Developmental origin of wiring specificity in the olfactory system of *Drosophila*

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Summary

In both insects and mammals, olfactory receptor neurons (ORNs) expressing specific olfactory receptors converge their axons onto specific glomeruli, creating a spatial map in the brain. We have previously shown that second order projection neurons (PNs) in *Drosophila* are prespecified by lineage and birth order to send their dendrites to one of ~50 glomeruli in the antennal lobe. How can a given class of ORN axons match up with a given class of PN dendrites? Here, we examine the cellular and developmental events that lead to this wiring specificity. We find that, before ORN axon arrival, PN dendrites have already created a prototypic map that resembles the adult glomerular map, by virtue of their selective dendritic localization. Positional cues that create this prototypic dendritic map do not appear to be either from the residual larval olfactory system or from glial processes within the antennal lobe. We propose instead that this prototypic map might originate from both patterning information external to the developing antennal lobe and interactions among PN dendrites.

Key words: Dendrites, Dendritic development, Antennal lobe, Projection neurons, *Drosophila*, Olfaction

Introduction

Neural maps are a key feature of brain organization. For example, many areas of the visual system maintain a retinotopic organisation corresponding to a two-dimensional projection of visual space onto the retina. The developmental origins of these maps have been extensively studied in the projection of retinal ganglion cells to the optic tectum / superior colliculus. Retinal ganglion cells initially overshoot their correct termination zone but then show site-specific collateral branching and pruning to the correct location of the developing tectum according to their position of origin in the retinal map (Simon and O'Leary, 1992; Yates et al., 2001). Gradients of axon guidance cues of the ephrin and Eph receptor families are believed to regulate both initial extension and the subsequent refinement. Final refinement of these maps seems to rely on activity-dependent mechanisms; spatially correlated firing in an input field might cause spatially correlated synaptic strengthening in the target field, leading to a smoothly graded map (reviewed in Katz and Shatz, 1996). However not all maps show the same graded organization – the map in the first olfactory processing area (the olfactory bulb in vertebrates) shows a discontinuous, punctate organization consisting of individual functional units, called glomeruli. Each glomerulus is the site of convergence for axons of olfactory receptor neurons expressing a specific seven-transmembrane-span olfactory receptor. Odorants typically bind multiple olfactory receptors, so the representation of olfactory stimuli is believed to be combinatorial: the activation of distinct groups of glomeruli signifies the presence in the external world of different odorants (reviewed in Axel, 1995; Buck, 2000).

The developmental origins of this punctate olfactory map remain somewhat mysterious. Experiments in mice have demonstrated an apparent role for the olfactory receptors in this process (Wang et al., 1998; Vassalli et al., 2002) but showed no substantial role for activity in the establishment of the map (Lin et al., 2000; Zheng et al., 2000). Recent studies in *Drosophila* (Gao et al., 2000; Vosshall et al., 2000) have highlighted the organizational similarities of vertebrate and insect olfactory systems (reviewed in Strausfeld and Hildebrand, 1999), opening up another model system to address the developmental origins of the olfactory map.

The antennal lobe (AL) is the first olfactory centre in the *Drosophila* brain (Fig. 1A). Structurally it is a flattened sphere of maximum diameter ~80 μm subdivided into about 50 glomeruli (Laissue et al., 1999; Kondoh et al., 2003), which are the functional units of the olfactory map. In cellular terms, it consists of the cytoplasmic processes (but not cell bodies) of four major cell types: the axonal terminals of olfactory receptor neurons (ORNs) and the dendrites of second-order projection neurons (PNs) (both of which usually invade single glomeruli),

local interneurons (LNs) [whose processes ramify through large areas of the lobe, synapsing in many glomeruli (Stocker et al., 1990)] and, finally, glial processes ensheathing the glomeruli (Jhaveri et al., 2000).

In *Drosophila*, the connectivity of the second-order neurons (i.e. PNs) has been well studied in comparison to vertebrate mitral/tufted cells. In particular, it has been possible to show that individual PNs are prespecified by lineage and birth order to form synaptic connections only with one specific class of incoming ORNs (Jefferis et al., 2001). How can the axons of one class of ORNs synapse specifically with one class of PNs? There are approximately 50 classes each of these pre- and postsynaptic neurons, so this represents a significant challenge in cell recognition. One can present three basic hypotheses as to how the eventual wiring specificity is achieved (Fig. 1B). (1) Presynaptic ORN axons are the first to pattern the developing AL and act as targets for partner dendrites. (2) Postsynaptic PN dendrites are the first to pattern the developing AL and act as targets for ORN axon guidance. (3) Patterning is created by coincident targeting of ORN axons and PN dendrites, presumably reacting to some common positional cues. It is important to realize that, in these three models, it is the order of spatial patterning rather than arrival time of ORN axons or PN dendrites that is most significant. Neuronal processes could initially be unpatterned and broadly distributed through the developing AL before their eventual restriction to a particular region (for an example, see Fig. 1C, which is a variant of the Model 1 in Fig. 1B).

Here, we present a systematic study of the development of PNs down to single-cell resolution. We find that specific connectivity in the AL develops in three phases. First, PN dendrites show active targeting to specific regions of the developing AL before the arrival of their partner ORN axons; a prototypic map resembling the adult AL therefore forms before any physical contact with ORN axons. Second, glomerular development proceeds rapidly after the arrival of ORNs and a largely mature glomerular organisation is reached before significant glial ingrowth. Finally, glomeruli increase uniformly in size and are wrapped by glia. These studies reveal two previously unexpected findings, which might be of general significance in neural development. First, PN dendrites are capable of autonomous growth and pattern formation in the absence of their future presynaptic partners. Second, our analysis does not support the existence of a third-party guidepost cells or processes within the developing AL that serve as origins of pattern formation; instead, we propose that the olfactory map originates both from patterning information external to the developing AL and from interactions among PN dendrites.

Materials and methods

Fly stocks

Labelling of ORNs in Fig. 2 was achieved by generating MARCM (Lee and Luo, 1999) clones in the antennal and eye discs/optic lobe with *ey-FLP* (Hummel et al., 2003). The genotype was *GAL4-C155 ey-FLP/+; FRT^{G13} tubP-GAL80/ FRT^{G13} UAS:mCD8-GFP*.

In Figs 3 and 4, PN MARCM clones were induced by 1-2 hour, 37°C heat shocks at approximately 0-4 hours after larval hatching unless otherwise specified; single cell PN clones generated at this time are from the anterodorsal lineage and will always send their dendrites to dorsolateral glomerulus 1 (DL1). For the initial time series experiments,

flies were of the genotype *y w hs-FLP UAS-mCD8-GFP/+ or Y; FRT^{G13} tubP-GAL80/FRT^{G13} GAL4-GH146 UAS-mCD8-GFP*.

The enhancer trap *GAL4-Mz19* was originally characterised by Ito et al. (Ito et al., 1998) and its PN expression was characterized by K. Tanaka (unpublished). A recombinant chromosome was generated to produce flies of the genotype y w; *GAL4-Mz19 UAS-mCD8-GFP*.

Immunochemistry

Fixation, immunochemistry and imaging were carried out as described (Jefferis et al., 2001). Additional antibodies used in this study were: the mouse monoclonal 9F8A9 and the rat monoclonal 7E8A10 against Elav, both at1:20 dilution; the mouse monoclonal 8D12 against Repo at 1:10 dilution (all DSHB, University of Iowa); rat monoclonal anti-N-cadherin extracellular domain, at 1:40 dilution (T. Uemura, Kyoto University); rabbit polyclonal antibody against green fluorescent protein (GFP), 1:800 (Molecular Probes); Cy5-conjugated goat anti-rat/mouse IgG at 1:400 dilution (Jackson). General nuclear staining was with TOTO-3 for 30 minutes at 1:1000 of the stock concentration (Molecular Probes).

Defining the border of the developing AL

N-Cadherin staining was used as the reference for defining the extent of the developing AL. When N-cadherin was not used, anti-fasciclin-II (FasII) antibody and/or TOTO-3 staining (for nuclei) were used to trace the outline of the lobe. In the case of TOTO-3, this generated a clear if slightly less accurate border. In the case of FasII, three axon tract branch points were used as fiducial points in conjunction with weak FasII staining in the core of the developing AL; this allowed the lobe outline to be estimated reliably. This approach was validated by blind labelling of the AL outline using the FasII channel in confocal stacks of brains triple labelled for GH146, N-cadherin and FasII.

Results

The adult AL develops during pupal stages. However, there is a larval AL (Cobb, 1999; Python and Stocker, 2002) composed of embryonically born ORNs and PNs; although these ORNs (hereafter called larva-specific ORNs) degenerate during metamorphosis, the PNs (hereafter called persistent PNs) actually persist through metamorphosis (G.S.X.E.J. and E. C. Marin, unpublished) and are integrated into the adult olfactory system (Fig. 1D). Thus, the adult AL, which is the focus of this paper, is composed of adult-specific ORNs born in early pupal stages, adult-specific PNs born in larval stages, and persistent PNs born in the embryo, which function both in the larva and the adult. Although this study focuses on the development of adult-specific PNs, we first describe the sequence of adult-specific ORN axon development because the precise timing of axon arrival is crucial to our study.

