Development of neuronal connectivity in *Drosophila* antennal lobes and mushroom bodies

Gregory SXE Jefferis*†§, Elizabeth C Marin†§, Ryan J Watts†§ and Liqun Luo*†‡

Recent advances in the study of the connectivity of *Drosophila* olfactory system include the demonstration that olfactory receptor neurons project to specific glomeruli according to the receptor type they express, and that their projection neuron partners are prespecified to innervate particular glomeruli by birth order or time. This same theme of sequential generation has been observed in the generation of the three major types of mushroom body neurons.

Addresses
*Neurosciences Program and †Department of Biological Sciences, Stanford University, Stanford, California 94305-5020, USA
‡e-mail: lluo@stanford.edu
§These authors contributed equally to this review

Current Opinion in Neurobiology 2002, 12:80–86

0959-4388/02/$ – see front matter

Abbreviations

AL	antennal lobe
APF	after puparium formation
DORs	*Drosophila* olfactory receptors
DPM	dorsal-paired medial neuron
GRs	gustatory receptors
LH	lateral horn
LNs	local interneurons
MARCM	mosaic analysis with a repressible cell marker
MB	mushroom body
ORs	olfactory receptors
ORNs	olfactory receptor neurons
PNs	projection neurons

Introduction

Olfactory systems are of interest to developmental biologists for a number of reasons. As for most sensory systems, we have some idea of the information processing that must be achieved for the olfactory system to function effectively. For researchers interested in cell specification, olfactory receptors are molecular markers for 1000 similar, but distinct, cell types in rodents. For others, the convergence of olfactory receptor neurons on single glomeruli, according to the receptor they express, is probably the most dramatic example of the precise wiring of neural networks yet discovered in developmental neurobiology. Furthermore, understanding the development and structure of these systems has provided, and will continue to provide, insights into how odours are recognised and how olfactory memories are formed.

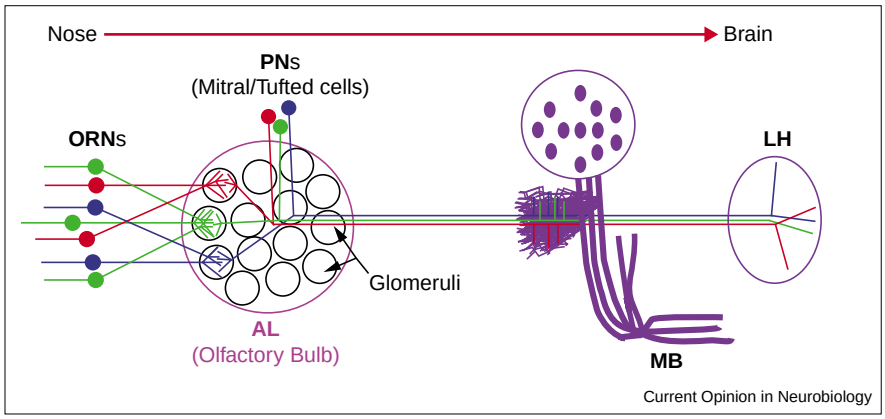
The *Drosophila* olfactory system has a number of distinctive features that make it a worthy complement to the study of vertebrate systems. In essence, the scale of the system is substantially reduced, compared to vertebrate systems, whereas the organisational logic remains similar [1]. A full description of the olfactory receptor repertoire is already available [2•], and a complete map of receptor neuron projection patterns into the brain will undoubtedly be available within the next year or two. Much progress has also been made in determining which odours are detected by different receptor neurons [3,4]. Additionally, the central targets of olfactory neurons, the olfactory glomeruli, are precisely identifiable in *Drosophila* by virtue of their size, shape and position alone [5] — a distinct advantage over more complex vertebrate systems. Furthermore, there are highly sophisticated molecular genetic techniques available for the investigation of both development and behaviour in *Drosophila* systems. Finally, by integrating form and function, the study of olfactory learning in *Drosophila* has provided key insights into the molecular basis of learning and memory [6].

Figure 1 depicts the insect olfactory system in simplified cartoon form and Figure 2 shows representative images of the relevant cells *in situ*. Olfactory information can be considered to flow linearly from the site of transduction on the dendrites of the olfactory receptor neurons (ORNs) to the stereotypically arranged olfactory glomeruli of the antennal lobe (AL). Interactions between glomeruli, mediated by local interneurons (LNs), reformat this information pattern, which is then transferred by projection neurons (PNs) to the dendritic region or calyx of the mushroom body (MB) and also to the lateral horn (LH). MB neurons have been shown to be a required site of plasticity in olfactory learning assays [7]. This mode of organisation is also found in the vertebrate olfactory system, in which the ORNs project to glomeruli in the olfactory bulb, where they synapse onto mitral/tufted cells that relay activity to the olfactory cortex.

