followed the Aristotelian tradition of defining the component parts of memory. The first mutagenesis studies identified genes controlling different phases of memory acquisition and consolidation (reviewed in [2]). Notably, *rutabaga*, which encodes a calcium-dependent adenylyl cyclase, was found to modulate short-term memory and was eventually proposed as a site of coincidence–detection in neurons, where intracellular signals induced by the conditioned stimulus (CS), such as an odor, and the unconditioned stimulus (US), such as an electric shock, coincide biochemically to alter the properties of a neuron, thereby laying down a memory trace. But where in the fly brain is the enzyme encoded by *rutabaga* doing this?

If Aristotle had been able to see the internal structure of the fly brain, he would probably have claimed that the mushroom bodies carry out an important function that is divided into three parts (Figure 1A). The mushroom bodies are paired structures composed of around 2500 neurons each that receive synaptic input about odors via their 'calyx' (mushroom cap) near the top of a fly's head, and then project down and front along a 'peduncle' (mushroom stalk) before projecting into three different directions giving rise to the $\alpha\beta$, $\alpha'\beta'$and γ lobes. Such a division into distinct lobes (Figure 1B) suggested the segregation of distinct functions in the mushroom body, likely pertaining to the processing of odors.

In several landmark studies spanning a decade, Martin Heisenberg and colleagues established that the *Drosophila* mushroom bodies are necessary for olfactory learning in the T-maze (reviewed in [3]). This suggested that mushroom body intrinsic neurons act as the single 'container' for memory storage, akin to the simple sensory-motor system of the mollusc *Aplysia* [4]. Following these initial insights into mushroom bodies, a whole host of tools have been used to detect and dissect memory traces in the fly brain. There have been calcium-imaging studies [5,6], and studies in which the neurons in mushroom body substructures were transiently silenced during specific phases of the experiment [7,8], and 'rescue' experiments where *rutabaga* function was restored in the mushroom body lobes while left defective in the rest of the animal [9,10]. What emerged from these experiments was a complex, and at times even contradictory picture, in which both intrinsic and extrinsic components of various mushroom body subsystems were shown to be necessary or sufficient for different phases of memory formation and consolidation.

Currently, the most consistent story suggests that immediate odor memories require the γ lobes of the mushroom bodies, while longer-lasting memory is consolidated in $\alpha\beta$ lobes. Memory consolidation in the $\alpha\beta$ lobes probably requires interactions among the lobes as well as between $\alpha'\beta'$ neurons and mushroom body-extrinsic cells, thereby making all three mushroom body lobes necessary for some aspect of olfactory memory [5,6,8,9,11,12]. To tackle this story from another angle by systematically addressing where in the mushroom bodies *rutabaga* action is sufficient, Blum *et al.* [1] investigated *rutabaga* function in the three mushroom body subsystems (the $\alpha\beta$, $\alpha'\beta'$, and γ lobes) by using distinct combinations of Gal4 drivers to rescue *rutabaga* mutants — by driving wild-type gene expression from a Gal4-controlled upstream activating sequence (UAS) in particular sites defined by the regulatory elements that control where *GAL4* is expressed in the fly — and then tested these different classes of flies either immediately after training (short-term memory, STM), or 3 hours after training (middle-term memory, MTM), or 24 hours after training (long-term memory, LTM).

The results of these experiments confirm the complexity of our current understanding of memory formation in the fly: during training, a short-lived, *rutabaga*-dependent memory (presumably involving G-protein and cAMP signalling) is formed in the γ lobes [9], while a distinct coincidence system (possibly also involving G-protein signalling [6]) originates in the $\alpha'\beta'$ lobes [8]. Memory is consolidated in parallel in the $\alpha\beta$ lobes for longer-term effects that again require *rutabaga* (Figure 1C). The recental finding of Blum *et al.*'s [1] study is that STM and LTM are functionally independent and require different neural circuits. Thus, more than two decades after the mushroom bodies were first suggested as the location where olfactory memory is formed and stored, it has become clear that the mushroom bodies are far from the equivalent of a molluscan sensory-motor synapse. Instead, there are parallel traces formed in different neurons at different times (some requiring *rutabaga*, some not) and these processes seem to interact dynamically with various extrinsic processes [8,12,13]. Aristotle would be confused.