Timing of ORN axon development

Adult-specific ORNs (hereafter referred to simply as ORNs) are born during early pupal development and send their axons towards the AL shortly thereafter. Two previous studies (Tissot et al., 1997; Jhaveri et al., 2000) have described the timing of ORN axon arrival at the developing AL using several enhancer-trap lines expressed in subsets of ORNs. Jhaveri et al. (Jhaveri et al., 2000) observed that pioneering ORN axons begin to contact the lobe 18 hours after puparium formation (APF) and start to invade the lobe 20 hours APF. Besides confirming that developmental staging was consistent between laboratories, we felt that a re-examination of the time of ORN axon arrival was important for two reasons. First, we have found that N-cadherin labels the developing AL (H. Zhu, personal communication),
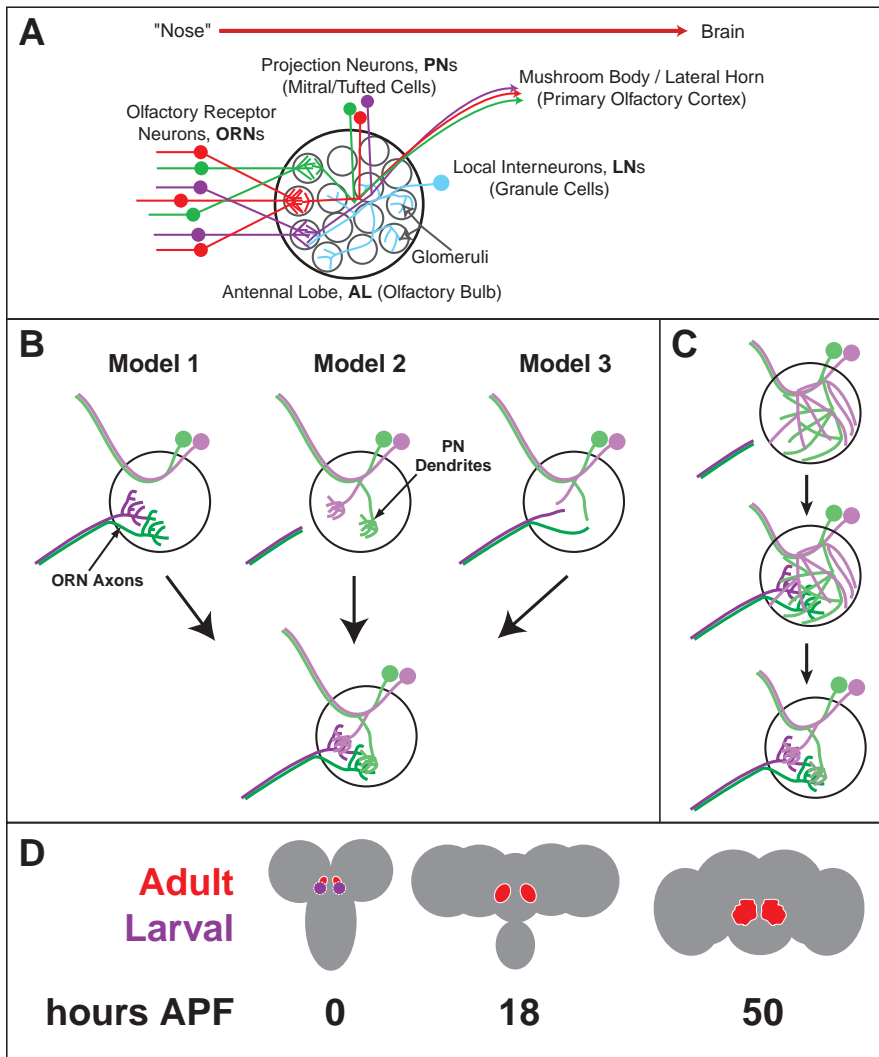
Fig. 1. AL organization and development. (A) Organization of the mature *Drosophila* antennal lobe (AL) with vertebrate counterparts in parentheses. Each colour represents olfactory receptor neurons (ORNs) expressing a particular seven-transmembrane-span receptor or their post-synaptic projection neuron (PN) partners. (B) Three hypotheses for the cellular basis of highly specific wiring between ORN axons and PN dendrites. (Model 1) ORNs pattern the lobe, then PN dendrites grow in. (Model 2) PN dendrites pattern the lobe, serving as a template for ORN axons. (Model 3) The lobe is patterned by the co-incident interaction of both cell types. (C) Model equivalent to Model 1 above, illustrating that the order of growth and patterning might be distinct: PNs could ramify extensively through the lobe but remain unpatterned until the arrival of ORNs – models 2 and 3 could be similarly extended. (D) Time course of adult lobe development and larval lobe degeneration. Red structures outline adult lobes; the larval lobes are shown in purple.

providing an objective criterion to define this structure that was not available in earlier studies. Second, we examined contributions from all ORNs to exclude the possibility that some particularly precocious ORNs would not be labelled by the enhancer trap lines used in earlier studies.

In order to label ORN axons of all types, we used *GAL4-C155*, an enhancer trap of the *elav* locus expressed in all post-mitotic neurons as soon as they are born (Robinow and White, 1988). However, because we did not wish to label neurons within the brain, we combined this with the MARCM strategy (Lee and Luo, 1999) and the *eyeless-FLP* element (Newsome et al., 2000); in addition to the eye disc, *ey-FLP* drives mitotic recombination in the antennal disc and maxillary palp where ORN cell bodies reside (Hummel et al., 2003) (Fig. 2A). This strategy results in random labelling of up to half of the ORNs in any particular antennal disc (Fig. 2B$_2$). Because *GAL4-C155* shows pan-neural expression and clone generation is random, by examining several samples we can be assured of studying all classes of ORN axons.

We found at 14 hours APF that there is already a thin axon bundle exiting the third antennal segment, where ORN cell bodies are located. Over the next 4 hours, the axon bundle becomes much thicker as more ORNs differentiate (data not shown). When do these axons reach their target? At 14 hours

APF, ORN axons (Fig. 2C-H green) have not yet contacted the developing AL (outlined by N-cadherin, Fig. 2C-E, red) (six hemispheres examined). By 16 hours APF, they have just reached the AL in most cases examined (10/14 hemispheres) and, at 18 hours APF, we observe contact of ORN axons with the ventral edge of the developing AL in all samples examined (12/12). This finding is consistent with those of Jhaveri et al. (Jhaveri et al., 2000). At 18 hours APF, ORN axons circumscribe the developing AL extending towards the midline; by 20 hours APF, axons reach the midline as they project to the contralateral AL. Significant ORN axon invasion of the AL is evident at 26 hours APF (Fig. 2F-H). As late as 26 hours APF, there is no evidence for the arrival of maxillary palp ORN axons, which should be distinguishable from antennal ORNs because they enter the lobe from a more ventral location. In summary, ORN axons reach the periphery of the lobe 16-18 hours APF and do not invade the lobe until 24-26 hours APF.

PNs extend axons and then dendrites

The enhancer trap *GAL4-GH146* (Stocker et al., 1997) labels ~90 of the estimated 150 PNs; the lineage (Jefferis et al., 2001), and mature organization (Marin et al., 2002; Wong et al., 2002) of *GH146*-positive PNs have been extensively characterized. To study a subset of these *GH146* PNs, we used the MARCM system (Lee and Luo, 1999) to generate labelled clones of two types: single cells (products of terminal division after heat-shock-induced mitotic recombination) and neuroblast clones (containing all the remaining cells within a neuroblast lineage after the time of heat shock). Earlier studies have identified two major PN lineages, each derived from a single neuroblast, and 2 corresponding groups of neurons with cell bodies anterodorsal and lateral to the mature AL (adPNs and lPNs, respectively)

