

MUSHROOM BODY MEMOIR: FROM MAPS TO MODELS

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Genetic intervention in the fly *Drosophila melanogaster* has provided strong evidence that the mushroom bodies of the insect brain act as the seat of a memory trace for odours. This localization gives the mushroom bodies a place in a network model of olfactory memory that is based on the functional anatomy of the olfactory system. In the model, complex odour mixtures are assumed to be represented by activated sets of intrinsic mushroom body neurons. Conditioning renders an extrinsic mushroom-body output neuron specifically responsive to such a set. Mushroom bodies have a second, less understood function in the organization of the motor output. The development of a circuit model that also addresses this function might allow the mushroom bodies to throw light on the basic operating principles of the brain.

For centuries, researchers in the functional brain sciences have been mapping properties of behaviour to areas of the brain. Recent non-invasive imaging techniques have initiated a new round in this exercise. But a mechanistic understanding of brain and behaviour is not satisfied by maps. Rather, it asks for models that describe behavioural functions in terms of algorithms, which should allow them to be implemented in artificial systems. The step from maps to models has proven exceedingly difficult — not even the simplest, generally accepted model of the brain is available. Without a firm idea of how the brain works as a whole, assigning functions to its parts is an open-ended quest. For example, local circuits in the cerebellum, hippocampus and amygdala have been extensively studied, but their contributions to a behavioural model of the brain are still little understood. This general problem is not restricted to mammals, but it might be less severe in insects, which have much smaller brains and distinctly less complex behaviour.

One of the best studied regions of the insect brain is the mushroom bodies (FIG. 1). In species such as the honeybee or cockroach, mushroom bodies are present as two massive stalks (peduncles) with large cup-shaped protruberances (calyces) at their dorsocaudal ends. They receive inputs from axon collaterals of projection neurons from the antennal lobes — the first neuropil of

the olfactory pathway — indicating a role for the mushroom bodies in olfaction. In support of this idea, the mushroom bodies are, relatively, largest in social insects, which excel in chemical communication.

The traditional Latin term *corpora pedunculata* (stalk-like bodies) refers not to the mushroom-like appearance of these structures in some insect species, but to the thick bundles formed by the long, densely packed parallel fibres of the intrinsic mushroom body neurons, the Kenyon cells. These bundles are seen in all types of mushroom body, even those with small or no calyces that are found in some of the earliest insect species and in species with secondarily reduced olfactory systems¹. The different connotations of the Latin and English terms reflect the dichotomy in our understanding of mushroom body function. Most of what is known about the mushroom bodies relates to the olfactory system; their contributions to other aspects of behaviour are only slowly beginning to emerge.

Fewer than a dozen insect species have contributed to our knowledge about mushroom bodies. Most prominent among these are the honeybee *Apis mellifera*, the fly *D. melanogaster*, the cockroach *Periplaneta americana* and, to a lesser extent, the locust, cricket, grasshopper, ant and wasp. This article is biased towards *Drosophila* and focuses on current developments (for additional background, see earlier reviews^{1–4}). I intend to show that

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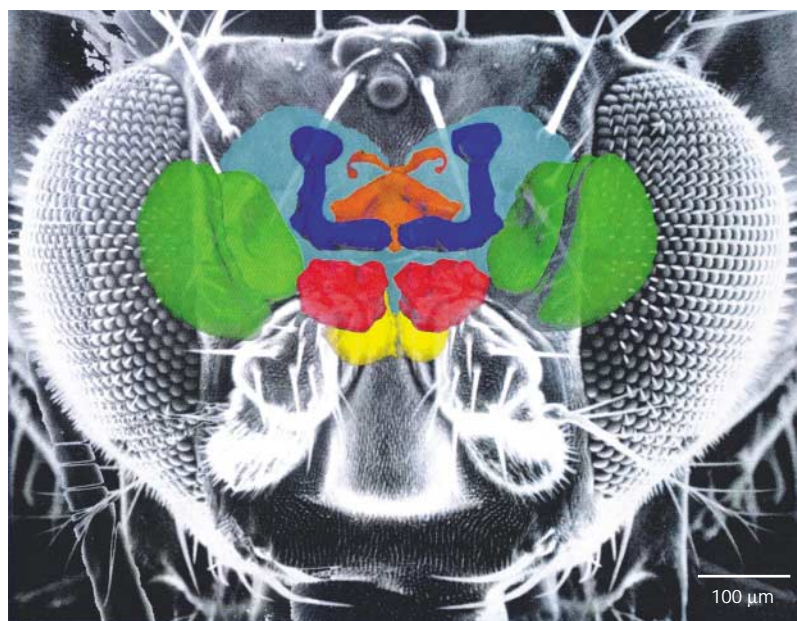


Figure 1 | *Drosophila* brain shown in the head capsule. The most prominent fibre assemblies are colour coded. Green, optic lobes; yellow, suboesophageal ganglion; red, antennal lobes; blue, mushroom bodies; orange, central complex. The various neuropil regions surrounding the mushroom bodies and central complex are shown in grey in the background.

mushroom body research is at the verge of moving from functional topology to a circuit model.

In the insect midbrain, the mushroom bodies and the central complex (FIG. 1) are clearly visible. They are separated by glial sheaths from the many discrete, but so far barely studied, neuropil regions surrounding them. This general neuropil, the mushroom bodies and the central complex might be the three principal components in a basic functional model of the (supraoesophageal) insect brain.

