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Sensory neurons of the Atonal lineage pioneer the formation of glomeruli within the adult *Drosophila* olfactory lobe

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SUMMARY

The first centers for processing of odor information by animals lie in the olfactory lobe. Sensory neurons from the periphery synapse with interneurons in anatomically recognizable units, termed glomeruli, seen in both insects and vertebrates. The mechanisms that underlie the formation of functional maps of the odor-world in the glomeruli within the olfactory lobe remains unclear. We address the basis of sensory targeting in the fruitfly *Drosophila* and show that one class of sensory neurons, those of the Atonal lineage, plays a crucial role in glomerular patterning. Atonal-dependent neurons pioneer the segregation of other classes of sensory neurons into distinct glomeruli. Furthermore, correct sensory innervation is necessary for the arborization of projection neurons into glomeruli and for the elaboration of processes of central glial cells into the lobe.

Key words: Atonal, Antennal lobe, Olfactory glomeruli, Projection neurons, Glia, *Drosophila*

INTRODUCTION

Diverse odors are detected by sensory neurons and represented in the olfactory lobes in anatomically identifiable regions called glomeruli. Functional imaging experiments in insects (Rodrigues, 1988; Hansson et al., 1992) and, more recently, in vertebrates have established that odor information is encoded as a spatial map of glomerular neural activity (Rubin and Katz, 1999; Uchida et al., 2000). Each sensory neuron in the fruitfly *Drosophila* typically projects to a single identifiable glomerulus and synapses on to interneurons of central origin (Stocker, 1994). Projection neurons from glomeruli target to higher centers in the mushroom body and the lateral horn. In addition, a number of local interneurons connect glomeruli either within the same lobe or between contralateral lobes. Each glomerulus is ensheathed by glial cell projections (Jhaveri et al., 2000a). What are the relationships between peripheral neurons and their central targets during development, resulting in the organized development of olfactory glomeruli?

Large families of candidate chemosensory receptors have been identified in several different animals by experimental approaches or by the use of novel computer algorithms (Buck, 1996; Vosshall et al., 1999; Clyne et al., 1999; Gao and Chess, 1999; Stortkuhl and Kettler, 2001). Results from in situ hybridization experiments in *Drosophila*, have shown that, with a few minor exceptions, each olfactory neuron expresses a single receptor gene (Vosshall et al., 1999; Clyne et al., 1999). Neurons expressing a given receptor type appear to converge largely onto a predominant glomerulus in each olfactory lobe (Vosshall et al., 2000; Gao et al., 2000). A similar topographic map has been described in the olfactory bulb of rodents (Ressler et al., 1994; Vassar et al., 1994; Royet et al., 1998). Receptor substitution experiments as well as mutation of receptor genes in mice has provided compelling evidence for the role of odorant receptors in the formation of the topographic map (Mombaerts et al., 1996; Wang et al., 1998).

A model in which the selective expression of receptor genes within olfactory neurons is itself instrumental in guiding axons to defined targets is an elegant explanation for generation of the odor map. However this mechanism is unlikely to be widely applicable across species. In the zebrafish, a population of transient, clonally distinct set of pioneer neurons, which do not express odorant receptors, prefigure the olfactory pathway (Whitlock and Westerfield, 1998). Similarly, in *Drosophila*, expression of odorant receptor genes occurs late in pupal development, well after patterning of the lobe is completed (Vosshall et al., 1999; Clyne et al., 1999; Jhaveri et al., 2000a). This suggests that novel and rather complex developmental mechanisms must operate in these systems to create odor-specific neural maps within the adult brain.

A large body of evidence that has accumulated from studies of the moth olfactory pathway has shown that the sensory neurons are instructive in determining glomerular patterns (Schneiderman et al., 1986; Oland and Tolbert, 1998; Rossler et al., 1999a; Rossler et al., 1999b). Transplantation of the male antennal disc into female pupae leads to formation of a male-specific macroglomerulus, which is important for sensing of sexual pheromones. This and other investigations suggest that the sensory neurons are essential for organizing the specificity

of glomerular formation during development. However, it seems unlikely that lobe interneurons are passive partners during glomerular formation; Malun et al. (Malun et al., 1994) ablated sensory neurons and demonstrated that a few 'glomerular-like' structures could still be formed. The olfactory lobe is therefore a unique system for the study of how interactions between sensory neurons, interneurons and glial cells can define functionally distinct neural structures.

We investigate the role of sense organ specificity in the choice of central targets. There are three morphologically distinct sense organs on the surface of the antenna: sensilla coeloconica, basiconica and trichoidea (Venkatesh and Singh, 1984). We have previously shown that the helix-loop-helix transcription factor, Atonal (Ato) is necessary and sufficient for formation of the coleoconic sensilla (Gupta and Rodrigues, 1997; Jhaveri et al., 2000b). A related proneural protein, Amos, is required for the specification of the other two types of sensilla (Goulding et al., 2000). Expression of *amos* is regulated by the runt domain transcription factor Lozenge (Lz), which appears to regulate the choice between basiconic and trichoid sensilla in a dose-dependent manner (Gupta et al., 1998). We show that neurons of the Ato lineage are the first to enter the nascent adult olfactory lobe and are necessary for the correct targeting of other sensory neurons into the lobe. In mutants where Ato neurons fail to form, the afferents of other sense organs at first remain stalled at the periphery and although they ultimately enter the lobe, they fail to target correctly. As a consequence, glomerular formation is disrupted. The projection of central glial cells within the lobe is affected and postsynaptic interneurons enter the lobe normally but fail to terminate in glomeruli. This suggests that an intimate interaction between sensory neurons, projection neurons and glia during development orchestrates glomerular patterning, with sensory neurons of one class pioneering the organization of all sensory inputs crucial for this process.

MATERIALS AND METHODS

Fly stocks

B. Shymala (Shymala and Chopra, 1999) kindly provided the SG18.1-Gal4 enhancer trap line, MZ317-Gal4 was a gift from Kei Ito and GH146 from Reinhard Stocker. The *ato* strains- ato^1/TM3, ato^2/FM6, Df(3R)p^{13}/TM3 and UAS-Ato were obtained from Andrew Jarman (Jarman et al., 1995). ato^1 is either a strong hypomorph or an amorphic allele; the strain carries a closely linked lethal, making it inviable as a homozygote. The phenotype can be examined by placing it in *trans* with a deficiency (Df(3R)p^{13}) for the region. The ato^2 allele is a hypomorph caused by an insertion into the region upstream of the gene with no change in the coding region. The strain carries a translocation of part of the X chromosome onto the autosome requiring that both X and third chromosomes must segregate together (White and Jarman, 2000). lz^3 was obtained from Reinhard Stocker; UAS dominant negative Cdc42 (UAS-DNCdc42) and UAS-Rac^{v12} were obtained from the Jan laboratory; and UAS-GFP (1010T2) was obtained from Barry Dickson. Balancer and marker stocks were kindly provided by the *Drosophila* stock center at Bloomington, Indiana (Lindsley and Zimm, 1992).

To visualize sensory neurons and glial cells in strong *ato* hypomorphs, stable stocks of SG18.1 UAS-GFP; ato^1/ CyO-Tb and MZ317 UAS-GFP; ato^1/CyO-Tb were generated and crossed to Df(3R)p^{13}/TM3-actin-GFP. ato^1/Df(3R)p^{13} pupae were selected based on the absence of balancers. Weaker *ato* heteroallelic combination was obtained by individually crossing SG18.1 UAS GFP; ato^1/CyO-Tb and Mz317 UAS GFP; ato^1/CyO-Tb to ato^2/FM6. The reduced ommatidial phenotype was used to select for ato^1/ato^2 animals.