Fig. 2. Time course of ORN axon development. (A) The approach used to label ORNs – the restricted expression of the *eyeless-FLP* construct generates GFP-labelled cells only in the antennal disc and eye disc/optic lobe, not in the central brain. (B1-2) Developing antennae at 20 hours APF. (left) *GAL4-C155* drives expression of membrane targeted mCD8-GFP (green) in all post-mitotic neurons, whereas a monoclonal anti-Elav antibody stains neuronal nuclei (magenta). (right) An antenna containing MARCM clones generated according to the scheme in (A). Up to half of the cells are randomly labelled. In both cases, the upper group of neurons [second antennal segment (2AS)] are auditory mechanoreceptors of the Johnston's organ, whereas the lower group are the ORNs of the third antennal segment (3AS). (C-F) Time course of ORN invasion of the developing ALs. Note that, at 14 hours APF, no axons touch the lobe (C), whereas, at 16 hours APF (D), axons in the left hemisphere just touch the lobe. C-E are stained for GFP (detecting the mCD8-GFP marker ORN axons, green), N-cadherin (which labels the developing AL in red; an example is outlined in C); in C, nc82 is in blue, whereas, in D and E, blue is FasII (which labels axon tracts). F-H are stained with anti-mCD8 (i.e. the mCD8-GFP marker) and anti-Elav antibody. The developing ALs (C-E) are devoid of Elav staining. Unless indicated otherwise, all images in this and subsequent figures are maximum-intensity *z* projections of confocal stacks; dorsal is uppermost, with the midline indicated by a dashed white line when within the field of view or located to the left of the panel otherwise.
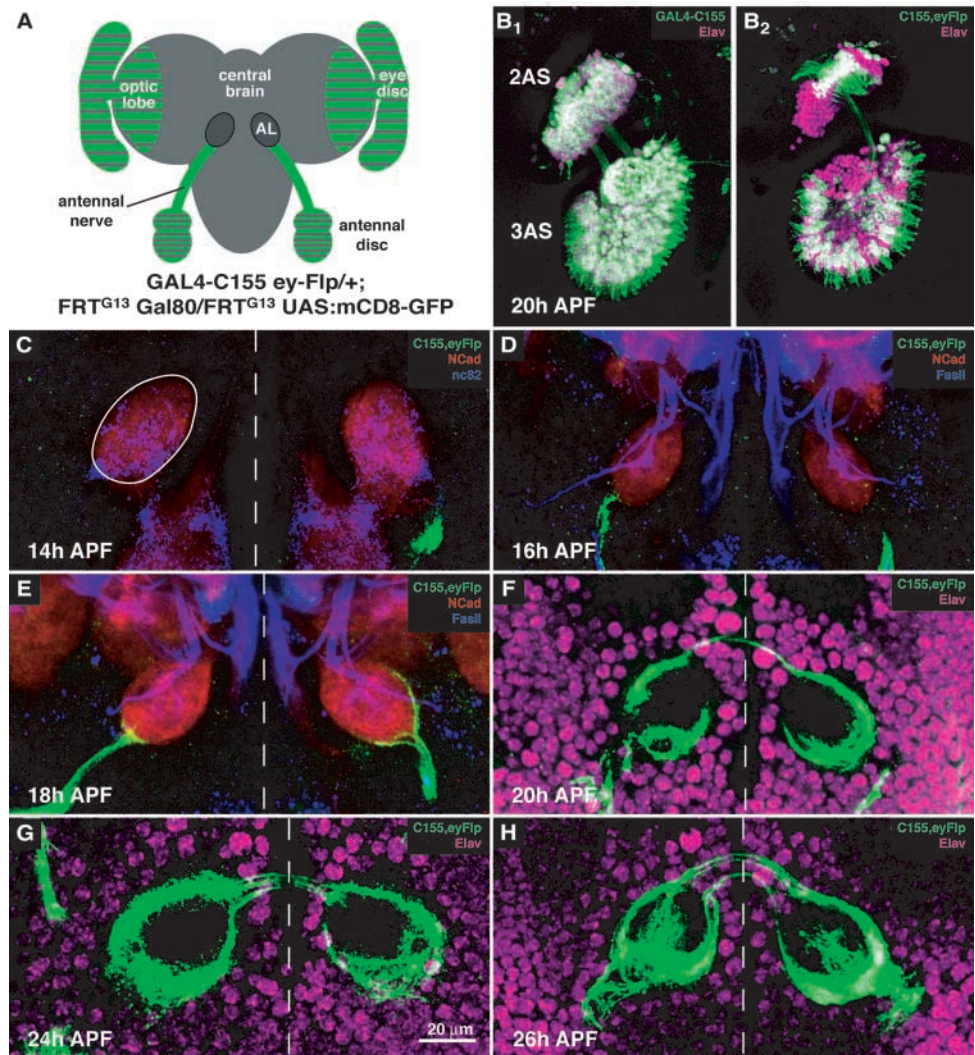
(Jefferis et al., 2001). Extensive studies of the adPN lineage demonstrated that different classes of PNs are born sequentially; for example, single cell clones generated within the first 24 hours of larval hatching always send their dendrites to the glomerulus DL1. This observation is of significant technical value because we can follow the development of these DL1 PNs long before any glomeruli are evident. In addition, it should be emphasized that neuroblast clones generated at this stage or later are exclusively composed of adult-specific PNs.

After their birth, these adult-specific PNs extend axons towards higher olfactory centres in the larval brain. At 72 hours after larval hatching, axons do not seem to have reached these structures but, by the wandering third instar stage, axons have extended to the mushroom body calyx and a presumptive lateral horn area (data not shown). At this stage, PNs have no dendritic or axonal terminal arborizations. In short, although adult-specific PNs might use the existing axonal tracts of persistent PNs for axon guidance, they are not integrated into the larval olfactory system.

The first signs of dendritic growth can be observed at the wandering third instar stage (Fig. 3A) and growth is evident by the time of puparium formation (Fig. 3B). At this point, dendrites appear as a swelling, grow radially outwards from the axonal bundle and lack any obvious organisation. Intriguingly, this growth is at a novel site outside the existing larval AL: there is a lack of overlap between the synaptic marker nc82, which stains the larval AL (Fig. 3B, dotted outline), and the new dendritic growth (Fig. 3B, arrowheads). In short, it appears that PN dendrites are generating a new structure rather than invading an existing one. By comparing single cell and neuroblast clones at this time, it appears that the single cell dendrites are diffuse but might extend through a large fraction of the volume occupied by an ~30 cell neuroblast clone (Fig. 3B1,B3). This observation applies both to DL1 PNs (Fig. 3B3) and to other classes of PNs labelled by later heat shocks (data not shown). Thus, at this stage, dendrites are not restricted into separate regions.

PN dendrites rapidly restrict into discrete areas of the developing AL

By 6 hours APF, there has been significant dendritic growth, so that the volume occupied by adPN dendrites is now similar to that occupied by the larval lobe (Fig. 3C1). However,

Fig. 3. Single representative specimens of (A_1-J_1) anterodorsal (adPN), (A_2-J_2) lateral (lPN) projection neuron neuroblast clone, or (A_3-J_3) single cell clones of the DL1 class at the indicated developmental stage. Because these are maximum-intensity z projections, structures that are in different z planes sometimes appear to overlap (e.g. B_1). Furthermore, because of unavoidable variation in the mounting angle of the brains, there is some variability in the degree of apparent overlap (e.g. compare nc82 staining in B_1 and B_3). Dotted circles indicate the larval lobe; continuous lines encircle the developing adult lobe. Asterisks in D_1 and D_2 indicate an area that is innervated by adPN but not lPN dendrites. Green is CD8-GFP, red is FasII and purple is nc82. (G_1) The nc82 channel is a single optical section. (I_2) Both channels are single optical sections. APF, after puparium formation; L3, wandering third instar larva.
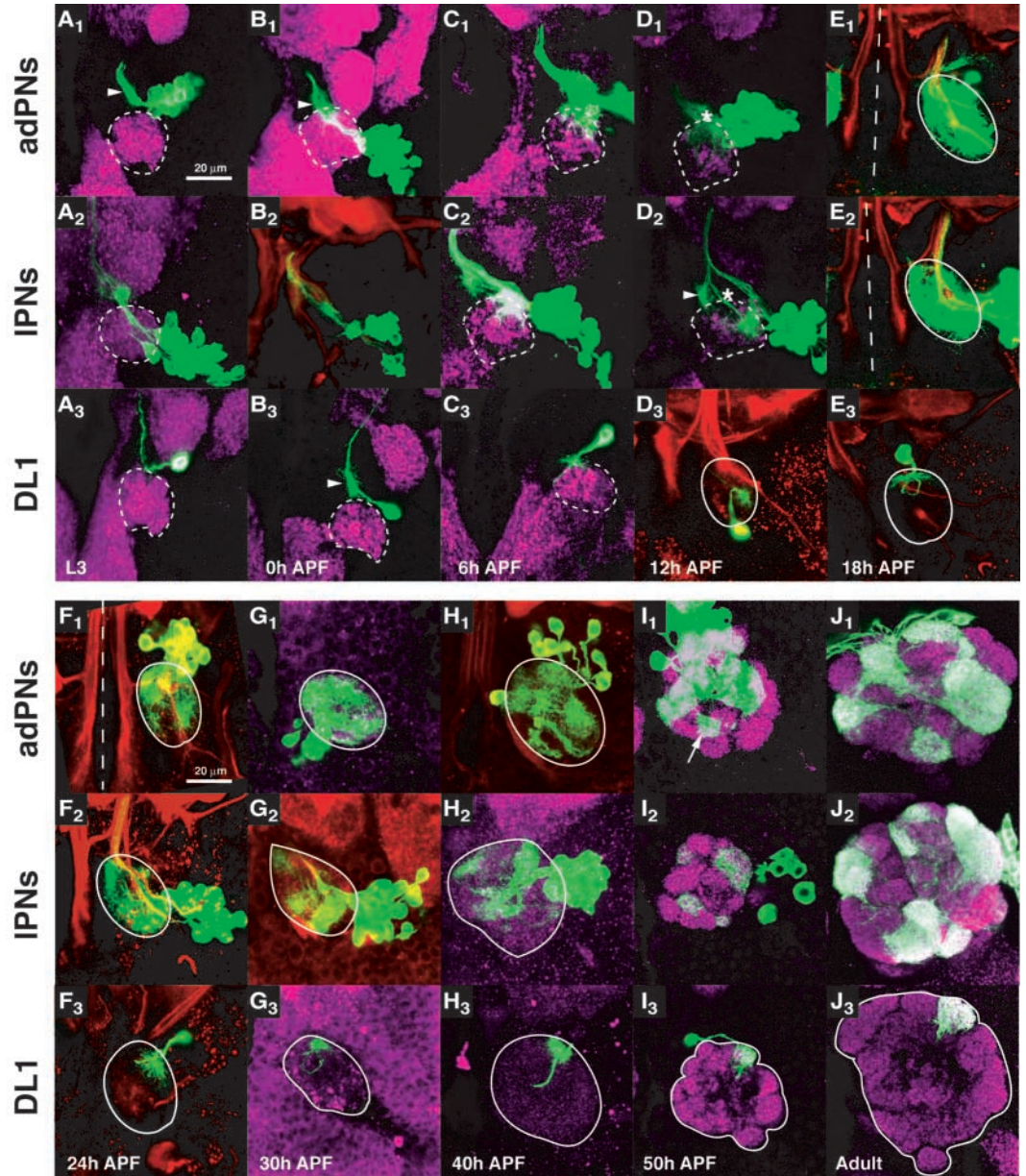
inspection of single cells does not show a large increase in the volume through which their dendrites extend (Fig. $3B_3$,C_3). A possible explanation is that the dendrites of individual neurons are starting to sort into individual regions, reducing their degree of overlap; a group of neurons would therefore occupy more space even though individual dendritic arbours might not change in extent. Evidence for this is provided by the appearance of lPNs at this time (6 hours APF) (Fig. $3C_2$). These are less homogeneous than adPN dendrites, showing discrete areas of extension and other uninnervated areas. This impression is strengthened by examining lPN dendrites at 12 hours APF (Fig. $3D_2$). Dendrites accumulate only at certain regions of the developing lobe, whereas large areas are almost devoid of lPN dendrites (e.g. asterisk in Fig. $3D_2$). In fact, it can be seen that this central unoccupied area in Fig. $3D_2$ corresponds roughly with the zone of innervation of adNb dendrites in Fig. $3D_1$. These reproducible patterns of

