Projections of *Drosophila* multidendritic neurons in the central nervous system: links with peripheral dendrite morphology

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Neurons establish diverse dendritic morphologies during development, and a major challenge is to understand how these distinct developmental programs might relate to, and influence, neuronal function. *Drosophila* dendritic arborization (da) sensory neurons display class-specific dendritic morphology with extensive coverage of the body wall. To begin to build a basis for linking dendrite structure and function in this genetic system, we analyzed da neuron axon projections in embryonic and larval stages. We found that multiple parameters of axon morphology, including dorsoventral position, midline crossing and collateral branching, correlate with dendritic morphological class. We have identified a class-specific medial-lateral layering of axons in the central nervous system formed during embryonic development, which could allow different classes of da neurons to develop differential connectivity to second-order neurons. We have examined the effect of Robo family members on class-specific axon lamination, and have also taken a forward genetic approach to identify new genes involved in axon and dendrite development. For the latter, we screened the third chromosome at high resolution in vivo for mutations that affect class IV da neuron morphology. Several known loci, as well as putative novel mutations, were identified that contribute to sensory dendrite and/or axon patterning. This collection of mutants, together with anatomical data on dendrites and axons, should begin to permit studies of dendrite diversity in a combined developmental and functional context, and also provide a foundation for understanding shared and distinct mechanisms that control axon and dendrite morphology.

**KEY WORDS:** *Drosophila*, Sensory map, Dendritic arborization neurons, Genetic screen, Axon targeting, Dendrite morphogenesis

INTRODUCTION

The functional distinctions between dendrites and axons impose different constraints on their morphology. Nevertheless, proper nervous system wiring would seem to require that their morphogenesis is coordinated so that the shape and position of dendritic input is appropriate for that of the axonal output, and vice versa. An example of the close link between dendritic and axonal morphology and/or function can be seen in the organization of certain sensory maps and connectivities in the brain. For example, in the *Drosophila* olfactory system, the glomerulus innervated by projection neuron (PN) dendrites is reliably predicted based on axon projection patterns in higher brain centers (Marin et al., 2002; Wong et al., 2002). POU-domain transcription factors act to specify both class-specific axon and dendrite morphotype among groups of PNs, suggesting that dendrite and axon morphogenesis can be regulated together by some of the same molecules (Komiya et al., 2003). Coordination between dendritic and axonal morphology and development within cells is likely to be a general feature of nervous system wiring. A major challenge is therefore to understand how, in the context of their distinct morphologies and cytoskeletons, dendrite and axon development can be linked with cell identity to ensure proper wiring of the nervous system.

The *Drosophila* dendritic arborization (da) sensory neurons provide a suitable system in which to study neuronal morphogenesis. These cells are positioned just below the transparent body wall in the peripheral nervous system (PNS) and branch in two dimensions (Bodmer and Jan, 1987), making it possible to study the growth and branching of whole dendritic arbors in vivo (Gao et al., 1999). The patterns of dendritic branching of da neurons have been characterized (Grueber et al., 2002; Sweeney et al., 2002) and based on branching morphology were grouped into four distinct classes (Grueber et al., 2002). The genetic programs that regulate the development and morphological diversification of these arbors are beginning to be elucidated (Brenman et al., 2001; Gao et al., 2000a; Grueber et al., 2003a; Li et al., 2004; Moore et al., 2002; Sugimura et al., 2004; Ye et al., 2004). As yet, however, there is no evidence of the functional relevance of these diverse dendritic morphologies. Establishing this connection would help to develop a system for studies of morphological development in the context of dendrite and circuit function, and perhaps allow investigations of the functional consequences of alterations in morphology produced in various mutant backgrounds.

If morphological and functional diversity are correlated, da neurons with different dendritic morphologies might project axons to different regions of the central nervous system (CNS) and thus present sensory information with spatial divergence. Elucidating the sensory axon pattern might begin to reveal the relationship between dendrite morphology and function, and also allow studies of coordinated and distinct molecular control of axonogenesis and dendritogenesis using this system. A subset of da neuron axons have

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been labeled in embryos and larvae and shown to project primarily to ventral regions of the neuropil, with a small subset of axons projecting more dorsally (Merritt and Whittington, 1995; Schrader and Merritt, 2000). Here we show that the axons of da neurons in different dendritic classes have distinct morphologies and organize into different layers in the CNS. To begin to examine the molecular mechanisms responsible for the specific patterns of da neuron axons and dendrites, we tested candidate genes and generated 3299 ethylmethanesulfonate (EMS)-induced lethal lines to screen axon and dendrite morphology simultaneously with high resolution. Our anatomical data, along with this collection of mutants, provide entry points for identifying novel genes and pathways that function during neuronal morphogenesis.