All flies were reared at 25°C on a standard cornmeal media containing yeast. For staging, white puparia [0 hours after puparium formation (APF)] were collected and allowed to develop on moist filter paper at 25°C unless otherwise mentioned. The white pupal stage lasts for 1 hour hence the error in staging is ±30 minutes. Wild-type Canton-Special (CS) pupae emerge within 100 hours after white puparium stage at 25°C. Developmental times in all other genotypes were normalized with respect to that of the CS strain.

Immunohistochemistry

Dissection and antibody staining of the pupal and adult brain wholemounts were carried out as described elsewhere (Jhaveri et al., 2000a). The primary antibodies used were 22C10 (1:50; from S. Benzer's laboratory), anti-Fas II, ID4 (1:5; from Corey Goodman), anti-Dachshund (1:25, Developmental Studies Hybridoma Bank at University of Iowa) and rabbit anti-Repo (1:250, from Gerd Technau). Secondary antibodies used were Alexa 568-coupled goat anti-mouse and anti-rabbit IgG (Molecular Probes), and Cy5-conjugated goat anti-mouse and anti-rabbit IgG (Amersham). The labeled samples were mounted in anti-fading agent, Vectashield (Vector Laboratories), imaged on BioRad Radiance 2000 at 1 μm intervals, and data were processed using Confocal Assistant and Adobe Photoshop 5.0.

Cuticle preparation

Adult antennae were placed in a drop of Faure's mountant (34% v/v chloral hydrate, 13% glycerol, 20 mg/ml gum Arabic, 0.3% cocaine chlorohydrate) and allowed to clear overnight on a heating block at 70°C. Olfactory sense organs on the third antennal segment were counted by projecting images on the video monitor. A minimum of 10 samples was analyzed in each case to compute the mean and standard deviation.

In situ hybridization

Digoxygenin-labeled riboprobes were generated for Or22b, which was cloned in pGEM-T Easyvector (a kind gift from John Carlson) using standard methods. RNA in situ hybridization was performed as described (Clyne et al., 1999) on 10 μm frozen antennal sections. Digoxigenin-RNA probe was visualized using alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim).

RESULTS

Each sensory neuron from the third antennal segment projects to a single glomerulus and, in the majority of cases, sends a collateral branch to the corresponding glomerulus on the contralateral side (Stocker et. al., 1990). The SG18.1-Gal4 enhancer-trap strain was used to drive a green fluorescent protein (GFP) reporter specifically in sensory neurons, allowing visualization of their presence during glomerular development (Brand and Perrimon, 1993). In the antenna, SG18.1-Gal4 is expressed in all olfactory neurons, although more strongly in the coeloconic and trichoid sense organs (Shymala and Chopra, 1999). In adult lobe, well-patterned identifiable glomeruli and the inter-antennal commissure can be clearly discerned (Fig. 1A,G,J). Synaptic maturation within the glomeruli can be visualized by staining with antibodies against the immunoglobulin superfamily member, Fasciclin II (Fas II) (Schuster et al., 1996; Jhaveri et al., 2000a). The glomerular pattern resembles that seen in the adult by 60 hours after formation of the white puparium (APF) (Fig. 1B). At

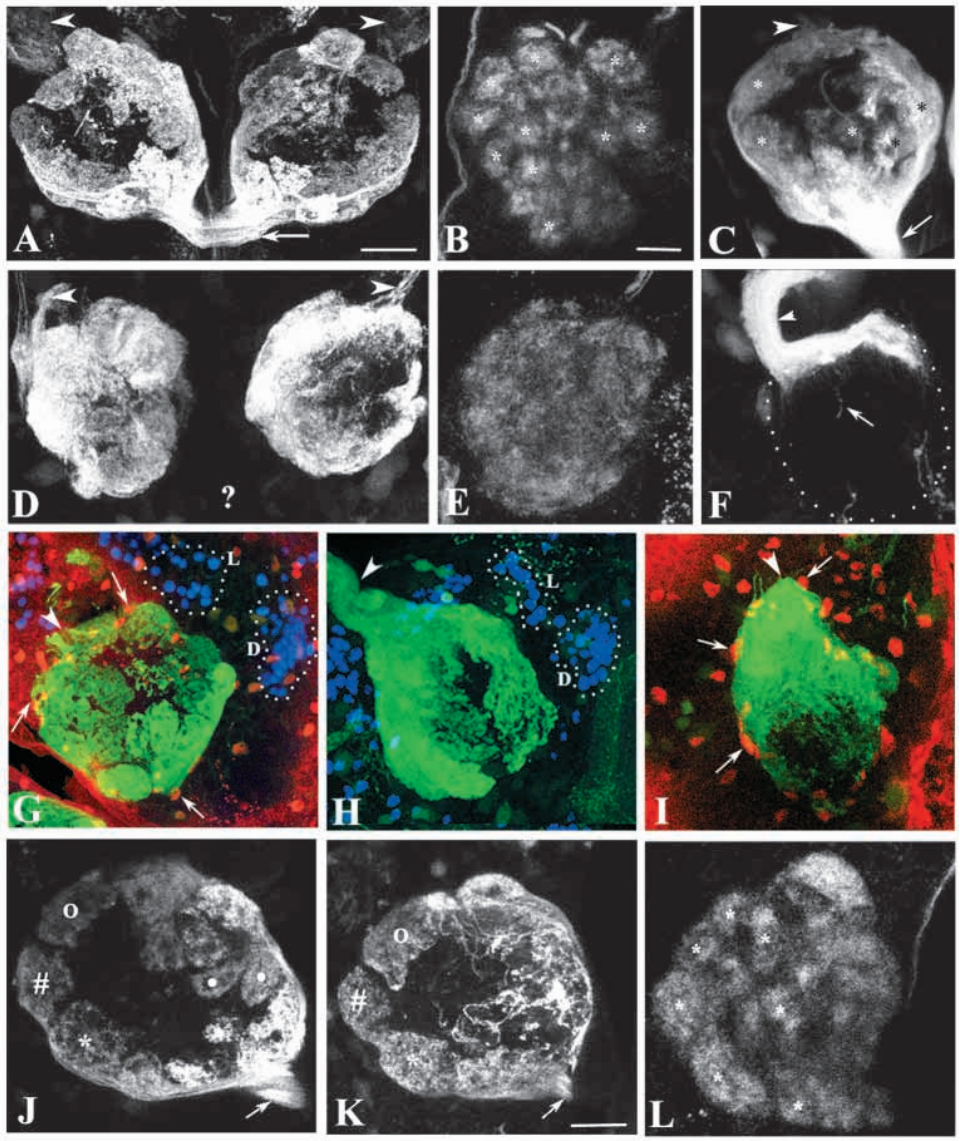

Fig. 1. Effect of sense organ specificity on olfactory lobe patterning. The olfactory neurons in wild type and mutants were traced using the SG18.1-Gal4;UAS-GFP strain (green). (A-C) Wild-type antennal lobes; arrowheads mark entry of the antennal nerve and arrows indicate the inter-antennal commissure. (A) Adult lobe. (B) 60 hour APF lobe stained with anti-Fas II. (C) 48 hour APF lobe at the same magnification as (B) showing several well formed glomeruli (*). (D) Adult SG18.1 UAS GFP; *ato*^{1}/Df(3R)*p*^{13} lobes at the same magnification as A. Antennal nerves enter the lobe (arrowheads) but no glomeruli can be discerned. The expected position of the inter-antennal commissure is indicated by (?). (E) 60 hour APF mutant lobe stained with anti-FasII; no glomeruli can be distinguished (compare with B, at the same magnification). (F) 48 hour APF SG18.1 UAS GFP; *ato*^{1}/Df(3R)*p*^{13} lobe. Most afferents are stalled immediately upon entry of the antennal nerve (arrowhead); only a few invade the lobe (arrow). (G-I) Adult wild-type (G) and *ato*$^{-}$ mutant (H,I) lobes stained with anti-Dachshund (blue in G,H) and anti-Repo (red in G,I). Dorsal (D) and lateral (L) clusters of interneurons are demarcated with dotted lines in G,H. Arrows in G,I indicate glial cells at the lobe periphery. (J) Adult SG18.1-Gal4 UAS GFP/+ (K) and *lz*^{3}; SG18.1-Gal4 UAS GFP/+ lobes. Well-formed glomeruli are indicated by *, # and o.; white dots indicate glomeruli in the wild type that are poorly innervated in the mutant. (L) 60 hours APF lobe stained with anti-FasII shows presence of several well-developed glomeruli. Scale bars: 25 μm in A,B; 20 μm in K. G-L are at the same magnification.

about this time, odorant receptor expression in the sensory neurons is first detected, making it unlikely that the receptors play any role in the targeting of sensory neurons to the olfactory lobe (Clyne et al., 1999).

