Integration of complex larval chemosensory organs into the adult nervous system of *Drosophila*

Nanaë Gendre[1], Karin Lüer[2], Sandrine Friche[1], Nicola Grillenzoni[1], Ariane Ramaekers[1], Gerhard M. Technau[2] and Reinhard F. Stocker[1,*]

[1]Department of Biology and Program in Neuroscience, University of Fribourg, CH-1700 Fribourg, Switzerland
[2]Institute of Genetics, University of Mainz, D-55099 Mainz, Germany
*Author for correspondence (e-mail: reinhard.stocker@unifr.ch)

Accepted 22 September 2003

Development 131, 83-92
Published by The Company of Biologists 2004
doi:10.1242/dev.00879

Summary

The sense organs of adult *Drosophila*, and holometabolous insects in general, derive essentially from imaginal discs and hence are adult specific. Experimental evidence presented here, however, suggests a different developmental design for the three largely gustatory sense organs located along the pharynx. In a comprehensive cellular analysis, we show that the posteriormost of the three organs derives directly from a similar larval organ and that the two other organs arise by splitting of a second larval organ. Interestingly, these two larval organs persist despite extensive reorganization of the pharynx. Thus, most of the neurons of the three adult organs are surviving larval neurons. However, the anterior organ includes some sensilla that are generated during pupal stages. Also, we observe apoptosis in a third larval pharyngeal organ. Hence, our experimental data show for the first time the integration of complex, fully differentiated larval sense organs into the nervous system of the adult fly and demonstrate the embryonic origin of their neurons. Moreover, they identify metamorphosis of this sensory system as a complex process involving neuronal persistence, generation of additional neurons and neuronal death. Our conclusions are based on combined analysis of reporter expression from P[GAL4] driver lines, horseradish peroxidase injections into blastoderm stage embryos, cell labeling via heat-shock-induced flip-out in the embryo, bromodeoxyuridine birth dating and staining for programmed cell death. They challenge the general view that sense organs are replaced during metamorphosis.

Key words: Pharyngeal sense organs, Sensory neurons, Metamorphosis, FLPout labeling, Embryonic labeling, Mitotic labeling

Introduction

Holometabolous insects have a unique way of generating their adult sensory apparatus. The vast majority of their larval sensilla is lost during metamorphosis and becomes replaced by adult sensilla that originate from imaginal discs (reviewed by Levine et al., 1995; Tissot and Stocker, 2000; Truman, 1996). This is certainly because many adult sense organs develop on adult-specific appendages. Yet, this design might also relate to the fact that larval and adult sense organs are often very different in terms of cell numbers, organization and function, reflecting the different life styles of the two stages. For example, in the creeping *Drosophila* larva, odor sensation is accomplished by no more than 21 sensory neurons. They are assembled in a single sensillum, which is part of a mixed olfactory/gustatory sense organ (Heimbeck et al., 1999; Python and Stocker, 2002; Singh and Singh, 1984). By contrast, in the flying adult, olfaction relies on 1300 sensory neurons allocated to 550-600 sensilla (Stocker, 2001) that are sited on two exclusively olfactory appendages. Hence, during ontogeny, it might be more economical to replace the simple larval olfactory apparatus than to reorganize it.

As a seeming exception to the rule of sensory replacement, small subsets of neurons associated with leg imaginal discs or abdominal segments have been shown to persist during metamorphosis (Jan et al., 1985; Shepherd and Smith, 1996; Tix et al., 1989a; Williams and Shepherd, 1999). However, experimental evidence suggests that these neurons might have a specialized function, serving as pioneers for growing adult afferents (Usui-Ishihara et al., 2000; Williams and Shepherd, 2002). Whether they become truly integrated in the adult nervous system is not known.

The external gustatory sensilla of the *Drosophila* larva appear to follow the general holometabolan fate: they degenerate during metamorphosis and are replaced by adult-specific sensilla that derive from the labial imaginal disc (Ray et al., 1993; Ray and Rodrigues, 1994; Wildermuth and Hadorn, 1965). Here, we study whether this rule also applies to the internal gustatory system that is located along the pharyngeal tube. Interestingly, the adult pharynx derives essentially from small, densely packed imaginal cells – the clypeolabral bud – that are closely associated with the larval pharyngeal skeleton (Bryant, 1978; Gehring and Seippel, 1967; Struhl, 1981). Does this imply that adult pharyngeal sensilla are born during metamorphosis, like their external counterparts, or do the anatomical similarities of certain larval (Python and Stocker, 2002; Singh, 1997; Singh and Singh, 1984) and adult pharyngeal organs (Nayak and Singh, 1983; Stocker, 1994; Stocker and Schorderet, 1981) rather suggest persistence of sensilla through metamorphosis?

To study possible links between the two sets of sense organs, we undertook a combined approach involving reporter expression from P[GAL4] driver lines, horseradish peroxidase (HRP) injections into syncytial blastoderm stage embryos, cell labeling through heat shock induced FLPout in the late embryo, bromodeoxyuridine (BrdU) birth dating and staining for programmed cell death. Our data include, neuron by neuron, the entire pharyngeal sensory system. They demonstrate that each of the three adult pharyngeal organs is of embryonic origin and derives from mature larval sense organs. Only a few sensilla are added through metamorphosis and few larval sensilla degenerate. This design is in marked contrast to nearly all other sense organs, which originate entirely from imaginal discs. The overall persistence of the larval sensory system is particularly striking because the pharynx undergoes extensive reorganization during this period.

Materials and methods

Fly strains and staging

Only female flies were used in this study. Flies were kept at 25°C in a 12:12 hours light:dark cycle under non-crowded conditions. Apart from the wild-type Canton S (CS) strain, the P[GAL4] strains *MJ94/FM7* (Joiner and Griffith, 1999) and *pros^{V1} (Voila^{1})/TM3* (Balakireva et al., 1998) were studied. Male *MJ94* flies were crossed with female *UAS-lacZ* (Brand and Perrimon, 1993), *UAS-GFP* (Yeh et al., 1995) (kindly provided by A. H. Brand, Wellcome/CRC, Cambridge, UK) or *UAS-mCD8-GFP* (Bloomington Stock Center). Expression of *pros^{V1}* was studied in the *pros^{V1}-UAS-GFP/TM3* strain. The *y w hs-flp; Sp/CyO*; *UAS>CD2 y^{+}>mCD8-GFP/CyO* stock (supplied by R. Axel, Columbia University) was used for the FLPout experiments (see below). Larval patterns were analyzed in feeding third instars. Pupal age is indicated as hours after puparium formation (APF). Adults were studied 3-5 days old.

Dissection

Larvae were cut at about half of the body length in PBS containing 0.2% Triton X-100 (PBST). Anterior halves were turned inside out. Gut, fat body and salivary glands were removed. Tissues were then fixed on ice for 1 hour in 4% paraformaldehyde (PA) in PBST. In the fragile pupae, spiracles and the posterior end of the pupal case were removed. An incision was made along the dorsal midline from posterior to the thoracic region. After fixation for 1 hour in PA on ice, pupae were transferred to PBST and dissected from the pupal case. Heads were cut, fat bodies were removed by gentle flow from a pipette and tissues were transferred back to PA for 1-2 hours. In adults, the proboscis was opened distally with a razor blade and cut into distal and proximal parts that were fixed in PA for 2-3 hours on ice. At all stages, tissues were prepared for whole mounts. In adults, cryosections (10-16 μm) were also made.

Tracing embryonic lineage

HRP was used as a cellular tracer of embryonic lineage, as described previously (Technau, 1986; Tix et al., 1989a). Briefly, HRP 10% in 0.2 M KCl was injected into stage 4 (syncytial blastoderm) embryos (wild-type CS or *MJ94/UAS-lacZ*). The injected embryos were allowed to develop to adulthood. 1-2-day-old adults were dissected, fixed, cryosectioned and processed for HRP and/or *lacZ* histochemistry (see below). A second method for tracing embryonic origin was to generate *mCD8-GFP*-labeled cells by heat-shock-induced FLPout. For this purpose, the progeny of the cross *MJ94* × *y w hs-flp; Sp/CyO; UAS>CD2 y^{+}>mCD8-GFP* (Wong et al., 2002) was exposed to 35°C for 1 hour. Owing to egg-laying periods of 6 hours, the age of these animals varied between 18-24 hours and 12-18 hours after egg laying (AEL).