Interestingly, a parallel *rutabaga* rescue story is unfolding for visual learning in the fly. For simple visual memories (involving single tethered flies in flight arenas), the mushroom bodies appear to be dispensible. Instead, *rutabaga* function is required in the central complex, specifically in substructures of the central complex called the 'fan-shaped body' [14] and the 'ellipsoid body' [15] (Figure 1D). Recent work by Pan *et al.* [15] has shown that *rutabaga* function must be restored in both the fan-shaped body and ellipsoid body simultaneously to

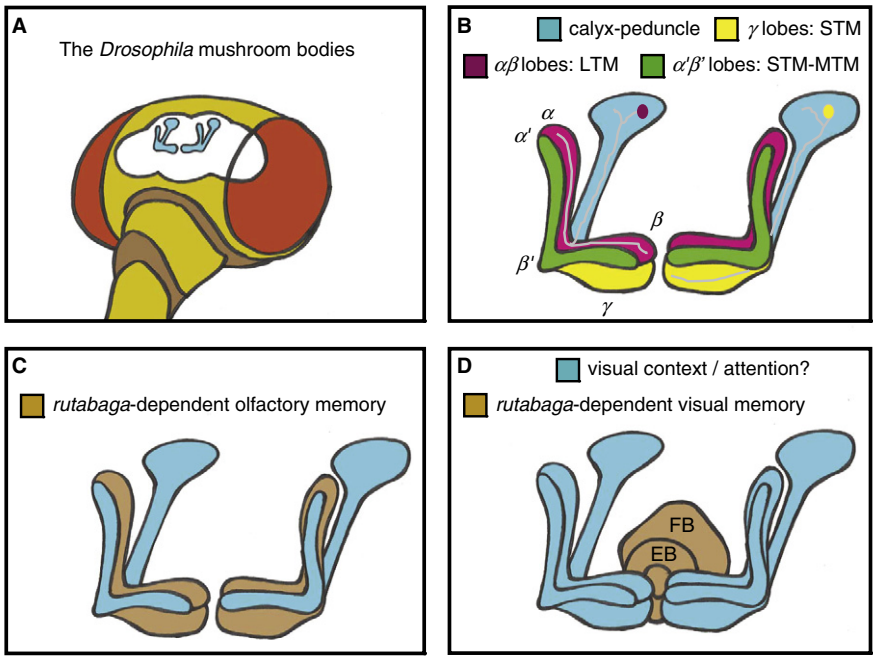

Figure 1. Memory in the *Drosophila* brain.

(A) Location of the paired mushroom bodies (mushroom body) in the fly brain. (B) Mushroom body parts and functions associated with olfactory learning. STM, short-term memory; MTM, middle-term memory; LTM, long-term memory. An αβ and a γ neuron are depicted. (C) The *rutabaga* gene is required in the γ lobes for STM and in the αβ lobes for LTM. (D) *rutabaga* is required in the fan-shaped body (FB) and ellipsoid body (EB) of the central complex for visual learning, but not in the mushroom body. Instead, the mushroom body seems to be required for visual attention-like behavior [17,18].

rescue the visual learning defects of a *rutabaga* null mutation. This result suggests that there exists a dynamic interaction between the fan-shaped body and the ellipsoid body for laying down visual memories, just as the work by Blum *et al.* [1] and others [8,12] suggests an interaction among mushroom body lobes for odor memories. In this way, fly memory increasingly resembles memory in higher-order animals where memory traces are moved through time, such as between the hippocampus and cortex in mammals.

To make matters even more complicated, the fly mushroom bodys are not only restricted to processing and remembering odors. These structures appear to also be crucial for motor control, as well as for more complex aspects of visual learning, such as context generalization [16] and visual attention-like behavior [17]. Indeed, the mushroom bodies are required for restoration of visual attention defects in *dunce* mutants [18] (the *dunce* gene has been shown to act with *rutabaga* to modulate cAMP signalling [2]). Somehow, the overlap of visual and olfactory processes in the mushroom bodies must be explained, even if the final repositories of memories for either modality might be distinct. It remains possible that the Gal4 strategy used to compartmentalize the fly brain provides results that are not as clear-cut as one might hope: *Drosophila* brain researchers have settled on a few key Gal4 strains to define each mushroom body or central complex subset, even though much else is often labelled in these driver lines, and some expression may just not be detectable.

