Perchance to Smell

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The identification of large families of odorant receptor (OR) genes in several different animals has generated a great flurry of research in the development and functioning of the olfactory system. Olfactory receptor neurons (ORNs) from the periphery project to the olfactory lobe where they synapse with interneurons in distinct glomeruli. Results from studies using a variety of imaging methods suggest that a glomerulus is a functional unit of odour coding and stimulus quality is represented as a spatial map of activation among different glomeruli. What are the mechanisms that create this highly stereotyped map? In vertebrates, targeting of sensory neurons to glomeruli is regulated, at least to some extent, by the ORS themselves. In *Drosophila*, this is probably not the case since OR expression occurs at a time during development well after glomerular patterning has been established. The facile genetics of *Drosophila* has allowed rapid progress in the rules governing axonal connectivity and synapse formation in other regions of the central nervous system. This information is of great value in deciphering mechanisms during olfactory pathway development. The specification of ORN identities and their targeting to the distinct glomeruli is a subject of intense investigation in *Drosophila.* The close parallels in organisation of the olfactory pathway between vertebrates and invertebrates validate the use of *Drosophila melanogaster* useful model system for investigation of the basic mechanisms of olfactory coding and development. From our current knowledge it is apparent that the development of neural circuits underlying olfactory behaviour involves a precisely choreographed interaction between ORN, glial cells lobe interneurons. How this is regulated to give rise to precisely sculpted and yet behaviourally plastic circuits is a fundamental question in neurobiology.

Key Words: **Olfactory lobe, Olfactory glomeruli, Olfactory receptors, Axon targeting, Synapse formation, Olfaction,** ***Drosophila***

Olfactory behaviour is well known for the richness and complexity of response generated to simple stimuli. Chemical cues are known to induce dramatic changes in physiology as well as behaviour of animals; the type of response varies with the context in which the stimulus is presented. Very striking examples of chemical control of behaviour are seen in response to pheromones in a variety of animals including humans (reviewed in McClintock 1998). How do neural circuits that underlie such complex processing tasks develop and function? This review attempts to highlight our current understanding of the development and functioning of the olfactory system. The discussion will focus largely on the olfactory system of *Drosophila* although we will refer to results from other animals where appropriate. Anatomical and genetic similarities between the olfactory systems of insects and vertebrates have been the subject of a number of several recent reviews and the reader is referred to these (Hildebrand & Shepherd 1997, Strausfeld & Hildebrand 1999).

Odour Coding

Organisation at the Periphery

Olfactory receptor neurons (ORNs) are present within epithelia of the nasal cavity in vertebrates and on various appendages in invertebrates. Antennae of insects are the major smell organs and are exquisitely designed trapping-devices that allow efficient interaction of the chemical world with the detection surface (Boeckh et al. 1965). The description of antennal anatomy and ultrastructure has been the subject of a large body of work reviewed by Keil (1992) and Halberg and Hansonn (1999). In *Drosophila*, the organisation of olfactory sense organs on the antenna and maxillary palps have been chartered in some detail (Venkatesh & Singh 1984, Stocker 1994, Shanbhag et al. 1999). There are approximately 450 sense organs on the antennal surface which are of three morphologically distinct sensillar types. These sensilla are located in a fixed pattern on

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the antennal surface which is conserved between animals. The diversity of sense organ types makes the *Drosophila* antenna an attractive model for the analysis of determination of sense organ identity and cell fate (Ray & Rodrigues 1995, Reddy et al. 1997, Gupta & Rodrigues 1997, Goulding et al. 2000, Jhaveri et al. 2000b, Sen et al. 2003).

Electrophysiological measurements from ORNs in insects, amphibians and some vertebrates suggest that they are rather broadly tuned, detecting a range of chemical stimuli (reviewed in Getchell 1986, Siddiqi 1987). A subset of neurons have narrow stimulus specificity and they may act as 'labelled lines' to the central nervous system. de Bruyne and his colleagues systematically measured the electrical responses of individual ORNs on the *Drosophila* antenna to a panel of odorants and suggested that sensory units of similar function are clustered together. Such an organisation could have important implications in the modulation of neuronal activity and generation of olfactory behaviour (Boeckh 1981). A similar distribution of response properties exists other insects (Grant et al. 1998) and in mammals (Araneda et al. 2000).

Odorant Receptors

In the last decade, experimental approaches and novel computer algorithms have identified large families of candidate ORs in different animals (Buck 1996, Vosshall et al. 1999, Clyne et al. 1999, Gao & Chess 1999). In *Drosophila* as well as all vertebrates studied, each ORN expresses a single OR gene; this needs to be reconciled with the broad stimulus selectivities of each ORN. In the nematode *C. elegans*, on the other hand, each chemosensory neuron expresses an average of about 20 chemoreceptor genes (Bargmann 1998). OR genes encode seven transmembrane domain G-protein coupled receptors with little structural homology between species (Troemel et al. 1995, Buck 1996, Clyne et al. 1999, Vosshall et al. 1999, Gao & Chess 1999, figure 1). Ligands for these candidate receptors are currently being deciphered using adenoviral mediated gene expression systems and elegant calcium imaging strategies (Zhao et al. 1998, Malnic et al. 1999). Targeted misexpression studies in *Drosophila* have identified ligands for one of the olfactory receptors, Or 43a which responds to cyclic

molecules such as benzaldehyde and cyclohexanone (Stortkuhl & Kettler 2001).

How does each neuron select one from among hundreds of OR genes for expression? This is clearly a very challenging issue in studies related to of gene regulation. In *Drosophila*, Clyne et al. (1999) have identified a POU domain protein Acj6 which is involved in OR gene regulation in a subset of ORNs. The gene was first identified in a screen for mutants defective in olfactory behaviour (reviewed in Carlson 1996).