innervation suggest that the dendrites of these two different groups already only innervate specific regions of the lobe. Significantly, this is not just because dendritic growth is restricted to a region immediately adjacent to the cell bodies of these two classes of projection neurons – the arrowhead in Fig. $3D_2$ indicates a group of dendrites some distance from the lPN cell bodies. These observations suggest active targeting of dendrites to regions of the developing AL.

Dendritic patterning before ORN axon arrival

From the data presented so far, it is clear that, by 12 hours APF, PN dendrites occupy restricted regions of the developing lobe. Furthermore, the gross innervation patterns of adPNs and lPNs, two specific sets of ~30 neurons, show signs of localizing to specific regions of the lobe. This touches on a key question: do the dendrites of any particular neuron occupy a restricted, specific portion of the lobe? Fig. 4 addresses this question at
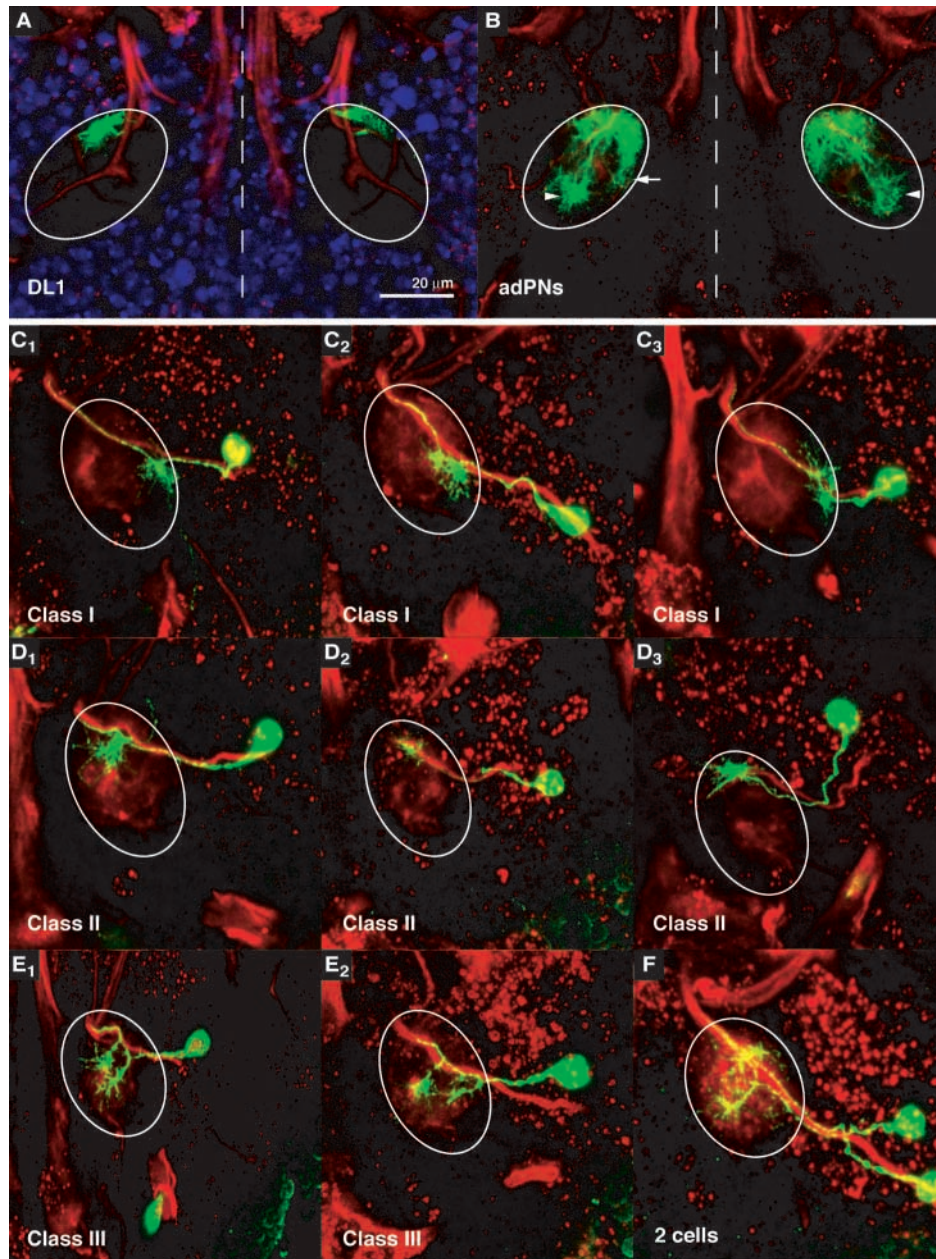
Fig. 4. PN dendrites occupy discrete regions of the lobe at 18 hours APF. (A) Two DL1 single cell clones in the same brain; both occupy a similar region of the dorsolateral corner of the developing AL. The green (CD8-GFP) and blue (TOTO-3-stained DNA) channels are from a single optical section about 12 μm into the lobe, whereas the red channel (FasII) is a maximum-intensity z projection of 46 1-μm sections. (B) Two anterodorsal neuroblast clones imaged at the same depth in the developing AL. Arrowheads indicate an example of a bilaterally symmetric focal concentration of dendrites, probably VA1d/VA1lm; an arrow indicates probable VA3 dendrites. (C-E) Single cell clones generated 72 hours after larval hatching can be divided into three morphological classes. (C_1-C_3) Class I have dendrites to the lateral edge of the lobe. (D_1-D_3) Class II have dendrites in the dorsomedial corner of the lobe. (E_1-E_2) Examples of single cell clones with two main dendritic branches. (F) A pair of single cell clones in the same hemisphere, with two distinct zones of innervation.

corner of the lobe – this is consistent with the eventual position of DL1 (Laissue et al., 1999) (Fig. 3J₃). We analysed the position of 20 such single cell clones and found that this location was consistent among all samples examined.

DL1 dendrites occupy a specific portion of the dorsal AL. However, this is relatively close to their cell bodies, so there is one trivial explanation that must be excluded – that these dendrites are localized because they have not yet been able to grow further into the AL. For comparison, therefore, a pair of anterodorsal neuroblast clones is presented in Fig. 4B, imaged at the same depth in the AL. The dendrites of these two neuroblast clones extend through a large area at this level of the lobe. This indicates that the DL1 dendrites in Fig. 4A are not just in a specific part of the AL because of a very restricted distribution of all adPN dendrites; DL1 dendrites actively target a distinct region of the AL compared with other neurons of this lineage.

Do all individual projection neurons behave in the same way as DL1 PNs? This is technically difficult to answer with precision, because DL1 PNs are the only class that we can unambiguously identify by their birth-date. Nevertheless, we generated a distinct set of clones by heat-shocking larvae at 72 hours after larval hatching and dissecting them at 18 hours APF; none of these clones will be DL1 PNs (Jefferis et al., 2001). All clones had spatially restricted dendrites in different parts of the AL, as exemplified by Fig. 4F from a brain in which two single cells were labelled. In fact, the other 27 single cell

18 hours APF, up to which point PN development is independent of ORN axons (Fig. 2).

As described earlier, MARCM clones generated within the first 24 hours after larval hatching always innervate the glomerulus DL1. We can therefore test whether, at this early stage in development, when no glomeruli can be discerned, these DL1 PNs target a specific region of the developing lobe.