MATERIALS AND METHODS

Fly stocks

MARCM analysis: (1) y w; FRT19A, (2) hsFLP, tubP-Gal80, FRT19A; Gal4109(2)80, UAS-mCD8::GFP, FLP-out analysis of axonal projections: (3) ppk-Gal4 (this study), (4) Gal4109(2)80 (Gao et al., 1999), (5) hsFLP; Sp/CyO; UAS>CD2+nCD8::GFP (Wang et al., 2004), (6) hsFLP; ppk-Gal4; UAS>CD2+nCD8::GFP, (7) hsFLP; Gal4109(2)80, UAS>CD2+nCD8::GFP, (8) Gal4109(2)80, ppk-GFP, Axon projections in embryos: (9) UAS-Kaede; ppk-eGFP, Ecotropic expression of Robo2 and Robo3; (10) UAS-robo2 (Rajagopalan et al., 2000), (11) UAS-robo3 (Rajagopalan et al., 2000), (12) yw/CyO; UAS-robo2 or robo3/CyO; ppk-GFP th st FRT2A/TM6 Ubx y+, (13) yw, hsFLP; Gal4109(2)80, UAS-mCD8::GFP, tubP-Gal80, FRT2A.

Generation of clones

MARCM (Lee and Luo, 1999) clones were produced as previously described (Grueneber et al., 2002) by mating stocks 1 and 2 or 13 and 12 above. FLP-out clones were produced by providing a 30 minute heat shock (38°C) to late embryonic and early larval progeny from matings of stocks 3 and 12 above.

Transgenic constructs

pickpocket (ppk)-Gal4 was constructed by replacing the eGFP portion of ppk-eGFP (Grueber et al., 2003b) with a Gal4 fragment. The Gal4 fragment was initially digested from pT7GAL (Sharma et al., 2002) as a HindIII fragment and was subsequently cloned into the HindIII site of pBlueScript (Stratagene). The final ppk-Gal4 construct was produced by performing a three-way ligation using: (1) the ppk promoter released from ppk-eGFP as an EcoRI-KpnI fragment; (2) Gal4 released from pBlueScript-Gal4 as a KpnI-XbaI fragment; and (3) pCasper vector released from pCasper hs-43 lacZ digested with EcoRI and XbaI.

To generate pUAST-Kaede, Kaede cDNA (Ando et al., 2002) was excised from pCS2+ Kaede (courtesy of Dr A. Miyawaki) at the BamHI and EcoRI sites and then ligated into pCDNA3.1 (Invitrogen, Carlsbad, California). It was subsequently excised from pCDNA3.1 and ligated into pUAST at KpnI and XbaI sites.

Immunohistochemistry and image analysis

Immunostaining and mounting of larvae harboring MARCM clones was performed as previously described (Grueneber et al., 2002; Lee and Luo, 1999). Animals with FLP-out clones were processed similarly, and immunolabeled with rabbit-anti-GFP (1:2000; kindly provided by Dr Yang Hong, UCSF), mouse anti-CD2 (1:200; Serotec, Raleigh, NC), and rat anti-mCD8 (1:100; Caltag/Invitrogen, Carlsbad, CA), and in some cases mouse anti-Fas II (Developmental Studies Hybridoma Bank, University of Iowa). Secondary antibodies used were Cy2-conjugated donkey anti-rabbit, Rhodamine Red X-conjugated donkey anti-mouse, and Cy5-conjugated donkey anti-rat all at 1:200 dilution (Jackson ImmunoResearch, West Grove, PA). Immunostained larvae were imaged on a TCS SP2 confocal microscope (Leica Microsystems, Bannockburn, IL), or a Zeiss 510 Meta (Carl Zeiss Inc., Thornwood, NY). Live imaging of mutant embryos was performed on an MRC-600 confocal microscope (Bio-Rad, Hercules, CA). Ortho views were created using LSM510 software (Carl Zeiss Inc.).