In this review, we summarise recent progress in our understanding of the neuronal connectivity of the *Drosophila* olfactory system and how such connectivity arises during development. In addition, we would like to make two points concerning the scope of this review. First, both *Drosophila* larvae and adults can smell. The larval olfactory system develops largely in the embryo; the adult olfactory system develops during metamorphosis in the pupa, although its constituent neurons are born during embryonic and larval stages. This review focuses on events contributing to the development of the adult olfactory system. Second, this review focuses on the central components of the olfactory system; for further details on the sensory receptor molecules and structures, readers are referred to other recent reviews [8,9].

Figure 1

Schematic of the *Drosophila* olfactory system, with mammalian counterparts in parentheses. The different colours represent ORNs expressing particular receptors and the PNs that synapse with these ORNs.



From sensory appendages to the antennal lobe: olfactory receptor neurons

Two families of candidate *Drosophila* chemosensory receptors have been reported: *Drosophila* olfactory receptors (DORs) [10,11] and gustatory receptors (GRs) [12]. 40 out of 57 DORs and 3 out of 56 GRs have been detected by *in situ* hybridisation in adults, either in the 1200 ORNs of the third antennal segment or in the 120 ORNs of the maxillary palp. The remainder were either not detectable by *in situ* hybridisation, or expressed only in larval tissue or gustatory receptor structures [2•,12]. Consequently, the total number of candidate olfactory receptors (ORs) expressed in the adult stands at 43, although this number should not be considered final. *In situ* hybridisation studies with pair-wise combinations of probes for three of the seven ORs expressed in the maxillary palp revealed no coexpression of ORs. Similar observations were made for antennal DORs. There is however one unusual point: OR83b is expressed in every ORN; one hypothesis is that this is a coreceptor [2•].

ORN dendrites and cell bodies are arranged in characteristic but overlapping zones on the maxillary palp and the third antennal segment, indicating that a neuron born at any particular location may express only one of a restricted subset of ORs [10,11]. Their axons, which travel down the antennal nerve into the brain, converge on stereotypical glomeruli in the antennal lobe. Six fusions of candidate OR promoters with reporter transgenes have been analysed for axonal projection patterns; five project to single glomeruli, and one to two small, close, but non-adjacent glomeruli [2•,12,13]. In cases in which an OR projects to two glomeruli, it will be of interest to determine whether single ORNs project

Figure 2

Visualising PNs and MB neurons.
(a) Schematic of a *Drosophila* head (anterior view) with the olfactory appendages marked in red and the outline of the brain in dark grey. **(b)** A brain in the same orientation stained with the monoclonal antibody nc82, which recognises neuropil. Note the position of the AL, the calyx of the MB (Calyx) and the LH. The midline is indicated with a dotted red line. **(c)** The same brain as in (b) showing, in green, an anterodorsal PN single cell clone on the left and an anterodorsal neuroblast clone on the right; nc82 stained neuropil now pseudocoloured red. Cell bodies are outlined by dotted lines. **(d)** An MB single cell clone of the α′/β′ type (left) and a neuroblast clone (right). The image is a composite of two original confocal stacks. (b) and (c) reproduced with permission from [21•].