A more optimistic view would suggest that the mushroom bodys and the central complex are not simply static 'filing cabinets' for different kinds of olfactory and visual learning, respectively, but instead that neurons in these structures are required for ongoing dynamic processes gating perception or motor learning [11]. Indeed, recent flight arena work by Björn Brembs [19] suggests that the mushroom bodies regulate habit formation in *Drosophila* by suppressing motor learning and thus promoting behavioral flexibility, as would be required for attention-like behavior. Whether the over-training required to produce olfactory LTM in the αβ lobes of mushroom bodies involves a loss of behavioral flexibility remains an open question. However, cumulative data from multiple labs using different techniques now all agree that the αβ lobes are where we should look for more permanent synaptic modifications underlying LTM (or motor learning) in the fly model.

It is unlikely that the lobes of the mushroom bodies or central complex act merely as containers storing different kinds of memories. Instead, most studies using the now classic strategy of rescuing *rutabaga* defects in the fly [1,9,10,14] suggest that more dynamic measuring tools will be required to further understand the nature of memory in this tiny brain. Ultimately, a thorough understanding of fly memory will require insight into neuronal events on much shorter time scales, such as has been shown in the mushroom body of the locust where local oscillations control spike-timing-dependent plasticity in the β lobes [20]. The cumulative effort by workers in the field seems to have taken fly learning and memory as far as it can go within the realm of Aristotelian thinking. The next step is to explain the apparently dynamic interactions among the parts [8,12], which will probably require better imaging and electrophysiology.

References

1. Blum, A., Li, W., Cressy, M., and Dubnau, J. (2009). Short and long-term memory in *Drosophila* require cAMP in distinct neuron types. Curr. Biol. *19*, 1341–1350.
2. Davis, R.L. (2005). Olfactory memory formation in Drosophila: from molecular to systems neuroscience. Annu. Rev. Neurosci. *28*, 275–302.
3. Gerber, B., Tanimoto, H., and Heisenberg, M. (2004). An engram found? Evaluating the evidence from fruit flies. Curr. Opin. Neurobiol. *14*, 737–744.
4. Hawkins, R.D., Greene, W., and Kandel, E.R. (1998). Classical conditioning, differential conditioning, and second-order conditioning of the Aplysia gill-withdrawal reflex in a simplified mantle organ preparation. Behav. Neurosci. *112*, 636–645.
5. Yu, D., Akalal, D.B., and Davis, R.L. (2006). Drosophila alpha/beta mushroom body neurons form a branch-specific, long-term cellular memory trace after spaced olfactory conditioning. Neuron *52*, 845–855.
6. Wang, Y., Mamiya, A., Chiang, A.S., and Zhong, Y. (2008). Imaging of an early memory trace in the Drosophila mushroom body. J. Neurosci. *28*, 4368–4376.
7. Dubnau, J., Grady, L., Kitamoto, T., and Tully, T. (2001). Disruption of neurotransmission in Drosophila mushroom body blocks retrieval but not acquisition of memory. Nature *411*, 476–480.
8. Krashes, M.J., Keene, A.C., Leung, B., Armstrong, J.D., and Waddell, S. (2007). Sequential use of mushroom body neuron

subsets during Drosophila odor memory processing. Neuron *53*, 103–115.
9. Zars, T., Fischer, M., Schulz, R., and Heisenberg, M. (2000). Localization of a short-term memory in Drosophila. Science *288*, 672–675.
10. McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., and Davis, R.L. (2003). Spatiotemporal rescue of memory dysfunction in Drosophila. Science *302*, 1765–1768.
11. Isabel, G., Pascual, A., and Preat, T. (2004). Exclusive consolidated memory phases in Drosophila. Science *304*, 1024–1027.
12. Keene, A.C., Krashes, M.J., Leung, B., Bernard, J.A., and Waddell, S. (2006). Drosophila dorsal paired medial neurons provide a general mechanism for memory consolidation. Curr. Biol. *16*, 1524–1530.
13. Schwaerzel, M., Monastirioti, M., Scholz, H., Friggi-Grelin, F., Birman, S., and Heisenberg, M. (2003). Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in Drosophila. J. Neurosci. *23*, 10495–10502.
14. Liu, G., Seiler, H., Wen, A., Zars, T., Ito, K., Wolf, R., Heisenberg, M., and Liu, L. (2006). Distinct memory traces for two visual features in the Drosophila brain. Nature *439*, 551–556.
15. Pan, Y., Zhou, Y., Guo, C., Gong, H., Gong, Z., and Liu, L. (2009). Differential roles of the fan-shaped body and the ellipsoid body in Drosophila visual pattern memory. Learn. Mem. *16*, 289–295.
16. Liu, L., Wolf, R., Ernst, R., and Heisenberg, M. (1999). Context generalization in Drosophila visual learning requires the mushroom bodies. Nature *400*, 753–756.
17. Xi, W., Peng, Y., Guo, J., Ye, Y., Zhang, K., Yu, F., and Guo, A. (2008). Mushroom bodies modulate salience-based selective fixation behavior in Drosophila. Eur. J. Neurosci. *27*, 1441–1451.
18. van Swinderen, B., McCartney, A., Kauffman, S., Flores, K., Agrawal, K., Wagner, J., and Paulk, A. (2009). Shared visual attention and memory systems in the Drosophila brain. PLoS One *4*, e5989.
19. Brembs, B. (2009). Mushroom bodies regulate habit formation in *Drosophila*. Curr. Biol. *19*, 1351–1355.
20. Cassenaer, S., and Laurent, G. (2007). Hebbian STDP in mushroom bodies facilitates the synchronous flow of olfactory information in locusts. Nature *448*, 709–713.