Spatial distribution of ORs among cells of olfactory epithelia is tightly programmed. In rodents, neurons expressing a given OR are confined to one of four zones, although the distribution within each zone is somewhat randomly dispersed (Ressler et al. 1993). The patterning of OR expression among cells in the epithelium attains an added degree of complexity in vertebrates where ORNs are being turned over throughout adult life. In *Drosophila in situ* hybridisation has demonstrated the expression of 32 OR genes on the antennae, 7 on the maxillary palp and 1 in both organs (Vosshall et al. 1999, Clyne et al. 1999). The OR that a given ORN expresses is related to its fate; this predicts a linkage between genetic cascades that regulate sense organ identity and receptor expression (Bhalerao et al. 2003, Sen et al. 2003).

The Central Odotopic Map

The organisation of insect olfactory lobes is remarkably similar in design to that of vertebrates (Hildebrand & Shepherd 1997, figure 2). ORNs from the periphery project to stereotypically arranged glomeruli in the olfactory lobes. Glomeruli are regions of high synaptic density formed by the terminal branches of sensory afferents and the dendritic arborisation of their target interneurons. In *Drosophila* there are 43 glomeruli which are characterised by their size, shape and invariant topographic position in the lobe (Stocker 1994, Laissue et al. 1999). Local interneurons connect glomeruli within the lobe and projection neurons (PNs) carry information out of the lobe to the calyx of the mushroom bodies and the lateral horn (figure 2A; Stocker 1994). The vertebrate equivalents of PNs are the mitral/tufted cells that relay information from the ~1800 glomeruli to the higher olfactory centres which comprise the piriform cortex, olfactory tubercle,

Figure 2 The organisation of the olfactory system in *Drosophila* (A) and mammals (B). In both cases the olfactory lobe is characterised by an array of glomeruli that receive axonal projections from the olfactory epithelia. In the fly, most of the receptor neurons arborize in the ipsilateral glomeruli and also send a collateral branch to corresponding glomeruli on the contralateral side. Local neurons and projection neurons (mitral cells) arborize within glomeruli and connect to → the higher centres in the brain.

|	Rodents	Nematode (*C. elegans*)	Fruit fly (*D. melanogaster*)
Size of the family (including pseudogenes)	~1000	~500	~60
Year of discovery	1991	1995	1999
Method of discovery	Selective single-cell PCR	Genetics and bioinformatics	Computer algorithms
Number of genes expressed/neuron	1?	~20	1?
Receptors with known ligands	Several e.g. OR 17/octanal	ODR-10/diacetyl	Or43a/benzaldehyde, cyclohexanol, cyclohexanone
Other related families	Vomeronasal receptors ~240 (1)		Gustatory receptors ~56 (2)
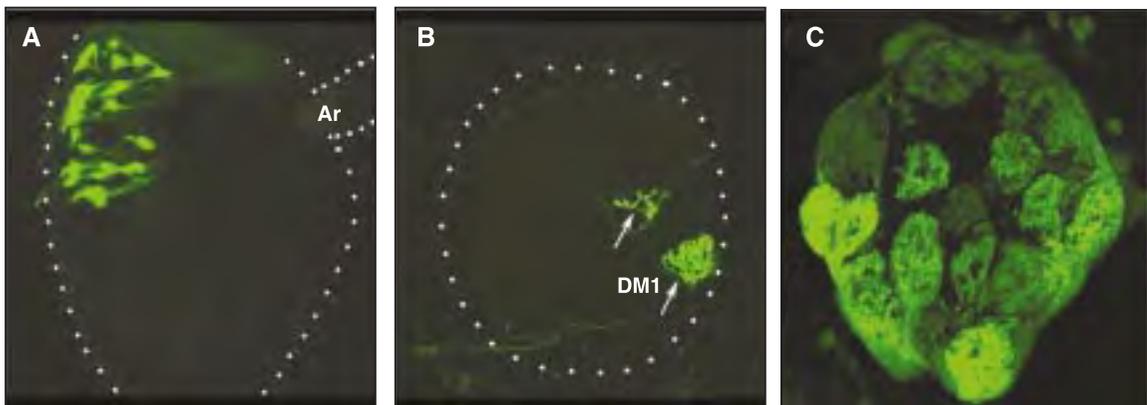
Figure 1A-C Olfactory receptor gene families. The Table above figures summarises information on the olfactory receptors in rodents, worms and flies. In addition to the ORs, families of vomeronasal receptors (1- Belluscio et al. 1999) and gustatory receptors have also been described (2- Scott et al. 2001). Figures A and B show the distribution of neurons expressing one of the *Drosophila* receptors Or22a. (A) Antenna (courtesy A. Sen) with arista indicated (Ar) **(B)** olfactory lobe showing projection of neurons to DM1 and an accessory glomerulus (arrows). **(C)** olfactory lobe showing multiple glomeruli marked with GFP using the Gal4 line SG18.1.
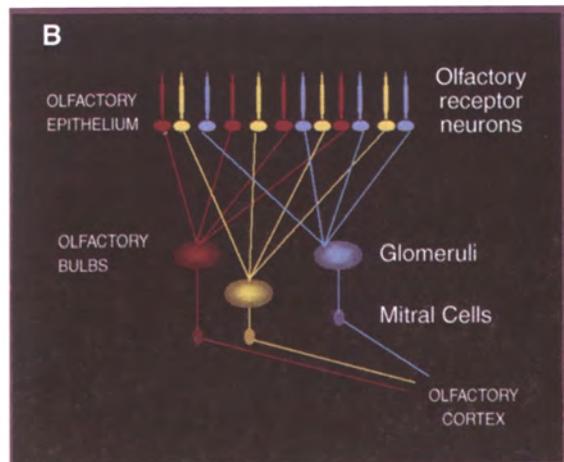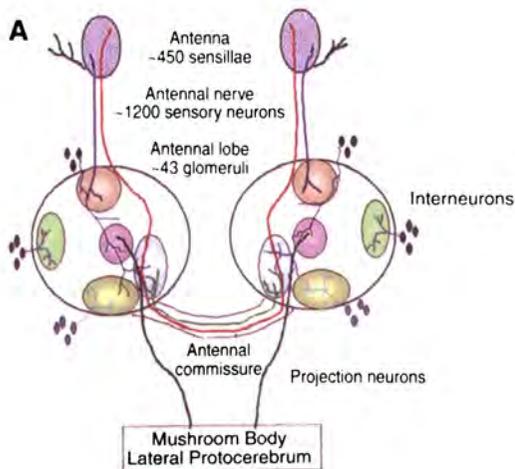
anterior olfactory nucleus and specific parts of amygdala. (Shepherd 1972, figure 2B).