Anatomy

The mushroom bodies of flies, honeybees and cockroaches share many design features. They are two mirror-symmetrical stalks (peduncles) extending from dorsocaudal to rostroventral through the midbrain and dividing frontally into a medial and a vertical lobe. Most of this structure is contributed by the Kenyon cells (about 200,000 in the cockroach, 170,000 in the honeybee and 2,500 in *Drosophila*), with their small cell bodies densely packed above and beside the calyces in the dorsocaudal cell body rind and their long thin axons forming the peduncle and lobes.

The close association of the mushroom bodies with the olfactory system is highlighted by a prominent tract (inner antennocerebral tract, iACT; FIG. 2) that projects from the antennal lobe to the calyx and the lateral protocerebrum. The tract is formed by projection neurons, which typically have their dendritic arbor in a single glomerulus of the antennal lobe and terminate in defined areas of the lateral protocerebrum^{5,6}, sending collaterals into the calyx of the mushroom body. Distinct projection zones in the calyx are most apparent in the honeybee, where an input area from the optic lobes

(the collar) is intercalated between two areas of chemosensory input (the lip and the basal ring)⁷.

The output from the mushroom body is less obvious. The lobes and the rostral part of the peduncle are innervated by extrinsic single neurons that provide input to, as well as output from, the mushroom bodies^{8,9}. These neurons have various extrinsic target areas, typically in circumscribed regions that lie medial and lateral to the lobes, and occasionally in the lateral protocerebrum where the projection neurons terminate. Some neurons connect to the lobes of the ipsilateral or contralateral mushroom body.

Subdivisions defined by different types of Kenyon cells and glial lamellae have been found in all mushroom bodies. These subtypes can be visualized by antibodies against transmitters and neuropeptides, and more directly by the expression patterns of genes and enhancer trap lines. In the peduncle they often appear as concentric rings, and in the lobes as laminae (for example, in the honeybee and cockroach). In *Drosophila*, Strausfeld¹⁰ has identified five subdivisions in the medial lobe using antibodies against glutamate, aspartate and taurine. Enhancer-GAL4 lines (BOX 1) often mark subsets of Kenyon cells that do not conform to the subdivisions that have been identified on the basis of morphology and transmitter immunocytochemistry, indicating that Kenyon cells have considerable molecular diversity. In this review I refer to only two Kenyon cell subsystems, the α/β -system and the γ -system. The latter was originally assumed to be a speciality of flies but recently has also been described in cockroaches and bees^{11,12}. Kenyon cells in the γ -system are the earliest to be formed, and are characterized by claw-like dendritic endings in all parts of the calyx. In flies and honeybees they predominantly or exclusively serve the medial lobe, whereas in the cockroach they branch to both lobes, like those of the α/β -system. I will summarize all other subsystems as α/β . Kenyon cells of these systems have spiny, varicose and other (not claw-like) dendritic morphologies, they contribute equally to the medial and vertical lobes, and they are formed later than the Kenyon cells of the γ -system^{10–13}.

Functional anatomy of the olfactory system

The identification of the odour receptor genes (OR genes) in flies has confirmed what functional anatomy had indicated: the axons of odour receptor neurons that express the same OR gene converge on a common glomerulus in the antennal lobe¹⁴. The glomeruli represent distinct chemical specificities, or, from the animal's perspective, primary odour qualities (POQs) and they are interconnected by local interneurons, many of which contain GABA (γ -aminobutyric acid). The glomeruli might provide a minimal degree of CONCENTRATION INVARIANCE¹⁵, adjust the number of activated glomeruli in complex odour mixtures¹⁶ and synchronize the firing of sets of projection neurons¹⁷. Although none of these functions is firmly documented, they serve as examples to show that processing in the antennal lobe might not interfere with the concept of the glomeruli representing POQs.

CONCENTRATION INVARIANCE
Consistency in response to
odourant molecules across
different concentrations.

