Central projections of persistent larval sensory neurons prefigure adult sensory pathways in the CNS of Drosophila

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SUMMARY

We have used a GAL4 enhancer-trap line driving the expression of a lacZ construct to examine the reorganisation of an identified group of proprioceptive sensory neurons during metamorphosis in Drosophila. The results show that whilst most larval sensory neurons degenerate during the first 24 hours of metamorphosis a segmentally repeated array of 6 neurons per segment persists into the adult stages to become functional adult neurons. These sensory neurons retain their axonal projections in the central nervous system intact and unchanged throughout. The adult sensory neuron axons enter the central nervous system at around 44 hours after puparium formation. Most of these axons grow along the pathways defined by the persistent larval sensory axons. The ordering of the adult sensory projections is, therefore, established upon the larval pattern of projections. The possibility that the larval neurons act as guidance cues for organising the ordered arrays of sensory neurons is discussed.

Key words: axon guidance, metamorphosis, pioneer neurons, enhancer-trapping, Drosophila

INTRODUCTION

A key feature of the life history of Drosophila is the profound change in body form that occurs during metamorphosis. This transition sees the animal change from a simple larva, with rudimentary appendages, to a complex adult bearing legs, wings, genitalia and sensory apparatus for flying. Concomitant with these changes in external appearance are changes to the internal organisation, most notably in the central nervous system (CNS). Work on a number of holometabolous insects (primarily Manduca and Drosophila) has shown that this transformation involves: differentiation of larvally produced central neurons (Booker and Truman, 1987a,b; Truman and Bate, 1988; Prokop and Technau, 1991), degeneration of obsolete larval neurons (Truman, 1983) and remodelling of other larval neurons to fulfil new functions in the adult (Truman and Reiss, 1976; Levine and Truman, 1982, 1985).

In addition to changes in the CNS there is a less well documented reconstruction of the sensory system. During metamorphosis the larval sensory system is essentially destroyed and replaced with a new adult-specific set of sensory neurons. These adult sensory neurons originate mostly de novo and since they arise preferentially are required to extend axons into the CNS in order to establish a pathway to their central targets (Bate, 1978). One important question to ask is how are these growing sensory axons able to navigate the relatively long distances to the pupal CNS in order to establish this pathway? This is a problem faced by all developing sensory neurons, even in the embryo, but in the larger and more complex adult body the potential problems are greater. Studies of the embryonic development of sensory neurons have shown that one mechanism for assisting axon guidance over long distances is to use pioneer neurons. Pioneer neurons arise early in embryogenesis and grow an axon to establish a pathway to the CNS whilst the distances to be navigated are still short and less complex. Thus, later developing axons can find the CNS by following the route established simply by the pioneer neurons (Bate, 1976; Keshishian and Bentley, 1983a).

It is conceivable that similar mechanisms may guide the growth of adult sensory axons during metamorphosis. In this situation larval sensory neurons could retain their links with the CNS through metamorphosis to provide a pathway for the adult sensory axons. Indeed some larval sensory neurons associated with the eye and leg imaginal disks have been shown to persist through metamorphosis and it has been argued that these axons could provide guidance cues for the adult sensory neurons (Tix et al., 1989a,b; Lakes-Harlan et al., 1991b). Despite the possibility, however, definitive proof that these neurons are necessary for correct axon guidance during metamorphosis has not been obtained.

In this work we have used a GAL4 enhancer-trap line driving expression of a UAS-linked lacZ construct (Brand and Perrimon, 1993; Yang et al., 1995; Smith and Shepherd, 1996) to analyse the development of a small, defined group of sensory neurons during metamorphosis in Drosophila. One important result of this study has been the discovery of a segmentally repeated array of persistent larval sensory neurons that are not associated with adult prestructures such as the imaginal disks.
Furthermore, these neurons retain their central axonal projections unchanged and adult sensory neurons appear to grow into their appropriate regions of neuropile by following these already established pathways. The results suggest two things: first, persistent sensory neurons are a general feature in the metamorphosis of the *Drosophila* nervous system; second, these neurons may channel growing axons into the correct region of neuropile and play a crucial role in establishing the ordering of the sensory axon arrays in the adult CNS (Murphey et al., 1989).

**MATERIALS AND METHODS**

**Collections and timing of pupae**

Stocks of the GAL4 enhancer-trap line C161 (Smith and Shepherd, 1996) were raised on standard medium at 25°C. White prepupae were collected from bottles and placed on moist filter paper in Petri dishes and cultured at 25°C. All times are given with this point as time zero, 0 hours after puparium formation (APF).