Queensland Brain Institute, The University of Queensland, St. Lucia, Queensland, 4072 Australia.
E-mail: b.vanswinderen@uq.edu.au

DOI: 10.1016/j.cub.2009.07.064

Bacterial Evolution: Dynamic Genomes and the Power of Transformation

Virulence and avirulence genes carried on large, unstable pathogenicity islands (PAI) strongly influence the course and fate of host–pathogen interactions. A recent study shows how one such PAI can be rapidly transferred between two closely related bacteria via transformation *in vivo*, and how this horizontal gene transfer affects the fitness of the recipient strain.

David S. Guttman

A contradiction exists between our view of organisms as extremely dynamic and responsive entities, and our common perception of these organisms' genomes as static structures that may change over long evolutionary periods, but which are largely isolated from the *sturm und drang* of daily life. While the explosion of new comparative genomic data is breaking down our typological view of genomes, and natural genetic variation has finally come out of the population genetics closet to be widely appreciated as a critical information source for clinical and functional studies, there is still a disconnect between the genomic variation and plasticity we see around us and our perception of genomes as blueprints written in permanent ink.

Natural selection can drive significant genomic changes in microbial populations over dramatically short time frames. Much of this genomic flux is associated with mobile elements such as insertion elements, transposons, plasmids, and phages. While the movement of these elements is often deleterious to the host bacterium, it can also result in the mobilization and acquisition of factors critical for the survival or success of the bacteria in specific environments. Few genomic elements demonstrate this as clearly as pathogenicity islands (PAIs), which are large, unstable chromosomal or plasmid-borne regions encoding virulence-associated or resistance genes [1]. PAIs typically carry genes that facilitate DNA movement, such as integrases and transposases, are flanked by direct repeats, have GC contents that differ from the genomic average, and include tRNAs that can act as the target sites for DNA integration. The more general term of 'genomic island' has been used to describe similar unstable genomic regions that carry loci other than those involved in pathogenicity, such as those required for symbiosis or adaptation to specific niches.

PAIs are widespread among pathogenic bacteria and commonly encode factors that are necessary and, in some cases, sufficient for pathogenesis. For example, the *Escherichia coli* locus of enterocyte effacement (LEE) encodes a type III secretion system required for the attachment and effacement of these pathogens to the intestinal lumen [2]. Similarly, the hrp/hrc cluster of the phytopathogen *Pseudomonas syringae* encodes a type III secretion system that can deliver an assortment of over seventy type III effectors into their plant hosts [3].

While PAIs are clearly acknowledged to vary among natural isolates, the evolutionary pressures and time required to generate this diversity is typically a matter of speculation. Retrospective and evolutionary studies have been performed to map the historical record of PAI transfer and the subsequent ecological and clinical consequences [4]. *In vitro* studies have been performed to show that PAI mobilization can be induced under laboratory conditions. Nevertheless, it has been much more difficult to study the process of acquisition, loss, and transfer of PAIs *in vivo*. This may be due in part to the common belief that these events work on a time scale incompatible with *in vivo* studies.

Important progress in understanding the *in vivo* pattern and process of PAI transfer was made by Dawn Arnold's group at the University of the West of England [5] who demonstrated how host–pathogen interactions can drive PAI mobilization. The dynamics of their system are