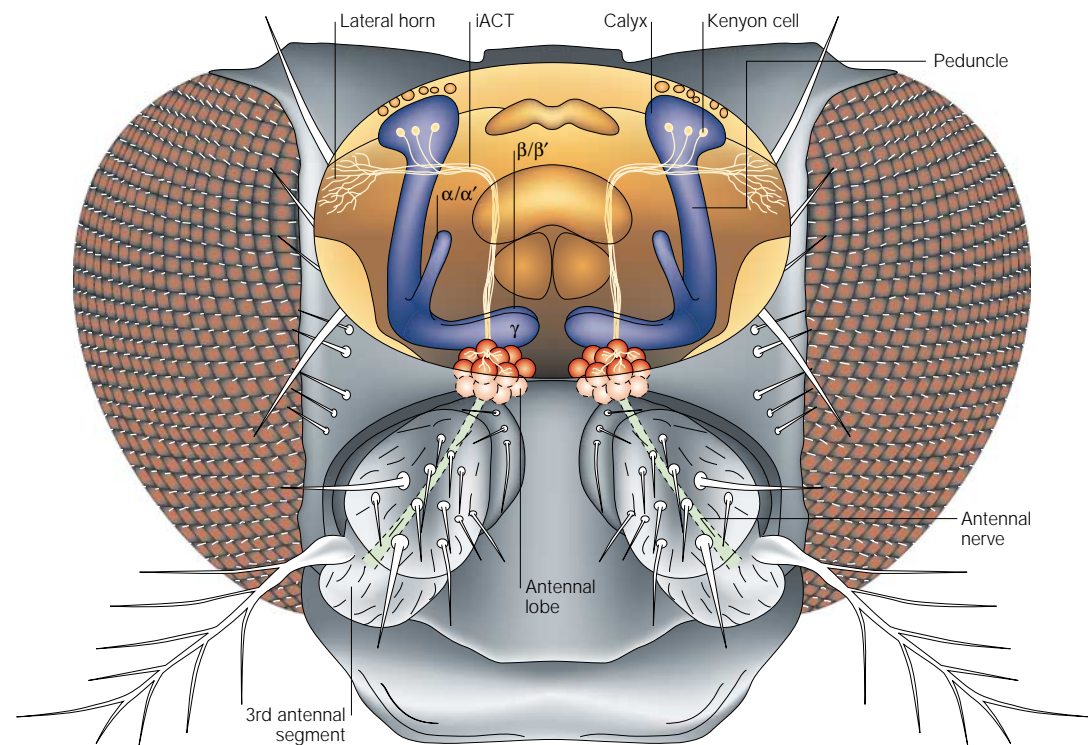


Figure 2 | **Olfactory pathway.** Odour information is carried from the third antennal segments and maxillary palps (not shown) to the antennal lobe, where receptor fibres are sorted according to their chemospecificities in about 40 glomeruli. These represent the primary odour qualities, which are reported to two major target areas in the brain, the dorsolateral protocerebrum (lateral horn) and the calyx of the mushroom body. The inner antennocerebral tract (iACT) connects individual glomeruli to both areas. α/α' , β/β' and γ mark the three mushroom body subsystems described by Crittenden *et al.* (REF. 64).

Several types of antennal lobe projection neurons have been found: 'on' and 'off', phasic and sustained, as well as more complicated ones¹⁸. This is in line with the observation that more than one projection neuron links each glomerulus to the calyx and lateral protocerebrum. We will not consider the fine temporal structure of the olfactory input — this might be more important for acute behaviour, which we assume to be processed in the lateral

horn, than for odour memories that normally do not represent the fine temporal structure of events. In addition to the projection neurons, most of which seem to be cholinergic, the calyx also receives GABA and octopamine inputs^{10,19}. Recurrent GABA neurons from the mushroom body lobes²⁰ and from the lateral protocerebrum²¹ to the calyx have been described in the honeybee and the locust. The octopaminergic input will be discussed later.

Box 1 | Genetic intervention in the brain

Drosophila is unique in its arsenal of genetic tools for intervention in the brain. Although similar techniques are also available for the mouse, the worm *Caenorhabditis elegans* and, with qualification, a few other organisms, in none of them is the versatility of techniques growing as fast. Mutants with reduced or altered mushroom bodies were described more than 20 years ago⁶². In several cases their brains were found to be otherwise intact, and many of them show surprisingly normal behaviour. They have been instrumental in establishing the role of the mushroom bodies in olfactory learning and memory²⁵. Moreover, several genes have been identified that are preferentially or even exclusively expressed in the mushroom bodies⁴ and their promoter sequences have been used to drive transgene expression in these tissues (reviewed in REF. 3).

The main thrust in *Drosophila*, however, derives from the enhancer-GAL4 technique⁶¹. A yeast transgene for the transcription factor GAL4 is inserted in an arbitrary location in the *Drosophila* genome. There, the expression of GAL4 is controlled by flanking *Drosophila* enhancers and suppressors that normally regulate a *Drosophila* gene in the neighbourhood. The resulting expression pattern of GAL4 might include certain cells and tissues under investigation. As GAL4 is itself a transcription factor, it can drive the expression of other genes (effectors) that are placed downstream of a DNA sequence that binds GAL4. Hence, the combination of GAL4-driver and effector gene is a way to target the effector to tissues that happen to express GAL4 in a particular driver line.

More than 40 driver lines with expression in parts of the mushroom bodies (among other brain neurons) and various effector genes that ablate, block, modify or just visualize the neurons expressing them, are available, providing an unprecedented finesse in the manipulation of the system.

How are odour qualities represented in the mushroom bodies? This is an open question. For our discussion of olfactory memory, I will simplify the problem (BOX 2). Let us assume that the glomeruli in the antennal lobe are all-or-none detectors of POQs, and that each odour is defined for an animal by a characteristic set of POQs. How, then, can a neuron be made to respond to this and only this set of POQs? The neuron would have to be connected to the appropriate projection neurons and designed as a coincidence detector for these inputs. This might happen in the direct olfactory pathway to the lateral protocerebrum, which might provide a fast but coarse odour analysis relying on only a few POQs.

If, however, the animal has to discriminate between complex odour mixtures that have many overlapping POQs, it might need to process the odour information more extensively, and this might occur in the mushroom bodies. Imagine that each Kenyon cell is connected to three projection neurons and that every possible combination of three projection neurons has a Kenyon cell. Let us also assume that Kenyon cells are coincidence detectors that generate an action potential only if they receive excitatory input from all three projection neurons simultaneously. With this assumption, all possible combinations of POQs would be represented by unique but overlapping sets of Kenyon cells. A neuron that detects fermenting apple would be connected to the appropriate set of Kenyon cells and would respond not exclusively, but with high specificity to fermenting apple and only a little to fermenting banana (BOX 2).

Few data are available to test this hypothesis. Recently, the transfer of olfactory information from projection neurons to Kenyon cells was investigated in the locust²¹. Although the locust does not follow the typical design of most other insect olfactory systems — its antennal lobe has about 10 times as many but smaller glomeruli and, correspondingly, its projection neurons have their dendrites in several of these glomeruli — it is worth noting the electrophysiological properties of projection neurons and Kenyon cells under comparable experimental conditions in this animal. Perez-Orive *et al.*²¹ gave the probability of an excitatory response to an odour measured over all projection neurons and all odours tested as $p = 0.64$; that of Kenyon cells under similar conditions was $p = 0.11$. (This included a subpopulation of 'generalist' Kenyon cells that responded to many of the odours.) Responses in projection neurons were typically between 5 and 30 spikes, whereas those of Kenyon cells were only one or two spikes. The authors attribute the reduction in response probability and strength between projection neurons and Kenyon cells to various mechanisms which together make the Kenyon cells effective coincidence detectors. One of these mechanisms is a recurrent feedforward inhibition that is mediated by the GABA projections from the lateral protocerebrum to the calyx. Although much still needs to be found out about the information transfer between projection neurons and Kenyon cells in different species, it seems reasonable to assume that odours are represented by sets of Kenyon cells. (For an earlier hypothesis regarding