**Fly stocks**

Details of the GAL4 enhancer-trap screen have been published elsewhere (Smith and Shepherd, 1996).

**X-gal staining and immunochemistry**

All histological procedures were performed as previously described (Smith and Shepherd, 1996). The results of this study are based on a sample of 211 successfully stained preparations.

**Photomicroscopy**

Stained preparations were examined on a Zeiss Axioskop FS microscope and photographed with Kodak Tech Pan and Ektachrome 160 films. Images were digitised by scanning onto PhotoCD (Kodak). Photomontages were assembled using Adobe Photoshop 3.0 on a Macintosh 2ci computer and optimised for contrast and brightness only.

**RESULTS**

Expression of the enhancer detector in line C161 is restricted to a small, identifiable set of sensory neurons in larvae and adults. All expressing neurons are of the same sensory modality (proprioception) and since reporter gene expression can be used to visualise axonal projections we have used the line to describe the central organisation of proprioceptive sensory axons in adults and larva (Fig. 1).

**Expression in the adult**

Details of the central projections in the adult have already been described (Smith and Shepherd, 1996). Briefly, reporter gene expression is seen in neurons associated with the femoral chordotonal organs (FCO); hair plates; a subset of campaniform sensilla on leg, wing and haltere; and wing hinge stretch receptor. Axons from these neurons form three major axonal projections in each thoracic hemineuromere (Fig. 1B). Axons from the external sensilla on the leg form a crescent-shaped projection which completely outlines the neuropile in each neuromere (les, Fig. 1B). Axons from the FCO project medially to terminate close to the midline (fco, Fig. 1B). Axons from the prothoracic stretch receptor (PSR), wing and haltere form complex projections restricted to dorsal neuropile (Fig. 1B).

**Expression in the larva**

In third instar larvae, *lacZ* expression is seen in a segmentally repeated array of sensory neurons (Fig. 2A); the pattern is bilaterally symmetrical and almost identical in all unfused trunk segments. All the neurons are internal sensory neurons and can be readily identified from the map of embryonic sensory neurons detailed by Merritt and Whitington (1995). Ventralmost is a cluster of 5 neurons (vc, Fig. 2A), four of which have branched and varicose dendrites running along the surface of the muscles and epidermis (arrowheads, Fig. 2B). These are
dendritic arborisation neurons (da) and can be identified as vdaA, vdaB, vdaC and vdaD (nomenclature after Merritt and Whitington, 1995). The fifth neuron is a bipolar dendrite neuron (bd) and can be identified as vbd (not shown). Posterior to the VC group is a single da neuron, this is the vpda neuron (vpda; Fig. 2A,B). In the anterior/lateral region of each segment there is a single large neuron (lbd; Fig. 2A) this is the lateral bipolar dendrite neuron (lbd) and is associated with muscle 8 (Fig. 2C) (see Crossley, 1978, for muscle numbering). Adjacent to lbd, is a small cell that is not visible in Fig. 2A which envelopes a trachea (t); this is the ltd neuron (Fig. 2D). There are two other groups of da neurons (da1,da2; Fig. 2A), constituting all other known da neurons. Expression is also seen in one scolopale in the lateral chordotonal organ (lch, Fig. 2E). Finally, there is a dorsal cluster of neurons (dc; Fig. 2A) which contains all five dorsal da neurons (ddaA-E; Fig. 2F), and the dorsal bipolar dendrite neuron (dbd) associated with muscle 3 (dbd; Fig. 2F). An identical array of expressing neurons was seen in first and second instar larvae. Expression was not detected in embryonic stages.

**The central projections of expressing neurons**

In the thoracic and abdominal neurones, the central projections of the expressing sensory neurons are relatively simple and easily described. A segmentally repeated array of axons is clearly visible (Fig. 1A) that is identical in almost all neurones (Fig. 1A). The only difference between neurones is that the thoracic neurones are slightly larger. For this reason the details of the projections are most easily described in these neurones. Thus, in each thoracic hemineuromere it is possible to recognise three distinct axon projections which enter the ganglion via the single fused nerve root (Fig. 1A). Within this nerve the axons form two distinct axon bundles, one anterior which corresponds with the intersegmental nerve (ISN) and the other posterior which corresponds with the segmental nerve (SN) (isn, sn; Fig. 1A).