In order to test whether sense organ type affects lobe patterning, we examined the projection patterns of the sensory neurons in mutants that lack specific sense organs. Mutations in *ato* affect specifically the coeloconic sense organs (Gupta and Rodrigues, 1997) while the *lz* locus affect both basiconica and trichoidea (Stocker et al., 1993).

Absence of cells of the Ato-lineage affects segregation of neural inputs from all olfactory sense organs

Flies of genotype *ato*^{1}/Df(3R)*p*^{13} lack all coeloconic sensilla on the antenna (Gupta and Rodrigues, 1997). These sense organs are each innervated by two or three sensory neurons (Shanbhag et al., 1999); as there are about 75 coeloconica, approximately 200 of the total of 1200 sensory axons would be absent in animals that lack *ato*$^{+}$ function. We traced the projection patterns of the residual (~1000) neurons projecting from sensilla basiconica and trichoidea on the antenna by examining GFP expression in animals of the genotype SG18.1 UAS-GFP/+; *ato*^{1}/Df(3R)*p*^{13} (Fig. 1D-F). In the wild type, the first sensory afferents to enter the brain are those of the Ato lineage (Jhaveri et al., 2000a). These neurons enter the brain at 20 hours APF and remain on the periphery of the lobe in close association with glial cells until about 30 hours APF. Soon after, these neurons, together with the projections from other sensilla, which have by then arrived within the brain, invade the lobe, and glomerular formation begins at about 36 hours APF. In *ato* mutants, the residual sensory afferents stall at the periphery of the lobe up until the mid-pupal stage (compare Fig. 1F with 1C) with only very few stray fibers actually projecting into the lobe (small arrows in Fig. 1F). Later in development, afferents do enter the lobe but remain disorganized with no obvious glomerular structures (Fig. 1D,E). The antennal commissure fails to form, suggesting that even though the sensory neurons of the basiconic and trichoid sensilla can enter the lobe in the

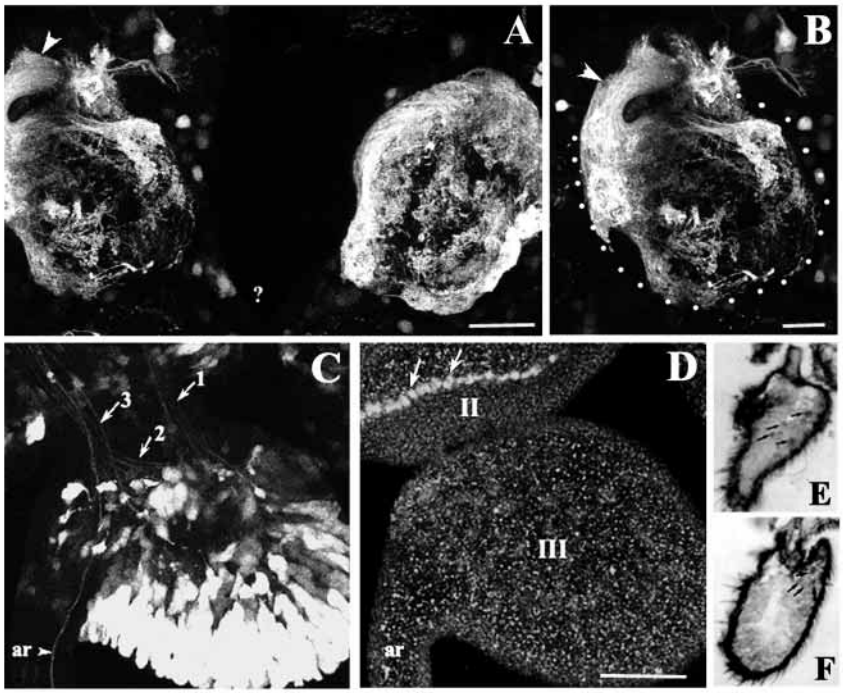

Fig. 2. Ectopic expression of Ato does not rescue the glomerular phenotype of *ato*^{1}/Df(3R)*p*^{13}. (A,B) The SG18.1-Gal4 line was used to drive UAS-Ato and UAS-GFP in the SG18.1 UAS GFP/UAS-Ato; *ato*^{1}/Df(3R)*p*^{13} genotype. The adult lobe morphology is similar to *ato*^{1}/Df(3R)*p*^{13} (see Fig. 1D). Inter-antennal commissure is absent (?). Arrowheads mark the entry of sensory neurons. The left lobe in A is enlarged in B for clarity. Dotted line marks lobe boundary. (C,D) Third antennal segments from 22 hours APF pupae. Sensory neurons from the third segment and the arista (ar) have differentiated and leave the antenna in three distinct fascicles (1, 2, 3). (C) Staining with anti-Ato (red) shows no immunoreactivity in the third segment (III). Cells of the Johnston's organ (arrows) in the second segment (II) express Ato (D). (E,F) RNA in situ hybridization on horizontal sections of the adult antenna with digoxygenin-labeled Or22b probe. Wild-type (E) and *ato*^{1}/Df(3R)*p*^{13} (F) antennae show the odorant receptor gene expression in a subset of large basiconica (arrows). The altered morphology of the *ato*$^{-}$ antenna makes the location of the sensilla appear somewhat different, but we verified that the same number of expressing cells were present in wild-type and *ato*^{1}/Df(3R)*p*^{13} antennae. Scale bars: 25 μm in A,B; in D, 25 μm in C,D.

absence of the Ato lineage, they do not send collateral branches to the contralateral lobe (Fig. 1D). These phenotypes could, in principle, arise from the lack of Ato function in the brain. We examined the presence of lobe associated interneurons by staining with anti-Dachshund (blue in Fig. 1G,H), and glia with anti-Repo (red in Fig. 1G,I) and find that these are normal in *ato*$^{-}$ mutants.

Our data demonstrate that the lack of coeloconic sense organs affects patterning of all sensory neurons within the antennal lobe, including those arising from other sensilla. The fact that the Ato-dependent sensilla are the first to appear within the antennal disc is consistent with this lineage playing a pioneering role during lobe development.

Absence of sense organs specified by Lz affect only those glomeruli which receive inputs from these neurons

Null alleles at the *lz* locus lead to an absence of all basiconic sensilla and a large subset of trichoid sensilla (Gupta et al., 1998). In *lz*^{3} animals only ~300 of the 1200 olfactory neurons form (Stocker, 1994; Shanbhag et al., 1999). Serial confocal sectioning of *lz*^{3}; SG18.1 UAS-GFP/+ adult lobes allowed the identification of most glomeruli seen in wild-type controls (Fig. 1J,K). The prominent 'V' glomerulus was absent, as described before (Stocker and Gendre, 1988; Laissue et al., 1999), and some central glomeruli (white dots in Fig. 1J), although present, were poorly formed in the mutants. Staining of 60 hour pupal lobes with anti-FasII antibodies revealed several well-defined glomeruli (Fig. 1L)

These observations demonstrate that glomerular formation proceeds relatively normally despite the complete absence of basiconic sensilla and most of the trichoids. It is intriguing to note that the lobe can be correctly patterned by only ~300 neurons, about two thirds arising from the Ato-lineage.