Box 2 | Number game

Let us assume that each Kenyon cell receives input from three primary odour qualities (POQs), each represented by a projection neuron. The Kenyon cell is activated only if all three input cells are active (coincidence detector). A hypothetical olfactory system with 40 POQs (only 10 are shown in FIG. 3) can form 9,216 triplets of POQs. (*Drosophila* has only 2,500 Kenyon cells, fewer than this scheme would require.) If an odour mixture A consists of 20 POQs, it will activate 1,035 Kenyon cells. If a second odour mixture B also has 20 POQs of which 10 are the same as in A, the two sets of Kenyon cells would overlap by only 11%. Two odours with 6 POQs each and 5 in common would each be represented by sets of 20 Kenyon cells, of which 10 would be activated by both odours. With a 50% overlap at the level of the POQs, the two sets of Kenyon cells would share only a single cell. The numbers show that because of the coincidence requirement, odour representations at the level of the mushroom body could be well separated.

the transition between projection neurons and Kenyon cells, see REF. 22.)

With little additional circuitry, this network would be well suited to store odour memories (FIG. 3). We would need a neuron to report a positive or negative event — the unconditioned stimulus (US) — to all Kenyon cells during the conditioning phase. We would also need an extrinsic mushroom-body output neuron for the conditioned response (CR neuron) that receives synapses from all Kenyon cells. Without conditioning, these synapses would all be latent and the CR neuron would not respond to any odour. In the course of conditioning, the CR neuron would come to act as an odour-specific neuron that reports the presence of a particular odour as an alerting signal for the expected US. Consider, for instance, appetitive conditioning of the proboscis-extension reflex (PER) of the honeybee²³, in which the animal learns to extend its proboscis in response to an odour that predicts food delivery. Odours A and B are the conditioned stimuli (CS⁺ and CS⁻, respectively) and sugar is the US. The two odours are (presumably) represented by distinct sets of Kenyon cells. At the onset of training, the (presumed) CR neuron does not respond to either odour because of low transmission in the synapses. If one of the odours (odour A, the CS⁺) coincides with sugar (reported to the Kenyon cells by the US neuron), those Kenyon cells that are activated simultaneously by the odour will upregulate their output synapses. This would make the CR neuron responsive to odour A.

This scheme is not purely theoretical. Maelshagen²⁴ studied a mushroom-body output neuron in the honeybee during PER conditioning. This exceptionally prominent cell, PE1, contacts many Kenyon cells in the α -lobe and projects to the lateral and medial protocerebrum. At the onset of the experiment, PE1 responded equally to both odours, but in the fifth conditioning trial its response to the CS⁺ was distinctly larger than to the CS⁻. So, PE1 could be a CR neuron that, in the conditioned honeybee, signals the expectation of food to other parts

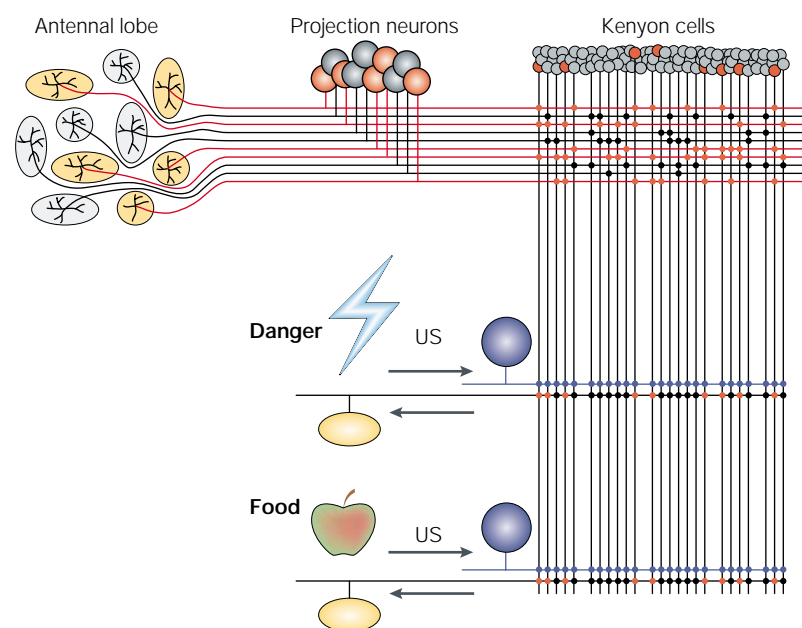


Figure 3 | Circuit model of odour memory. Odours are represented in the mushroom bodies by sets of Kenyon cells. Extrinsic mushroom-body output neurons are connected to the Kenyon cells by latent synapses. The output neurons are accompanied by modulatory input neurons presenting the unconditioned stimulus to the Kenyon cells. Simultaneous arrival of the conditioned (CS) and unconditioned stimulus (US) strengthens the synapses from Kenyon cells to output neurons (see also FIG. 4).

of the brain in response to odour A. In the fly, few mushroom-body output neurons have been identified⁹. If each one represents a 'matter of concern' such as food, egg laying, danger or a mate, a fly might survive with a limited number.