Fig. 4A shows a single optical section through the middle of a pair of ALs in a rare brain with DL1 single cell clones in both hemispheres. The margin of the lobe can be traced out by the border of cell nuclei stained with TOTO-3 (blue); orientation is also provided by staining for the FasII marker, which dynamically stains axon tracts during the first third of pupal development along with some weak staining of processes within the AL. It can be seen that the dendritic arbours of both DL1 neurons are specifically localized in the dorsolateral
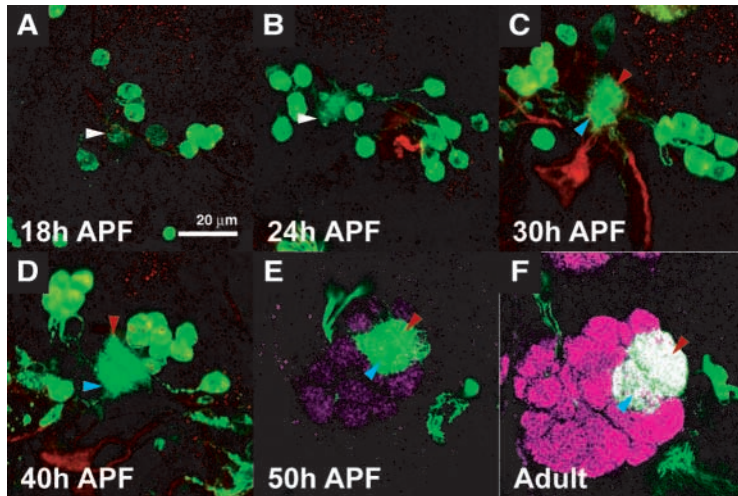
DL1 clones, which also have a compact dendritic tree restricted to a single glomerulus that can be identified by nc82 staining alone.

The major change from 50 hours APF to adult appears to be a large increase in the size of the AL. The increase is about 50% in linear dimensions or fourfold in volume. A large proportion of this increase results from local increases in the density of existing neuronal processes, with additional contribution from ingrowth of glial processes (see later).

clones that we generated could be classified into three main classes, according to their dendritic location. 19/27 cells (Fig. 4C, Class I) had dendrites on the lateral aspect of the lobe; 5/27 cells (Fig. 4D, Class II) innervated the dorsomedial corner of the AL; 3/27 cells (Fig. 4E, Class III) actually had two branches. These observations are consistent with the notion that individual PNs occupy a restricted, specific portion of the AL. This is further supported by the mirror symmetry of neuroblast clones that occupy specific regions of the AL (e.g. Fig. 4B, arrowheads).

Maturation of the glomerular map after ORN axon invasion

From 18-40 hours APF, there is a progressive refinement of the projection pattern of PN dendrites, although no glomeruli can be detected by the nc82 synaptic marker. In fact, even in neuroblast clones at 18 hours APF, tentative assignments can even be made for some dendritic foci – for example, the arrowheads in Fig. 4B are probably an accumulation of dendrites from VA1d and VA1lm PNs. Similarly, glomerulus VA3, which is a ventral glomerulus well separated from other adPN glomeruli (e.g. arrow in Fig. 3I$_1$), is the probable target of the ventromedial accumulation of dendrites that can be seen in the left hemisphere in Fig. 4B (arrow). By 40 hours APF, nearly all dendritic accumulations can be identified in confocal stacks (compare, for example, Fig. 3H$_1$ with Fig. 3J$_1$).

During the first 18 hours of pupal development, the intensity of nc82 staining diminishes rapidly so that, by 18 hours, it is no longer detectable; this reflects the degeneration of the larval AL (see below). At 30 hours and 40 hours, there are signs of a reappearance of specific nc82 staining; however, distinct glomeruli are only detectable using this marker by 50 hours APF. At this time, the dendritic organization is essentially mature. In Fig. 3I$_1$, for example, all of the major adNb glomeruli can be matched to their adult counterparts in Fig. 3J$_1$; in this *z* projection, however, it is hard to get an idea of how well contained dendrites are within a single glomerulus. Fig. 3I$_2$ is a single optical section through a lateral neuroblast clone. All four glomeruli (DA1, VA5, VA7m, DA2 clockwise from top right) are clearly identifiable and innervated without any obvious dendritic spill-over to neighbouring glomeruli. These results are corroborated by the appearance of single cell

Following PN development using a subtype-specific PN marker

Our initial studies make use of GH146, which labels a large subset of PNs. Although we were able to focus on small numbers of GH146-positive neurons using the MARCM system, we sought to complement these studies by identifying enhancer trap lines labelling specific subsets of PNs. After testing a panel of GAL4 enhancer trap lines (Hayashi et al., 2002), we chose to focus particularly on the line *GAL4-Mz19*; in adults, it specifically labels PNs that innervate two large glomeruli, VA1d and DA1, on the anterior surface of the AL, along with a less distinct and more posterior glomerulus DC3 (Fig. 5F). We can then ask when specific innervation of these glomeruli can be observed during development.

We found that Mz19-driven marker expression could be detected as early as 18 hours APF (Fig. 5A). Because ORNs have only just reached the AL periphery at this time, it seems that PN subtypes show distinct patterns of gene expression before contact with their ORN partners; this complements the results presented above showing that they are morphologically distinct at this time. At 24 hours APF, marker expression is strong enough to see two overlapping dendritic arbours corresponding to the two major protoglomeruli, VA1d and DA1. Because VA1d and DA1 are directly adjacent, we could also ask when adjacent proto-glomeruli become clearly resolved. At 30 hours APF, we saw that the VA1d and DA1 regions were clearly separable (Fig. 5C, arrowheads).

Development of axon terminal arborizations

In the adult fly, PNs of the same glomerular class have stereotyped axon terminal arborization patterns in a higher olfactory centre, the lateral horn (Marin et al., 2002; Wong et al., 2002). What is the relative timing of the development of specific dendritic targeting and specific axonal arborizations? Fig. 6 presents a time course of axonal development for single cell DL1 clones. At early pupal stages, axonal development appears to lag somewhat: at 0 hours and 6 hours APF, there is no sign of terminal extension in the mushroom body calyx or lateral horn. By 12 hours APF, however, there are signs of sprouting in these regions (Fig. 6C, blue and red asterisks). At 18 hours APF in the mushroom body, this early sprouting has resolved itself into several distinct collaterals (Fig. 6D, blue
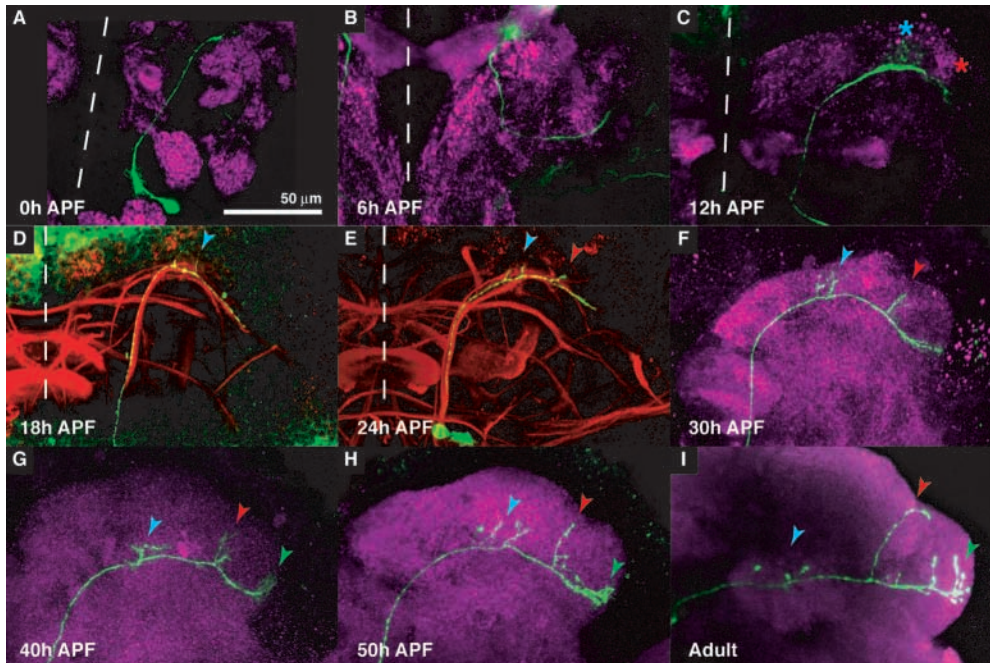
Fig. 6. Development of DL1 projection neuron axons. (A-J) Maximum intensity *z* projections of confocal stacks of representative DL1 single cell clones at successive developmental time points. Asterisks in C indicate diffuse outgrowths from the existing axon in the mushroom body calyx (blue) and lateral horn (red) areas; arrowheads in D-I indicate collaterals in these same areas. Green is mCD8-GFP, red FasII and magenta nc82.

swelling in the form of an ovoid of ~20×10×10 μm at 0 hours APF to a sphere of ~30 μm diameter at 18 hours APF), these cues are more likely cell-surface bound than freely diffusible, and that at least some of these cues should be internal to the developing AL. Because we found that there are no cell nuclei within the lobe (Fig. 4A), any internal positional cues must be cytoplasmic processes of either neurons or glia. We first tested whether remnants of the degenerating larval AL could contribute to these positional cues.

We determined the time of degeneration of the larval lobe using nc82, a presumed synaptic marker, and N-cadherin, a marker enriched in the developing adult-specific lobe. In addition, we used GH146 directly, without generating MARCM clones, so both persistent and adult-specific PNs are labelled. At 0 hours APF (Fig. 7A), the larval lobe (dotted outline) is strongly stained by nc82 and also by N-cadherin; this is also where most GH146-positive dendrites are concentrated (Fig. 7A$_2$). However, there is a second area (solid outline) dorsal and posterior to the larval lobe with a lower concentration of GH146-positive dendrites, higher levels of N-cadherin and no nc82. This area matches in position the location of newly developing dendrites described in the neuroblast and single cell clones in Fig. 3B. Thus, at this stage the intact larval lobe and newly developing adult lobes are clearly distinct.