A variety of functional imaging methods have revealed that olfactory information is encoded as an activity map among the lobe glomeruli (Jourdan et al. 1980, Lancet et al. 1982, Rodrigues 1988, Hansson et al. 1992, Rubin & Katz 1999, Galazia et al. 1999a,b, Uchida et al. 2000, Fiala et al. 2002, Sachse & Galizia 2002, Ng et al. 2002, Wang et al. 2003). These methods are advantageous over odour-evoked electrical measurements since they allow monitoring of populations, rather than individual neurons. The earliest studies in *Drosophila* used 2-deoxyglucose uptake to establish the presence of an overlapping pattern of activation among lobe neurons in response to odours (Rodrigues 1988). This method has the disadvantage of poor temporal resolution coupled with the limitations inherent to methods where fixation of post-mortem tissue is required. More recent techniques have great merit in allowing visualisation of neural activity active in live animals, hence providing spatial as well as temporal information (Falia et al. 2002, Ng et al. 2002 and Wang et al. 2003). High resolution imaging using a calcium-sensitive fluorescent protein in ORNs and PNs suggests a faithful transmission of information from the sensory input to higher brain centres (Wang et al. 2003).

PNs innervating specific glomeruli show stereotyped axon branching patterns and terminate in a defined region of the lateral horn (Marin et al. 2002, Wong et al. 2002). Neurons receiving input from neighbouring glomeruli arborize in similar domains in the calyx and lateral horn. This architecture provides a neuronal substrate for integration of information from glomeruli in the higher centres. In rodents, transneuronal tracing using barley lectin has shown the existence of a stereotyped map in the olfactory cortex (Zou et al. 2001). Inputs from different ORs map onto partially overlapping clusters of neurons in the olfactory cortex and those from the same receptor are targeted to multiple cortical areas. This would, in principle, permit parallel and differential integration of olfactory information.

Recent advances in Functional Magnetic Imaging (fMRI) and Positron Emission Tomography (PET) technology have greatly advanced out knowledge of odour coding in human brains (Savic 2002). Analysis of imaging data from human subjects coupled with psychophysical experiments is likely to provide very valuable information about modulation of complex behaviour by chemical stimuli. A growing body of imaging data suggests that perception of odorants is mediated by a set of core regions which are distributed across different brain centres.

Wiring the Olfactory System

Two pathways for processing of olfactory information exist in mammals- the main and the accessory olfactory bulb. The accessory bulb receives predominant input about pheromones from the vomeronasal organ. In the main olfactory bulb, ORNs expressing a single OR gene project largely to a single glomerulus in the olfactory lobe (Mombaerts et al. 1996), ORNs expressing vomeronasal receptors, on the other hand, project to multiple glomeruli in the accessory bulb (Rodriguez et al. 1999, Belluscio et al. 1999). In *Drosophila*, Vosshall et al. (2000) and Gao et al. (2000) suggest that ORNs of a single OR-type project to the same glomerulus.

Where does the instruction for this wiring come from? This issue has been addressed in variety of experimental systems ranging from rodents, zebrafish and amphibian systems to moths and flies (reviewed in Tolbert 1998, Lin & Ngai 1999).

Vertebrates

In rodents, compelling evidence exists for the role of the ORs themselves in providing guidance information during development (Mombaerts et al. 1996, Wang et al. 1998, Mombaerts 2001). In support of this hypothesis, *in situ* hybridisation experiments detected early embryonic expression of receptors and revealed presence of specific receptor mRNAs at axon termini within the olfactory lobe (Ressler et al. 1994, Vassar et al. 1994). Receptor substitution, as well as mutation of receptor genes in mice demonstrated that ORs are key players in establishing the topographic map (Mombaerts et al. 1996, Wang et al. 1998). The mechanisms underlying this process are still elusive. Vassalli et al. (2002) misexpressed of OR transgenes and found that these occasionally gave rise to additional glomeruli innervated by ectopic neurons as well as those expressing endogenous OR. This provides a clue that the transmembrane receptors could act by promoting homotypic interactions between sensory neurons of similar type.

It is unlikely, however, that ORs could provide all the information necessary for patterning sensory neurons within the antennal lobe. Cell adhesion molecules-olfactory cell adhesion molecule (OCAM), neural cell adhesion molecule (NCAM), and neuropilin-1, - are expressed in ORNs (Yoshihara et al. 1997, Treloar et al. 1997). Dominant-negative and mutant strategies in

chick and mice respectively, suggested a role for Sema3A/Neuropilin-1 mediated chemorepulsive signalling in preventing premature innervation in the bulb (Renzi et al. 2000, Schwarting et al. 2000). Different Rb8 NCAM isoforms could also regulate broad zonal distribution of neurons by homophilic cell adhesion (Alenuius & Bohm 2003).

Analysis of lobe development in the zebrafish is likely to reveal developmental principles distinct from those in rodents. Whitlock and Westerfield (1998) identified a population of transient, clonally distinct set of pioneer neurons, which do not express ORs and prefigure the olfactory pathway Ablation of these 'pioneers' severely disrupts axonal targeting of follower neurons, suggesting that these provide essential guidance cues for pathfinding by later sensory neurons.

Insects

Studies on olfactory lobe development have been most extensively been carried out in the moth *Manduca sexta.* In a classic series of experiments, Hildebrand and his colleagues (reviewed in Hildebrand 1996) transplanted the antennal disc from a male to a female pupa. This resulted in the formation of a male-specific glomerulus in the female lobe. This structure allowed the female to show behavioural responses to pheromones comparable to that in males (Schneiderman et al. 1986, Oland et al. 1998). These experiments provide evidence for the instructive role of ORNs in glomerular patterning. In addition to sensory neurons, glial cells have been implicated to act as intermediaries in developmental interactions between pre and post-synaptic populations of neurons within the lobe (Bailey et al. 1999). Glial cells present around the lobe prior to the entry of sensory neurons provide sorting cues and also help to stabilise axonal proto-glomeruli within the developing lobe (Baumann et al. 1996, Rossler et al. 1999). The timing of invasion of the ORNs into the lobe anlage is an important factor in proper development, implying the presence of temporally defined cues in the olfactory lobe (Rossler et al. 2000). Thus, accurately orchestrated interactions between sensory neurons, glial cells and interneurons are crucial during formation of structural units underlying the olfactory coding.