Mutagenesis

Males were fed 0.025 mol/l EMS in 1% sucrose solution according to a modified method from Lewis and Bacher (Lewis and Bacher, 1968). Mutations were mapped by standard recombination mapping using the multiply marked ru h th st ca sr e ca line, followed by deficiency mapping and complementation analysis with known mutant alleles.

RESULTS

Morphology of da neuron axons correlates with dendritic class

Drosophila dendritic arborization (da) neurons have been segregated into four classes (classes I-IV) that reflect arbor complexity, arbor size and the length of terminal branches (Grueber et al., 2002). The cell bodies are distributed in ventral, ventral’, lateral and dorsal clusters between the epidermis and muscles, spreading dendrites across the body wall, and axons to the ventral nerve cord (Fig. 1A). We reasoned that if morphological classes correspond to at least partially distinct sensory systems, then their axons may have divergent morphologies and target non-overlapping regions of the ventral nerve cord, where information will be relayed to second-order neurons. Previous studies have found that most da neurons arborize together in a common fascicle in the ventral CNS, with a subset, including at least some class I da neurons, projecting to more dorsal neuropil (Merritt and Whittington, 1995; Schrader and Merritt, 2000). In light of studies showing distinct morphological types of da neurons, we used mosaic analysis with a repressible cell marker (MARCM) (Lee and Luo, 1999) to examine the morphology of da neuron dendrites and axons in third instar larvae. As a MARCM driver we used Gal4109(2)80 (Gao et al., 1999), which labels all multidendritic (md) sensory neurons, including those belonging to the da subgroup (Fig. 1B). Owing to the sparse labeling of central neurons, the 109(2)80 driver combined with MARCM allowed us to resolve axon morphology of individual neurons (Fig. 1B).

We collected data from wild-type da neuron clones in segments A2-A7 to identify their axon projections in the CNS (n=128). Different da classes showed distinctive types of central projections. Class I neurons were unique in their projection to the dorsal neuropil (see below; n=19). Class II axons showed collateral branches (branches exiting from the main axonal trunk, although the timing of their emergence has not been determined) that were not observed in class III and IV neurons (Fig. 1C; n=27). The class I neuron vpda also showed such a branch (Fig. 1C). Class IV neurons projected axon branches across the midline, but these were only rarely observed for the other classes (Fig. 1C; n=39; we observed one case in which the ‘v’ pda class III neuron crossed the midline). Dorsal and ventral’ class IV axons crossed the midline, but axons from the ventral neuron did not. Each class IV neuron also showed a large accumulation of branches medial to the commissural/longitudinal branch bifurcation (Fig. 1C). The class III terminals extended in an anteroposterior (AP) orientation and were relatively unbranched, showing neither the collateral branches observed in class II neurons, nor contralateral projections observed among class IV neurons (Fig. 1C; n=43).

The axons of class I and class IV neurons also showed evidence of somatotopic arrangements in the CNS. The trajectory of class I neurons in the CNS mirrored the polarity of their dendrites in the periphery. Dorsal class I neurons have distinct polarity with respect to the AP body axis: dendrites of ddaD extend anteriorly and ddaE extend posteriorly (Grueber et al., 2003b) with a dIII site of pBlueScript (Stratagene). The final ppk-Gal4 construct was produced by performing a three-way ligation using: (1) the ppk promoter released from ppk-eGFP as an EcoRI-KpnI fragment; (2) Gal4 released from pBlueScript-Gal4 as a KpnI-XbaI fragment; and (3) pCasper vector released from pCasper hs-43 lacZ digested with EcoRI and XbaI.

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and ventral regions of the body wall, but not the lateral region, extended axons across the midline (Fig. 1C), fitting with principles of somatotopy established for body wall bristle neurons (Levine et al., 1985; Merritt and Whitington, 1995). These data together demonstrate that da neuron classes have distinguishing axon terminals, and that neurons in the same class show evidence of somatotopic organization.