BrdU labeling

BrdU labeling (Stocker et al., 1995; Truman and Bate, 1988) was performed in the progeny of the cross *MJ94* × *UAS-lacZ* or *MJ94* × *UAS-mCD8-GFP* at 0 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 12 hours and 18 hours APF. BrdU was applied topically (Winberg et al., 1992): 2-3 μg of a BrdU solution (100 mg ml^{-1}) in a mixture of dimethylsulfoxide and acetone (1:1) was applied onto the animals' intact anterior surface. In pupae at more than 12 hours APF, the puparium was partially removed. Animals were then allowed to undergo metamorphosis and were sacrificed as 2-3-day-old adults.

Immunocytochemistry and histochemistry

Green fluorescent protein (GFP) and anti-CD8 antibody (Caltag) coupled with green fluorescence label Alexa 488 (Molecular Probes) were used as fluorescent markers for the confocal microscope. This was done often in combination with the neuronal nuclear marker anti-Elav 9F8A9 (Developmental Studies Hybridoma Bank; http://www.uiowa.edu/~dshbwww) coupled with red fluorescence Cy3 (Jackson ImmunoResearch) or Alexa 568 (Molecular Probes). These double labelings were performed in both whole mounts and cryosections (Python and Stocker, 2002). The embryonic tracer HRP was identified in cryosections using the diaminobenzidine method. For *lacZ* labeling or HRP/*lacZ* and BrdU/*lacZ* double labeling, β-galactosidase was visualized histochemically using X-Gal (Gibco) (Brand and Perrimon, 1993). BrdU immunocytochemistry with an anti-BrdU antibody (G3G4; Developmental Studies Hybridoma Bank) was performed as described by Stocker et al. (Stocker et al., 1995).

Programmed cell death

Apoptosis was studied in flies *MJ94/UAS-mCD8-GFP* by applying an antibody against *Drosophila* caspase (α-active Drice), generously supplied by B. Hay (California Institute of Technology, Pasadena, CA). Double labeling was accomplished by applying two secondary antibodies, tagged with green fluorescence Alexa 488 (Molecular Probes) for CD8 and with red fluorescence Alexa 568 for caspase, respectively.

Confocal and light microscopy

Multiple series of confocal images were taken at 0.5-1 μm intervals with a BioRad MRC 1024 microscope (equipped with a Kr/Ar laser). Image analysis was performed on a Macintosh computer using the public domain NIH Image program (http://rsb.info.nih.gov/nih-image/). Color selection of images was done using Adobe PhotoShop. Light microscopic images were taken with a Zeiss AxioCam digital camera at 1300×1030 resolution and stored and processed with the AxioVision program.

Results

Larval and adult pharyngeal sense organs

Two P[GAL4] enhancer trap lines were used to investigate the pharyngeal sensory system. *MJ94* (Joiner and Griffith, 1999) expresses GAL4 in the entire set of pharyngeal sensory neurons, and *pros^{V1}* (Balakireva et al., 1998) in subsets of accessory cells. *mCD8-GFP* or *lacZ* were used as reporters and anti-Elav served as a pan-neuronal marker (Robinow and White, 1988). The essentials of this study are shown in Fig. 5.

Three paired sense organs consisting of several sensilla each are located along the larval pharynx (Fig. 1A) (Python and Stocker, 2002). Sensilla contain between one and nine sensory neurons, collected below a common terminal pore or bristle (Singh and Singh, 1984). The dorsal and ventral pharyngeal sense organs (dps, vps) sit behind the mouth-hooks; a smaller posterior organ (pps) is located considerably further back. For