Axons in the SN bundle project medi ally and ventrally to terminate close to the midline (Fig. 1A). Within this bundle it is possible to resolve at least two axons. The axons in the ISN form two distinct medi ally directed projections, one running ventrally (v-ant, Fig. 1A) and the other dorsally (d-ant, Fig. 1A). In the v-ant projection it is possible to resolve a number of axons (>7). The d-ant projection appears to contain a single, large diameter axon.

The same three projections are evident in each abdominal hemineuromere (1 to 7) (post, v-ant and d-ant; Fig. 1A). It is not possible to make accurate estimates of axon number but the d-ant projection is clearly a single axon and the post and v-ant projections contain several axons with v-ant containing more axons than post.

**Metamorphosis of the sensory axon projection**

The onset of expression in C161 occurs early in sensory neuron differentiation and persists at high levels until the apparent death of the neuron. Thus expression can be used to analyse the transformation of the larval sensory projections into the adult form.

**0-20 hours APF**

At puparium formation (0 hours APF) the central projections are indistinguishable from those of the third instar larvae (not shown) and during the first 20 hours APF undergo few detectable changes. Thus, at 20 hours APF it is possible to recognise the typical larval projections in all thoracic and abdominal neurones (v-ant, d-ant and post; Figs 3, 4A).

In thoracic neurones the v-ant pathway can be identified running around the anterior edge of the neuropile and is still composed of a number of axons (Figs 3A, 4A). The post projection is still present (Figs 3A, 4A) and d-ant is still made up of a single axon and appears unchanged (Fig. 3A).

In the abdominal neurones the pattern of axonal projections (Figs 3A, 4B) is identical to that seen in the larval CNS (Fig. 1A) and appears unchanged.
During this period there is a clear decrease in the complexity of the central axon projections (Figs 3B, 4C,D). Comparison of this 24 hours APF preparation with a 20 hours APF CNS shows that the central arborisations are markedly decreased but three remain, corresponding to the three larval projections; d-ant, v-ant and the medial (m-post) and posterior (p-post) branches of post. (D) 40 hours APF, the central axons remain unchanged but expansion of the thoracic neuromeres is more pronounced. At this stage the separation of the segmental (sn) and intersegmental (Isn) nerves in the thoracic neuromeres also becomes apparent. It is also evident that the v-ant and post projections remain within the segmental nerve and that the d-ant projection separates off with the intersegmental nerve. (E) 48 hours APF, adult sensory axons begin to arrive in the CNS. The earliest adult axons appear in the anterior abdominal neuromeres (large arrows) and originate from a large scolopidial structure in the anterior abdomen. Axons from thoracic sensory neurons follow the pathways established by the larval axons. Leg sensory neurons enter via the leg nerves (ln1-3) and grow along the pathways defined by the v-ant, m-post, p-post axons. Axons from the femoral chordotonal organ (fco) form a projection that does not correspond with a visible larval pathway. Axons from dorsal sensory neurons enter via the dorsal nerve roots and follow the pathway defined by the d-ant axon. (F) 72 hours APF, the adult pattern of projections is established and complete. The axons from the abdominal scolopidial structure have grown into the prothoracic neuromere (large arrows) and the relationship of the major adult projections with the larval axons is evident. psn, prosternal nerve; admn, anterior dorsal median nerve; hn, haltere nerve. All drawings are to the same scale. Scale bar, 75 μm.

**Fig. 3.** Camera lucida drawings of the central projection of proprioceptive sensory neurons showing the progressive changes in sensory projections during metamorphosis. All times are hours After Puparium Formation (APF). (A) 20 hours APF the larval pattern is still evident, with the three projections per hemineuromere evident, (v-ant, d-ant and post). (B) 24 hours APF, there is evidence of a reduction in the extent of projections but the three main projections per hemisegment are still evident. (C) 36 hours APF, most larval axons have disappeared but three remain, corresponding to the three larval projections; d-ant, v-ant and the medial (m-post) and posterior (p-post) branches of post. (D) 40 hours APF, the central axons remain unchanged but expansion of the thoracic neuromeres is more pronounced. At this stage the separation of the segmental (sn) and intersegmental (Isn) nerves in the thoracic neuromeres also becomes apparent. It is also evident that the v-ant and post projections remain within the segmental nerve and that the d-ant projection separates off with the intersegmental nerve. (E) 48 hours APF, adult sensory axons begin to arrive in the CNS. The earliest adult axons appear in the anterior abdominal neuromeres (large arrows) and originate from a large scolopidial structure in the anterior abdomen. Axons from thoracic sensory neurons follow the pathways established by the larval axons. Leg sensory neurons enter via the leg nerves (ln1-3) and grow along the pathways defined by the v-ant, m-post, p-post axons. Axons from the femoral chordotonal organ (fco) form a projection that does not correspond with a visible larval pathway. Axons from dorsal sensory neurons enter via the dorsal nerve roots and follow the pathway defined by the d-ant axon. (F) 72 hours APF, the adult pattern of projections is established and complete. The axons from the abdominal scolopidial structure have grown into the prothoracic neuromere (large arrows) and the relationship of the major adult projections with the larval axons is evident. psn, prosternal nerve; admn, anterior dorsal median nerve; hn, haltere nerve. All drawings are to the same scale. Scale bar, 75 μm.