Late expression of Ato in neurons does not rescue antennal lobe patterning defects

Hassan et al. (Hassan et al., 2000) have shown that Ato plays a regulatory role in the arborization of neurons during pupal life, perhaps by an interaction with Notch. We tested whether the patterning defects observed in the olfactory lobe of *ato*$^{-}$ mutants could be explained by a later role in arborization, rather than being a consequence of the lack of proneural function leading to the absence of coeloconic sensilla. We expressed Ato in sensory neurons that still formed in *ato*^{1}/Df(3R)*p*^{13} animals using the SG18.1 UAS-GFP/UAS-Ato; *ato*^{1}/Df(3R)*p*^{13} genotype. The aglomerular phenotype could not be rescued by Ato ectopic expression and the lobes closely resembled those of strong *ato* hypomorphs (compare Fig. 2A,B with Fig. 1D). In the wild type, ectopic expression of Ato in all olfactory neurons using Neuralised-Gal4 or SG18.1-Gal4 did not affect glomerular formation (data not shown).

Gupta and Rodrigues (Gupta and Rodrigues, 1997) showed that Ato expression begins in the antennal disc of the third larval instar and decays by about 12 hours APF. This is consistent with a role for *ato* as a proneural gene. We stained pupal antenna between 18 hours and 36 hours APF, a period when sense organs undergo differentiation (Fig. 2C). No Ato immunoreactivity was detected at these times in the third antennal segment, although cells of the Johnston's organ in second segment showed expression (Fig. 2D). No Ato immunoreactivity was seen in the developing antennal lobe at any pupal age (not shown).

In order to test whether differentiation of sensory neurons was affected by lack of *ato*$^{+}$ function, we examined the expression of candidate odorant receptor genes in the antenna. In situ hybridization to sections of adult *Drosophila* antenna using a digoxygenin-labeled Or22b probe showed normal expression in a subset of large basiconica in wild type as well

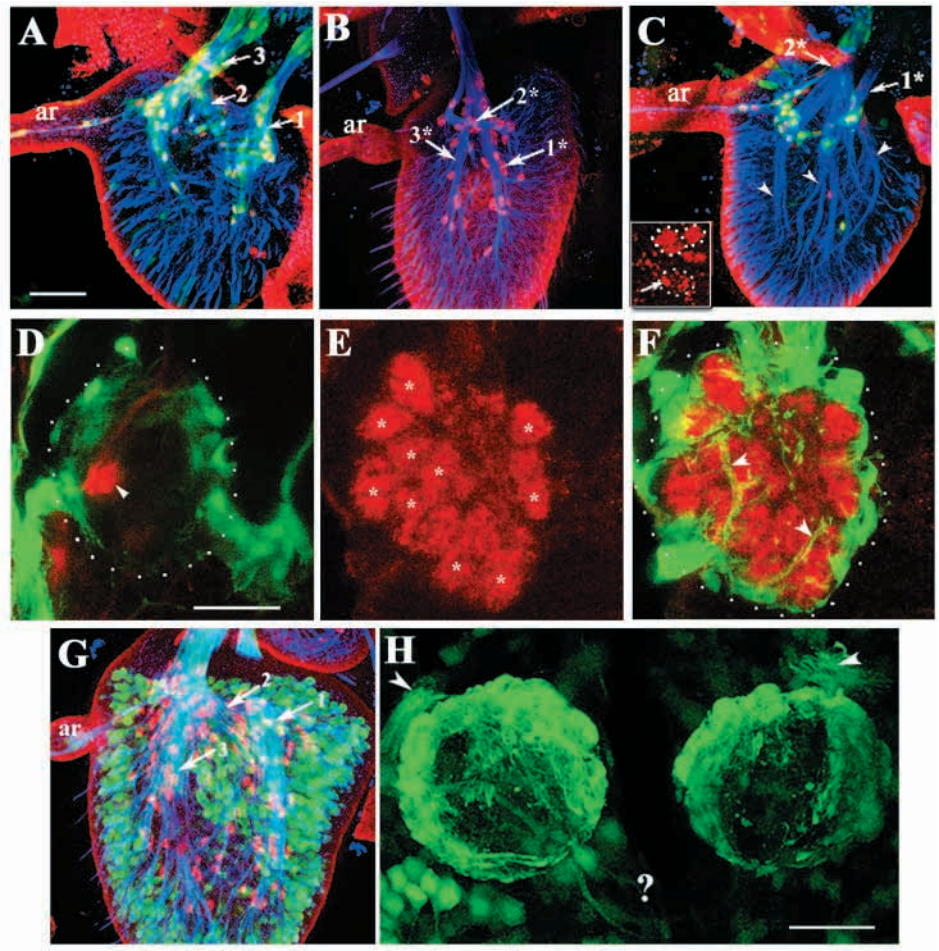

Fig. 3. Peripheral glia direct fasciculation of olfactory neurons in the antenna but do not influence patterning of the lobe. (A-C) Pupal antennae stained with mAb 22C10 (blue) to visualize sensory neurons; glia are labeled with anti-Repo antibodies (red). In the wild type (A) at 25 hours APF, Repo-positive glial cells associate with the axons of the sensory neurons in three fascicles (1, 2, 3). Expression of GFP driven by MZ317-Gal4 GAL4 shows glial cell bodies and their processes wrapping each of the fascicles (green). (B) In 36 hour APF *ato*^{1}/Df(3R)*p*^{13} antennae, there is a reduction of glia (~ 35 Repo-labeled cells in *ato* hypomorphs compared with ~100 in the wild type) and the three fascicles (1*, 2* and 3*) merge into a single bundle. (C) 25 hours APF; MZ317-Gal4 UAS-GFP/UAS-DNCdc42; *ato*^{1}/Df(3R)*p*^{13} antenna. The MZ317-Gal4 enhancer trap line drives expression of DN-Cdc42 as well as GFP in glia. Glial cells (red) are reduced in number; remaining cells are aggregated in the proximal region of the antenna. Only two exiting fascicles (1* and 2*) can be recognized. Inset shows glial cells stained with anti-Repo; cell bodies are demarcated by dotted lines. Dying cells show a fragmented staining. (D-F) Olfactory lobe morphology of the genotype described in (C). (D) 36 hours APF; a single glomerulus shows Fas II staining; lobe associated glia (green) are present normally surrounding the lobe (dotted line). (E,F) 60 hours APF; glomerular formation proceeds normally and several glomeruli can be recognized (* in E). Ectopic expression of DN-Cdc42 in the glial cells associated with the lobe does not appear to affect their morphology and processes can be seen entering lobe (arrowheads in F). (G,H) Ectopic expression of constitutively active form of Rac in sensory neurons in SG18.1-Gal4 UAS GFP/+; UAS Rac^{V12}/+ animals. (G) 36 hour APF antenna stained with mAb22C10 (blue) and anti-Repo (red); the SG18.1-Gal4 GFP is shown in green. Sensory neurons exit normally in three fascicles (1, 2, 3) (ar, arista). (H) Adult antennal lobe from genotype described in G. The entry of sensory neurons into the antennal lobe is shown by arrowheads, but neurons within the lobe are completely disorganized. The inter-antennal commissure (?) fails to form. Scale bars: in A, 30 μm in A-C; in D, 30 μm in D-F; in H, 20 μm.

as *ato*^{1}/Df(3R)*p*^{13} animals (Fig. 2E,F) (Clyne et al., 1999). This demonstrates that the onset of receptor gene expression is not affected in situations where central connectivity is compromised.