This is also the case at 6 hours APF (Fig. 7B), when the adult lobe has expanded significantly in size. However, the relative intensity of GH146 staining is reversed – now the adult lobe shows stronger staining. This suggests that the dendrites of larval GH146-positive PNs have started to be pruned back – consistent with results obtained with clonal analysis of embryonically born PNs (E. C. Marin and L.L., unpublished). Staining with nc82 is also somewhat weaker at this point, suggesting disassembly of the larval structure.

By 12 hours APF, nc82 staining is very much reduced (Fig. 7C$_4$) and the larval lobe is no longer clearly demarcated, although there is an area of nc82 and weaker N-cadherin staining along the ventrolateral margin of the lobe (dotted lines in Fig. 7C). There are some GH146-positive dendrites within this marginal zone that are continuous with those in the rest of lobe (Fig. 7C$_{1-4}$, arrowhead), suggesting that the two structures

arrowhead). In the lateral horn, there is further extension of the main axon branch but no extension of the dorsal collateral, which is very characteristic of DL1 projection neurons (compare Fig. 6D and 6I). At 24 hours APF, however, there is the first sign of this dorsal collateral at this point, a stump of about 5 μm (Fig. 6E, red arrowhead). By 30 hours APF, this has become more substantial – about 15 μm in the example shown. It was possible to discern a clear collateral in 8/10 samples at this stage (Marin et al., 2002). In the 40 hours APF sample, there is actually a second dorsal collateral present; this is sometimes observed in pupal stages but never persists in the adult. There is also substantially enhanced terminal sprouting (Fig. 6G, green arrowhead). By 50 hours APF, the axon projection pattern is quite mature – mushroom body collaterals have formed a terminal ball, as observed in the adult, and the lateral horn dorsal collateral has reached almost its full extent. However, the terminal arborizations of the main branch in the lateral horn have not yet obtained their final form. This time point is significant because it is just before the earliest known expression of an olfactory receptor in *Drosophila* (Clyne et al., 1999) and before mature synapses have been detected in the lobe (Devaud et al., 2003). Patterned sensory activity is therefore unlikely to play an instructive role in development of either PN dendritic targeting or, at least for DL1 PNs, most aspects of axon terminal arborisation.

Cellular cues for PN dendritic development – larval neurons

We have characterized an early phase of PN dendritic development (0-18 hours APF), in which PN dendrites show very specific targeting in the absence of their presynaptic partner ORNs. This unexpected observation raises an intriguing question: what are the spatial cues that allow these dendrites to target specific regions of the developing lobe? We reasoned that, given the small space within which dendritic patterns have to be created (expanding from a dendritic

Fig. 7. Relationship between larval and adult ALs in early pupal stages. (A-C) Images of *GAL4-GH146* driving membrane-targeted GFP. Each panel is a single confocal section through the developing AL stained with anti-GFP antibody (green), anti-N-cadherin antibody (red) and nc82 (blue). Dotted lines indicate the larval area of the lobe; solid lines indicate the adult-specific zone. Notice that, in C_4, nc82 is extremely weak in spite of an increase in laser power from 3% to 10% (A_4 and B_4, respectively). (D-F) *z* projections of confocal images of *GAL4-MJ94* driving *mCD8-GFP* in larval ORNs. Asterisks indicate the ORN axons of the antennal nerve entering the larval AL; the arrow in F indicates the 'stump' of remaining ORN axon terminals and the arrowheads indicate examples of nc82-positive densities that seem to lie along the few remaining ORN processes. Dotted lines indicate the extent of the larval AL. Green is mCD8-GFP; (D,F) magenta is nc82; (E) red is N-Cadherin and blue is nc82.
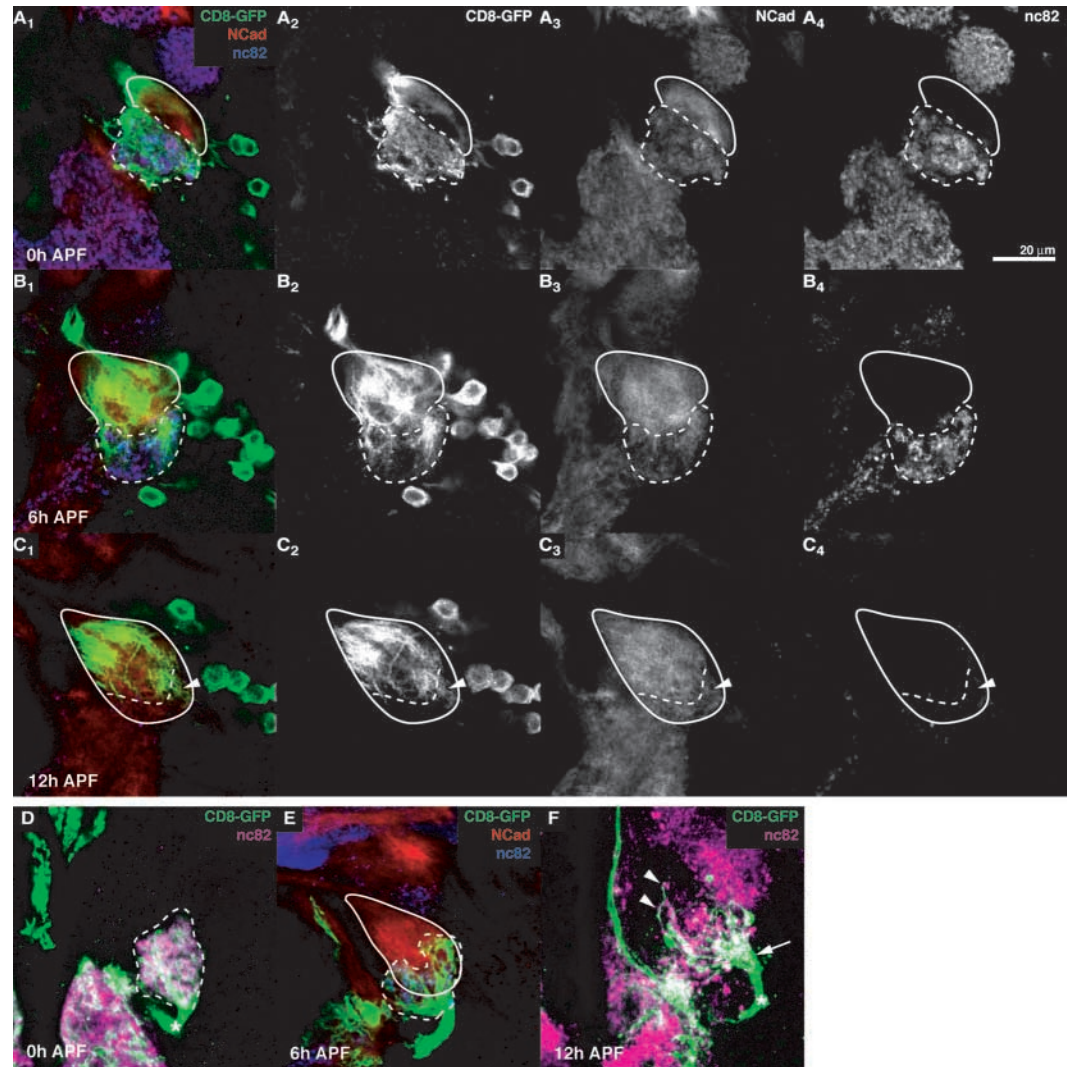
might be effectively fused at this stage. This is consistent with the results in Fig. 3D, in which the dendrites of adPN and lPN clones showed some overlap with the remaining nc82-positive larval area. In summary, the larval lobe is intact at 0 hours APF, whereas an adjacent adult structure, posterior and dorsal to the larval lobe, starts to show dendritic elaboration; by 6 hours APF, larval dendrites have degenerated significantly whereas adult-specific dendrites have grown significantly; at 12 hours the adult lobe is largely continuous with what remains of the larval structure.

What is the status of the larval ORNs during this time? We examined this question using the enhancer trap line *GAL4-MJ94* (Joiner and Griffith, 1999), which labels all the receptor neurons of the larval olfactory and gustatory organs (R.F.S., unpublished); similar results (data not shown) were obtained with a second line *GAL4-JFF-4551*, which labels two-thirds of larval ORNs (Python and Stocker, 2002). At 0 hours APF, larval ORNs densely innervate the nc82-positive larval lobe (dotted outline in Fig. 7D). MJ94 driven CD8-GFP can also be seen in the ORN axons in the antennal nerve, indicated by an asterisk as it reaches the lobe. At 6 hours APF (Fig. 7E), larval ORNs extend through a similar area but now showing fingers of innervation (which are coincident with weaker nc82

staining) separated by uninnervated areas. The zone of larval innervation is largely distinct from the N-cadherin-positive adult-specific lobe but there is some overlap along the anterior face of the ventral part of the new lobe. By 12 hours APF, ORN axons have mostly pruned back to a stump in the ventrolateral corner of the lobe. There are still, however, about 10-15 neurites, which are bare except for occasional blebs of up to 3 μm in diameter. Although these neurites extend in a few instances up to 20 μm, their position on the anterior surface of, rather than within, the adult-specific lobe indicates that they have limited contact with adult-specific structures.