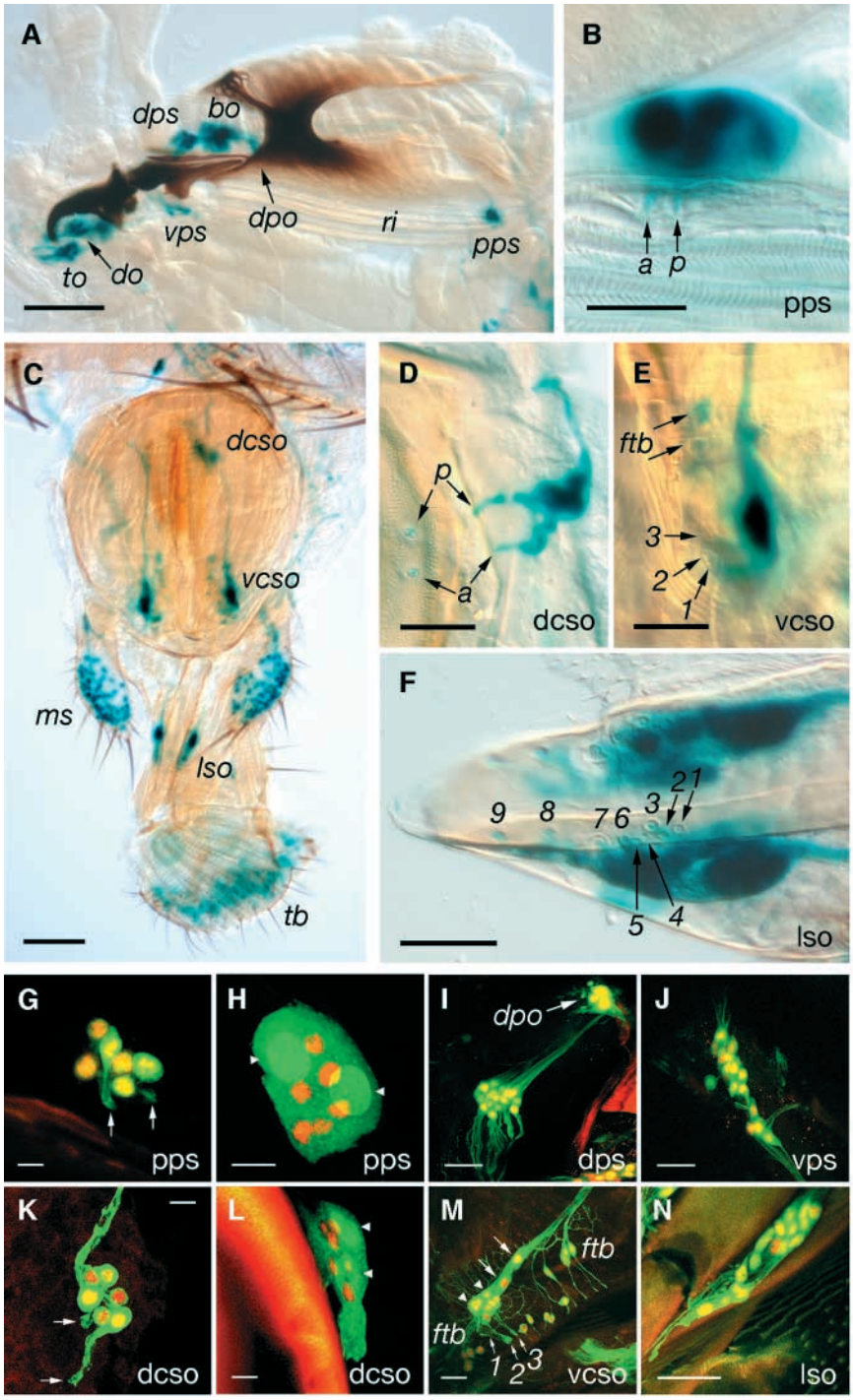

Fig. 1. Pharyngeal sense organs in third instar larvae (A,B,G-J) and adults (C-F,K-N) of *D. melanogaster*, as shown by the P[GAL4] driver line *MJ94*, expressed in all pharyngeal neurons (all panels except H,L) and the *pros*^{V1} line, which labels subsets of accessory cells (H,L). Expression patterns are shown by the reporters *lacZ* (A-F: blue) or *mCD8-GFP* (G-N: green). Neuronal nuclei in G-N are labeled with anti-Elav antibody (red). (A) The larval pharynx is equipped with a dorsal and a ventral pharyngeal sense organ located behind the mouth-hooks (dps, vps), and a smaller sense organ far more posterior (pps), in a region characterized by cuticular ridges (ri). Additional expression is seen in Bolwig's organ (bo), in the presumed dorsal pharyngeal organ (dpo) and in the dorsal and terminal organs (do, to). (B) The pps is composed of an anterior and a posterior sensillum (a, p). (C) The adult pharynx comprises the dorsal and ventral cibarial sense organs (dcso, vcso) and the labral sense organ (lso). *MJ94* drives expression also in maxillary palp sensilla (ms) and in labial palp taste bristles (tb). Higher magnifications reveal an anterior and a posterior sensillum in the dcso (D: a, p), three sensilla in the vcso (E: 1-3) and nine sensilla in the lso (F: 1-9; opposite lso shown on top). Label occurs also in the neurons of the fishtrap bristles (E: ftb). Both the larval pps (G,H) and the adult dcso (K,L) are composed of two sensilla (G,K: arrows) of three neurons each. In both sense organs, *pros*^{V1} labels two accessory cells (H,L: arrowheads). The larval dps (I) and vps (J) contain 17-18 and 16 neurons, respectively. The dps is accompanied further back by the dorsal pharyngeal organ (I: dpo). (M) In the adult, the vcso includes three sensilla (1-3) comprising eight neurons (between arrowheads). The vcso is accompanied by two rows of mononeuronal fishtrap bristles (ftb) and by a few extra neurons (arrows). (N) The nine sensilla of the lso contain a total of 18 neurons. (A,B,F,G) Lateral view, distal to the left. (C-E,H-N) Frontal view, distal to the bottom. Scale bars, 100 μm (A,C), 50 μm (N), 40 μm (D-F), 20 μm (B,G-M)

possible homologies with embryonic sense organs (Campos-Ortega and Hartenstein, 1997; Schmidt-Ott et al., 1994), see Python and Stocker (Python and Stocker, 2002). The nerves associated with the three organs are shown in Fig. 5A. The pps consists of two sensilla with three neurons each (*MJ94*, *n*=14; *pros*^{V1}, *n*=18) (Fig. 1B,G, Fig. 5A). In *M94*, we established 17-18 anti-Elav-antibody-stained neurons for the dps (*n*=18) and 16 for the vps (*n*=15) (Fig. 1I,J, Fig. 5A). An additional five neurons which probably correspond to the dorsal pharyngeal organ (dpo) (Campos-Ortega and Hartenstein, 1997; Schmidt-Ott et al., 1994) are located about 100 μm behind the dps (Fig. 1A,I).

In the adult fly, dorsal and ventral cibarial sense organs (dcso, vcso) occupy the upper and middle part of the pharynx, respectively, whereas the distalmost labral sense organ (lso) extends along the hypopharynx (Fig. 1C-F) (Nayak and Singh, 1983; Stocker, 1994; Stocker and Schorderet, 1981). The larval ridge-like cuticular pattern of the pharynx (Fig. 1A,B) is replaced by a rugged pattern (Fig. 1D), indicating shedding and regeneration of the larval cuticle. The dcso and vcso were reported to include two sensilla each, containing three and three gustatory neurons in the dcso and two and four in the vcso (Nayak and Singh, 1983). We confirm this for the dcso but find an additional proximal sensillum with two neurons in the vcso (Fig. 1D,E,K,M; Fig. 5B; Table 1). Two rows of singly innervated mechanosensory 'fishtrap'

Table 1. Adult pharyngeal sense organs: neuronal composition, function and expression pattern of P[GAL4] lines used

Sense organ	Sensillum type	Number of neurons	Function	Total number of Elav-positive cells		Number of GAL4-expressing cells	
				CS	*MJ94*	*MJ94*	*pros^{V1}*
dcso	Posterior	3	Gustatory	6 (*n*=4)	6 (*n*=15)	6	0
	Anterior	3	Gustatory				
Accessory cells						0	2
	Proximal*	2*	Gustatory*?				
vcso	Middle	2	Gustatory	8 (*n*=11)	8 (*n*=9)	8	0
	Distal	4	Gustatory				
Accessory cells						0	3
lso	1-6	6×1	Mechanosensory				
	7	8	Gustatory	18 (*n*=11)	18 (*n*=7)	18	0
	8	2	Gustatory and mechanosensory	or 19 (*n*=2)			
	9	2	Gustatory and mechanosensory				
Large/small accessory cells						0	3/8
Fishtrap bristles (two rows)	Eighteen to 23 sensilla	One each	Mechanosensory	One each	One each	One each	0
Accessory cells						0	One each

Data in columns 2-4 are from Nayak and Singh (1985), except * (this paper).

bristles accompany the vcso distally and proximally (Nayak and Singh, 1983) (Fig. 1E,M). The lso consists of a heterogeneous group of nine sensilla (Fig. 1F, Fig. 5B). According to fine structural criteria, the distalmost sensilla (8 and 9) each possess a gustatory and a mechanoreceptive neuron, sensillum 7 comprises eight gustatory neurons, and the remaining sensilla are singly innervated mechanoreceptors (Nayak and Singh, 1983). The total of 18 neurons is confirmed by our Elav-binding data (Fig. 1N, Table 1). The nerves carrying the afferents from the adult organs are shown in Fig. 5B.

Interestingly, the larval pps appears to be anatomically equivalent to the adult dcso. Similarity refers to the position on the pharynx (cf. Fig. 1A,C), the presence of two sensilla with three neurons each (cf. Fig. 1G,K) and the labeling of two large accessory cells in *pros^{V1}* (cf. Fig. 1H,L). Hence, the dcso might derive directly from the pps (Fig. 5), suggesting embryonic birth dates for its neurons. For the other sense organs, the anatomy was found to be different at the two stages.

Most of the adult pharyngeal neurons are persisting larval neurons born in the embryo

To study whether some of the adult sensory neurons were indeed born during embryonic development, we followed two different approaches. First, we injected the lineage tracer HRP at the syncytial blastoderm stage (Technau, 1986). Using this technique, adult cells born during embryogenesis remain labeled, whereas adult cells born during postembryonic stages undergo an almost complete dilution of the marker owing to massive proliferation and growth (Tix et al., 1989a). Second, we generated *mCD8-GFP*-labeled pharyngeal neurons by heat-shock-induced recombination in the progeny of a cross between *MJ94* and *y w hs-flp; Sp/CyO; UAS>CD2 y$^+$>mCD8-GFP* (cf. Wong et al., 2002). When the heat shock is applied late during embryogenesis, the presence of labeled single neurons in adult multineuronal sensilla should prove their embryonic origin. This is because multineuronal sensilla derive from a common sensory mother cell, the neurons being born last in the cell lineage (Ray et al., 1993).

In the adult, the embryonically injected HRP was observed in many cells, except in tissues that are known to derive from imaginal discs, such as the antennae. Remarkably, many cells in all three pharyngeal sense organs showed the HRP product, mostly in their cytoplasm. In the lso, strong HRP staining was present in sensillum 7 (Fig. 2A), but label was weak or absent in the other sensilla. Whether HRP resided in neurons or in sheath cells was not clear. Intense HRP staining was also present in the vcso and dcso. In the vcso, the label was strongest in what seemed to be dendritic extensions (Fig. 2B). In the dcso, HRP product was clearly present in neurons, as judged by the labeling of dendritic and axonal processes. All six neurons were probably HRP positive (Fig. 2C,C′). No label was found in fishtrap bristle sensilla (Fig. 2D).