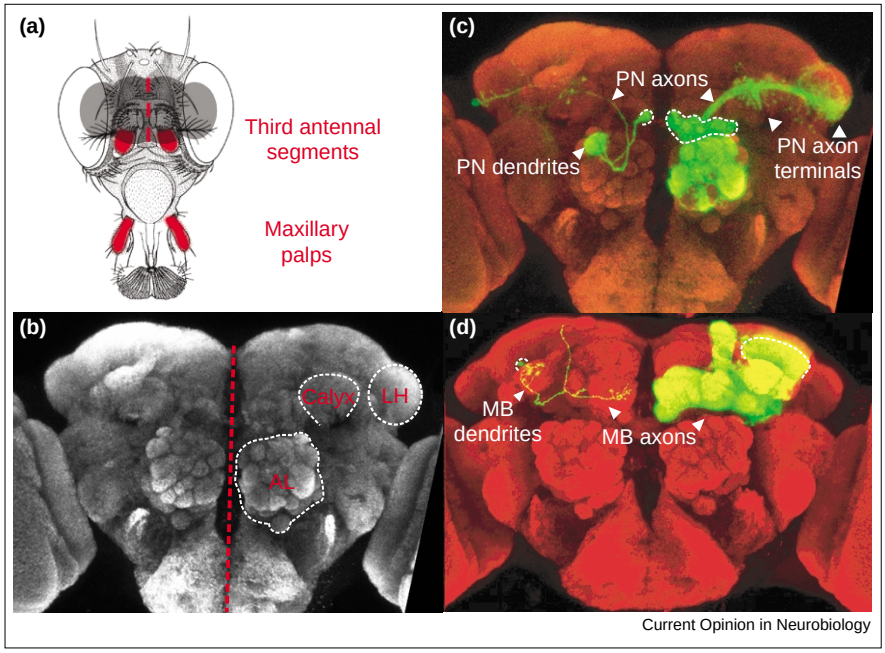
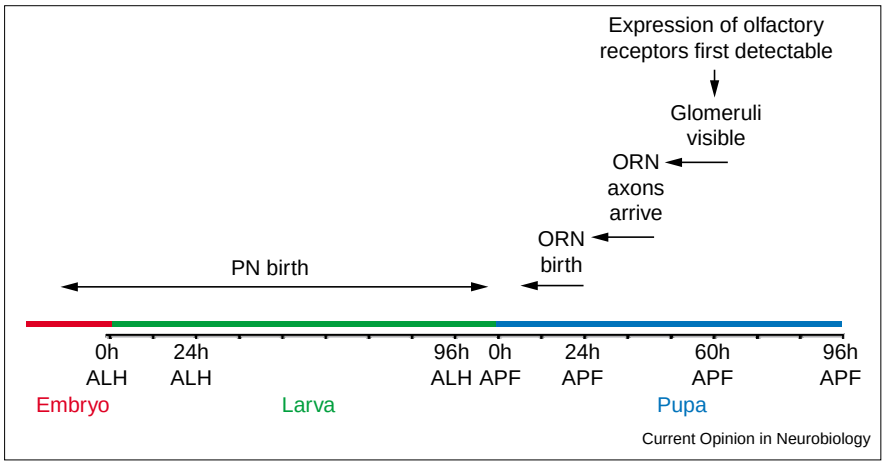


Figure 3



Time course of developmental events contributing to the adult AL. ALH: after larval hatching.

to both glomeruli, or rather whether two subpopulations exist, each projecting to a single glomerulus.

Adult ORNs are born early in pupal development in the eye/antennal disc. GAL4 enhancer lines driving expression of a reporter gene labelling large subsets of these ORNs have been used to describe the behaviour of ORNs during AL formation [14•]. The first differentiated ORNs are detectable ~16 hours after puparium formation (APF). ORN axons first reach the AL area ~18 hours APF, with processes entering the lobe by 22 hours. Some of these axons have converged into neuropil knots by 36 hours APF; a number of these have taken on a glomerular appearance by 48 hours APF; by 72 hours APF, ORNs have essentially adopted their adult structure (Figure 3).

Although ORN axons seem to extend rapidly to the AL, to date, expression of ORs has only been reported later in development — *in situ* hybridisation experiments performed on three DORs showed that one was first detectable at 60 hours APF and the other two at 90 hours APF [11]. Although more direct evidence is awaited, *Drosophila* ORs may not have the critical role in ORN axon convergence that has been found in vertebrates [15].

From antennal lobe to mushroom body and lateral horn: antennal lobe projection neurons

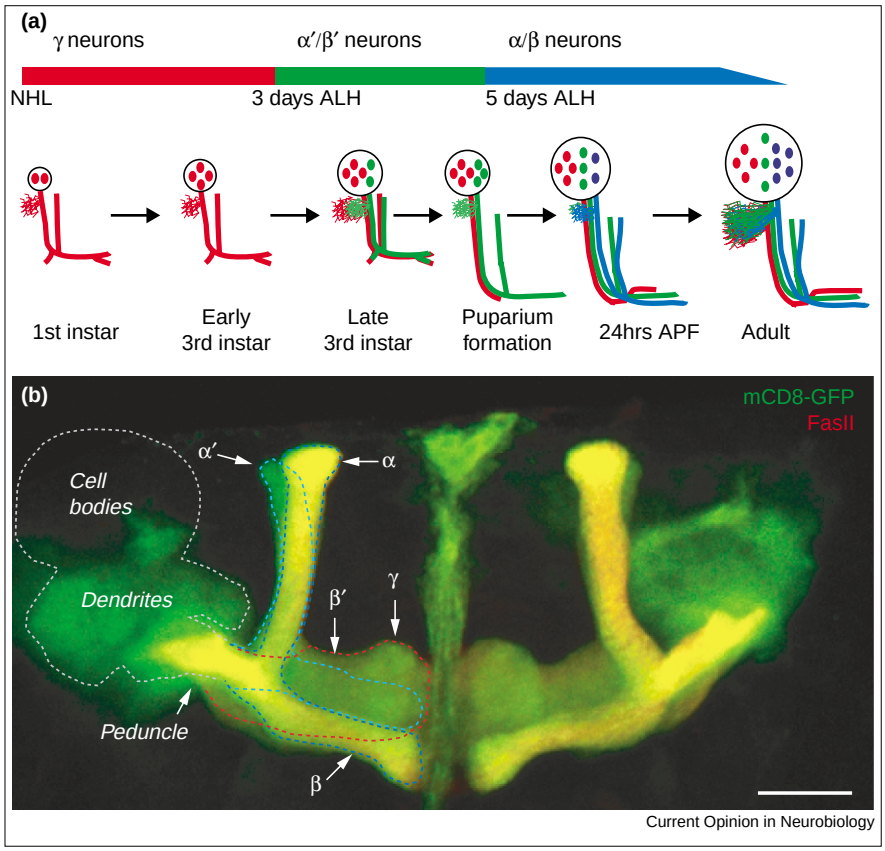
The *Drosophila* antennal lobe is composed of ~43 identified glomeruli [5], meaning that there are about the same number of glomeruli as ORs (see above). Each glomerulus consists of ORN axon termini and the dendrites of PNs and LNs, all of which are ensheathed by glia, giving rise to its characteristic shape (Figure 2). In the AL, olfactory information from the ORNs is processed by LNs and relayed to higher brain centres by PNs.