**20-24 hours APF**

During this period there is a clear decrease in the complexity of the central axon projections (Figs 3B, 4C,D). Comparison of this 24 hours APF preparation with a 20 hours APF CNS shows that the central arborisations are markedly decreased (Figs 3A,B, 4A,C). This is most evident in the thoracic neuromeres but can be detected in the abdominal neuromeres. The loss of branching does not, at this stage, appear to be the consequence of a loss of axons entering the CNS. Examination of the peripheral nerves shows no detectable change in the number of axons entering the CNS (not shown).

Despite the loss of axonal arborisations there is no change in the pattern of the projections. All three larval projections (v-ant, d-ant and post) are still recognisable in all hemineuromeres (Figs 3B, 4C,D). The only other observed change is the appearance of the ganglion, which shows enlargement of the thoracic neuromeres (Fig. 3B).

**24-36 hours APF**

During this period there is a significant loss of larval axons. By 36 hours APF most axons have disappeared and it is possible to recognise only 3 axons in each hemineuromere. Each of these axons corresponds to one of the three major larval projections. For example, in thoracic neuromeres the v-
ant projection is evident as a single axon (Figs 3C, 4E) which follows the original trajectory of the v-ant pathway and curves round the anterior edge of the segmental neuropile, extending posteriorly to the posterior margin of the neuromere (Figs 3C, 4E). An axon with this exact projection can be distinguished within v-ant at earlier stages (<20 hours APF) when v-ant contained a number of axons (Figs 3A-C, 4C).

The post projection is also detectable as a single axon but, due to the expansion of the thoracic neuromeres it is evident that this axon has two branches, both run medially but via different routes; p-post projecting slightly more posterior than m-post (Figs 3C, 4E). This branching is evident at earlier stages but is not as pronounced (Fig. 3A,B). d-ant is also still visible (Figs 3C, 4E).

Similar changes are evident in the abdominal neuromeres. The basic three projections per hemineuromere are still recognisable. v-ant projection is composed of fewer axons than at earlier stages (Figs 3C, 4F). The post and d-ant paths are both evident as single axons (Figs 3C, 4F).

36-40 hours APF
This period is characterised by a change in the structure of the ganglion which begins to assume an adult appearance with enlarged thoracic and reduced abdominal neuromeres (Fig. 3D). At this stage it also becomes apparent that the peripheral nerve roots from the abdominal neuromeres are beginning to fuse to form the single abdominal nerve root typical of the adult (Fig. 3D-F).

There are no significant changes in the central projections of the sensory neurons. The three larval axons are evident in each hemineuromere (v-ant, d-ant and post; Fig. 3D). In the thoracic neuromeres these axons can be identified despite their relative positions having changed due to expansion the neuromeres. The abdominal projections remain unchanged.

Changes in the structure of the ganglion
40-48 hours APF
Prior to this stage the persistent axons in each hemineuromere entered the ganglion as part of a single fused peripheral nerve root, which includes the SN and ISN (sn, isn; Fig. 3A-D). During the 40- to 48-hour period, however, there is a large expansion of the thoracic neuromeres and in the thorax the SN and ISN become separated into two distinct nerve roots. These two nerve roots become increasingly separated as expansion of the ganglion progresses. One nerve, the ISN, comes to lie dorsally and the other, the SN, ventrally (Fig. 3D,E). The SN is, therefore, the precursor of the ventral leg nerves (LN1-3) and the ISN forms the prosternal nerve (PSN, in prothorax), anteriordorsalmedian nerve (ADMN, in mesothorax) and haltere nerve (HN, in metathorax). The persistent sensory axons are also separated by this morphological change, v-ant and the m-post /p-post axons remain together in the SN but d-ant separates off with the ISN.