FLPout clones performed in the *MJ94* line were fully compatible with these results. In all three pharyngeal organs, we observed single *mCD8-GFP*-labeled cells when the 1 hour heat treatment was done between 12 hours and 24 hours AEL. These cells were clearly neurons, as judged by the neuron-specific expression of the *MJ94* line, the presence of dendrites and axons, and simultaneous labeling by anti-Elav antibody. More precisely, of the 64 GFP-labeled neurons observed, 52 were single cells (Table 2). Exclusively single labeled neurons were found in sensilla 1-7 of the lso (Fig. 2E-G) (with one exception; see below) and in all five sensilla of the vcso and dcso (Fig. 2K,L). Neurons arranged in pairs were observed five times in sensilla 8 and 9 of the lso (Fig. 2H,I, Table 2). The fact that in none of the multiply innervated sensilla, except sensilla 8 and 9 of the lso, were more than one labeled neuron found indicates that these neurons had undergone their terminal mitosis during embryogenesis. By contrast, when the FLPout was performed earlier (6-18 hours AEL), a clone of three labeled neurons was observed in sensillum 7 of the lso (Fig. 2J) and a clone of two neurons in the vcso (not shown). This suggests that, during this interval, cell divisions were still going on. The frequency of FLPouts in late embryos was relatively

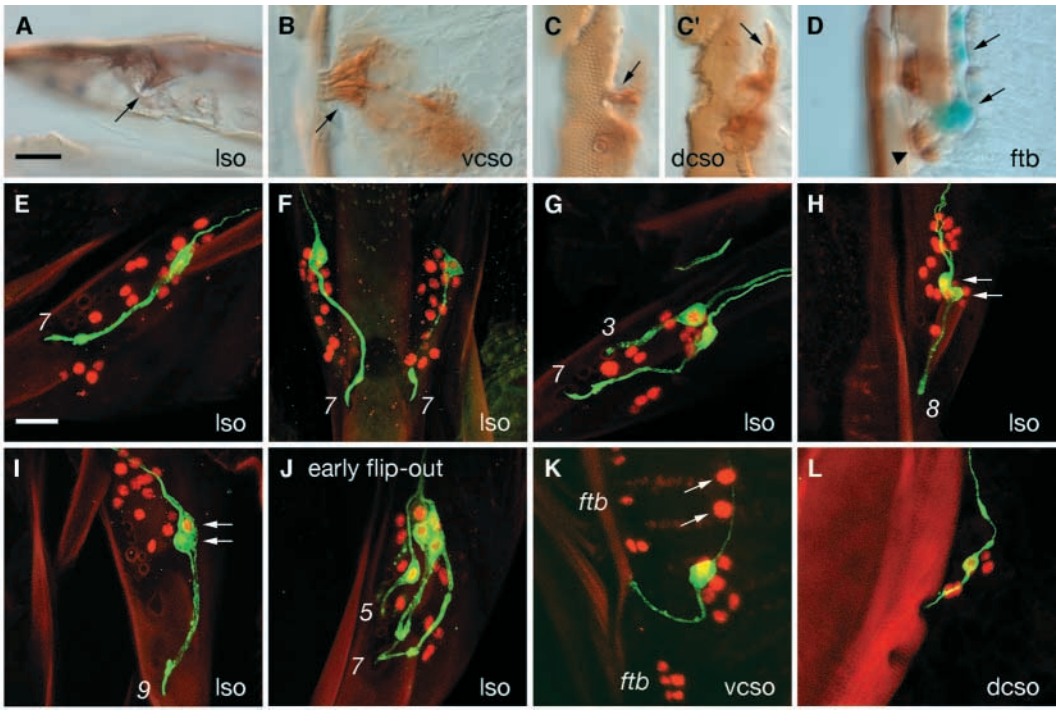

Fig. 2. Embryonic origin of subsets of neurons and non-neuronal cells in the pharyngeal sense organs, as shown by HRP injection at the syncytial blastoderm stage (A-D) and by FLPout (Wong et al., 2002) induced in F1 embryos of the cross *MJ94* × *y w hs-flp; Sp/CyO; UAS>CD2 y*$^{+}$*>mCD8-GFP* (E-L). (E-L) Neuronal nuclei are labeled with anti-Elav antibody (red). (A-C′) HRP product is present in dendritic tips of the lso sensillum 7 (A: arrow) and of the vcso (B: arrow), as well as in dendrites, cell bodies and axons of dcso neurons (consecutive sections C,C′: arrows). (D) Fishtrap bristles labeled by *MJ94/UAS-lacZ* (arrows) lack HRP staining, in contrast to the vcso (arrowhead). (E-I) When FLPouts are induced in the *MJ94* line at 12-24 hours after egg laying (AEL), single neurons are labeled in the lso, in both sensillum 7 (composed of eight neurons) (E-G: 7) and the mononeuronal sensilla 1-6 (G: 3). By contrast, the bineuronal sensilla 8 and 9 always exhibit paired labeled neurons (H,I: arrows). Simultaneous labeling of two sensilla suggests the occurrence of two independent FLPout events (F,G). (J) FLPouts induced earlier (6-18 hours AEL) lead to simultaneous label in many neurons (e.g. three in sensillum 7). (K,L) FLPout induced at 12-24 hours AEL invariably leads to single labeled neurons in the multineuronal sensilla of the vcso (K) and dcso (L). The two large neurons in K (arrows) are outside the vcso. ftb, fishtrap bristles. Distal is to the bottom except in (A) in which distal is to the left. F shows both left and right lso. Scale bars, 20 μm (A shows scale for A-D; E shows scale for E-L).

high, as shown by several observations of two putative independent events in left and right sense organs (Fig. 2F) or in different sensilla of the lso (Fig. 2G). A similar explanation might apply to an exceptional case of two labeled neurons in the multineuronal sensillum 7 (see above).

Both the HRP and FLPout data suggest that most of the adult pharyngeal neurons are persisting larval neurons that have become fully integrated into the adult system. For the monoinnervated sensilla 1-6 of the lso, FLPouts with *MJ94* are not conclusive because a clone extending to accessory cells would go undetected. The lack of driver lines expressing GAL4 reliably in every cell of a mature sensillum renders such an experiment unfeasible for the moment. However, the gradual appearance of Elav expression in most of these neurons at mid-pupal stages (see below) points to a postembryonic origin of these sensilla.

Extra neurons are added during metamorphosis

Interestingly, late embryonic FLPouts always led to pairs of labeled neurons in sensilla 8 and 9 of the lso (Fig. 2H,I, Table 2), suggesting that cell divisions occurred after the FLPout. To discover whether the terminal cell divisions of these neurons had taken place during metamorphosis, we used the mitotic marker BrdU (Truman and Bate, 1988; Stocker et al., 1995) to *MJ94* and wild-type CS animals, in experiments ranging from 0 hours to 18 hours APF. Indeed, in adult flies that had been treated in this way as pupae, subsets of neuronal and non-neuronal cells of the pharyngeal sense organs were labeled. In

Table 2. Clones induced in pharyngeal sensilla by hs flip-out in the *MJ94* line

Sense organ	Sensillum type (neuron number)	Flip-out induced 12-24 hours AEL Number of labeled neurons observed: 1	2	Flip-out induced early (6-18 hours AEL)
dcso	Posterior (3)	5×	0×	
	Anterior (3)	4×	0×	
vcso	Proximal (2) Middle (2) Distal (4)	9×	0×	1×: 2 neurons
lso	1 (1)	1×	0×	
	2 (1)	1×	0×	
	3 (1)	5×	0×	
	4 (1)	3×	0×	
	5 (1)	3×	0×	
	6 (1)	6×	0×	
	7 (8)	14×	1×	1×: 3 neurons
	8 (2)	0×	3×*	
	9 (2)	1×†,‡	2×†	
Fishtrap bristles	Proximal (1)	0×	0×	
	Distal (1)	0×	0×	

The three sensilla of the VCSO were not identified in the flip-outs.
*Two of the cases were induced at 12-18 hours after egg laying (AEL), one case at 18-24 hours AEL.
†Induced at 12-18 hours AEL.
‡Perhaps caused by abnormal loss of second neuron.
Total number of left/right sides observed is 86.