LNs, which are equivalent to vertebrate olfactory granule cells, lack an axonal process and are intrinsic to the AL, and have extensive arborisations that terminate in multiple glomeruli [16]. Little is known about the development of LNs in *Drosophila*, but hydroxyurea experiments suggest that they derive from one of five neuroblasts that have been observed to divide actively in each hemisphere of the early larval brain [17,18].

PNs, whose cell bodies are located at the periphery of the AL, project their dendrites to single or multiple glomeruli and relay activity to higher brain centres [16,19]; they are therefore analogous to vertebrate mitral/tufted cells. Mosaic analysis with a repressible cell marker (MARCM), is a genetic technique that makes use of inducible mitotic recombination in a dividing precursor cell to turn on a reporter gene of choice, thereby positively labelling one daughter cell and all of its progeny [20]. This technique allows detailed visualisation of neurons in lineage tracing experiments and allows the study of gene functions in various aspects of neuronal development. Recent lineage tracing studies using the MARCM system in combination with the GAL4 driver GH146 [18] reveal that these GH146 positive PNs (~60% of total PNs) are generated by three major neuroblasts: anterodorsal, lateral, and ventral. PNs derived from the anterodorsal or lateral neuroblast project their axons via the inner antennocerebral tract to the MB and LH, whereas those derived from the ventral neuroblast project via the medial antennocerebral tract, bypassing the MB calyx (dendritic field) [21•]. Uniglomerular PNs derived from the anterodorsal and lateral neuroblasts innervate distinct, intercalated, but non-overlapping sets of glomeruli, demonstrating that the neuroblast of origin restricts PN dendrites to a specific subset of glomeruli. In addition, lineage tracing experiments reveal that anterodorsal PNs innervating particular glomeruli are born during characteristic developmental windows. In fact, analysis of partial neuroblast clones allows the inference of an invariant PN birth sequence, suggesting that birth time or order determines which glomerulus a PN will innervate [21•].

PNs are born in a period extending from early embryogenesis to the late third instar (Figure 3) [21•]. Embryonically born

Figure 4



Development of the MBs. **(a)** Diagram showing the sequential generation of three distinct types of MB neurons on the basis of birth order: γ neurons (red), α′/β′ neurons (green), and α/β neurons (blue). NHL: newly hatched larvae; ALH: after larval hatching; **(b)** Composite confocal image of adult MBs expressing *UAS-mCD8-GFP* (green) driven by *GAL4-OK107* and stained with anti-Fas II (red), showing differential expression of Fas II in the α/β neurons (strong) and γ neurons (weak). Fas II is not detectable in α′/β′ neurons in adults.

PNs have recently been shown to arborise in specific subregions of the larval AL, as have both olfactory and gustatory afferents, suggesting that the larval AL possesses a degree of functional compartmentalisation similar to that of the adult, despite its relative numeric simplicity (R Stocker, personal communication). During metamorphosis, the size of the AL vastly increases as additional ORNs and interneurons differentiate and innervate the future adult structure. At the time of ORN axon invasion of the AL, larvally born PNs have already projected their axons to the MB and LH and have initiated dendritic branching [21•].