Genetic intervention

With this model in mind, we now turn to genetic intervention. In *Drosophila*, the olfactory system and in particular the mushroom bodies can be genetically and developmentally manipulated (BOX 1). Such experiments have led to the conclusion that odour discrimination learning requires the mushroom bodies. This result is particularly trustworthy as it was obtained by various learning tests and by three different intervention methods^{25–27}. Moreover, aversive and appetitive learning were similarly affected. As early as the larval stage, animals that lack mushroom bodies are impaired in olfactory learning²⁵.

Appropriate controls are needed to show that it is learning and memory, rather than sensorimotor processing, that is affected in flies without mushroom bodies. It has become routine to show that a fly's spontaneous responses to the CS (odours) and the US (electric shock, sugar) are not affected. However, in a differential test with a CS⁺ and a CS[−], one cannot be sure that the flies can distinguish between them. In the model shown in FIG. 3, conditioned odour discrimination is possible without spontaneous odour discrimination. Arguments that will be presented later together with preliminary experiments indicate that both the spontaneous discrimination of complex odours and

the formation and retrieval of odour memories require the mushroom bodies.

Flies can also be trained to discriminate two concentrations of the same odour²⁸, although they perceive the same odour quality¹⁵. Learning concentration differences also requires the mushroom bodies (M. Schwärzel and M. Heisenberg, manuscript in preparation). For the model shown in FIG. 3 this would imply that the olfactory pathway upstream of the mushroom bodies provides only little concentration invariance, and that different concentrations of an odour activate different patterns of POQs and therefore sufficiently different sets of Kenyon cells to allow the differential upregulation of the respective Kenyon cell–CR neuron synapses. (If, at the higher concentration, all the POQs of the lower concentration are also active but in addition further POQs are recruited, this would imply that, in the frame of the model, only the higher concentration of an odour could form a memory template, whereas with the lower concentration as the CS⁺ no association would be formed.)

Not all aspects of odour-related learning and memory require the mushroom bodies. If the odour has the role of an aversive reinforcer (US), as can be studied in conditioned flight control at the flight simulator (REF. 29, but using the abdomen steering apparatus described in REF. 30), memory formation is not affected by the absence of mushroom bodies (P. Masek and M. Heisenberg, manuscript in preparation). This is in line with the anatomy showing that the mushroom body is only a side route of the olfactory pathway. Flies without mushroom bodies can also categorize odours as aversive and attractive, as shown by their spontaneous osmotactic behaviour.

Are the mushroom bodies the site of the memory trace in odour discrimination learning? Showing that the mushroom bodies are necessary for learning is not enough to answer this question — they might instead be involved in an indispensable step of data processing during acquisition or retrieval. To address this problem, we have to go to the molecular level.

In many organisms, cyclic AMP has been shown to be a crucial second messenger in learning and memory as well as in synaptic plasticity³¹. *Drosophila* mutants carrying mutations in genes that affect cAMP metabolism or regulation are impaired at various types of learning, including odour discrimination learning, and these genes are expressed in the mushroom bodies at an elevated concentration⁴. These mutants also show disrupted synaptic plasticity at the larval neuromuscular junction on the neuronal side³². One of these genes, *rutabaga* (*rut*), codes for a calcium/calmodulin (Ca/CaM)-dependent adenylyl cyclase (Rut-AC)³³, which has been considered to be the molecular site of convergence of the US and CS pathways in associative learning³⁴. Rut-AC is thought to be stimulated by the US through a G-protein-coupled transmitter receptor, and by the CS through Ca/CaM. Coincident activation of both pathways would be required for effective stimulation of cAMP synthesis (FIG. 4).

If the above model of mushroom body function were true, Rut-AC would be needed exclusively in the

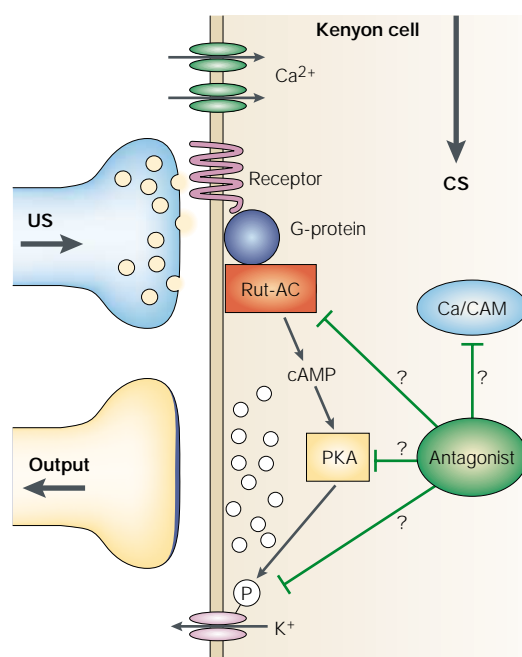


Figure 4 | Presynaptic modulation of transmission at Kenyon cell-to-output neuron synapses is thought to underlie short- and middle-term memory of odours in flies. Simultaneous arrival of the conditioned stimulus (CS) and the unconditioned stimulus (US) in the Kenyon cells activates adenyl cyclase, which in turn increases cyclic AMP synthesis. Elevated levels of cAMP activate protein kinase A (PKA), which might phosphorylate target proteins at the synapse. To explain extinction, an antagonist is postulated that could interfere with any of the steps in the cAMP signalling pathway. Local, independently modulated synaptic domains for different USs might reside in the same Kenyon cells. Ca/CAM, calcium/calmodulin; Rut-AC, Ca/CAM-dependent adenyl cyclase. Adapted, with permission, from REF. 36 © (2002) Elsevier Science.