These results together provide strong evidence that the cells of the Ato lineage, probably the neurons themselves, play a pioneering role in glomerular patterning.

The *ato* lineage is the source of peripheral glial cells, required for the fasciculation of all sensory neurons, but not for lobe development

Jhaveri et al. (Jhaveri et al., 2000a) had shown that sensory progenitors selected by *ato*$^{+}$ function give rise not only to neurons and support cells of the sensilla coleoconica but also to the majority of the antennal glia. Repo-positive glial cells migrate along the developing neurons and their process ensheath the sensory axons into three distinct fascicles (designated 1, 2, 3 in Fig. 3A). In *ato*^{1}/Df(3R)*p*^{13} animals, peripheral patterning is affected; only one major fascicle is observed as the axons exit the antenna (Fig. 3B). There is also a striking reduction in the number of peripheral glia; we could count only 35±1 glia compared with 100±6 in the wild type. The majority of the antennal glia therefore arise from the gliogenic Ato lineage, while a small population are Ato independent and could have a central origin (Jhaveri et al., 2000a).

As *ato* mutants affect both glial as well as sensory neurons, the primary cause of the fasciculation defect in the antenna is unclear. In order to test the possible causal role of glia in sensory fasciculation, we specifically perturbed development of these cells using the Gal4/UAS system. Mis-expression of a dominant negative form of Cdc42 (DN-Cdc42) using the glial-specific MZ317-Gal4 line, would be expected to block cell growth because of disruption of cytoskeletal polarity (Jhaveri et al., 2000a; Dickson, 2001). We observed a reduction in glial cell number, presumably because of cell death, and residual cells were aggregated with no apparent processes (Fig. 3C and inset). Sensory neurons showed a range of defects in fasciculation, some of which closely resembled that of *ato* mutants (Fig. 3C).

These observations demonstrate a role for glia in segregation of sensory axons in the adult peripheral nervous system. One

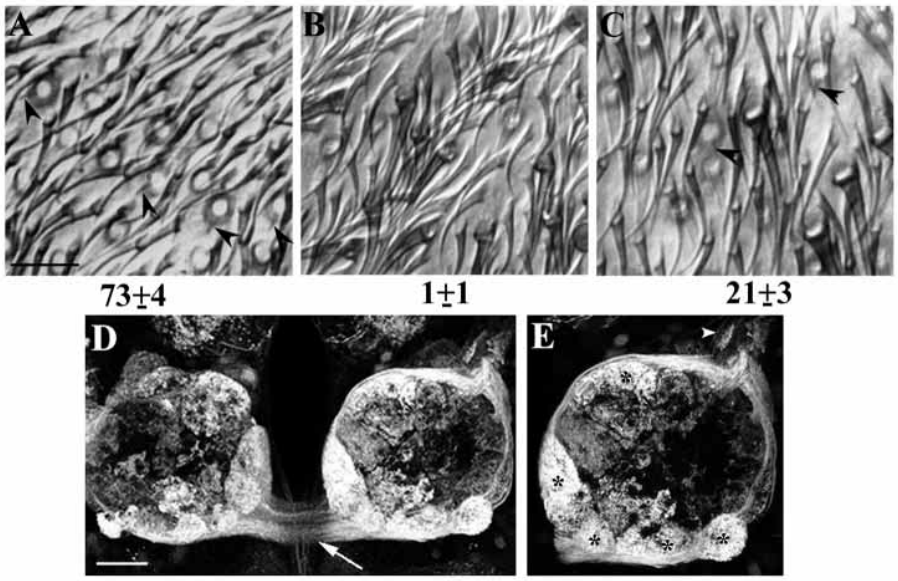

Fig. 4. A subset of coeloconic sense organs which form in weak hypomorphic mutants are sufficient to pattern the antennal lobe. (A-C) Region of the adult antenna showing coeloconic sensilla (arrowheads). Numbers given below are based on counts from at least 10 antenna. Similar numbers were obtained from the canton-S wild-type strain (A) as well as *ato*^{1}/+ and Df(3R)*p*^{13}/+ heterozygotes. (B) *ato*^{1}/Df(3R)*p*^{13}. (C) *ato*^{1}/*ato*^{2}. (D,E) GFP expression in the sensory neurons using SG18.1 in *ato*^{1}/*ato*^{2} background. (D) Adult antennal lobe showing almost normal looking lobe with identifiable glomeruli and presence of commissure (arrow) (compare with Fig. 1A). (E) A single lobe enlarged to show the presence of distinct glomeruli (*). Arrowhead marks entry of antennal nerve to the lobe. Scale bars: in A, 10 μm in A-C; in D, 20 μm in D,E.

possibility is that these glia, which ensheath the peripheral axons, also play a role in the sorting of afferents into glomeruli. The presence of glial sorting zones that direct migration of olfactory neurons to target glomeruli has been shown in the moth *Manduca sexta* (Rossler et al., 1999a). We stained the lobes of MZ317-Gal4 UAS-GFP/UAS-DN-CDC42 with an antibody against FasII (Fig. 3D-F). Glomeruli developed normally in spite of a reduction of peripheral glial numbers and defective organization of the sensory neurons at the periphery. MZ317-Gal4 also expresses in the glial cells around the lobe (green in Fig. 3D,F). Somewhat surprisingly, we did not observe any obvious defects in the location of the lobe-associated glia or their extensions into the lobe (arrow in Fig. 3F).

Hence, while peripheral glia instruct patterning of the olfactory neurons at the periphery into distinct fascicles, they play no role in the organization of the antennal lobe. We conclude that the focus of the defect in lobe patterning seen in *ato* hypomorphs lies within the neurons arising from the coeloconic sense organs.

Expression of constitutively active Rac in neurons does not affect peripheral fasciculation but phenocopies the *ato* defect in the lobe

Rho GTPases form an important link between signaling cues and the actin cytoskeleton; thus, ectopic expression of constitutively active or dominant negative forms in neurons could lead to defects in growth cone motility (Dickson, 2000). We mis-expressed activated Rac in olfactory neurons using SG18.1-Gal4 and observed no defect in the peripheral organization of the olfactory neurons (Fig. 3G). Interestingly, the lobes of SG18.1-Gal4 UAS-GFP/UAS-Rac^{v12} appeared remarkably similar to those of *ato* loss-of-function animals (compare Fig. 3H with Fig. 1D). The neurons were disorganized within the olfactory lobe and the inter-antennal commissure was not formed. The phenotype that we observe could result from an abrogation of proper signaling between neurons during lobe formation or be due to a failure of growth cone extension of Ato-dependent neurons into the lobe.

The patterning defect in the antennal lobe can be 'rescued' by the presence of a small subset of Ato neurons

Antenna from *ato*^{1}/Df(3R)*p*^{13} animals completely lacked coeloconic sensilla (compare Fig. 4B with 4A). Weaker hypomorphic allelic combinations - *ato*^{1}/*ato*^{2} - allowed formation of a smaller number of coeloconic sensilla (arrowheads in Fig. 4C). The antennal lobes of these animals showed normal glomeruli and the presence of the inter-antennal commissure (compare Fig. 4D,E with Fig. 1A,G). The presence of about 20 coeloconic sensilla in these mutants would contribute to about 50 sensory neurons. This means that the presence of even a small fraction of Ato-dependent neurons is sufficient to provide instruction to the rest of the afferents for correct targeting.