How is olfactory information organised as it is transmitted to higher brain centres? It appears that PNs projecting dendrites to specific glomeruli in the adult AL exhibit stereotypical axon branching patterns in the lateral horn (R Axel, personal communication; G Jefferis, E Marin, T Komiyama, H Zhu, L Luo, unpublished data). Identification of the target cells of olfactory PNs in the MB and LH would greatly improve our understanding of the circuitry involved in odour interpretation, learning, memory, and behaviour.

Although little is known about the organisation of the *Drosophila* MB or LH dendritic field, studies in other insects suggest that sensory inputs are organised in the MB dendritic field. In the honeybee, the MB calycal neuropil is subdivided into the lip, collar, and basal ring fields [22]. Functional divisions in the MB are evident by the first pupal stage, prior to arborisation of PN dendritic processes in the AL: olfactory PNs project to the prospective lip, whereas visual afferents project to the prospective collar [23]. Moreover, anterograde labelling of olfactory PNs results in varied staining intensities in the MB lip, suggesting that different PN groups project to different zones [24]. These findings are consistent with studies in the cockroach, in which the calycal neuropil is segmented distoproximally into four successive zones, each of which receives input from a specific combination of afferents from discrete populations of olfactory glomeruli, as well as from the optic lobes and/or protocerebrum [25].

It is not yet known how olfactory information is organised in the *Drosophila* MB, and it will be of great interest to find out whether there is detectable compartmentalisation of inputs, as in other insects. However, genetic and molecular techniques have permitted a great deal to be learned about the organisation and development of the MB itself.

Development of the *Drosophila* mushroom bodies

The intrinsic neurons (Kenyon cells) of the *Drosophila* mushroom bodies, referred to hereafter as the MB neurons, were shown to originate from four neuroblasts per hemisphere of the embryonic brain, with each neuroblast giving rise to

an indistinguishable set of MB neurons and glia [17]. MB neuroblasts begin to divide during embryonic stage 9 [26] and continue until late pupal stages [17], giving rise to at least three distinct types of MB neurons [27] in succession: γ, α′/β′, and α/β neurons, respectively [28] (Figure 4). All three types of MB neurons initially exhibit similar projections. Each neuron possesses dendrites composing the calycal neuropil, which receives input from the AL via the PNs (Figure 2). Additional inputs to the calycal neuropil have yet to be defined in *Drosophila*. A single axon from each neuron is sent in a ventro-anterior direction, fasciculating with other MB axons in a concentric fashion, with younger axons lying interior to older ones [29]; this collection of axons constitutes the peduncle (Figure 4b). After the peduncle, axons of different types of MB neurons enter their distinct axonal lobes (Figure 4b). There, all axons bifurcate into dorsal and medial branches, except those of the γ neurons in adult (Figure 4). Observation of embryonic MB neurons suggests that the neurons achieve their bifurcated axons by projecting to the medial lobe first and then sending collaterals to the dorsal lobe [26]. It is not known whether later born neurons also follow this pattern, or if their axon growth cones split and send projections simultaneously to both the dorsal and medial lobes.

Earlier studies of *Drosophila* brains during metamorphosis suggested that large scale reorganisation takes place in the MBs [30,31]. More recent systematic lineage analysis using the MARCM system revealed that during metamorphosis, the early-born γ neurons prune their dorsal and medial projections back to the pedunculus. Later, they reextend projections towards the midline, forming the γ lobe in adults [28]. Interestingly, the α′/β′ neurons born during the late third instar larval stage maintain their axonal projections in both the dorsal and medial lobes during metamorphosis, which make up the α′/β′ lobes in adults. Lastly, the α/β neurons are born during metamorphosis and project to form the tightly bundled α/β lobes with their bifurcating axons (Figure 4) [28].

Previous studies using P[GAL4] enhancer-trap or immunohistochemical approaches have shown heterogeneity between the larval and adult MBs and within the dorsal and medial axonal bundles [27,32,33]. For example, antibody staining against Fas II strongly stains the α/β neurons, weakly stains the γ neurons and does not stain the α′/β′ neurons in adults [27] (Figure 4). As a result of mosaic analysis, it is now clear that the majority of this heterogeneity is the result of the three distinct types of MB neurons [28]. Not only have differences in development and projection patterns been identified among the MB neurons, but recent work showing the presynaptic role of MB neurons in memory retrieval [34,35] has also suggested functional differences among these types of neurons in memory processing [35]. Many questions still remain about the role of each neuronal type in higher cognitive processes.