Growth of sensory axons
At 40-44 hours APF, the first adult axons become visible in the
anterior abdominal neuromeres (Fig. 3E). These axons enter the ganglion via the anterior abdominal nerve roots and form a knot of new axons around the junction of the first abdominal and metathoracic neuromeres (arrows, Fig. 3E). Over the next 12 hours these axons extend anteriorly into the thoracic neuromeres forming a bilaterally symmetrical pair of axon projections which terminate in the prothoracic neuromere (arrows, Fig. 3F). These axons originate from a large scolopidial structure in the anterior abdomen (Smith and Shepherd, 1996).

The first axons from thoracic sensory neurons appear in the CNS at approximately 44 hours APF. There is no detectable order to the arrival times, axons enter from all peripheral routes simultaneously. Axons from leg sensory neurons enter the ganglion via the leg nerves (In1-3, Fig. 3E). Axons from dorsally located sensory neurons enter the ganglion via the dorsal nerve roots. Thus the axons from the PSR enter via the PSN; wing sensory neurons via ADMN; and the haltere, via HN (Fig. 3E).

**Persistent axons prefigure growth routes**

The adult axons grow along specific pathways that coincide exactly with the pathways defined by the persistent larval axons. Thus, by 46 hours APF, whilst it is clear that there are many more axons projecting into the CNS, most of these axons project along pathways that correspond to persistent larval axons. Thus, most axons from leg sensory neurons grow along the pathways defined by v-ant, m-post and p-post (Figs 3E, 4G) and axons from dorsal sensory neurons follow the d-ant axon (Figs 3E, 4G). Differences in the detail of the projections are caused by changes in the overall structure of the ganglion.

Not all new sensory axons follow the preexisting pathways. For instance, axons from the FCO project medially to terminate at the midline (fco; Figs 3E, 4G) (Smith and Shepherd, 1996) along a pathway that does not correspond to any preexisting neuron we have seen. Similarly, whilst most axons from wing sensory neurons follow the d-ant projection (Fig. 3E), a small subset form novel pathways (Figs 3F, 4G).

The abdominal neuromeres have now become much reduced and resolution of individual axons is difficult. It is possible to still resolve, relatively unchanged, the three larval projections in each hemineuromere (Figs 3E, 4H). There does not appear to be any significant addition of new axons.

**Eclosion**

48 hours APF

Over the next 20 hours more axons join these pathways (Fig. 3F) until approximately 70 hours APF, when the pattern of projections appears complete and all of the major pathways described in the adult can be seen. The majority of these pathways can be directly related to the larval pattern of axons (Fig. 3F). Thus, for the axons arising from the leg sensory neurons v-ant, m-post and p-post form the basis of the crescent shaped projection that is typical of hairplate axons and some campaniform sensilla in the adult (Fig. 1B). For the dorsal sensory neurons, the situation differs in each thoracic neuromere: in the prothoracic neuromere, d-ant prefigures the route taken by axons of the PSR which enter the ganglion via the PSN (psn, Fig. 3E); in the mesothorax, d-ant prefigures the projections of sensory neurons from the wing and, in the metathoracic neuromere, d-ant prefigures the path taken by sensory neurons on the haltere (Fig. 3F).

**Fig. 5.** The fate of sensory neurons during metamorphosis revealed with X-gal staining. (A) At puparium formation (0 hours APF) a low power image of the body wall of a single trunk segment shows that all sensory neurons identified in the larva are still evident. (B) By 24 hours APF there is evidence of cellular breakdown. Amongst this cellular debris is X-gal stained material in positions that correspond with the locations of sensory neurons identified earlier (arrows). Some sensory neurons show no sign of degeneration, lbd can be still seen intact. (C) Low power view of two adjacent abdominal segments at 40 hours APF reveals a segmentally repeated array of three cells per hemisegment. These cells occur in fixed locations and can be identified as being part of the original larval array of sensory neurons. ml, ventral midline; vc, ventral cluster; lbd, lateral bipolar dendrite neuron; vnpa, vnpa neuron; dc, dorsal cluster. The ventral midline is to the left in all figures. Scale bar, 65 μm (A, C); 20 μm (B).