Sensory inputs act to organize central glia and interneurons within the lobe

Our data so far have shown that the presence of Ato-dependent neurons is crucial for organization of the rest of the afferents within the lobe. Does the defect in sensory neurons also affect the targeting of central glial cells and interneurons? Most of the glial cell bodies lie at the periphery of the lobe (arrowheads in Fig. 5A) with only a few within the lobe itself (not shown). In lobes from *ato*^{1}/Df(3R)*p*^{13}, we had observed a normal distribution of Repo-positive glial cells associated with the lobe (Fig. 1I). The glial processes visualized in MZ317 UAS-GFP/+; *ato*^{1}/Df(3R)*p*^{13}, however, showed aberrant hyper-branching within the lobe (Fig. 5B). We had previously shown that in the wild type, glial process invade the lobe at approximately the same time as those from the sensory afferents (Jhaveri et al., 2000a). The phenotype in *ato* mutants suggests that the correct extension of glial processes to ensheath individual glomeruli is regulated by interaction with the sensory neurons. The elaboration of glial processes is normal in the weaker hypomorphic combination *ato*^{1}/*ato*^{2} (Fig. 5C).

Do the sensory neurons influence arborization of the lobe interneurons into specific glomeruli? The interneurons are of two broad classes: (1) the projection neurons that arborize within

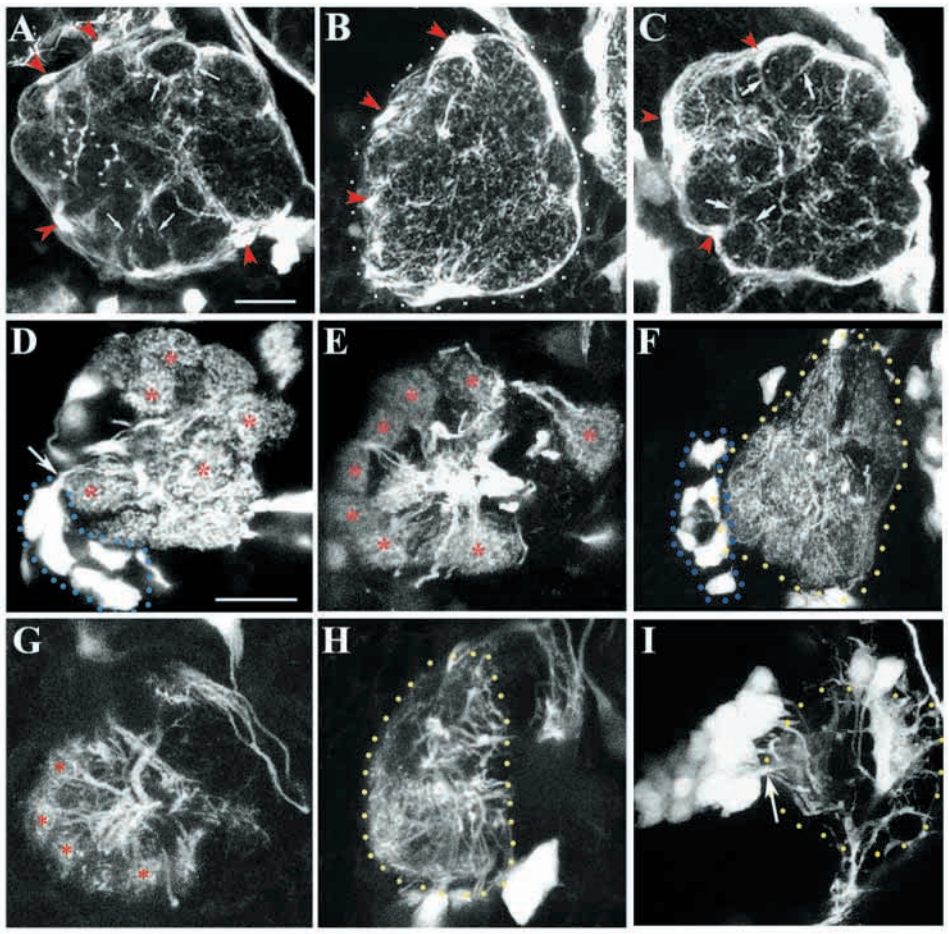

Fig. 5. Formation of glial processes and dendritic arborization of interneurons within the lobe are influenced by sensory inputs. (A-C) MZ317-Gal4 driven expression of GFP in the adult antennal lobes. Glial cell bodies lie at the periphery of the lobe (red arrowheads). (A) Wild type; processes extend into to lobe and ensheath individual glomeruli (small arrows). (B) Glial cells in *ato*^{1}/Df(3R)*p*^{13} lobes show excessive branching. (C) *ato*^{1}/*ato*^{2}; glial organization is comparable with wild type. (D-I) GH146 expression in pupal lobes from wild-type (D,E,G,I) and *ato*^{1}/Df(3R)*p*^{13} (F,H) lobes. (D-F) Late pupae (~90 hours APF). Dotted blue lines demarcate the lateral cluster of projection neurons cell bodies in the wild-type (D) and mutant (F) pupae. Projection neurons enter the lobe (arrow in D) and terminate in well defined glomeruli (* in D,E). (F) In *ato*^{1}/Df(3R)*p*^{13}, projection neurons are present within the lobe (boundaries indicated by dotted yellow lines); but these are unpatterned. (G,H) Mid-pupal (~45 hours APF) lobes from wild type (G) and mutant (H). At this time, wild-type projection neurons have arborized within well-defined glomeruli (* in G). No such organization is visible in the mutant. (I) 14 hour APF projection neurons invade the lobe anlage (dotted yellow lines), although patterning has not yet occurred. Scale bars: in A, 20 μm for A-C; in D, 20 μm in D-I.

single glomeruli and send axons into higher brain centers including the mushroom body and the lateral horn; and (2) the local interneurons that connect the glomeruli within the same lobe and also connect corresponding glomeruli in both lobes (Stocker, 1994). The GH146-Gal4 line coupled with GFP allows the visualization of about 90 projection neurons that lie in three clusters anterodorsal, lateral and ventral to the antennal lobe (Stocker et al., 1997; Jefferies et al., 2001).

We used the GH146 line to study the effect of sensory innervation on projection neuron targeting. In late pupae, these neurons target to well defined glomeruli (asterisk in Fig. 5D,E). Patterning of these interneurons is temporally correlated with the arrival of sensory neurons into the lobe; hence, at the mid-pupal stage (~45 hours APF), several glomeruli become recognizable (Fig. 5G, asterisk). In *ato*^{1}/Df(3R)*p*^{13} pupae, projection neurons enter the lobe but fail to target to proto-glomerular sites (Fig. 5F,H). These neurons invade the lobe well before sensory neurons have differentiated (Fig. 5I), although dendritic arborization requires the presence of sensory afferents. From our experiments, we are unable to ascertain whether the putative instructive signals come from the Ato-dependent neurons or are a general property of all olfactory neurons.

DISCUSSION

Our results from the genetic manipulation of sensory neurons on the antenna can be interpreted in the context of the events that take place during normal development. The adult olfactory lobe in *Drosophila* is built upon a pre-existing larval structure by addition of interneurons that arise by proliferation of identified neuroblasts during larval life (Tissot et al., 1997; Stocker et al., 1997; Jefferies et al., 2001). The glomerular innervation of these interneurons is pre-specified by lineage and birth order, and they enter the pupal lobe well before sensory neurons are chosen from cells on the antennal disc (Ray and Rodrigues, 1995; Reddy et al., 1997) (Fig. 6A). At this stage, however, there is no evidence of glomerular patterning and dendrites of the projection neurons have not terminated into their appropriate proto-glomeruli. The first olfactory neurons to enter the brain are those of the Ato-lineage, which remain at the periphery of the lobe for several hours after arrival (Fig. 6B). These afferents, together with those of the basiconic and trichoid sense organs, which arrive later, invade the lobe at around 30 hours APF. Glial processes are elaborated and dendritic arborization of projection neurons occurs after targeting of sensory afferents is initiated (Fig. 6C). Neurons of the Ato lineage are key players in these patterning events; in their absence, none of the other sensory afferents or the projection neurons or glia can target correctly to glomeruli (Fig. 6D). This leads us to propose a functional role as pioneers to neurons projecting from the Ato-derived sense organs.