Apart from the afferents to the calyces, MB neurons connect to other parts of the brain via extrinsic neurons that arborise within the MB calyces and/or axonal lobes [22]. Ito *et al.* have identified several neural structures that innervate, or are innervated by, the MB neurons [36]. One such afferent, known as the dorsal-paired medial neuron (DPM), was characterised by Waddell *et al.* [37•] while identifying the subcellular localisation of the *amnesiac* gene product. They also showed that blocking neurotransmission of the DPM neurons mimicked the *amnesiac* mutant learning phenotype. The two independent cell bodies of the DPM neurons lie medial to the MB neurons. Each sends out a single anterior projection, which bifurcates and innervates both the dorsal and medial lobes of the ipsilateral MB. With the exception of the DPM neurons, the development and function of the other extrinsic neurons remains to be elucidated and the link between the MB and motor output is completely unknown.

Genes that affect MB development have been identified through genetic screens for abnormal adult MB structure [38,39]. Two such genes have recently been cloned: *mushroom body defect* (*mud*) encodes a coiled-coil protein [40], whereas *mushroom bodies tiny* (*mbt*) encodes a p21-activated kinase-like protein serine/threonine kinase [39]. These genes, as well as *enoki mushroom* (*enok*), which encodes a histone acetyltransferase [41], appear to control MB neuronal number by regulating MB neuroblast number or proliferation and/or MB neuronal survival. The transcription factor, *Eyeless*, and another nuclear protein gene, *Dachshund*, have also recently been shown to regulate MB development by acting independently of each other to influence axon pathway selection [36,42,43]. Analysis of candidate gene function using MB neurons as a model system will not only shed light on the function of these genes, but may also contribute to our understanding of the myriad developmental processes that shape the adult MB and eventually contribute to its important functions.

Conclusions

Studies of AL and MB development have already shed light on general neural developmental mechanisms. Lineage tracing using the MARCM method [20] has allowed the initial description of the development of both PNs and MBs. Interestingly, sequential assignment of neuronal fates from birth order [21•,28] appears to be a common theme for the specification of axonal and/or dendritic targets for both PNs and MB neurons. A similar logic is used in *Drosophila* embryonic ventral nerve cord, where sequential expression of transcription factors by a neuroblast and its progeny has been shown to confer neuronal cell fate [44]. In the future, it will be interesting to see whether this logic is used in assembling neural circuits both in other parts of the *Drosophila* brain and in the brains of other animals.

With wild-type developmental events characterised, and the ability to genetically manipulate both PNs and MB neurons in mosaic animals, the olfactory system will be useful for studying questions of general interest in developmental neurobiology. These include mechanisms of

axon growth, guidance and branching, dendritic targeting, and pruning of axon and dendritic processes [45]. For example, the ecdysone receptor has been shown to be cell-autonomously required for MB γ axon pruning [46•], a developmentally controlled process that permits the study of the molecular mechanisms of neural remodelling.

What makes the study of the development of *Drosophila* AL and MB neurons even more attractive is that it can be placed in the context of a neural network whose function is clearly defined. The input to the network is the olfactory world. With the recent molecular characterisation of olfactory receptors and the description of their axonal projections, soon we expect to have a complete map correlating odours, their receptors, and their glomerular targets in the AL for ORNs expressing specific receptors. Moreover, the function of MB neurons in olfactory learning and memory has already been well defined. PN dendrites connect with ORN axons in the AL and, as second order interneurons, transfer olfactory information to the MB and LH (Figure 1). Delineating the output neurons from MB and LH and their eventual connections to motor control systems remains an important task for the future. Once the wiring of the normal olfactory system is established, it will be possible to address whether, how, and where in the neural network, sensory experience modifies this wiring programme. Studying the logic of the assembly of this circuit promises to link two exciting fields: olfactory information processing and learning and memory.

Acknowledgements

We thank K Ito, R Stocker and L Vosshall for comments. G Jefferis and E Marin are Howard Hughes Medical Institute predoctoral fellows, R Watts is supported by a National Institutes of Health predoctoral training grant. Work in our lab is supported by grants from the National Institutes of Health.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hildebrand JG, Shepherd GM: Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu Rev Neurosci* 1997, 20:595-631.

2. • Vosshall LB, Wong AM, Axel R: An olfactory sensory map in the fly brain. *Cell* 2000, 102:147-159.

The authors of this paper provide an essentially complete description of the DOR family. In the maxillary palp, *in situ* hybridisation with pools of probes to a number of receptors was used to show that several receptors were not coexpressed in the same ORN; analogous experiments were performed in the antenna. OR:Gal4 fusions were used to demonstrate that receptor neurons project to discrete glomeruli in the AL; four receptors projected to single glomeruli and one projected to a pair of close but non-adjacent glomeruli. Gao *et al.* [13] also made two of these fusions and obtained the same result.

3. de Bruyne M, Clyne PJ, Carlson JR: Odor coding in a model olfactory organ: the ***Drosophila*** maxillary palp. *J Neurosci* 1999, 19:4520-4532.