Kenyon cells at the synapses to the output neuron. Using the enhancer-GAL4 technique (BOX 1), Zars *et al.*³⁵ expressed a *rut*⁺ cDNA in a *rut* mutant background specifically in subpopulations of Kenyon cells. This manipulation restored odour discrimination learning. The effect is particularly striking at three hours after acquisition when remaining memory in the *rut* mutant has declined to near zero, and the full wild-type memory score is recovered by expression of Rut-AC exclusively in the mushroom bodies. Both aversive (electric shock) and appetitive learning (sugar) can be rescued using the same GAL4-driver lines (REF. 36 and M. Schwaerzel *et al.*, manuscript in preparation). This result is consistent with the study of Connolly *et al.*²⁷, who used the GAL4 enhancer trap technique to express, in the mushroom bodies, a constitutively active mutant $G\alpha_s$ -protein that is expected permanently to upregulate Rut-AC. Four of the eight/nine driver lines were the same in the two studies. The same two expression patterns in subsets of Kenyon cells that blocked learning in the experiments of Connolly *et al.*, rescued it in those of Zars *et al.*³⁵. The GAL4 driver 201y gave an intermediate result in both studies, as if only some of the Kenyon cells representing the odours used were targeted. The fourth line had no

effect in both studies. These results indicate that for odour discrimination learning, Rut-AC is required only in the appropriate Kenyon cells. In as much as Rut-AC is part of the memory trace and regulates synaptic plasticity presynaptically, the memory trace is located at the synapses to the (as yet unidentified) mushroom-body output (CR) neurons.

These conclusions have been confirmed and extended using a refined method of intervention. Kitamoto³⁷ adapted a mutant dynamin gene called *Shibire^{ts1}* (*Shi^{ts1}*) as an effector to the enhancer-GAL4 technique. *Shi^{ts1}* reversibly blocks synaptic transmission at an elevated temperature that is still within the permissive range of odour learning in the wild type. The switch between normal function and blockade takes only a few minutes, allowing the dissection of dynamic processes such as acquisition and retrieval. Three studies have found that synaptic output from the Kenyon cells is required during retrieval but not during acquisition or storage of memories^{36,38,39}. In some of the experiments, the same GAL4-driver lines were used as in the rescue studies, indicating that Kenyon cell output synapses can be modulated while being blocked. In these lines, memory can still be fully suppressed at three hours if the temperature is increased just before the test, indicating that during this time the memory trace remains confined to the Kenyon cells. The finding that the Kenyon cells can be 'muted' during acquisition and storage without an impairment of the memory trace precludes more dynamic models of odour memory involving the Kenyon cells in a larger network. This is remarkable because frequent synapses are found between Kenyon cell fibres in the peduncle⁸, and recurrent inhibition by GABA neurons from the lobes to the calyces has been reported in the honeybee^{20,40}. It is possible that these parts of the circuitry become relevant only under special (more extreme) conditions. These conclusions rely on the assumption that *Shi^{ts1}*, which codes for a dominant negative form of dynamin, exerts its effect presynaptically. So far, this has been shown only for the neuromuscular junction³⁶.

Memories fade. Flies can be made to show less avoidance of an aversively conditioned odour if the CS⁺ is repeatedly presented without the US (extinction). It has been debated whether animals erase their memories or learn something new during extinction⁴¹. In the fly, this process requires input to the Kenyon cells but, again, no synaptic output from them. Probably, the same synapses that increase their efficacy of transmission during memory formation decrease it during extinction³⁶ (FIG. 4). All these findings are remarkably consistent among each other and with the above circuit model based on functional anatomy (FIG. 3).

Memory phases

Memories change their properties over time. Early on, they are sensitive to anaesthesia and other treatments; later, memory is consolidated, which might require transcription and protein synthesis. This has led to the distinction of memory phases. In *Drosophila*, four such phases have been described: short-term memory

(STM), which decays in less than an hour; middle-term memory (MTM), lasting from one to three hours; and two forms of long-term memory that are distinguished by training procedures⁴². Extensive training elicits memory components that last for 24 hours or more. One is independent of protein synthesis (anaesthesia-resistant memory, ARM) and a second one, observed after training that has been repeatedly interrupted by rest periods, requires it (long-term memory, LTM). If the same amount of training is given without the intermissions, only ARM, but not LTM, is formed.

The model above so far accounts for memories lasting up to three hours. This would include MTM. Such an intermediate memory phase was proposed after the discovery of a gene called *amnesiac* (*amn*). Mutant *amn* flies forget the odour within one to three hours, depending on the conditions of the experiment. The gene codes for a putative preproneuropeptide involved in the regulation of cAMP synthesis⁴³. Recently, Waddell *et al.*⁴⁴ identified two *Amn*-containing neurons that profusely innervate the mushroom body lobes. Using the enhancer-GAL4 technique they showed that only these two neurons need to express the *amn* gene for normal MTM to occur, and that the crucial phase for this requirement is during the behavioural experiment. Presumably, *Amn* or a processed peptide is secreted and stimulates the Kenyon cells to keep their *Rut*-AC in an upregulated state for hours during memory consolidation. Whether the secretion is stimulated by the US or is due to other behavioural circumstances (such as stress) is unknown. These findings support the conclusion above that cAMP regulation is involved in the formation of the memory trace and that this is located in Kenyon cell axons.

Recently, LTM has also been linked to the mushroom bodies. Pascual and Pr  at⁴⁵ investigated a mutant (α -lobe absent, *ala*) in which individual flies are lacking (on a seemingly statistical basis) either the α - or the β -lobes, whereas the γ -system seems to be intact. Over all *ala* flies, short- and long-term memory seemed normal. But when

the authors performed post-mortem brain histology and grouped the animals according to their brain phenotype, they discovered that LTM at 24 hours was entirely missing in the small group of flies that lacked α -lobes on both sides. STM and 24-hour ARM, however, were normal in all groups. Flies with only one α -lobe or without β -lobes showed normal LTM. To fit these findings into the mushroom body model described earlier, one would like to assume that the output neuron for LTM in electric-shock avoidance learning is located in the α -lobe. It must be emphasized, however, that the experiment of Pascual and Pr  at does not allow us to draw this conclusion. In a network, memory loss as a consequence of 'ablating' a structure does not assign the memory trace to this structure. An experiment in which LTM was rescued by locally restoring a molecular component of the LTM trace would be required.