**Changes at the periphery**

Observations of the CNS imply that some larval sensory neurons persist through metamorphosis. To identify which neurons survive we examined the fate of the larval sensory neurons during metamorphosis.

0-24 hours APF

At puparium formation all the sensory neurons identified in the larva are evident and show no signs of modification or degeneration (Fig. 5A). This situation remains unchanged until approximately 18 hours APF, when degeneration of larval tissues is apparent. During the subsequent period, 18-22 hours APF, many larval sensory neurons disappear. The likely reason for their disappearance is degeneration. At 20 hours APF (Fig. 5B) there is evidence of the degeneration of larval tissues and amongst this material there is β-galactosidase-positive debris in locations that coincide with the positions at which larval sensory neurons were seen earlier in pupation (arrows, Fig. 5B). Since the only cells that stained positive for β-galactosidase-positive cellular debris.
2381 Persistent sensory neurons
dase prior to this stage were sensory neurons it is likely that this material derives from degenerating larval sensory neurons. This positively stained material remains evident until approximately 24 hours APF when the degeneration of larval tissues is largely complete.

Not all larval sensory neurons degenerate. Amongst the cell debris shown in Fig. 7B, the lbd neuron is still evident and shows no sign of degeneration (lbd, Fig. 5B). At 24 hours APF, after the degeneration is complete it is possible to identify three larval sensory neurons in each hemisegment (Fig. 5C). These three cells lie in characteristic positions and can be identified at all subsequent stages of metamorphosis. At no stage do these three neurons show signs of degeneration.

Two of these neurons can be unequivocally identified as larval neurons by their maintained association with identified larval muscles (Fig. 6A). These are dbd and lbd which are associated with larval muscles 3 and 8 respectively in larva and pupa (Fig. 6A). The third more ventrally located neuron (vn) cannot be definitively identified. These three neurons (vn, lbd and dbd) can be seen in the same positions in all trunk segments at 35 hours APF (B), 48 hours APF (C) and 55 hours APF (D). The ventral midline is to the left in all figures. Scale bar, 40 µm (A); 65 µm (B); 50 µm (C); 40 µm (D).

24-40 hours APF
At 35 hours APF all three neurons are still visible in each hemisegment and retain the same locations (Fig. 6B) and appearance. By careful dissection of the nerve roots and CNS it is possible to demonstrate that these cells are neurons and have axons that project to the CNS. Fig. 7 shows a pupal CNS at 28 hours APF and attached to each nerve root it is possible to identify the three surviving larval neurons (Fig. 7, inset). Axons can be seen to project from the cells into the CNS to form the central projections described.

40-50 hours APF
By 48 hours APF the adult musculature is apparent and the three persisting larval neurons can still be seen in each hemisegment (Fig. 6C). There are, however, other β-galactosidase staining sensory neurons now visible (not shown). These include a pair of neurons associated with an external sensillum on the sternites and the large scolopidial organ in the anterior of the abdomen (Smith and Shepherd, 1996). This is the first appearance of adult specific neurons. The association of lbd with the persisting larval muscle 8 is still evident (Fig. 6C). The dbd neuron, however, also now associates with one of the adult dorsal muscles (Fig. 6C).
50 hours APF – eclosion

By 55 hours APF the full complement of adult sensory neurons can be recognised. The larvally derived neurons are still apparent in their characteristic locations but remain undifferentiated and appear relatively simple (Fig. 6C).

By 60 hours APF the persistent neurons begin to change in appearance as they mature into their adult specific forms and it is possible to identify the adult fate of each neuron (not shown; see Smith and Shepherd, 1996 for details). Thus, in the adult abdomen the ddb retains a bipolar dendritic appearance and associates with a dorsal muscle fibre to become an internal stretch receptor. The lbd cell retains a bipolar structure and remains associated with larval muscle 8. vn becomes multidendritic with processes that extend over the lateral muscles and epidermis.

DISCUSSION

The results demonstrate that a segmentally repeated array of larval sensory neurons persists through metamorphosis to become apparently functional adult neurons. These neurons maintain their central axonal projections intact through metamorphosis and the pathways defined by their axons prefigure the pathways taken by adult sensory axons. The results suggest that persistence of larval sensory neurons is more widespread than previously thought and that their axons may guide adult sensory axon growth. This guidance role is more refined than previously supposed, suggesting that rather than simply guiding peripheral sensory axons to the CNS the persistent neurons may play a role in controlling the assembly of sensory axon arrays within the CNS. Thus the order of the adult sensory systems may be an elaboration of a basic pattern established during embryonic development.