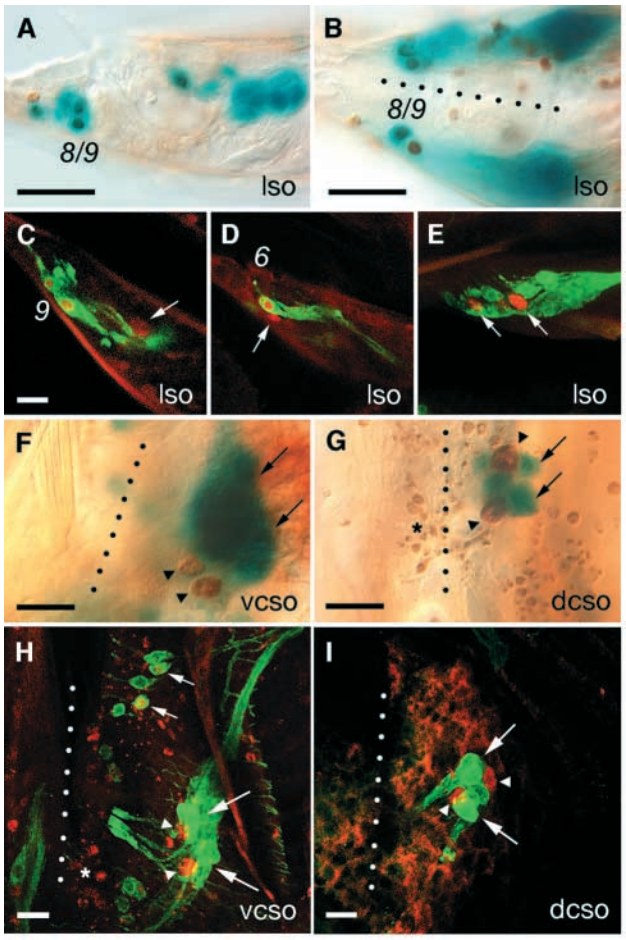

Fig. 3. Early pupal origin of subsets of neuronal and non-neuronal cells in the adult pharyngeal sense organs, as shown by BrdU incorporation at 3 hours APF in *MJ94* (G: 18 hours APF). The patterns were visualized by *lacZ* expression (blue) in the light microscope (A,B,F,G) or by *mCD8-GFP* expression (green) in the confocal microscope (C-E,H,I). The anti-BrdU product is shown in brown (A,B,F,G) or red (C-E,H,I). (A-E) In the lso, BrdU label is particularly obvious in the neurons of the distal sensilla 8 and 9. Additional BrdU take-up is visible in the neuron of the mononeuronal sensillum 6 (D) and in subsets of accessory cells (C-E: arrows). (F-I) In both vcso (F,H) and dcso (G,I), two accessory cells (arrowheads) but none of the neurons (large arrows) are BrdU positive. However, BrdU label is present in the neurons of the fishtrap bristles (H: small arrows). Asterisks in G,H indicate BrdU uptake in cells of the pharyngeal wall. (A,C,D,E) Sagittal sections; all others panels represent frontal views. Dots indicate midline. Distal is to the left (A-E) or to the bottom (F-I). Scale bars, 10 μm (F,G), 20 μm (A,B,H), 40 μm (C-E,I).

the lso, we observed BrdU-positive neuronal and non-neuronal cells at the distal tip, belonging to sensilla 8 and 9 (Fig. 3A-C). Additional take-up had occurred in cells situated more proximally in the lso (Fig. 3A-E), for example in the neuron associated with sensillum 6 (Fig. 3D). In the vcso and dcso, none of the preparations revealed any labeled neurons. However, two accessory cells were clearly BrdU positive in both of these organs (Fig. 3F-I). Also, we observed labeling in the neurons of fishtrap bristles (Fig. 3H) and massive take-up in cells of the pharyngeal wall (Fig. 3G,H). Thus, the BrdU studies suggest that subsets of cells in the three sense organs undergo terminal division during metamorphosis. In summary, the adult pharyngeal sense organs appear to consist of both embryonically and postembryonically derived neurons and accessory cells.

Fate of pharyngeal sense organs through metamorphosis

Having established the embryonic origin of most of the adult pharyngeal sensory neurons, we wanted to study the relations between larval and adult pharyngeal organs directly. To this end, we investigated the expression pattern of the *MJ94* line in progressively older pupal stages (Fig. 4). Until 20 hours APF, development was studied at hourly intervals; thereafter, it was studied at 24 hours, 48 hours, 64 hours and 68 hours APF. As expected, the most stable in terms of anatomy was the pps/dcso, which always exhibited six GAL4-positive and Elav-positive neurons, characterized by dendritic and axonal processes (Fig. 4A-C), and two GAL4-positive accessory cells in $pros^{V1}$ (not shown). This conserved organization strongly supports developmental continuity between the two organs (Fig. 5).

The fate of the vps was very different. A few hours APF, its neurons began to lose coherence and Elav- and *mCD8*-positive cellular debris appeared (Fig. 4D). The antibody α-active Drice, which displays a pattern in the pupal retina similar to the known pattern of apoptosis (Brachmann and Cagan, 2003) (not shown), labeled the neurons of the vps at early pupal stages (Fig. 4E,E′) but not those of the dps (Fig. 4F,F′) or the pps (not shown). These data suggest that the vps is subject to programmed cell death (Fig. 5).

In contrast to the pps and vps, the dps underwent complex transformation. Initially, its dendrites appeared to be collected in two clusters, anterior and posterior (a, p1) (Fig. 4G). Two neurons of the dpo (Fig. 1A,I, Fig. 4G) then joined the dps, leading to an additional cluster p2 (Fig. 4H,I). From 14 hours APF onward, this entire complex began to split into two distinct organs (vcso and lso) in parallel with the distal elongation of the pharynx (cf. Fig. 4J,K,M). The posterior organ, vcso, was derived from p1 and p2; p1 gave rise to the distal and middle sensilla of the vcso, p2 to the proximal sensillum (Fig. 4K,L). Five to six additional neurons, probably remnants of the dps and dpo, were found scattered on the associated nerve, outside the vcso proper (Fig. 4J-L). They persisted up to the adult stage (Fig. 1M, Fig. 2K). At 24 hours APF, Elav staining revealed for the first time the two rows of fishtrap bristles (Fig. 4L).

The anterior organ, lso, derived mostly from cluster a. It gave rise to two sensilla: a complex one with eight neurons (sensillum 7) and a mononeuronal sensillum (Fig. 4M), perhaps sensillum 3, judged by its distinct size. Around 20 hours APF, an additional four cells in the distalmost part of the lso, encompassing bineuronal sensilla 8 and 9, began to express Elav and GAL4, and another five neurons appeared more proximally (Fig. 4M-O). The latter formed mononeuronal sensilla 1-6 together with the persisting putative sensillum 3 (see above).