Localization of STM in relation to the Kenyon cell subsystems is still tenuous. The work of Zars *et al.*³⁵ indicates that the γ -system is necessary and sufficient for STM, as the GAL4 line H24, which drives *rut*⁺ expression exclusively in the γ -system, rescues STM in *rut* mutant flies. A final localization within the mushroom bodies would require a detailed anatomical analysis of expression patterns of the various GAL4 driver lines. If STM and LTM for the same odours were indeed localized in different subsystems this would raise the question of how they were related. Would the memory trace be transferred from one location to the other, or would STM and LTM be two parallel, independent memory systems? These issues can now be addressed.

So far, the pathway of olfactory memory is lost in the neuropil of the midbrain where the mushroom body output neurons have their presynaptic terminals. Formally, these neurons can be regarded as CR neurons that can report, to the decision stage of the brain, the presence of an odour that was previously paired with electric shock or food. The effect of these cells is to change the probabilities of behaviours. If, for instance, the output neuron for electric-shock conditioning (FIG. 5) is primed by 3-octanol, this cell will subsequently make it likely that an escape response is triggered in the presence of this odour. But how specific is the effect? Does it apply only to the special situation of the conditioning experiment or would it transfer to any situation? Moreover, where are all these probabilities integrated? What are the target cells of the CR neurons?

Other mushroom body functions

So far, only olfactory learning has been shown to require the mushroom bodies. Several kinds of visual, tactile and motor learning occur normally without them. Hence, the memory traces of colours and patterns must reside elsewhere in the brain. Altogether, flies without mushroom bodies are remarkably normal. They court and copulate, feed, lay eggs, are alert and seem to be well oriented in space. Even their deficit in olfaction is not obvious unless explicitly tested. This is probably not due to a peculiarity of the mushroom bodies, but rather it reflects the role of the brain as a higher control centre and, therefore, the challenges that face behavioural research.

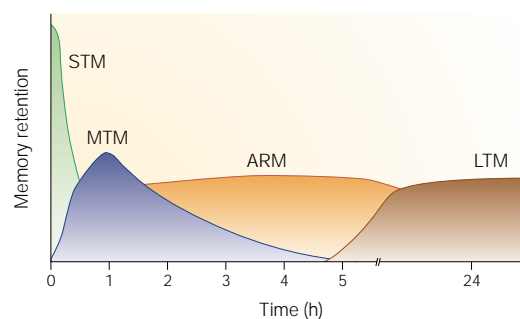


Figure 5 | Memory phases after electric-shock conditioning. Long-term memory (LTM) extends beyond two days and requires protein synthesis. Anaesthesia-resistant memory (ARM) is found together with LTM at 24h, does not require protein synthesis and cannot be erased by anaesthesia after memory consolidation. Middle-term memory (MTM), if it is a separate memory phase, can be observed in a mutant lacking ARM (*radish*) between one and three hours after training. Short-term memory (STM) remains in mutants lacking the *amnesiac* gene, which is required for MTM, ARM and LTM. Adapted, with permission, from REF. 65 © (1995) Elsevier Science.

Mushroom bodies are not needed only in olfaction. Huber⁴⁶, in his early work on crickets and grasshoppers using electrical stimulation and physical lesions, suggested that the mushroom bodies and central complex might be opponent players in the regulation of behavioural activity. It is one of the most basic tasks of the brain to 'decide' whether to start something new, to continue an ongoing behaviour, or to stop and rest. (We use the anthropomorphic term 'decide' as this type of brain activity might be homologous to deciding in humans.) Huber's observations have been paralleled and extended in *Drosophila*. Flies with blocked or missing mushroom bodies show increased spontaneous walking activity⁴⁷. Closer analysis of the data reveals that walking activity is clustered in bouts and that neither the number nor the distribution of bouts over time are altered in flies lacking mushroom bodies, but that bout duration is extended. In other words, the flies are less able to stop walking.

Similarly, when flies lacking mushroom bodies are met with a water moat while trying to reach a nearby, inaccessible landmark, they have difficulties in stopping their approach⁴⁸. The nearer the landmark, the longer they keep trying. As with odour discrimination learning, different methods of intervention show the same effect. Therefore, it seems justified to attribute this behaviour to the mushroom bodies.

Two particularly telling experiments have been performed with tethered *Drosophila* in a flight simulator. The flies are firmly attached by their thorax and head to a torque meter, and their intended turns (yaw torque) are transformed electronically into the appropriate rotatory movements of the panorama. With this visual feedback, they can virtually stabilize their flight against rotations and choose relative flight directions^{49,50}. Although the situation is highly artificial, flies can be conditioned (for example, by exposure to heat) to avoid certain orientations with respect to the panorama. They quickly learn and keep avoiding these orientations even after the heat is permanently switched off⁵¹. This memory lasts for more than 48 hours⁵². Immediately after the conditioning, the memory is still in a labile form, as changing contextual visual cues can readily extinguish it. In the new context memory fades within 30 seconds⁵³. Interestingly, the mushroom bodies contribute to the stability of visual memory traces⁵⁴. Wild-type flies tolerate moderately strong context changes that, in flies without mushroom bodies, interfere with visual memory. But the mushroom bodies have no influence on the dynamics of memory suppression⁵³.