4. de Bruyne M, Foster K, Carlson JR: Odor coding in the ***Drosophila*** antenna. *Neuron* 2001, 30:537-552.

5. Laissue PP, Reiter C, Hiesinger PR, Halter S, Fischbach KF, Stocker RF: Three-dimensional reconstruction of the antennal lobe in ***Drosophila melanogaster***. *J Comp Neurol* 1999, 405:543-552.

6. Waddell S, Quinn WG: Flies, genes, and learning. *Annu Rev Neurosci* 2001, 24:1283-1309.

7. Roman G, Davis RL: Molecular biology and anatomy of ***Drosophila*** olfactory associative learning. *BioEssays* 2001, 23:571-581.

8. Vosshall LB: Olfaction in ***Drosophila***. *Curr Opin Neurobiol* 2000, 10:498-503.

9. Vosshall LB: The molecular logic of olfaction in ***Drosophila***. *Chem Senses* 2001, 26:207-213.

10. Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R: A spatial map of olfactory receptor expression in the ***Drosophila*** antenna. *Cell* 1999, 96:725-736.

11. Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR: A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in ***Drosophila***. *Neuron* 1999, 22:327-338.

12. Scott K, Brandy R Jr, Cravchik A, Morozov P, Rzhetsky A, Zuker C, Axel R: A chemosensory gene family encoding candidate gustatory and olfactory receptors in ***Drosophila***. *Cell* 2001, 104:661-673.

13. Gao Q, Yuan B, Chess A: Convergent projections of ***Drosophila*** olfactory neurons to specific glomeruli in the antennal lobe. *Nat Neurosci* 2000, 3:780-785.

14. • Jhaveri D, Sen A, Rodrigues V: Mechanisms underlying olfactory neuronal connectivity in ***Drosophila*** – the atonal lineage organizes the periphery while sensory neurons and glia pattern the olfactory lobe. *Dev Biol* 2000, 226:73-87.

The authors of this paper describe when ORNs enter the developing lobe and how they progress to glomerular organization. A number of suggestive observations concerning the role of glia in AL development are made.

15. Mombaerts P: Molecular biology of odorant receptors in vertebrates. *Annu Rev Neurosci* 1999, 22:487-509.

16. Stocker RF, Lienhard MC, Borst A, Fischbach KF: Neuronal architecture of the antennal lobe in ***Drosophila melanogaster***. *Cell Tissue Res* 1990, 262:9-34.

17. 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 neurons and glial cells. *Development* 1997, 124:761-771.

18. Stocker RF, Heimbeck G, Gendre N, de Belle JS: Neuroblast ablation in ***Drosophila*** P[GAL4] lines reveals origins of olfactory interneurons. *J Neurobiol* 1997, 32:443-452.

19. Stocker RF: The organization of the chemosensory system in ***Drosophila melanogaster***: a review. *Cell Tissue Res* 1994, 275:3-26.

20. Lee T, Luo L: Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* 1999, 22:451-461.

21 • Jefferis GSXE, Marin EC, Stocker RF, Luo L: Target neuron prespecification in the olfactory map of ***Drosophila***. *Nature* 2001, 414:204-208.

Genetic mosaic analysis of *Drosophila* olfactory PNs allows correlation of lineage and birth order with glomerular target. Here, PNs are shown to be prespecified to synapses with specific incoming ORNs and therefore carry specific olfactory information to higher brain centres.

22. Mobbs PG: The brain of the honeybee ***Apis mellifera***. I. The connections and spatial organization of the mushroom bodies. *Philos Trans R Soc London Ser B* 1982, 298:309-354.

23. Schroter U, Malun D: Formation of antennal lobe and mushroom body neuropils during metamorphosis in the honeybee, ***Apis mellifera***. *J Comp Neurol* 2000, 422:229-245.

24. Gronenberg W: Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *J Comp Neurol* 2001, 435:474-489.

25. Strausfeld NJ, Li Y: Organization of olfactory and multimodal afferent neurons supplying the calyx and pedunculus of the cockroach mushroom bodies. *J Comp Neurol* 1999, 409:603-625.

26. Noveen A, Daniel A, Hartenstein V: Early development of the ***Drosophila*** mushroom body: the roles of ***Eyeless*** and ***Dachshund***. *Development* 2000, 127:3475-3488.

27. Crittenden JR, Sloulakis EMC, Han KA, Kalderon D, Davis RL: Tripartite mushroom body architecture revealed by antigenic markers. *Learn Mem* 1998, 5:38-51.

28. 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, 126:4065-4076.