Do larval sensory neurons persist?

Absolute proof that the sensory neurons identified in this study persist through metamorphosis is difficult to achieve. Evidence is based on the ability to identify these neurons at all stages of metamorphosis, which shows that: (1) There is a segmentally repeated array of sensory neurons that do not degenerate along with most larval tissues; (2) Sensory neurons with identical morphology can be seen at the locations occupied by these cells at every stage of metamorphosis; (3) Two of the sensory neurons can be positively identified by their association with identified persistent larval muscles (Currie and Bate, 1991). Further evidence for persistence is seen in their central axonal projections which are visible at all stages of metamorphosis and remain almost unchanged throughout.

The unbroken chain of observation is strong support for the conclusion that these neurons persist into the adult. There is, however, one caveat. It is impossible to eliminate the possibility that these neurons are replaced by similarly located adult neurons in such a way as to be undetectable. Although theoretically possible, this would seem unlikely.

In a detailed study of the development of wing sensory neurons in Drosophila, Whitlock and Palka (1995) identified a number of axons which they called early transient axons because they were visible only between 7 and 12 hours APF. These neurons look similar to the neurons described here and it is likely that they are the same neurons. Whitlock and Palka (1995) were, however, unable to identify these neurons at later stages and concluded that they degenerated. Our data suggest that these neurons do survive and that their transience in this earlier study may be a consequence of the dye staining technique. It now seems likely that the sensory neurons that extend these axons are not in the wing disk but closely associated and are revealed by dye staining at early stages. At later stages, however, these neurons become separated from the wing disk and are not labelled.

Other examples of persistent larval neurons

This is not the first demonstration of persistent larval sensory neurons in Drosophila or any other holometabolous insect. The best examples are those associated with the leg (Tix et al., 1989a; Lakes-Harlan et al., 1991b) and eye imaginal disk (Tix et al., 1989b) of diptera and the sensory neurons involved in the ‘gin trap’ reflex in the moth, Manduca (Bate, 1973; Levine et al., 1985); although whether the ‘gin trap’ sensory neurons persist into the adult is unclear. In these examples the persistent neurons are associated with either complex adult structures such as eyes and legs or in the case of Manduca a specialised pupal behaviour. The neurons we have described are not associated with adult structures or specialised behaviours but are widely distributed, even in the unspecialised abdominal segments. This suggests that persistent sensory neurons, like persistent central neurons (Truman, 1992), are not special cases but a general feature with a fundamental role.

What is the function of the persistent neurons?

From a fundamental point of view there could be two explanations for persistence of sensory neurons. First, these neurons perform an adult function and persist because they are required. There is no a priori reason to expect all larval sensory neurons to be eliminated during metamorphosis. Sensory neurons may die simply because they are no longer required. For instance, since most external sensilla are destroyed during metamorphosis it is possible that their associated sensory neurons die because they are of no further use. One feature of the neurons we have described is that they are all internal receptors and not associated with an external sensillum. Thus, these neurons need not die and can be retained to function in the adult. It is, therefore, not inconceivable that persistence could be regarded as the preferred fate and that death is restricted to neurons that have no adult function. One caveat is that the persistent neurons associated with the leg disk in Phormia are associated with external sensilla in the larva (Lakes-Harlan et al., 1991a) and, therefore, association with external sensilla need not necessitate the death of the neuron.

A second possibility is that persistent neurons provide guidance cues for adult sensory neuron growth. During metamorphosis, newly generated sensory neurons arise far from the CNS and this distance can present an enormous challenge for growing axons. It has been argued that persistent larval neurons could provide a ready made route to the CNS for these neurons. This role was originally ascribed to pioneer neurons in locust embryos (Bate, 1976; Keshishian and Bentley, 1983a,b) and latterly applied to persistent neurons in holometabolous insects (Tix et al., 1989a,b; Lakes-Harlan et al., 1991b). For example, Tix et al. (1989b) suggest that persistent neurons are likely to provide a useful guide for directing newly generated neurons from the imaginal discs to the CNS.
Steller et al. (1987) showed that flies homozygous for mutant alleles of the disco gene show defects in the projection of the larval Bolwig’s nerve, which prefigures the projections of the adult retinular neurons, and results in failure of the retinular neurons to innervate the optic lobe implying that disruption of the adult axons is a direct consequence of the failure of Bolwig’s nerve to form correctly (Steller et al., 1987). A study using the beetle Tenebrio shows that deletion of larval sensory neurons prior to the development of adult sensory neurons disrupts the growth of adult sensory neurons in a manner consistent with a guidance role (Breidbach, 1990). Clearly there is evidence to support the idea that the persistent neurons may play a role in guiding growing axons to the CNS, although this role has not been demonstrated conclusively.