These observations are fully compatible with the experimental data regarding the persistence of most of the larval neurons and the birth of a new, smaller set during

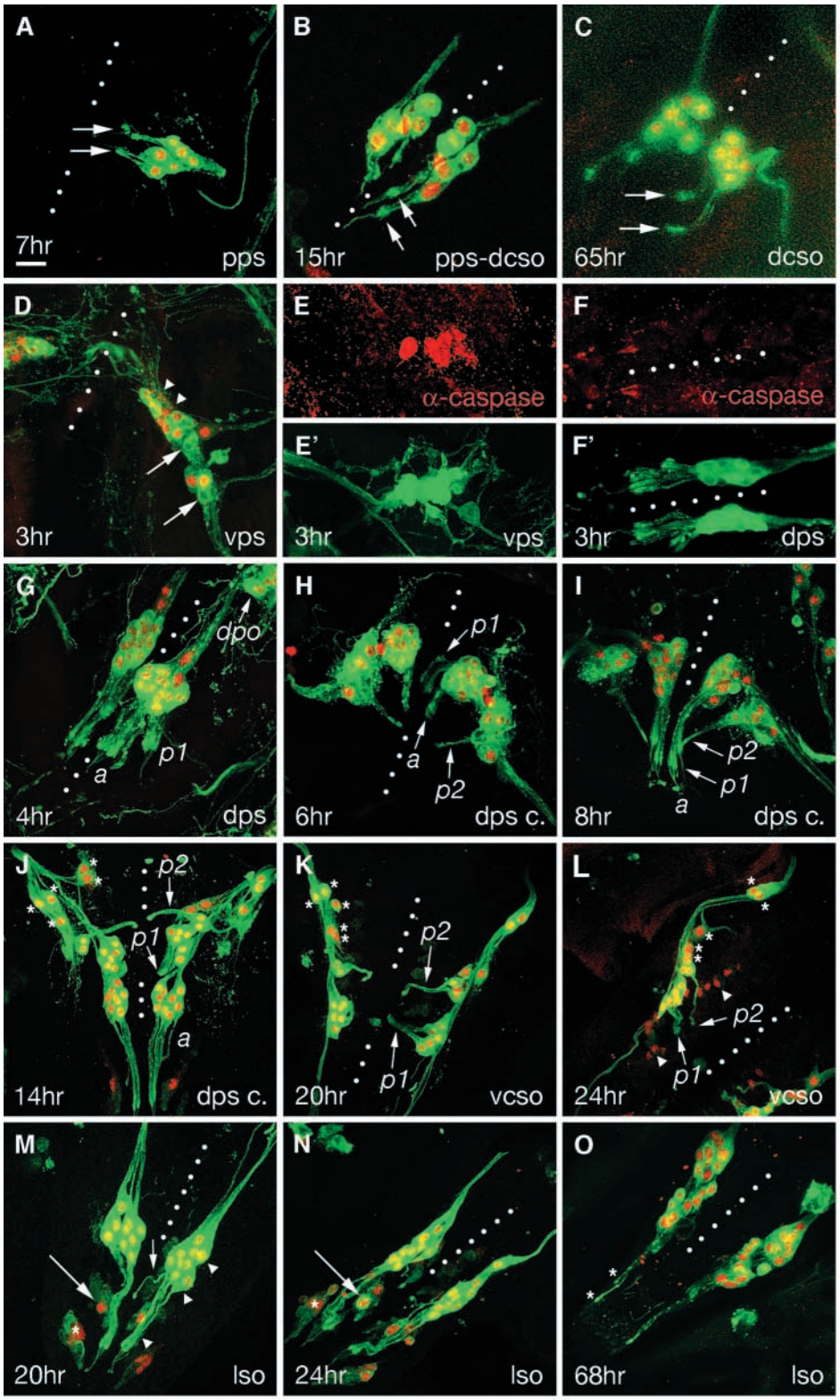

Fig. 4. Metamorphic transformation of pharyngeal sense organs, as shown by *MJ94/UAS-mCD8-GFP* (green). Red indicates the Elav marker except in E,F, in which it indicates α-caspase antibody. Stages are indicated in hours APF. (A-C) The pps maintains two sensilla throughout metamorphosis (arrows) each comprising three differentiated neurons, and becomes the dcso. (D-F) By contrast, the vps disintegrates in the early pupa and its neurons lose Elav immunoreactivity (D: arrows) or give rise to Elav-positive debris (D: arrowheads). Evidence for apoptosis is shown by staining with anti-caspase antibody (E). Neuronal identity of the apoptotic cells is indicated by *mCD8* expression in the same optical section (E′). By contrast, anti-caspase antibody does not bind to the neurons of the dps (F,F′). (G-I) The dps with its clusters a and p1 is joined by two additional neurons (p2) from the dpo, forming a dps complex (dps c.). (J-O) Later on, the dps c. splits into two distinct sense organs, vcso and lso. The vcso includes p1 and p2, with six and two neurons, respectively (J-L). The p1 cluster comprises the distal and middle sensilla of the vcso, and the p2 cluster comprises the proximal sensillum. Five to six additional neurons deriving from dps and dpo remain outside the vcso (J-L: asterisks). At 24 hours APF, the neurons of the fishtrap bristles appear (L: arrowheads). In the lso, the a cluster (consisting of nine neurons) will form sensillum 7 (eight neurons; M: arrowheads) and an extra mononeuronal sensillum (M: small arrow). An additional nine neurons appear in the distal part of the lso (M,N: large arrows), four of which are associated with bineuronal sensilla 8 and 9 (M-O: asterisks). White dots indicate midline; distal is to the bottom except in F,F′, in which distal is to the left. Scale bar, 20 μm.

metamorphosis. In addition they demonstrate the conservation through metamorphosis of entire sense organs, pps and dps, the existence of complex morphogenetic movements including the split of a larval sense organ in two, and the degeneration of the vps.

Discussion

Most of the larval pharyngeal neurons persist through metamorphosis

Our data prove that most of the neurons of the three major adult pharyngeal sense organs are persisting larval neurons that were born in the embryo (Fig. 5). This is unlike other adult sensory neurons, nearly all of which derive from imaginal discs. Our interpretation relies on two independent experimental approaches for demonstrating embryonic birth dates (the use of the embryonic lineage tracer HRP and cell labeling by FLPout at late embryonic stages, a novel use of this technique). The experimental data are supported by anatomical

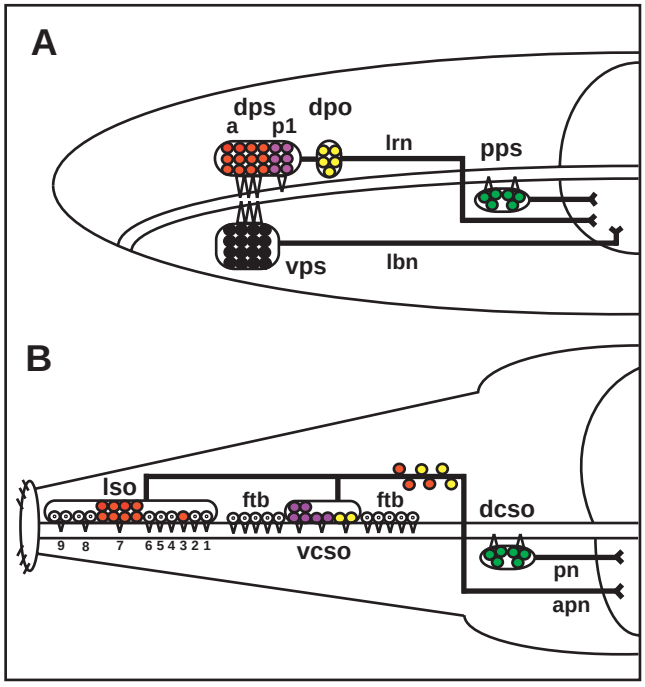

Fig. 5. Fate of pharyngeal sensory neurons through metamorphosis. Persisting neurons are shown in color, neurons undergoing apoptosis in black, and newly born neurons with a dot. Individual sensilla are shown as arrowhead symbols. (A) Third larval instar: the dorsal pharyngeal sense organ (dps) consists of two groups of neurons, a and p1. Precise numbers of sensilla in the dps, dpo (dorsal pharyngeal organ) and vps (ventral pharyngeal sense organ) are unclear. lbn, labial nerve; lrn, labral nerve; pps, posterior pharyngeal sense organ. (B) Adult: the dorsal cibarial sense organ (dcso) corresponds to the conserved pps. The labral sense organ (lso) and the ventral cibarial sense organ (vcso) derive essentially from the dps. However, the vcso also includes two neurons of the dpo. Seven small sensilla of the lso as well as the fishtrap bristles (ftb; numbers in the diagram reduced) are born during metamorphosis. Remaining neurons of the dps and dpo are located along the accessory pharyngeal nerve (apn). pn, pharyngeal nerve.

observations showing: (i) an almost identical organization of the larval pps and the adult dcso; (ii) the presence of the pps and dps sensilla continuously through metamorphosis; (iii) an uninterrupted expression of the P[GAL4] lines used in these two organs; and (iv) the persistence of dendrites and axons in all surviving neurons.