29. Verkhusha VV, Otsuna H, Awasaki T, Oda H, Tsukita S, Ito K: An enhanced mutant of red fluorescent protein DsRed for double labeling and developmental timer of neural fiber bundle formation. *J Biol Chem* 2001, 276:29621-29624.

30. Technau G, Heisenberg M: Neural reorganization during metamorphosis of the corpora pedunculata in ***Drosophila melanogaster***. *Nature* 1982, 295:405-407.

31. Armstrong JD, de Belle JS, Wang Z, Kaiser K: Metamorphosis of the mushroom bodies; large-scale rearrangements of the neural substrates for associative learning and memory in ***Drosophila***. *Learn Mem* 1998, 5:102-114.

32. Yang MY, Armstrong JD, Vilinsky I, Strausfeld NJ, Kaiser K: Subdivision of the ***Drosophila*** mushroom bodies by enhancer-trap expression pattern. *Neuron* 1995, 15:45-54.

33. Tettamanti M, Armstrong JD, Endo K, Yang MY, Furukubo-Tokunaga K, Kaiser K, Reichert H: Early development of the ***Drosophila*** mushroom bodies, brain centres for associative learning and memory. *Dev Genes Evol* 1997, 207:242-252.

34. Dubnau J, Grady L, Kitamoto T, Tully T: Disruption of neurotransmission in ***Drosophila*** mushroom body blocks retrieval but not acquisition of memory. *Nature* 2001, 411:476-480.

35. McGuire SE, Le PT, Davis RL: The role of ***Drosophila*** mushroom body signaling in olfactory memory. *Science* 2001, 293:1330-1333.

36. Ito K, Suzuki K, Estes P, Ramaswami M, Yamamoto D, Strausfeld NJ: The organization of extrinsic neurons and their implications in the functional roles of the mushroom bodies in ***Drosophila melanogaster*** meigen. *Learn Mem* 1998, 5:52-77.

37. • Waddell S, Armstrong JD, Kitamoto T, Kaiser K, Quinn WG: The amnesiac gene product is expressed in two neurons in the ***Drosophila*** brain that are critical for memory. *Cell* 2000, 103:805-813.
While characterising the expression pattern of the *amnesiac* gene product, AMN, the authors identified two extrinsic neurons of the MBs, the DPM cells, which act as afferents to the MB neuropil. Strikingly, inhibition of synaptic release in DPM neurons mimics the mutant *amnesiac* learning phenotype.

38. Heisenberg M: Mutants of brain structure and function: what is the significance of the mushroom bodies for behavior? *Basic Life Sci* 1980, 16:373-390.

39. Melzig J, Rein KH, Schafer U, Pfister H, Jackle H, Heisenberg M, Raabe T: A protein related to p21-activated kinase (PAK) that is involved in neurogenesis in the ***Drosophila*** adult central nervous system. *Curr Biol* 1998, 8:1223-1226.

40. Guan Z, Prado A, Melzig J, Heisenberg M, Nash HA, Raabe T: ***mushroom body defect***, a gene involved in the control of neuroblast proliferation in ***Drosophila***, encodes a coiled-coil protein. *Proc Natl Acad Sci USA* 2000, 97:8122-8127.

41. Scott EK, Lee T, Luo L: ***enok*** encodes a ***Drosophila*** putative histone acetyltransferase required for mushroom body neuroblast proliferation. *Curr Biol* 2001, 11:99-104.

42. Kurusu M, Nagao T, Walldorf U, Flister S, Gehring WJ, Furukubo-Tokunaga K: Genetic control of development of the mushroom bodies, the associative learning centers in the ***Drosophila*** brain, by the ***eyeless***, ***twin of eyeless***, and ***dachshund*** genes. *Proc Natl Acad Sci USA* 2000, 97:2140-2144.

43. Martini SR, Roman G, Meuser S, Mardon G, Davis RL: The retinal determination gene, ***dachshund***, is required for mushroom body cell differentiation. *Development* 2000, 127:2663-2672.

44. Isshiki T, Pearson B, Holbrook S, Doe CQ: ***Drosophila*** neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. *Cell* 2001, 106:511-521.

45. Lee T, Luo L: Mosaic analysis with a repressive cell marker (MARCM) for ***Drosophila*** neural development. *Trends Neurosci* 2001, 24:251-254.

46. • Lee T, Marticke S, Sung C, Robinow S, Luo L: Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for mushroom body neuronal remodeling in ***Drosophila***. *Neuron* 2000, 28:807-818.
Using a genetic mosaic screen to identify proteins required for MB γ neuron remodelling, the authors found that *ultraspiracle* (*usp*) and the ecdysone receptor isoform, EcR-B1, are necessary for γ neurons to prune both dendrites and axons during metamorphosis.