In *Drosophila*, the adult olfactory system arises during pupal life by modification of its larval counterpart as well as by *de novo* neurogenesis (Tissot & Stocker 2000). In the larvae, about 21 sensory neurons from the dorsal organ (figure 3A) project via the larval antennal nerve to the antennal lobe (Tissot et al. 1996). The lobe is approximately 30 μm in diameter and is

composed of glomerular-like structures (Python & Stocker 2002, figure 3B). This simple architecture is capable of detecting and discriminating a variety of chemical stimuli even though the behaviour generated is less sophisticated than that of adults (reviewed in Carlson 1996, Siddiqi 1987).

Adult ORNs develop from cells of the antennal disc and are guided to the brain by persistent larval neurons (Ray & Rodrigues 1995, Tissot et al. 1996, Reddy et al. 1997). The expression of OR genes in sensory neurons occurs late in pupal life well after lobe development is completed (Vosshall et al. 1999, Clyne et al. 1999, Jhaveri et al. 2000). This makes it unlikely that the ORs play a role in targeting to the lobe suggesting the presence of other mechanisms. We have demonstrated the presence of a distinct subset of neurons- those specified by the proneural gene *atonal* – which guide the rest of the neurons to the olfactory lobe (Jhaveri & Rodrigues 2002). Recent work from Hummel et al. (2003) has demonstrated that ORN classes require the Down Syndrome Cell Adhesion Molecule (*Dscam*) to synapse in the correct glomeruli. This finding is exciting since the *Dscam* locus could potentially generate 38,016 isoforms by alternative splicing (Schmucker et al. 2000).

Prior to sensory neuron entry, the lobe anlage contains remnant larval neurons as well as PNs. surrounded by glial cells probably of larval origin (figure 4A-C). Afferent innervation from the antenna arrives at the brain within the first 30% of pupal life, but remain along the periphery of the lobe also crossing over to the contralateral side (figure 4D,E). Sensory neurons remain in close association with glial cells around the lobe and processes from these two cell types invade the lobe simultaneously (figure 4F, Jhaveri et al. 2000). Glomerular formation first becomes apparent as loose structures after about 40% of pupal life; these protoglomeruli become more compact
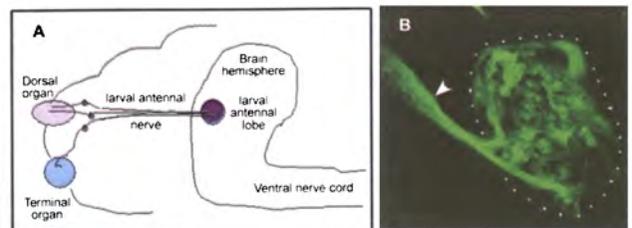

Figure 3 The larval olfactory system. A, Schematic showing the larval chemosensory system. Neurons from the dorsal organ project via the larval antennal nerve to the larval antennal lobe. The terminal organ is believed to be involved in contact chemoreception; B, Green Fluorescent Protein (GFP) is expressed in the larval olfactory neurons thus labelling the larval antennal nerve (white arrowhead) and the lobe. The larval neuropile outlined with dotted lines shows glomerular organisation.
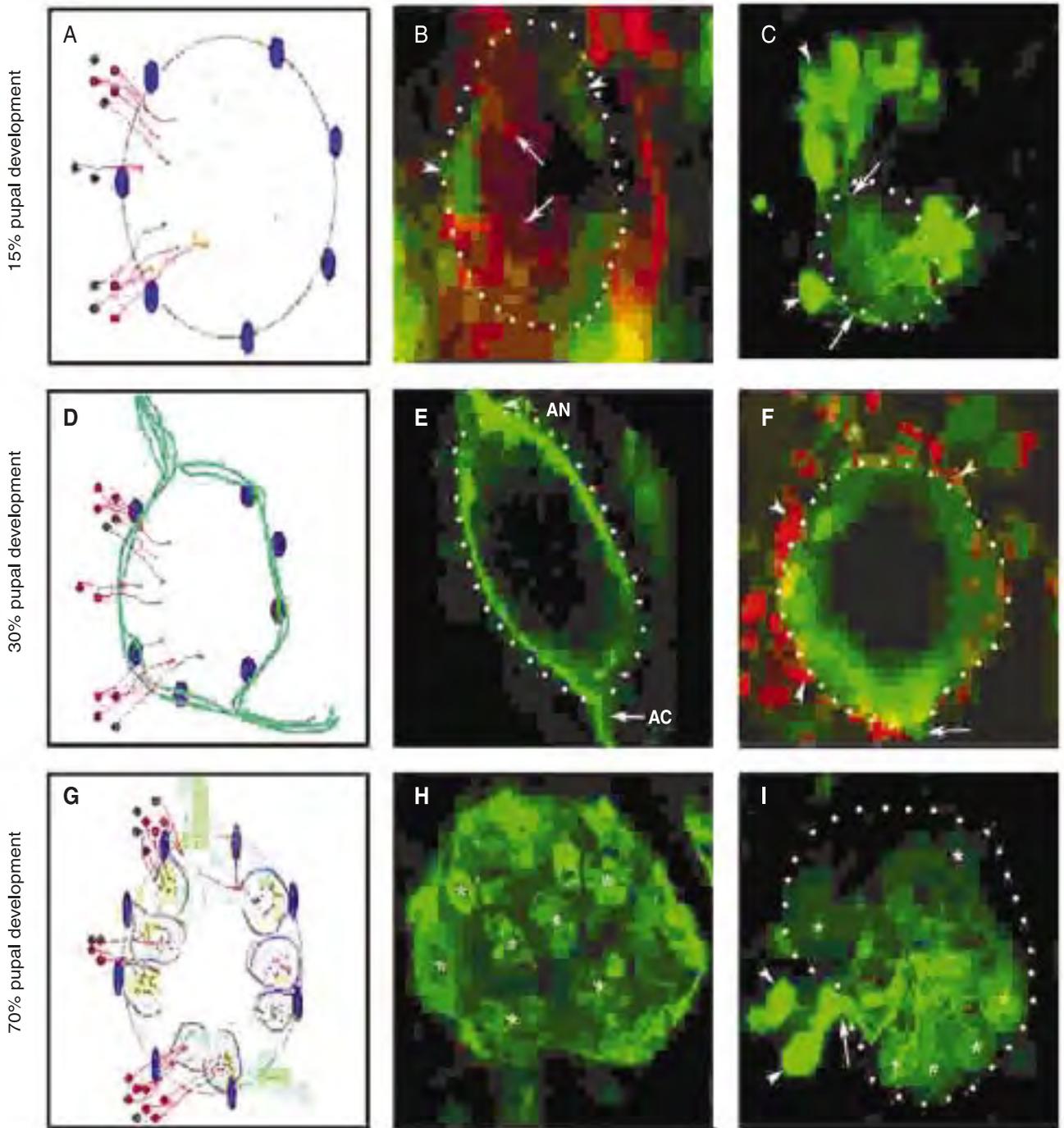
Figure 4 Development of the *Drosophila* olfactory lobe showing the interplay between sensory neurons, projection neurons and glial cells. In the schematic diagrams in A,D,G glial cells are depicted in blue, sensory neurons in green, PNs in red and larval remnant neurons in yellow. Pupal development lasts for about 100 hours at 25°C. At about 15% of pupal life, the sensory neurons in the antenna have not yet differentiated (A-C). The anlage of the olfactory lobe (dotted lines) is surrounded by glial cells (green in B) and is invested by larval remnant neurons (red in B). Cell bodies of the PNs (arrowheads in C) have already projected their axons (arrows in C) into the lobe (demarcated with dotted lines). By 30% of pupal life (D-F), the sensory neurons (green in E, F) have entered the lobe through the antennal nerve (AN), but remain on the periphery of the lobe and cross over to the contralateral side via the antennal commissure (AC). The sensory neurons remain in close association with glial cells (red in F). Late in pupal development when about 70% of pupal development is completed (G-I) glomerular formation is complete. The glomeruli can be visualised as tightly packed synaptic structures (* in H). At this time the cell bodies of the PNs (arrowheads) can be seen projecting axons (arrow in I) terminals to individual glomeruli (* in I).