HRP injected at the syncytial blastoderm stage becomes incorporated into every cell upon cellularization. During subsequent development, the marker remains at high levels in cells that divide only a few times but becomes diluted in cells that undergo repeated divisions (Technau, 1986). Consequently, labeling in the adult is expected in many neurons of the central nervous system known to be persisting larval neurons [e.g. optic lobe pioneers (Tix et al., 1989b)] but should be absent from tissues derived from imaginal discs (Levine et al., 1995; Tissot and Stocker, 2000; Truman, 1996). This corresponds to what we observe and allows us to postulate an embryonic origin for the elements containing high HRP levels in adult pharyngeal sense organs.

This interpretation is supported by the FLPout experiments (cf. Wong et al., 2002) performed at late embryonic stages with the neuron-specific *MJ94* line. In adults deriving from this treatment, we detected exclusively single labeled neurons in sensillum 7 of the lso (containing eight neurons) and in the five multiply innervated sensilla of the vcso and dcso (Table 2). Although we did not study the cell lineage of these sensilla, they are probably homologous to other multineuronal terminal-pore gustatory sensilla, which derive from a common sensory mother cell (Ray et al., 1993). Indeed, apart from its eight neurons, sensillum 7 of the lso corresponds to a typical insect gustatory sensillum in terms of fine structural and cellular organization, containing no more than three accessory cells (Nayak and Singh, 1983). Hence, the single labeled neurons in this sensillum and in all sensilla of the vcso and dcso must have been postmitotic during FLPout. This agrees with the observation that formation of head nerves is complete by embryonic stage 15 (Campos-Ortega and Hartenstein, 1997).

Could these neurons have remained immature during larval life, differentiating only during metamorphosis, similar to subsets of postmitotic cells in the larval central nervous system CNS (Booker and Truman, 1987; Truman, 1990)? We believe this rather unlikely because it would require either the entire sensillum or subsets of neurons in multineuronal sensilla to remain immature. Moreover, there is no indication for immature neurons from tracing their development with the marker line *mCD8-GFP*. Thus, we suggest that all the neurons of the dcso and vcso, and sensillum 7 of the lso derive from mature, functional larval neurons. Also, continuous reporter expression through metamorphosis suggests that one of the mononeural lso sensilla (perhaps sensillum 3) might be another persisting larval sensillum.

Cell proliferation and cell death in pharyngeal sense organs through metamorphosis

The persistence of mature larval neurons does not exclude the addition of cells during larva-adult transition. Indeed, BrdU applied during metamorphosis labeled subsets of cells in all three sense organs and in the fishtrap bristles. In agreement with these data, late embryonic FLPouts displayed pairs of labeled neurons in the sensilla 8 and 9 of the lso. Neuronal identity of the labeled cells was also established for the lso sensillum 6 as well as for the fishtrap bristles. Consequently, these neurons must have arisen by a terminal division after the FLPout and after BrdU application (i.e. during metamorphosis) (Fig. 5). Hence, of the total of 32 sensory neurons of the three main adult sense organs, only the birth dates of four monoinnervated lso sensilla remain unclear. Yet, their metamorphic origin is suggested by BrdU uptake in sensillum 6 and the gradual appearance of Elav staining at 24 hours APF in all four sensilla.

Taken together, these data suggest that the lso is composed of both larval and adult-specific sensilla, whereas the vcso and dcso consist exclusively of larval sensilla. Seen from a larval perspective, both the pps and the dps are conserved through metamorphosis, although with considerable modification of the dps (Fig. 5). By contrast, the vps undergoes programmed cell death, and the fishtrap bristles are entirely adult specific, lacking any larval counterpart. In conclusion, metamorphosis of the internal gustatory sensory system is an intricate process involving neuronal persistence, generation of additional neurons and neuronal death.

Larval sense organs persist despite extensive reorganization of the pharynx

The fact that the pps and dps persist through metamorphosis is remarkable given the origin of the adult labrum and cibarium from imaginal cells of the clypeolabral bud (Bryant, 1978; Gehring and Seippel, 1967; Struhl, 1981). In agreement with these reports, we observe massive labeling of pharyngeal epithelial cells after early pupal BrdU application. Moreover, the pharyngeal cuticle is shed and regenerates, a process that includes the cuticular part of the sensilla in question. Perhaps the birth of additional accessory cells during metamorphosis (e.g. in the dcso or vcso, containing exclusively persisting neurons) is related to this modification. Formation of new cuticular structures is also known from persisting external sensilla during larval molts, but the survival of pharyngeal sensilla during the extensive remodeling of the pharynx remains stunning. The morphogenetic movements we observe in the sensory system certainly reflect these dramatic changes.

Why is the larval pharyngeal sensory apparatus largely conserved through metamorphosis?

Small subsets of neurons associated with leg imaginal discs or with abdominal segments have previously been shown to persist through metamorphosis (Jan et al., 1985; Shepherd and Smith, 1996; Tix et al., 1989a; Williams and Shepherd, 1999). In the fly *Phormia*, such leg-disc-associated neurons remain immature (Lakes-Harlan et al., 1991a; Lakes-Harlan et al., 1991b), implying that they are non-functional. Laser ablation studies suggest that persisting neurons might help adult afferents to navigate from the imaginal discs to their central targets (Usui-Ishihara et al., 2000; Williams and Shepherd, 2002). Whether they become truly integrated in the adult nervous system or die after reaching adulthood (having completed their pathway role) remains to be shown. Recently, tracing the expression pattern of a *Kr*-driven reporter line suggested the incorporation of four receptor neurons of the larval eye into the so-called adult eyelet (Helfrich-Förster et al., 2002; Hofbauer and Buchner, 1989), but this was not tested experimentally.

Our data demonstrate for the first time experimentally the integration of larval sensory neurons into the adult nervous system of *Drosophila*. Particularly striking and novel is the fact that entire, fully differentiated larval sense organs become incorporated. Also, this is the first observation of metamorphic survival in the chemosensory system.

Concerning the persisting neurons of the lso, a pathway function for the newly developing afferents towards and inside the central nervous system is certainly possible. However, the integration of the surviving pharyngeal neurons into the adult sensory system invites other interpretations. For example, these neurons and/or their central projections might be particularly precious, allowing, for example, the persistence of specific feeding-associated gustatory tasks through metamorphosis. As an alternative explanation, survival might be due to reasons of economy, a principle that governs the metamorphosis of the nervous system (Tissot and Stocker, 2000). Although neuronal reorganization is indispensable owing to the changing demands of larval and adult life, it is kept at a minimum, as shown by the survival of most larval interneurons and motor neurons (Truman, 1996). Sophisticated adult sense organs, however, might be easier to build de novo than by the transformation of simple larval organs, explaining the almost complete replacement of the larval sensory system. Why pharyngeal sense organs do not follow this general rule might relate to their largely conserved function at the two stages of life (analyzing the quality of ingested food of similar composition). The presence of larva-specific and adult-specific sensilla, however, suggests the existence of stage-specific gustatory tasks.

Using the genetic potential of the fly, it will be intriguing to dissect the functions of the different types of sensilla. Moreover, our analysis invites us to study at single cell level the genetic basis of many essential developmental processes, including cell determination, differentiation and apoptosis.

This work was supported by Swiss National Funds (grants 31-52639.97 and 31-063447.00), the Roche Foundation and the DFG (grant TE 130/7-4). We thank P. Gehr and B. Rothen-Rutishauser (Institute of Anatomy, University of Bern) for providing confocal facilities, and B. Gerber (Würzburg), F. Python (Fribourg) and K. Störtkuhl (Bochum) for valuable comments on the manuscript.

References

Balakireva, M., Stocker, R. F., Gendre, N. and Ferveur, J. F. (1998). *Voila*: a new *Drosophila* courtship variant that affects the nervous system: behavioral, neural and genetic characterization. *J. Neurosci.* **18**, 4335-4343.