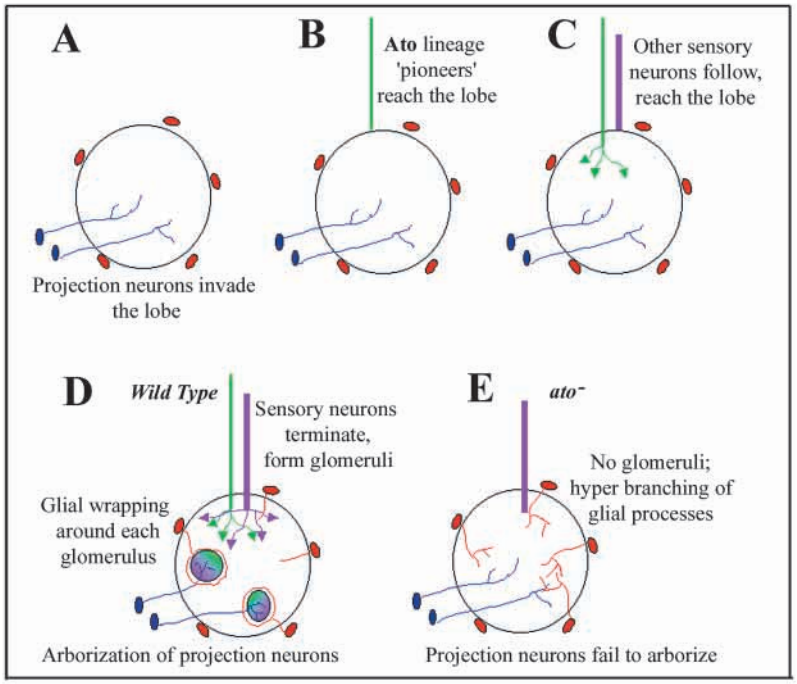

Fig. 6. Antennal lobe development in *Drosophila*. In the wild type, the projection neurons (blue) enter the lobe at the pupal stages before sensory afferents arrive in the lobe (A). (B) Ato-lineage neurons (green) arrive at the lobe at ~20 hours APF and remain at the periphery in close association with glial cells (red). (C) Other sensory neurons (purple) arrive later. (D) Between ~30 and ~60 hours APF, glomerular formation occurs. Sensory neurons invade the nascent lobe; projection neurons arborize at glomerular sites and glial processes ensheath the developing glomeruli. (E) In ato^1/Df(3R)p^{13} animals, the residual sensory neurons enter the lobe but do not pattern glomeruli. These interneurons, although present in the lobe, do not target specific glomeruli and the glial cells produce ectopic branching within the lobe.

The adult olfactory lobe is built upon a pre-existing larval template using instructive cues from sensory inputs

Drosophila larvae have rather well developed olfactory behavior, although the level of discrimination is not as sophisticated as that in the adult (Monte et al., 1989; Ayyub et al., 1990). Exposure to chemical stimuli at the larval stage can influence olfactory behavior in the adult (Thorpe, 1939; Tully et al., 1994). It is tempting to speculate that the larval olfactory lobe provides a pre-pattern upon which adult structures are remodeled. There are about 21 larval olfactory neurons that project from the dorsal organ to glomerular-like terminals within the lobe (Heimbeck et al., 1999). Before histolysis, the larval antennal nerve serves to guide axons of the adult olfactory neurons, which arise in the antennal disc, to the brain (Tissot et al., 1997).

Evidence in a variety of insect species has shown that in the adult, sensory inputs are instructive for glomerular development. (Tolbert and Sirianni, 1990; Oland and Tolbert, 1996). We have shown that the instructive role of afferents can be divided into two steps. First, one in which one class of sensory neurons plays a crucial role and, next, a possible general role for sensory inputs. It is remarkable that the Ato-lineage neurons, which comprise only a small percentage of the total number (approximately 200 out of a total of 1200), exert a disproportionate effect on glomerular development. In addition, the presence of only a small fraction of neurons of this lineage (~50) observed in a weak hypomorphic (ato^1/ato^2) allelic combination are sufficient to guide normal glomerular formation. In *lz* nulls, however, only the coeloconic and a few trichoid sensilla still remain, accounting for about 300 sensory afferents, i.e. about 900 neurons are absent (Gupta et al., 1998). These are capable of patterning the majority, if not all, glomeruli.

Cobalt fills from the coeloconic-rich region of the third antennal segment provided evidence for projections, although weak, to a large number of glomeruli (Stocker et al., 1983). This is supported by results from experiments in which the afferents from Ato-dependent neurons were traced in an Ato-Gal4; UAS-GFP strain (D. J. and V. R., unpublished). This would support a hypothesis that Ato-neurons project to most/all glomeruli and serve to guide neurons from other lineages to these glomeruli.

Neurons of the Ato lineage are both necessary and sufficient for glomerular patterning

While glomerular patterning fails in *ato* mutants, indicating that the Ato-lineage neurons are essential for this process, it could be argued that they are not sufficient to pattern glomeruli. In *lz* nulls, about 300 olfactory sensory neurons are seen, about half of which arise from Lz-independent trichoidea (Gupta et al., 1998). Could these remnant sensilla trichoidea be involved in a pioneer role similar to those of the Ato-lineage? Our results cannot exclude such a role for these neurons. However, there are several reasons, taken together, that suggest Ato-lineage neurons are sufficient for the process of glomerular patterning. First, neurons of the Ato-lineage arrive at the olfactory lobe before other sensory afferents. Second, mis-expression of Ato in all lineages using Neuralised-Gal4 converts most neurons to coeloconica (Jhaveri et al., 2000a; Jhaveri et al., 2000b). In this situation, glomerular patterning is normal. Finally, mutants in the proneural gene *amos* lack all basiconic and trichoid sensilla and, here too, glomerular formation takes place (Goulding et al., 2000) (Anindya Sen and Andrew Jarman, personal communication). Thus, with the current availability of reagents and methods, it appears that Ato-lineage neurons are sufficient for glomerular development.

What is the role played by Ato-dependent neurons in setting up the functional map in the antennal lobe?

Functional mapping experiments have shown that odor quality is represented as a spatial map of activity among the olfactory glomeruli (Rodrigues, 1988; Hansson et al., 1992). Vosshall et al. (Vosshall et al., 2000) and Gao and Chess (Gao et al., 2000) have shown that neurons expressing a given olfactory receptor terminate within the same glomeruli in the *Drosophil*a antennal lobe. A similar topographic mapping been described in vertebrates; evidence from these systems suggests that odorant receptors themselves play a role in guiding neurons to their targets (Mombaerts et al., 1996; Wang et al., 1998). The

mechanisms that underlie such guidance phenomena are unclear and, in addition, raise the question of how receptor gene regulation is specifically regulated. In *Drosophila*, the onset of receptor expression relative to the time of lobe development makes it very unlikely that odorant receptors play a role in the primary events in receptor axon targeting. Moreover, we find that ubiquitous expression of some of the olfactory receptors in all the sensory neurons throughout development does not affect glomerular patterning (D. J. and V. R., unpublished).