In conclusion, the larval lobe degenerates over the period from 0-12 hours APF; growth of the developing adult lobe occurs at a site adjacent to but distinct from this degenerating structure as long as the larval lobe remains identifiable. The spatial information allowing early dendritic patterning within the adult structure must therefore derive from cellular constituents other than remnants of the larval olfactory system.

Cellular cues for PN dendritic development – constituents of the developing adult-specific lobe

We next investigated whether glia could provide positional cues for PN dendritic targeting using an enhancer trap line
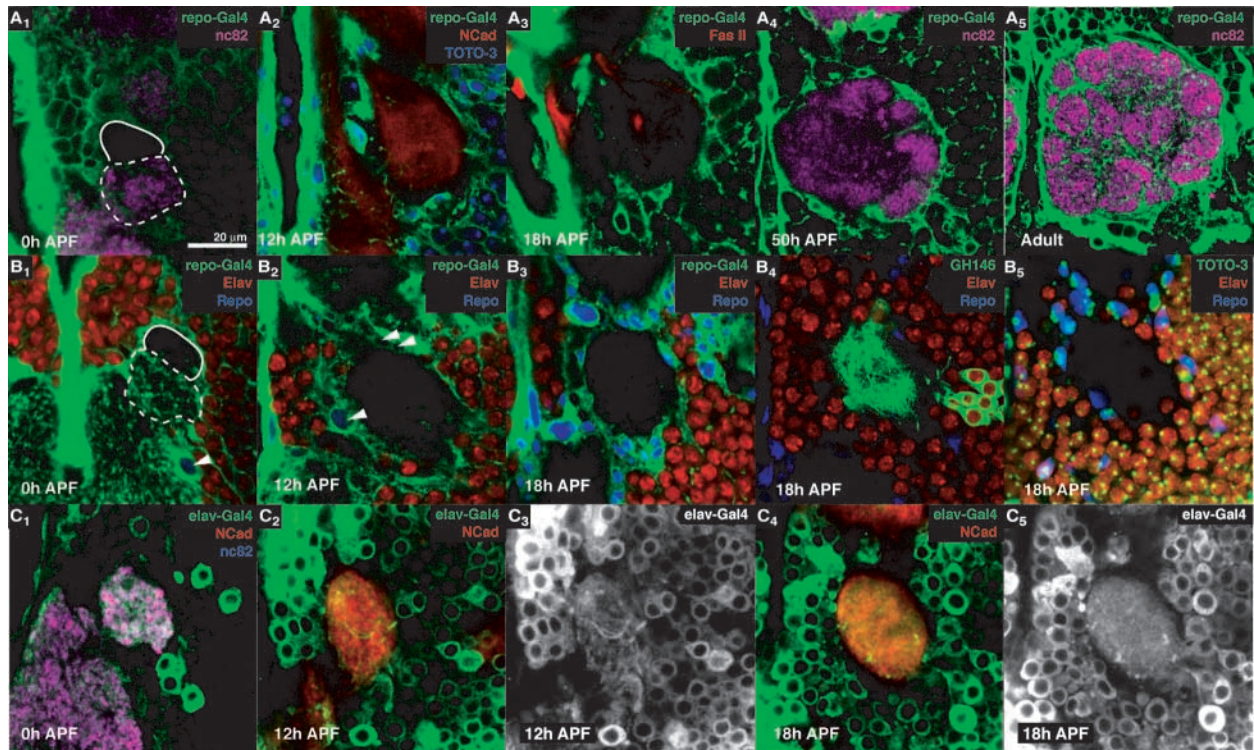
Fig. 8. Candidate cellular components of the developing adult AL. (A_1-A_5) Time course of repo-GAL4 driving mCD8-GFP expression, marking glial cell membrane. Each panel is a single optical section through the developing AL. (B_1-B_3) Comparison of repo-GAL4-driven marker expression, and anti-Repo antibody. Repo-GAL4 strongly labels all glia but has weak background staining in all cells. (B_4) Location of Repo-positive nuclei in relation to GH146-positive dendrites. (B_5) Triple stain of Repo, Elav and TOTO-3 (a DNA stain, most prominent in nucleoli) showing that Repo- and Elav-stained cells (glia and neurons, respectively) account for all nuclei in the vicinity of the AL. (C_1-C_5) Time course of *GAL4-C155* driven UAS:*mCD8-GFP* (single sections); C_3 and C_5 correspond to the green channel of C_2 and C_4, respectively.

GAL4-M1B, in which GAL4 is under the control of the *repo* promoter and therefore expressed in all central nervous system glia (Xiong et al., 1994). We confirmed that, near the developing AL, all nuclei are either Elav positive (a pan-neural nuclear marker) or Repo positive (e.g. Fig. 8B$_5$), and therefore all non-neuronal cells are Repo-positive glia. In addition, all Repo-positive nuclei are surrounded by strong membrane staining when *GAL4-M1B* is crossed to UAS-*mCD8-GFP*, a membrane marker; however, there is some additional weak background staining in most other cells in the brain. Therefore, *mCD8-GFP* driven from *GAL4-M1B* should strongly label all glial processes. As expected, in adults, glial processes can be seen wrapping individual glomeruli (Fig. 8A$_5$). Are these processes present in the developing AL during the early phase of PN dendritic targeting?

Although the larval lobe did have intercalated processes at 0 hours APF (Fig. 8A$_1$,B$_1$), which persisted in reduced number up to 12 hours, there were no glial processes in the developing adult-specific lobe between 0 hours and 18 hours (Fig. 8A$_{1-3}$). At 50 hours APF, the first signs of glial invasion could be observed (Fig. 8A$_4$) but, by this stage, PN dendrites have reached an almost mature organization (Fig. 3). Similar results regarding the development of glial processes were obtained (data not shown) using staining with anti-Draper antibody, which is a glia-specific cytoplasmic protein (Freeman et al., 2003).

Despite the lack of glial processes within the developing

adult-specific lobe, there are glial processes outside the lobe (Fig. 8A) and double labelling of *GAL4-M1B*-driven *mCD8-GFP* and anti-Repo antibody confirms that, during the period 0-18 hours APF, there are scattered Repo-positive nuclei surrounding the developing AL (Fig. 8B$_{1-3}$). Glial processes could therefore contribute to PN dendritic patterning, but only from outside the AL. In contrast to the lack of glial processes, we found that *mCD8-GFP* driven by the pan-neural *GAL4-C155* fills the developing AL between 0 hours and 18 hours APF (Fig. 8C). Because we have ruled out a significant contribution by larval ORNs (Fig. 7) and adult ORNs are yet to enter the lobe (Fig. 2), the remaining possibilities are LNs and PNs themselves. We could not conclusively determine the contribution of LNs owing to lack of appropriate reagents. Neither of the two LN GAL4 lines we have examined (*GAL4-189Y* and *GAD1-GAL4*) drives marker expression in the adult-specific AL between 0 hours and 12 hours APF, although both label the larval lobe (data not shown). However, *GAL4-189Y* only labels a few larval LNs (Python and Stocker, 2002) and *GAD1-GAL4*, which is derived from the promoter of the glutamic acid decarboxylase gene and should therefore label all GABAergic inhibitory neurons (Ng et al., 2002), might not turn on during early stages of LN development. Nevertheless, our inability to find LN processes in the developing adult-specific AL in early stages of pupal development is consistent with previous observations in moth that LN dendrites invade protoglomeruli later than PNs (Oland et al., 1990).

Thus, although we cannot rule out a contribution of LNs, the evidence accumulated so far suggests that PN dendrites are a major constituent of the developing AL. Indeed, when comparing *GAL4-C155* and *GAL4-GH146* (labelling ~90 of an estimated 150 total PNs), in anterior sections GH146-positive PN processes appear to occupy most of the space labelled by *GAL4-C155* (compare Fig. 8B$_4$ with 8C$_{4,5}$). We have argued that structures internal to the AL participate in patterning PN dendrites, and so we propose that PN-PN interactions contribute significantly to the initial patterning of the developing AL.

Discussion

The creation of an ordered spatial map for olfactory information processing in the vertebrate olfactory bulb and its insect equivalent, the AL, provides a striking example of wiring specificity in the developing nervous system. Although significant progress has been made since the discovery of convergent ORN axon projection in mammals and in flies (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Gao et al., 2000; Vosshall et al., 2000), a clear picture has yet to emerge concerning the logic that leads to connection specificity between ORN axons and the dendrites of second order neurons. In particular, the sequence of events in the assembly of the olfactory bulb or AL has not been described with sufficient temporal and spatial resolution to constrain possible models of pattern formation. In this paper, we performed a systematic developmental study to examine the sequence of events that lead to the assembly of the *Drosophila* AL.

Dendritic growth and patterning independent of presynaptic axons

A key finding in this study is that PN dendritic development is surprisingly independent of presynaptic ORN axons. It is generally thought that dendritic growth and maturation are coupled with presynaptic axon invasion and synapse formation, which might be significantly shaped by electrical activity (reviewed by Cline, 2001). We found that, before ORN axons reach the developing AL, PN dendrites have undergone significant growth and branching. More strikingly, dendrites of different PN classes have created a pattern in the AL at 18 hours APF that resembles the adult glomerular map, in the complete absence of their presynaptic partners (Fig. 4). This observation is in contrast to the prevailing view of olfactory development (see below) and underlines a very significant dendritic contribution to the origins of wiring specificity.