The data presented suggest that the proposed guidance role of persistent neurons could be extended to include a more refined role in controlling the orderly assembly of sensory axon projections. It is widely observed that the central axonal projections of insect sensory neurons are ordered such that axons segregate in CNS according to modality and peripheral position (Murphey et al., 1989; Merritt and Murphey, 1992; Merritt and Whitington, 1995; Shepherd and Smith, 1996). Whilst the functional significance of this order is understood the developmental mechanisms that control the precise assembly of these axon arrays remain unclear.

We have shown that each of the major adult sensory projections seen in this study is prefigured by a persistent larval sensory axon. These larval axons therefore provide an axonal scaffold that enables incoming adult axons to innervate the correct region of neuropile. The development of the order in the adult sensory projection can thereby be achieved by sensory axons choosing the appropriate larval axon to follow. Thus, the axons that form the two post projections (p-post and m-post) and the v-ant projection prefigure the projections of the external proprioceptors of the leg. This establishes the crescent shaped projection in the neuropile of sensory neurons associated with the hair plates (Merritt and Murphey, 1992; Smith and Shepherd, 1996). The dorsal projection prefigures the pathway taken by axons from dorsally located sensory neurons. In the prothorax this pathway is taken by the PSR axons (Smith and Shepherd, 1996), in the mesothorax by axons associated with the wing and in the metathorax by axons from the haltere. This scenario is comparable with the scaffold of central axon pathways, established during embryogenesis, that guide the growth of the later developing central neurons (Raper et al., 1983a-c). Here, however, the scaffold provides cues to ensure that sensory axons find the appropriate neuropile and assemble an orderly projection. Continuing the parallel with the CNS framework it may also be that selection of the correct pathway may be mediated by selective fasciculation (Raper et al., 1983a-c).

In addition to providing a developmental mechanism for assembling complex sensory arrays the persistent sensory neurons also reveal the fundamental design principles of the adult sensory system. As a basic scaffolding for the adult system these neurons show the adult sensory system in its most minimal form. Thus, from the distribution and central projections of these neurons we can identify the essential elements of the adult projections and define its rules of organisation. In this respect the neurons revealed by this study show several interesting features. Not only are these neurons segmentally repeated, but in each hemisegment their cell bodies are found in key locations. Each persistent neuron is located in one of the major sectors of the body wall, i.e. ventral, lateral and dorsal. Since the axons from these neurons also define specific central pathways it is possible that the positions of the neurons may not be coincidence but reflect functional constraints. Each neuron may, therefore, represent the minimal manifestation of the region of neuropile to be occupied by the axons of adult sensory neurons that originate from the same body sector. So the dbd neuron, for example, defines the region of neuropile that dorsal proprioceptors will innervate. In this way the somatotopic order is reflected by the location of these three neurons and their central projection. We can, therefore, see that the adult projections can be resolved as an elaboration of this three neuron projection with neurons from each sector of the body wall following their respective persistent neuron. If this analysis can be applied to all sensory neurons it would be possible to define the details of the entire adult sensory array in the same simple terms and allow examination of the mechanisms that generate the ordered adult sensory arrays.

This work has, however, only identified three persistent neurons per hemisegment and we cannot explain the central projections of all adult sensory neurons on this basis. Indeed there are adult pathways seen in this study which have no visible precursor axon e.g. axons from the FCO. There are, however, other larval neurons that could prefigure these pathways but are not revealed by this line, e.g. the persistent neurons of the leg disk (Tix et al., 1989a, Lakes-Harlen et al., 1991a). It is possible that there are other persistent sensory neurons to be uncovered whose axons could reveal the complete groundplan of the adult sensory system.

This interpretation implies that the order of the adult sensory system is established during embryonic development when the pioneer neurons that prefigure the adult pattern undergo axonogenesis. From this point the adult pattern of projections is established in miniature. Thus, if we are to understand how the adult sensory system of Drosophila is assembled we need to examine its embryonic origins.

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REFERENCES
Crossley, A. C. (1978). The morphology and development of the Drosophila...


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