Neurons of the Ato lineage have the capacity to target the lobe and project to most glomeruli. They may then serve as somewhat general guideposts for other sensory neurons that then distribute themselves to specific glomeruli in response to short-range signals. How does such a developmental model incorporate the invariant distribution of odorant receptors among sense organs on the antennal surface and their stereotypic projection to olfactory glomeruli? One possibility is that the Ato-dependent pioneers project specifically to targets of glomerular formation in the lobe primordium. These neurons attract sensory neurons from other lineages that are fated to express the same receptor gene. This, however, begs the question of how groups of pioneers acquire distinct properties to enable their specificity of projection and ability to select discrete populations of neurons. A somewhat similar phenomenon occurs during development of the zebrafish olfactory system where antigenically and clonally distinct pioneer neurons guide other neurons to specific targets within the lobe (Whitlock and Westerfield, 1998). These pioneers are not completely analogous to the Ato neurons, as they are transient and do not eventually express odorant receptors.

The targeting of pioneer neurons and subsequent patterning of a functional map must involve combinatorial cues that could exist as a pre-pattern in the developing lobe. Identification of molecules that set up such a developmental field is now possible because of identification of a large number of putative cell surface molecules from *Drosophila* genome analysis (Schmucker et al., 2000). We suggest that in response to these cues, Ato-dependent neurons not only project correctly but also play an active role in guiding other sensory neurons to defined glomeruli.

Jefferies et al. (Jefferies et al., 2001) have postulated that neurons from the input and output fields of the olfactory lobe are independently specified and that choice of glomerular targets of the projection neurons is predetermined early in development. In such a scenario, projection neurons should target and arborize correctly in the lobe, even in situations where sensory input is lacking. We found that this was not the case; in *ato*$^{-}$ mutants, the interneurons though present within the lobe failed to terminate within defined glomerular sites. We propose that despite the possible existence of autonomous developmental programs within the interneurons, cues from sensory inputs are still essential to trigger proper patterning.

The complex interaction between Ato-lineage pioneers, sensory neurons, projection neurons and glia underlies a novel mechanism that operates during development of a functional neural map within the olfactory lobe. While we do not yet know what the operative mechanism is, we now have a genetic handle that will allow the design of experiments that seek to identify it.

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REFERENCES

Ayyub, C., Paranjape, J., Rodrigues, V. and Siddiqi, O. (1990). Genetics of olfactory behaviour in *Drosophila melanogaster*. *J. Neurogenet.* **6**, 262-285.

Brand, A. H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

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

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.

Dickson, B. (2001). Rho GTPase in growth cone guidance. *Curr. Opin. Neurobiol.* **11**, 103-110.

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

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

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 Cells* **2**, 225-233.

Gupta, B., Flores, G. V., Banerjee, U. and Rodrigues. V. (1998). Prepatterning an epidermal field: *Drosophila* Lozenge, a member of the AML-1/Runt family of transcription factors, specifies olfactory sense organ type in a dose-dependent manner. *Dev. Biol.* **203**, 400-411.

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

Hassan, B. A., Bermingham, N. A., He, Y., Sun, Y., Jan, Y. N., Zoghbi, H. Y. and Bellen, H. (2000). Atonal regulates neurite arborization but does not act as a proneural gene in the *Drosophila* brain. *Neuron* **25**, 549-561.

Heimbeck, G., Bugon, V., Gendre, N., Haberlin, C. and Stocker, R. F. (1999). Smell and taste perception in *Drosophila melanogaster* larva: Toxin expression studies in chemosensory neurons. *J. Neurosci.* **19**, 6599-6609.

Jarman, A. P., Sun, Y., Jan, L. Y. and Jan, Y. N. (1995). Role of the proneural gene *atonal* in formation of Drosophila chordotonal organs and photoreceptors. *Development* **121**, 12019-12030.

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.

Jhaveri, D., Sen, A., 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.

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**, 543-552.

Lindsley, D. L. and Zimm, G. G. (1992). *The genome of* Drosophila melanogaster. San Diego, CA: Academic Press.

Malun, D., Oland, L. A. and Tolbert, L. P. (1994). Uniglomerular projection neurons participate in early development of the olfactory glomeruli in the moth Manduca sexta. *J. Comp. Neurol.* **350**, 1-22.

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.

Monte, P., Woodward, C., Ayer, R., Lilly, M., Sun, H. and Carlson, J. (1989). Characterization of the larval olfactory response in *Drosophila* and its genetic basis. *Behav. Genet.* **19,** 198-283.

Oland, L. A. and Tolbert, L. P. (1996) Multiple factors shape development of olfactory glomeruli: insights from an insect model system. *J. Neurobiol.* **30**, 92-109.

Oland, L. A. 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.

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.

Ressler, K. J., Sullivan, S. L. and Buck, L. B. (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**, 299-307.

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

Rossler, W., Randolph, P. W., Tolbert, L. P. and Hildebrand, J. G. (1999b). Axons of olfactory receptor cells of transsexually grafted antennae induce development of sexually dimorphic glomeruli in Manduca sexta. *J. Neurobiol.* **38**, 521-541.

Royet, J., Distel, H., Hudson, R. and Gervais, R. (1998). A re-estimation of the number of glomeruli and mitral cells in the olfactory bulb of rabbit. *Brain Res.* **788**, 35-42.

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

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.

Schuster, C. M., Davis, G. W., Fetter, R. D. and Goodman, C. S. (1996). Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls synaptic stabilization and growth. *Neuron* **17**, 641-654.

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.

Shymala, B. V. and Chopra, A. (1999). *Drosophila melanogaster* chemosensory and muscle development: identification and properties of a novel allele of *scalloped* and a new locus SG18.1, in a Gal4 enhancer trap screen. *J. Genet.* **78**, 87-97.

Stocker, R. F., Singh, R. N., Schorderet, M. and Siddiqi, O. (1983). Projection patterns of different types of antennal sensilla in the antennal glomeruli of *Drosophila melanogaster*. *Cell Tissue Res.* **232**, 237-248.

Stocker, R. and Gendre, N. (1988). Peripheral and central nervous effects of lozenge^3: A Drosophila mutant lacking basiconic antennal sensilla. *Dev. Biol.* **127**, 12-24.

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

Stocker, R. F., Lienhard, M. C., Borst, A. and Fischbach, K. F. (1990). Neuronal architecture of the antennal lobe in *Drosophila melanogaster*. *Cell Tissue Res.* **262**, 9-34.

Stocker, R. F., Gendre, N. and Batterham, P. (1993). Analysis of the antennal phenotype in the *Drosophila* mutant *lozenge*. *J. Neurogenet.* **9**, 29-53.

Stocker, R. F., Heimbeck, G., Gendre, N. and de Belle, J. S. (1997). Neuroblast ablation in Drosophila P(GAL4) lines reveals origins of olfactory interneurons. *J. Neurobiol.* **32**, 443-456.

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

Thorpe, W. H. (1939). Further studies on pre-imaginal conditioning in insects. *Proc. R. Soc. London Ser. B* **127**, 424-433.

Tissot, M., Gendre, N., Hawken, A., Stortkuhl, K. F. and Stocker, R. F. (1997). Larval chemosensory projections and invasion of the adult afferents in the antennal lobe of *Drosophila*. *J. Neurobiol.* **32**, 281-297.

Tolbert, L. P. and Sirianni, P. A. (1990). Requirement for olfactory axons in the induction and stabilization of olfactory glomeruli in an insect. *J. Comp. Neurol.* **298**, 69-82.

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. *Nat. Neurosci.* **3**, 1035-1043.

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**, 981-991.

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.

Vosshall, L. B., 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.

White, N. M. and Jarman, A. P. (2000). Drosophila atonal controls photoreceptor R8-specific properties and modulates both receptor tyrosine kinase and Hedgehog signalling. *Development* **127**, 1681-1689.

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