as glial cells wrap around them and synaptic consolidation takes places by 70% of pupal life (figure 4G-I).

Although PNs invade the lobe early in development, they only target glomerular sites after sensory neuron entry (figure 4C, I; Jhaveri & Rodrigues 2002). The glomerular targets of projection neurons are pre specified depending on their lineage and birth order (Jefferis et al. 2001). The wiring of the PNs are specified by the POU domain transcription factors Acj6 and Drifter (Komiyama et al. 2003).

Even though a great deal of progress in our understanding of lobe development has been made in recent years, the mechanisms that determine selective targeting and synapse formation are far from understood in the olfactory lobe of insects. Lessons can be learned in *Drosophila* from our knowledge of development of specificity in other systems in the fly- notably the embryonic central nervous system (Tessier-Lavigne & Goodman 1996, Dickson 2001). It would be interesting to test whether the rules of connectivity that have been uncovered in studies in the *Drosophila* embryonic central nervous system also operate in the olfactory lobe of the adult.

Does neural activity play a role in organisation and maintenance of the olfactory system?

The development of cortical circuits has been shown to occur first by the generation of a coarse pattern of connections that is subsequently refined by activity-dependent processes (Tessier-Lavigne & Goodman 1996, Katz & Shatz 1996). What is the role of activity in the development of olfactory circuits? In the moth, injection of tetrodotoxin, a sodium channel blocker, prior to the onset of glomerular formation failed to affect patterning indicating that glomerular formation is largely independent of neuronal activity (Oland et al. 1996). Consistent with this finding, odour-evoked activity in the receptor neurons was detected only during the last stages of pupal development, well after the formation of glomeruli (Schweitzer et al. 1976). More recent *in vitro* data provides some evidence for the role of electrical activity in neurons for development of connectivity and cell fate (Mercer & Hildebrand 2002a,b).

In mice, odour-evoked neural activity was blocked by disruption of the α subunit of cyclic nucleotide gated channel (Lin et al. 2000, Zheng et al. 2000). Projections of ORNs and the formation of glomeruli were differentially affected; the glomeruli receiving input from the OR-P2 expressing neurons formed normally whereas those of OR-M72 failed to converge on defined glomeruli. Taking advantage of the

phenomenon of monoallelic expression of OR genes in rodents, it was shown that the axonal projections of channel-positive (functional) and channel-negative (non-functional) sort out into separate glomeruli, suggesting that activity-dependent neuronal competition modulate the sensory map (Zhao & Reed 2001).

A hard-wired circuit implies that olfactory information from the environment would need to be matched against a preexisting perceptual map in the brain. This suggests a limited repertoire of behaviour which is contrary to what has been observed in olfaction. The changes in olfactory circuitry in response to previous exposure to odorants is the subject of great interest (Galizia & Menzel 2000). In a recent study, it was shown that prolonged exposure to odorants lead to central adaptation and provided some evidence for the structural changes in glomerular morphology in an odour-specific manner (Devaud et al. 2001). Thorpe (1939) had shown that larvae reared on some chemicals showed altered behavioural responses to these stimuli. Similarly, associative learning in the larvae has been shown to be preserved into the adult (Tully et al. 1994). The availability of a range of genetic and imaging techniques in vertebrates and invertebrates now makes it possible to investigate plastic changes that may occur during olfaction.