Finally, flies lacking mushroom bodies seem to have difficulties in resolving 'conflicting' situations. Flies are trained in the flight simulator to prefer, for instance, a blue T to a green inverted T. In the subsequent memory test they are confronted with a green T and a blue inverted T. Which one should they prefer? Avoidance of colour and pattern cues balance each other. Flies in this 'paradoxical' situation resolve the dilemma by taking the reliability of the current sensory information during retrieval into account⁵⁵. They evaluate the actual 'sensory strengths' of the parameters (colour and height of centre of gravity) corresponding to the two memory traces,

and respond only to the memory trace for which the current parameter has the higher saliency. In other words, at high colour saturation during retrieval they obey only the colours and disregard the patterns, whereas if colour saturation is low they respond only to the patterns. A sharp transition between 'high' and 'low' colour saturation is observed. As in the context experiment⁵⁴, flies need between 15 and 30 seconds to suppress one or the other memory trace (S. Tang, personal communication). Flies without mushroom bodies do not show this suppression⁵⁵ — irrespective of colour saturation, they show no avoidance of colours or patterns.

It would be difficult to explain these behavioural abnormalities by defects in the olfactory system. But if they reflect independent mushroom body functions, what is their common denominator? It seems that decision-like processes are involved in all four situations. Increased perseverance in flies lacking mushroom bodies^{47,48} indicates that the balance between maintaining the existing behaviour and switching to a new behaviour is offset. Decision-like processes might also be involved when the mushroom bodies protect short-term visual memory traces against context changes⁵⁴ and when they contribute to making the strength of the current sensory signals the arbitrator between two competing memory templates⁵⁵. If, indeed, ancient mushroom bodies had no calyces and no prominent olfactory input¹, one would like to assume that their involvement in decision making is their original function and that they acquired their contribution to olfactory processing as a secondary trait. What, then, is this role in decision making, and what made the ancient mushroom bodies suited for their second task?

Beyond the model

Mushroom body research is in a turbulent state. Several experimental findings about mushroom body function have been reported that are difficult to reconcile with the circuit model of odour discrimination learning described earlier. For instance, Strausfeld and colleagues have mechanically severed the mushroom bodies in the cockroach and found that visual place memory was impaired in these animals⁵⁶. Moreover, they recorded, in the behaving cockroach, single units assumed to be mushroom body extrinsic neurons⁵⁷. Some of these reflected head posture, others responded to reafferent but not exafferent mechanosensory stimulation, and others increased their activity just before the animal started moving. At present, these properties cannot be incorporated into the simple model sketched here.

What is more problematic, despite the properties of the PE1 neuron and a similar study of GABA-containing mushroom-body output neurons⁴⁰, is that the model does not easily accommodate PER conditioning in the honeybee. Hammer⁵⁸ identified a profusely arborizing neuron, VUMmx1, which bilaterally innervates the antennal lobes, calyces and lateral protocerebrum (among other brain regions). VUMmx1 mediates the US in PER conditioning and contains octopamine as its putative transmitter⁵⁹. In addition, Hammer and Menzel⁶⁰ could replace the US with its

temporal specificity by octopamine injection into the calyx or antennal lobe, but not the lateral protocerebrum or optic lobes, indicating that the antennal lobes and calyces are sites of convergence between US and CS in this kind of learning.

Although octopamine also seems to be a crucial transmitter in appetitive odour conditioning in flies (M. Schwärzel *et al.*, manuscript in preparation), neither of these locations can be reconciled with the genetic intervention data in *Drosophila*. The antennal lobe can be formally excluded^{35,36}. If the calyx were the convergence site of CS and US, this would imply that cAMP regulation would be far removed from the target synapses that were to be modified. Some intracellular messenger of the cAMP signalling cascade would have to be transported or passively diffuse from the calyx to the lobes. As in *Drosophila*, olfactory memory is found immediately after training, and physical displacement of a molecule along the entire length of the Kenyon cells between training and test seems unlikely. Moreover, a wide separation between the site of CS/US convergence and the synapse modulated would be incompatible with a further aspect of the model of FIG. 3. If each odour is represented in the mushroom body by a single set of Kenyon cells (as opposed to the notion of several equivalent sets of Kenyon cells representing the same odour), the same two sets will be involved in appetitive and aversive conditioning with the same two odours. To modulate the respective output neurons separately, the synapses of the US-carrying neurons and the mushroom-body output

neurons need to be close together, to assure the strictly local modulation of Kenyon cell output synapses. Finally, the low concentration of Rut-AC in the calyx⁶¹ argues against the calyx as the site of CS/US convergence in *Drosophila*. This discrepancy between honeybee and fly, at present, cannot be resolved. Possibly, the memory traces of PER conditioning and conditioned osmotaxis reside in different regions of the brain.

Final remarks

The model of odour memory sketched here is simple. A single layer of synapses and in the extreme possibly only a single synapse represent the memory trace of an odour. But, if the sketch is correct it should capture some basic features that have been attributed to olfactory systems. In a modest sense this is the case. For instance, storage of odours does not interfere with ongoing odour perception. Moreover, any odour, even one that has never occurred in nature, can be stored in the mushroom bodies if it is detected by the olfactory receptors. The model can be tested and possibly falsified, as shown in the discussion of honeybee PER conditioning. Furthermore, it predicts the outcome of experiments. As mentioned earlier, conditioned discrimination of two concentrations of one odour should give an associative response only for the pairing of the high concentration with the US. This asymmetry should show in the data. The scheme might serve as the layout for a quantitative model incorporating all the known properties of neurons and relays in the olfactory pathway.

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