It is important to realize that this finding is not a natural prediction of our previous findings that PNs are prespecified to synapse with a specific class of ORNs by lineage and birth order (Jefferis et al., 2001). For instance, all three models depicted in Fig. 1B are consistent with the prespecification data. Our current study clearly favours the second model, that PN dendrites are patterned first. It is probable that gradual invasion of ORN axons from 24 hours APF (Fig. 2) refines and consolidates this prototypic dendritic map (see below), allowing rapid development of the AL to essentially adult form by 50 hours APF.

Site selection of one synaptic partner before the other has also been described recently in *Caenorhabditis elegans* (Shen and Bargmann, 2003). Interestingly, in that case, the presynaptic partner selects the synaptic site in the absence of its postsynaptic partner by interacting with a third party guidepost cell.

Origin of patterning information

What positional cues allow dendritic patterning before ORN axon arrival? The first leading candidate to provide positional cues is the larval lobe because it is presumably patterned for larval olfaction, albeit in a form simpler than that of the adult AL (Tissot et al., 1997; Python and Stocker, 2002). However, we find that, at early pupal stages, the developing adult AL is clearly distinct from the larval lobe and is minimally invaded by remnants of the degenerating larval lobe. Glia are a second candidate and, indeed, glia have been suggested to play important roles in sorting ORN axons into individual classes in the moth (Rossler et al., 1999) and are the leading candidates in the mouse olfactory bulb to provide positional cues for ORN axon targeting (Bulfone et al., 1998). However, we did not find significant glial processes within the developing AL between 0 hours and 18 hours APF, the critical period for PN dendritic patterning. Although it is likely that glial cells and processes surrounding the AL contribute to PN dendritic patterning, it is difficult to imagine that the surrounding tissues could contribute all the positional cues that allow dendrites of a specific class to occupy specific regions of the AL, which is a three-dimensional sphere.

These analyses lead us to speculate that PN dendrite-dendrite interaction might contribute significantly to the eventual patterning of PN dendrites for the following reasons. First, PN dendrites appear to be a major constituent of the developing adult-specific AL, which is composed predominantly, if not exclusively, of neuronal processes (Fig. 8). Indeed, preliminary electron microscopic analysis using genetically encoded electron microscopy markers suggests that the developing AL is composed entirely of neuronal profiles and that GH146-positive PN dendritic profiles are clustered together without intermingling with other neuronal profiles (R. Watts and L.L., unpublished). Second, different classes of PNs are probably endowed with molecular differences because of their lineage and birth order (Jefferis et al., 2001; Komiyama et al., 2003), which could be used to create heterogeneities in the developing lobe. LN processes do remain a possible source of information that we could neither find positive evidence for nor rule out. However, it is more difficult to imagine how heterogeneity is created by LNs, because processes from each LN occupy a large proportion of the AL, rather than individual glomeruli (Stocker et al., 1990).

Based on these considerations, how do we envisage that PN patterning could occur in early pupa before ORN arrival? We propose a hierarchy of positional cues. Global cues, which could be diffusible or contact-mediated guidance molecules from outside the lobe, could allow dendrites to target to an approximate region of the developing lobe with respect to the body axes, causing, for example, glomerulus V to form ventrally and glomerulus D to form dorsally. Local dendrite-dendrite interactions would then allow PN dendrites of the same class to adhere tightly; dendrites destined to occupy neighbouring glomeruli would associate more weakly and/or be repelled. This hierarchy of cues seems to be essential for generating a structure that has global order and highly

reproducible local spatial relationships among neighbouring PN classes. Our observations at 18 hours APF (Fig. 4) suggest that, although the global targeting of dendrites to specific regions of the AL is largely complete, the sorting process is not, because dendrites from neighbouring classes can still exhibit significant overlap. Continuing dendrite-dendrite interaction after 18 hours APF and interaction with ORN axons (see below) would further refine this prototypic dendritic map.

It is noteworthy that axon-axon interaction has already been shown to contribute to the specificity of connections between *Drosophila* photoreceptors and their targets (Clandinin and Zipursky, 2000). Although we are not aware of a proposal for such mutual dendrite sorting, the molecular mechanisms could be related to dendrite tiling (Wassle et al., 1981; Grueber et al., 2002; Grueber et al., 2003). In tiling, dendrites of the same neuronal class show homotypic repulsive interactions; we would propose that homotypic attraction and/or heterotypic repulsion from neighbouring dendritic classes could contribute to dendritic sorting in the developing AL.

Contributions of ORNs to the developing olfactory system

The observation that in *Drosophila* PN patterning precedes that of ORNs seems at odds with existing observations in insects and vertebrates, emphasizing the primary role of ORN axons in organising glomerular development (e.g. Oland et al., 1990; Valverde et al., 1992; Malun and Brunjes, 1996; Treloar et al., 1999). In all these studies, glomerular formation was first evident in the accumulation of ORN axons in protoglomeruli. This organizational function of ORNs is further supported by several perturbation experiments. For instance, in the moth *Manduca sexta*, developmental deantennation prevents normal glomerular formation (Boeckh and Tolbert, 1993), whereas surgical removal of a subset of PNs still permits ORNs to form relatively normal glomerular terminations outlined by glial cells (Oland and Tolbert, 1998). Similarly, genetic ablation of most mitral or granule cells in mutant mice still permits axonal convergence of specific ORN classes, although the structure of the olfactory bulb is disorganized (Bulfone et al., 1998). In *Drosophila*, it was reported that in *atonal* mutants, formation of a proposed pioneer class of ORNs is disrupted, axonal targeting of other ORNs is delayed and glial processes are disturbed. It was further suggested that PN development must depend on ORNs because, in *atonal* mutants, PN patterning [as visualized by GH146 staining (labelling ~90 PNs)] is disrupted (Jhaveri and Rodrigues, 2002).

Many of these apparent contradictions can be resolved by treating spatial patterning and glomerular formation as two distinct processes. An important technical improvement of our study is that we are able to visualize the dendritic fields of identifiable PNs down to the single cell level with MARCM or small groups of cells with *GAL4-Mz19*. We can therefore describe much earlier developmental events with higher anatomical resolution, demonstrating the existence of spatial patterning well before glomeruli are morphologically distinct and, indeed, before one major component of the glomeruli – the ORN axons – is even present. For example, in Fig. 4A, we can see that dendrites of DL1 PNs occupy a discrete and specific location in the developing lobe. However, because their dendrites still overlap with other PN classes, this patterning is not that obvious when looking at larger numbers

of neurons (e.g. Fig. 4B) and is scarcely apparent at all when more cells are visualized (Fig. 8B$_4$). Indeed, some existing single cell labelling studies have shown that PNs in *Manduca* have restricted dendritic arborizations before any glomerular patterning is evident (Malun et al., 1994). However, without being able to label specific classes of PNs, such studies cannot determine whether PN dendrites are in a spatially appropriate location.

How do ORN axons come into register with the prototypic PN map when they reach the lobe? At one extreme, ORN patterning might be completely dependent on PN patterning. ORN axons could simply recognize specific classes of pre-patterned PN dendrites through receptor-ligand or homophilic interactions. Alternatively, PN patterning could generate a third-party map, which is recognized by ORNs. Although certainly consistent with the developmental studies described here, these models are not supported by experiments in other organisms such as the target neuron ablation experiments of Oland and Tolbert (Oland and Tolbert, 1998) in moth and Bulfone et al. (Bulfone et al., 1998) in mice. In addition, transplantation experiments in moth (Rossler et al., 1999) or the formation of a novel glomerulus upon expression of a rat olfactory receptor in mice (Belluscio et al., 2002), indicate that ORNs might have substantial autonomous patterning ability. At the other extreme, patterning of ORN axons could be completely independent of the prototypic map created by PN dendrites. In theory, ORN axons could recognize third-party cues previously used to pattern PN dendrites. However, we are unable to find such third-party cues within the developing AL. Of course, ORNs could still be patterned by PN independent cues external to the lobe and axon-axon interactions, in a manner directly analogous to our hypothesis for PN patterning. In the case of such strict independence, ORNs expressing a particular receptor would form specific connections with partner PNs, rather than inappropriate adjacent PNs, only because both sets of neurons target with great precision to exactly the same spatial location. This degree of independence seems both implausible and inefficient.

Instead, we propose that both ORN axons and PN dendrites have substantial autonomous patterning ability – for instance through PN-PN or ORN-ORN mutual interactions and interactions with cellular cues surrounding the developing AL – but that the two resultant proto-maps interact during development to generate the final mature glomerular organisation. This proposal is the most parsimonious explanation for the existing data supporting the organizational functions of ORNs as summarized above, our data described in this study and our preliminary observations that ORN axon targeting appears to be resistant to PN perturbation (T. Chihara, H. Zhu and L.L., unpublished). It also has the advantage of robustness – each map could reinforce and refine the other, resulting in a precise match between pre- and postsynaptic partners without the necessity for extreme targeting precision. Furthermore, although two maps might seem to be more complicated than one, they need not be molecularly more complex. Many of the molecules used for PN patterning could also be used for ORN patterning or for interactions between ORNs and PNs. Having described with great precision the cellular and developmental events that lead to the patterning of the AL, we are now in a good position to attack the molecular basis of wiring specificity in the *Drosophila* olfactory system.

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