Conclusions and Future Directions

The last decade has seen great advances in our understanding of how the olfactory system develops and functions. Several OR receptor families have been identified and we are beginning to identify the ligands for several of these molecules (Kajiya et al. 2001). We still need to understand how OR genes are regulated to express in specified ORNs during development. The mechanisms that specify the precise wiring between the peripheral receptors and the central neurons still await a complete description. Odour-induced behaviour suggests a major role for plasticity in the olfactory system. This is an area of research that requires attention using a variety of different experimental methods. The extent to which circuitry can be modulated both by previous exposure to stimuli as well as by environmental conditions and lifestyle of the animal remains a very exciting unsolved question in neurobiology.

References

Araneda R C, Kini A D and Firestein S 2000 The molecular receptive range of an odorant receptor; *Nat. Neurosci.* **3** 1248-55

Alenius M and Bohm S 2003 Differential function of RNCAM isoforms in precise target selection of olfactory sensory neurons; *Development* **130** 917-927

Bailey M S, Puche A C and Shipley M T 1999 Development of the olfactory bulb: evidence for glia-neuron interactions in glomerular formation; *J. Comp. Neurol.* **415** 423-48

Bargmann C I 1998 Neurobiology of the *Caenorhabditis elegans* genome; *Science* **282** 2028-2033

Baumann P M, Oland L A and Tolbert L P 1996 Glial cells stabilize axonal protoglomeruli in the developing olfactory lobe of the moth Manduca sexta; *J. Comp. Neurol.* **373** 118-28

Belluscio L, Koentges G, Axel R and Dulac C 1999 A map of pheromone receptor activation in the mammalian brain; *Cell* **97** 209-220

Bhalerao S, Sen A, Stocker R and Rodrigues V 2003 Olfactory neurons expressing identified receptor genes project to subsets of glomeruli within the antennal lobe of *Drosophila melanogaster*; *J Neurobiol.* **54** 577-592

Boeckh J, Kaissling K E and Schneider D 1965 Insect olfactory receptors; *Cold Spring Harb. Symp. Quant. Biol.* **30** 263-280

_____ 1981 Chemoreceptors: their structure and function; in *Sense Organs*, eds. M S Laverack and P J Costens (Glasgow, London Blackie & Son), pp 86-99

Buck L B 1996 Information coding in the vertebrate olfactory system; *Annu. Rev. Neurosci.* **19** 517-544

Carlson J R 1996 Olfaction in *Drosophila*: from odor to behavior; *Trends in Genetics* **12** 175-180

Clyne P J, Warr C G, Freeman M R, Lessing D, Kim J and Carlson J R 1999 A novel family of divergent seven transmembrane proteins: candidate odorant receptors in *Drosophila*; *Neuron.* **22** 327-338

de Bruyne M, Foster K and Carlson J R 2001 Odor coding in the *Drosophila antenna*; *Neuron.* **30** 537-552

Devaud J M, Acebes A and Ferrus A 2001 Odor exposure causes central adaptation and morphological changes in selected olfactory glomeruli in *Drosophila*; *J. Neurosci.* **21** 6274-6282

Dickson B J 2001 Rho GTPases in growth cone guidance; *Curr. Opin. Neurobiol.* **11** 103-110

Dulac C and Axel R 1995 A novel family of genes encoding putative pheromone receptors in mammals; *Cell* **83** 195-206

Fiala A, Spall T, Diegelmann S, Eisermann B, Sachse S, Devaud J M, Buchner E and Galizia C G 2002 Genetically expressed cameleon in *Drosophila melanogaster* is used to visualize olfactory information in projection neurons; *Curr. Biol.* **12** 1877-1884

Galizia C G and Menzel R 2000 Odour perception in honeybees: coding information in glomerular patterns; *Curr. Opin. Neurobiol.* **10** 504-510

_____, _____ and Holldobler B 1999a Optical imaging of odor-evoked glomerular activity patterns in the antennal lobes of the ant *camponotus rufipes*; *Naturwiss.* **86** 533-537

_____, Sachse S, Rappert A and Menzel R 1999b The glomerular code for odor representation is species specific in the honeybee *Apis mellifera*; *Nature Neurosci.* **2** 473-478

Gao Q and Chess A 1999 Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence; *Genomics* **15** 31-39

_____, Yuan B and Chess A 2000 Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe; *Nat. Neurosci.* **3** 780-785

Getchell T V 1986 Functional properties of vertebrate olfactory receptor neurons; *Physiol. Rev.* **66** 772-818

Goulding S E, zur Lage P and Jarman A 2000 *Amos* is a proneural gene for *Drosophila* olfactory sense organs that is regulated *lozenge*; *Neuron* **25** 69-78

Gupta B P and Rodrigues V 1997 Atonal is a proneural gene for a subset of olfactory sense organs in *Drosophila*; *Genes to Cells* **2** 225-233

Grant A, Riendeau C and O'Connell R 1998 Spatial organization of olfactory receptor neurons on the antenna of the cabbage looper moth; *J. Comp. Physiol. A* **183** 433-442

Hallberg E and Hansson B S 1999 Arthropod sensilla: morphology and phylogenetic considerations; *Microsc. Res. Tech.* **47** 428-439

Hansson B S, Ljungberg H, Hallberg E and Loftstedt C 1992 Functional specialization of olfactory glomeruli in a moth; *Science* **29** 1313-1315

Hildebrand J G 1996 Olfactory control of behavior in moths: central processing of odor information and the functional significance of olfactory glomeruli; *J. Comp. Physiol.* [A] **178** 5-19

_____ and Shepherd G M 1997 Mechanisms of olfactory discrimination: converging evidence for common principles across phyla; *Annu. Rev. Neurosci.* **20** 595-631

Hummel T, Vasconcelos M L, Clemens J C, Fishilevich Y, Vosshall L B and Zipursky S L 2003 Axonal targeting of olfactory receptor neurons in *Drosophila* is controlled by Dscam; *Neuron* **37** 221-231

Jourdan F, Duveau A, Astic L and Holley A 1980 Spatial distribution of ^{14}C-2-deoxyglucose uptake in the olfactory bulbs of rats stimulated with two different odors; *Brain Res.* **188** 139-154

Jefferies G S X E, Marin E C, Stocker R F and Luo L 2001 Target neuron prespecification in the olfactory map of *Drosophila*; *Nature* **414** 204-208