Booker, R. and Truman, J. W. (1987). Postembryonic neurogenesis in the CNS of the tobacco hornworm, *Manduca sexta*. I. Neuroblast arrays and the fate of their progeny during metamorphosis. *J. Comp. Neurol.* **255**, 548-559.

Brachmann, C. B. and Cagan, R. L. (2003). Patterning the fly eye: the role of apoptosis. *Trends Genet.* **19**, 91-96.

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

Bryant, P. J. (1978). Pattern formation in imaginal discs. In *The Genetics and Biology of* Drosophila, Vol. 2c (ed. M. Ashburner and T. R. F. Wright), pp. 229-335. London, New York, San Francisco: Academic Press.

Campos-Ortega, J. and Hartenstein, V. (1997). The embryonic development of *Drosophila melanogaster*. Berlin, Heidelberg, New York: Springer.

Gehring, W. and Seippel, S. (1967). Die Imaginalzellen des Clypeo-Labrums und die Bildung des Rüssels von *Drosophila melanogaster*. *Rev. Suisse Zool.* **74**, 589-596.

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

Helfrich-Förster, C., Edwards, T., Yasuyama, K., Wisotzki, B., Schneuwly, S., Stanewsky, R., Meinertzhagen, I. A. and Hofbauer, A. (2002). The extraretinal eyelet of *Drosophila*: development, ultrastructure, and putative circadian function. *J. Neurosci.* **22**, 9255-9266.

Hofbauer, A. and Buchner, E. (1989). Does *Drosophila* have seven eyes? *Naturwissenschaften* **76**, 335-336.

Jan, Y. N., Ghysen, A., Barbel, C. I. and Jan, L. Y. (1985). Formation of neuronal pathways in the imaginal discs of *Drosophila melanogaster. J. Neurosci.* **5**, 2453-2464.

Joiner, M. A. and Griffith, L. C. (1999). Mapping of the anatomical circuit of CaM kinase-dependent courtship conditioning in *Drosophila*. *Learn. Mem.* **6**, 177-192.

Lakes-Harlan, R., Pollack, G. S. and Merritt, D. J. (1991a). From embryo to adult: anatomy and development of a leg sensory organ in *Phormia regina* Meigen (Insecta: Diptera). I. Anatomy and physiology of a larval 'leg' sensory organ. *J. Comp. Neurol.* **308**, 188-199.

Lakes-Harlan, R., Pollack, G. S. and Merritt, D. J. (1991b) From embryo to adult: anatomy and development of a leg sensory organ in *Phormia regina* Meigen (Insecta: Diptera). II. Development and persistence of sensory neurons. *J. Comp. Neurol.* **308**, 200-208.

Levine, R. B., Morton, D. B. and Restifo, L. L. (1995). Remodeling of the insect nervous system. *Curr. Opin. Neurobiol.* **5**, 28-35.

Nayak, S. V. and Singh, R. N. (1983). Sensilla on the tarsal segments and

mouthparts of adult *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). *Int. J. Insect Morphol. Embryol*. **12**, 273-291.
Python, F. and Stocker, R. F. (2002). Adult-like complexity of the larval antennal lobe of *D. melanogaster* despite markedly low numbers of odorant receptor neurons. *J. Comp. Neurol.* **445**, 374-387.
Ray, K. and Rodrigues, V. (1994). The function of the proneural genes *achaete* and *scute* in the spatio-temporal patterning of the adult labellar bristles of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **203**, 340-350.
Ray, K., Hartenstein, V. and Rodrigues, V. (1993). Development of the taste bristles on the labellum of *Drosophila melanogaster*. *Dev. Biol.* **155**, 26-37.
Robinow, S. and White, K. (1988). The locus *elav* of *Drosophila melanogaster* is expressed in neurons at all developmental stages. *Dev. Biol*. **126**, 294-303.
Schmidt-Ott, U., González-Gaitán, M., Jäckle, H. and Technau, G. M. (1994). Number, identity, and sequence of the *Drosophila* head segments as revealed by neural elements and their deletion patterns in mutants. *Proc. Natl. Acad. Sci. USA* **91**, 8363-8367.
Shepherd, D. and Smith, S. A. (1996). Central projections of persistent larval sensory neurons prefigure adult sensory pathways in the CNS of *Drosophila*. *Development* **122**, 2375-2384.
Singh, R. N. (1997). Neurobiology of the gustatory systems of *Drosophila* and some terrestrial insects. *Microsc. Res. Tech.* **39**, 547-563.
Singh, R. N. and Singh, K. (1984). Fine structure of the sensory organs of *Drosophila melanogaster* Meigen larva (Diptera: Drosophilidae). *Int. J. Insect Morphol. Embryol.* **13**, 255-273.
Stocker, R. F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tissue Res.* **275**, 3-26.
Stocker, R. F. (2001). *Drosophila* as a focus in olfactory research: mapping of olfactory sensilla by fine structure, odor specificity, odorant receptor expression and central connectivity. *Microsc. Res. Tech.* **55**, 284-296.
Stocker, R. F. and Schorderet, M. (1981). Cobalt filling of sensory projections from internal and external mouthparts in *Drosophila*. *Cell Tissue Res.* **216**, 513-523.
Stocker, R. F., Tissot, M. and Gendre, N. (1995). Morphogenesis and cellular proliferation pattern in the developing antennal lobe of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol*. **205**, 62-75.
Struhl, G. (1981). A blastoderm fate map of compartments and segments of the *Drosophila* head. *Dev. Biol*. **84**, 386-396.
Technau, G. M. (1986). Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster*. I. The method. *Roux's Arch. Dev. Biol.* **195**, 389-398.
Tissot, M. and Stocker, R. F. (2000). Metamorphosis in *Drosophila* and other insects: the fate of neurons throughout the stages. *Prog. Neurobiol*. **62**, 89-111.
Tix, S., Bate, M. and Technau, G. M. (1989a). Pre-existing neuronal pathways in the leg imaginal discs of *Drosophila*. *Development* **107**, 855-862.
Tix, S., Minden, J. S. and Technau, G. M. (1989b). Pre-existing neuronal pathways in the developing optic lobes of *Drosophila*. *Development* **105**, 739-746.
Truman, J. W. (1990). Metamorphosis of the central nervous system of *Drosophila*. *J. Neurobiol.* **21**, 1072-1084.
Truman, J. W. (1996). Metamorphosis of the insect nervous system. In *Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cells* (ed. L. I. Gilbert, J. R. Tata and B. G. Atkinson), pp. 283-320. San Diego: Academic Press.
Truman, J. W. and Bate, M. (1988). Spatial and temporal patterns of neurogenesis in the central nervous system of *Drosophila melanogaster*. *Dev. Biol.* **125**, 145-157.
Usui-Ishihara, A., Simpson, P. and Usui, K. (2000). Larval multidendrite neurons survive metamorphosis and participate in the formation of imaginal sensory axonal pathways in the notum of *Drosophila*. *Dev. Biol.* **225**, 357-369.
Wildermuth, H. and Hadorn, E. (1965). Differenzierungsleistungen der Labial-Imaginalscheibe von *Drosophila melanogaster*. *Rev. Suisse Zool.* **72**, 686-694.
Williams, D. W. and Shepherd, D. (1999). Persistent larval sensory neurons in adult *Drosophila melanogaster. J. Neurobiol.* **39**, 275-286.
Williams, D. W. and Shepherd, D. (2002). Persistent larval sensory neurones are required for the normal development of the adult sensory afferent projections in *Drosophila*. *Development* **129**, 617-624.
Winberg, M. L., Perez, S. E. and Steller, H. (1992). Generation and early differentiation of glial cells in the first optic ganglion of *Drosophila melanogaster*. *Development* **115**, 903-911.
Wong, A. M., Wang, J. W. and Axel, R. (2002). Spatial representation of the glomerular map in the *Drosophila* protocerebrum. *Cell* **109**, 229-241.
Yeh, E., Gustafson, K. and Boulianne, G. L. (1995). Green fluorescent protein as a vital marker and reporter of gene expression in *Drosophila. Proc. Natl. Acad. Sci. USA* **92**, 7036-7040.