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

_____, _____, Reddy G V and Rodrigues V 2000b Sense organ identity in the *Drosophila* antenna is specified by the expression of the proneural gene atonal; *Mech. Dev.* **99** 101-111

_____ and Rodrigues V 2002 Sensory neurons of the Atonal lineage pioneer the formation of glomeruli within the adult *Drosophila* olfactory lobe; *Development* **129** 1251-1260

Kajiya K, Inaki K, Tanaka M, Haga T, Kataoka H and Touhara K 2001 Molecular bases of odor discrimination: reconstitution of olfactory receptors that recognize overlapping sets of odorants; *J. Neurosci.* **21** 6018-6025

Katz L C and Shatz C J 1996 Synaptic activity and the construction of cortical circuits; *Science* **274** 1133-1138

Keil T A 1992 Fine structure of a developing insect olfactory organ: morphogenesis of the silkmoth antenna; *Microsc. Res. Tech.* **22** 351-371

Komiyama T, Johnston W A, Luo L and Jefferies G S X E 2003 From lineage to wiring specificity: POU domain transcription factors control precise connections of *Drosophila* olfactory projection neurons; *Cell* **112** 157-167

Laissue P P, Reiter C, Hiesinger P R, Halter S, Fischbach K F and Stocker R F 1999 Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*; *J. Comp. Neurol.* **22** 543-52

Lancet D, Greer C A, Kauer J S and Shepherd G M 1982 Mapping of odor-related neuronal activity in the olfactory bulb by high resolution 2-deoxyglucose autoradiography; *Proc. Natl. Acad. Sci. USA* **79** 670-674

Lin D M and Ngai J 1999 Development of the vertebrate main olfactory bulb; *Curr. Opinion in Neurobiol.* **9** 74-78

_____, Wang F, Lowe G, Gold G H, Axel R, Ngai J and Brunet L 2000 Formation of precise connections in the olfactory bulb occurs in the absence of odorant-evoked neuronal activity; *Neuron* **26** 69-80

Malnic B, Hirono J, Sato T and Buck L B 1999 Combinatorial receptor codes for odors; *Cell* **96** 713-723

Marin E C, Jefferis G S X E, Komiyama T, Zhu H and Luo L 2002 Representation of the glomerular olfactory map in the *Drosophila* brain; *Cell* **109** 243-255

McClintock M K 1998 On the nature of mammalian and human pheromones; *Ann. N Y Acad. Sci.* **30 855** 390-392

Mercer A R and Hildebrand J G 2002a Developmental changes in the electrophysiological properties and response characteristics of Manduca antennal-lobe neurons; *J. Neurophysiol.* **87** 2650-2663

Mercer A R and Hildebrand J G 2002b Developmental changes in the density of ionic currents in antennal-lobe neurons of the sphinx moth, *Manduca sexta*; *J. Neurophysiol.* **87** 2664-2675

Mombaerts P, Wang F, Dulac C, Chao S K, Nemes A, Mendelsohn M, Edmondson J and Axel R 1996 Visualizing an olfactory sensory map; *Cell* **87** 675-686

_____ 2001 How smell develops; *Nat. Neurosci.* **4** 1192-1198

Ng M, Roorda R D, Lima S Q, Zemelman B V, Morcillo P and Meisenbock G 2002 Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly; *Neuron* **36** 463-474

Oland L A, Pott W M, Bukhman G, Sun X J, Tolbert L P 1996b Activity blockade does not prevent the construction of olfactory glomeruli in the moth Manduca sexta; *Int. J. Dev. Neurosci.* **14** 983-996

_____ and Tolbert L P 1998 Glomerulus development in the absence of a set of mitral-like neurons in the insect olfactory lobe; *J. Neurobiol.* **36** 41-52

Python F and Stocker R F 2002 Adult-like complexity of the larval antennal lobe of *D. melanogaster* despite markedly low numbers of odorant receptor neurons; *J. Comp. Neurol.* **445** 374-387

Ray K and Rodrigues V 1995 Cellular events during of the olfactory sense organs in *Drosophila melanogaster*; *Dev. Biol.* **167** 426-438

Reddy G V, Gupta B P, Ray K and Rodrigues V 1997 Development of the *Drosophila* olfactory sense organs utilizes cell-cell interactions as well as lineage; *Development* **124** 703-712

Renzi M J, Wexler T L, Raper J A 2000 Olfactory sensory axons expressing a dominant-negative semaphorin receptor enter the CNS early and overshoot their target; *Neuron.* **28** 437-447

Ressler K J, Sullivan S L and Buck L B 1993 A zonal organization of odorant receptor gene expression in the olfactory epithelium; *Cell* **73** 597-609

_____, _____ and _____ 1994 Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb; *Cell* **73** 597-609

Rodrigues V 1988 Spatial coding of olfactory information in the antennal lobe of *Drosophila melanogaster*; *Brain Res.* **453** 299-30

_____, Feinstein P and Mombaerts P 1999 Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system; *Cell* **97** 199-208

Rossler W, Oland L A, Higgins M R, Hildebrand J G and Tolbert T P 1999 Development of a glia-rich axon-sorting zone in the olfactory pathway of the moth Manduca sexta; *J. Neurosci.* **19** 9865-9877

_____, Tolbert L P and Hildebrand J G 2000 Importance of timing of olfactory receptor-axon outgrowth for glomerulus development in *Manduca sexta*; *J. Comp. Neurol.* **425** 233-243

Rubin B D and Katz L C 1999 Optical mapping of odorant representations in the mammalian olfactory bulb; *Neuron* **23** 499-511

Sachse S and Galizia C G 2002 Role of inhibition for temporal and spatial odor representation in olfactory output neurons: a calcium imaging study; *J. Neurophysiol.* **87** 1106-1117

Savic I 2002 Imaging of brain activation by odorants in humans; *Curr. Opin. Neurobiol.* **12** 455-461

Schmucker D, Clemens J C, Shu H, Worby C A, Xiao J, Muda M, Dixon J E and Zipursky S L 2000 *Drosophila* Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity; *Cell* **101** 671-684

Schneiderman A, Hildebrand J G, Brennan M M and Tomlinson J H 1986 Trans-sexual grafted antennae alter pheromone-directed behaviour in a moth; *Nature* **323** 801-803

Schwarting G A, Kostek C, Ahmad N, Dibble C, Pays L and Puschel A W 2000 Semaphorin 3A is required for guidance of olfactory axons in mice; *J. Neurosci.* **20** 7691-7697

Schweitzer E S, Sanes J R and Hildebrand J G 1976 Ontogeny of electroantennogram responses in the moth, *Manduca sexta; J. Insect. Physiol.* **2** 955-960

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

Sen A, Reddy G V and Rodrigues 2003 Combinatorial expression of Prospero, Seven-up and Elav, identify progenitor cell types during sense-organ differentiation in the *Drosophila* antenna; *Develop. Biology* **In press**

Shanbhag S R, Müller B and Steinbrecht R A 1999 Atlas of olfactory organs of *Drosophila melanogaster*. 1. Types, external organization, innervation and distribution of olfactory sensilla; *Int. J. Insect. Morphol. Embryol.* **28** 377-397

Shepherd G M 1972 Synaptic organization of the mammalian olfactory bulb; *Physiol. Rev.* **52** 864-917

Siddiqi O 1987 Neurogenetics of olfaction in *Drosophila melanogaster; Trends in Genetics* **3** 137-142

Strausfeld N J and Hildebrand J G 1999 Olfactory systems: common design, uncommon origins; *Curr. Opin. Neurobiol.* **9** 634-639

Stocker R F 1994 The organization of the chemosensory system in *Drosophila melanogaster*: a review; *Cell Tissue Res.* **275** 3-26

Stortkuhl K F and Kettler R 2001 Functional analysis of an olfactory receptor in *Drosophila melanogaster; Proc. Natl. Acad. Sci. USA.* **98** 9381-9385

Tessier-Lavigne M and Goodman C S 1996 The molecular biology of axon guidance; *Science* **274** 1123-1133

Thorpe W H 1939 Further studies on pre-imaginal conditioning in insects; *Proc. Roy. Soc. Lond. B* **127** 424-433

Tissot M, Gendre N, Hawken A, Stortkuhl K F and Stocker R F 1996 Larval chemosensory projections and invasion of the adult afferents in the antennal lobe of *Drosophila; J. Neurobiol.* **32** 281-297

_____ amd Stocker R F 2000 Metamorphosis in *Drosophila* and other insects: the fate of neurons throughout the stages; *Prog. Neurobiol.* **62** 89-111

Tolbert L P 1998 Olfactory development in invertebrates. On the scent of central developmental issues; *Ann. N Y Acad. Sci.* **855** 95-103

Treloar H, Tomasiewicz H, Magnuson T and Key B 1997 The central pathway of primary olfactory axons is abnormal in mice lacking the N-CAM-180 isoform; *J. Neurobiol.* **32** 643-58

Troemel E R, Chou J H, Dwyer N D, Colbert H A and Bargmann C I 1995 Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans; Cell* **83** 207-218

_____, Sagasti A and Bargmann C I 1999 Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C elegans; Cell* **99** 387-398

Tully T, Cambiazo V and Kruse L 1994 Memory through metamorphosis in normal and mutant *Drosophila; J. Neurosci.* **14** 68-74

Uchida N, Takashashi Y K, Tanifuji M and Mori K 2000 Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features; *Nature Neurosci.* **3** 1035-1043

Vassalli A, Rothman A, Feinstein P, Zapotocky M and Mombaerts P 2002 Minigenes impart odorant receptor-specific axon guidance in the olfactory bulb; *Neuron.* **35** 681-696

Vassar R, Chao S K, Sitcheran R, Nunez J M, Vosshall L B and Axel R 1994 Topographic organization of sensory projections to the olfactory bulb; *Cell* **79** 981-99

Venkatesh S and Singh R N 1984 Sensilla on the third antennal segment of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae); *Int. J. Insect Morphol. Embryol.* **13** 51-63

Vosshall L B, Amrein H, Morozov P S, Rzhetsky A and Axel R 1999 A spatial map of olfactory receptor expression in the *Drosophila* antenna; *Cell* **96** 725-736

_____, Wong A M and Axel R 2000 An olfactory sensory map in the fly brain; *Cell* **102** 147-159

Wang F, Nemes A, Mendelsohn M and Axel R 1998 Odorant receptors govern the formation of a precise topographic map; *Cell* **93** 47-60

Wang J W, Wong A M, Flores J, Vosshall L B and Axel R 2003 Two-Photon Calcium Imaging Reveals an Odor-Evoked Map of Activity in the Fly Brain; *Cell* **112** 271–282

Whitlock K E and Westerfield M 1998 A transient population of neurons pioneers the olfactory pathway in the zebrafish; *J. Neurosci.* **18** 8919-8927

Wong A M, Wang J W and Axel R 2002 Spatial representation of the glomerular map in the *Drosophila protocerebrum*; *Cell* **109** 229-241

Yoshihara Y, Kawasaki M, Tamada A, Fujita H, Hayashi H, Kagamiyama H and Mori K 1997 OCAM: A new member of the neural cell adhesion molecule family related to zone-to-zone projection of olfactory and vomeronasal axons; *J. Neurosci.* **17** 58 30-42

Zhao H and Reed R R 2001 X inactivation of the OCNC1 channel gene reveals a role for activity-dependent competition in the olfactory system; *Cell* **104** 651-660

_____, Ivic L, Otaki J M, Hashimoto M, Mikoshiba K and Firestein S 1998 Functional expression of a mammalian odorant receptor; *Science* **279** 237-242

Zheng C, Feinstein P, Bozza T, Rodriguez I and Mombaerts P 2000 Peripheral olfactory projections are differentially affected in mice deficient in a cyclic nucleotidegated channel subunit; *Neuron* **26** 81-91

Zou Z, Horowitz L F, Montmayeur J P, Snapper S and Buck L B 2001 Genetic tracing reveals a stereotyped sensory map in the olfactory cortex; *Nature* **414** 173-179