Dorsoventral distinctions between axon terminals

The position of sensory axons defines the population of possible second-order targets and thus contributes strongly to sensory information processing in the CNS. Axons of tactile receptors typically project to ventral areas of the neuropil, whereas strain-sensing or proprioceptive neurons usually project to more dorsal regions (Murphey et al., 1989; Pfluger et al., 1988). Fasciclin II-labeled axon tracts provide a frame of reference for assessing dorsoventral (DV) position in the CNS (Landgraf et al., 2003) (Fig. 2A). We studied the DV positions of axons in 42 ventral nerve cords (VNCs) using MARCM, and 18 VNCs using the FLP-out system (Basler and Struhl, 1994; Wang et al., 2004; Wong et al., 2002). Both techniques revealed that each class I neuron extended axons to dorsal regions of the neuropil, terminating just lateral to the dorsomedial (DM) fascicle (Fig. 2B). The position of class I axons was therefore indistinguishable at this level of resolution from the position of the dbd terminal arbor (see Fig. 2D,E) (Zlatic et al., 2003), implying that information from class I neurons and the putative stretch-sensing dbd neuron might be processed similarly in the CNS. Class II, III and IV axons targeted the ventral CNS without obvious class-specific dorsoventral lamination of terminal position (Fig. 2C-E). The positions of the class II collateral branches were somewhat variable, either terminating on the ventrolateral (VL) fascicle (Fig. 2C), or slightly lateral to VL (vdaA often had a more lateral termination; data not shown). These data together provided anatomical support for distinct functions among different da neurons, fitting with their distinct dendritic arbor morphologies. Class II, III and IV axons project similarly to known tactile afferents, while class I neurons have projections like known proprioceptive or strain-sensing neurons.

Mediolateral lamination of axon terminals

We next examined whether the terminal positions of the ventral-projecting class II, III and IV neurons could be further distinguished by their position. Short pickpocket (ppk) enhancer sequences can drive gene expression strongly in all class IV neurons and weakly in class III neurons (Grueber et al., 2003b). Viewing all class IV neurons together revealed that they crossed the midline in a single fascicle, that the stereotyped branching at the commissural-longitudinal junction overlapped for all neurons, and that longitudinal projections were not always tightly fasciculated (Fig. 3A, Fig. 5B). In ppk-eGFP and ppk-Gal4, UAS-CD8::GFP animals, we observed a strongly labeled set of medial axons and a weakly labeled, slightly more lateral, layer of terminals (Fig. 3A). We suspected that the weakly labeled axons were class III axons, which may form a layer next to class IV axons. To test this idea, we introduced ppk-Gal4 into the FLP-out mosaic system. We examined the relative locations of all class III axons except ddaF (whose axon was labeled too weakly) and found that their major longitudinal projections terminated immediately lateral to the scaffold of class IV axons (Fig. 3B).

The ppk reporter lines alone do not label the class II axons, and thus did not allow us to determine whether all da classes form a laminar organization or only the class III and IV neurons. However, examination of FLP-out clones produced with Gal4109(2)80, UAS-mCD8::GFP+, with or without ppk-eGFP to label class IV neurons, permitted labeling of
different axon groups. We found that class II neurons with a significant longitudinal projection formed a third layer of sensory axons that was lateral to both class III (Fig. 3C) and class IV axons (Fig. 3D), with class II collateral branches terminating in a distinct, even more lateral, position (Fig. 2C).

We confirmed the FLP-out data by mapping the relationships of individual pairs of sensory afferents using the MARCM technique. Within hemisegments, or in adjacent hemisegments, having two or more da neuron clones ($n=15$) axons were organized (medial>lateral) class IV>class III>class II (Fig. 3E). Laminar patterning was independent of peripheral cell body position (Fig. 3E). These data together indicate the presence of a laminar arrangement of somatosensory axons in the Drosophila CNS. These data also suggest that somatosensory information carried by different classes of da neurons might be distinguished by sensory axon connectivity to second-order targets.

Embryonic formation of the laminar pattern

The above FLP-out and MARCM data were collected from third instar larval stages, so we next examined when during development we could observe layering of the different classes of axons. To achieve live two-color discrimination of different neuronal classes in embryonic and early larval stages we generated transgenic flies expressing a photoconvertible fluorescent protein, Kaede (Ando et al., 2002), and placed expression under the control of $Gal4^{109(2)80}$ in the presence of $ppk-eGFP$ (Fig. 4A,A'). We converted the Kaede protein from green to red fluorescence using a 10-30 second UV pulse and examined the position of all da axons relative to $ppk-eGFP$-labeled class IV axons. As early as the sensory axon scaffold could be visualized (stage 17), class IV axons occupied a medialmost layer with respect to other classes (Fig. 4B,C). These data indicate that a laminar pattern develops at least by late embryonic stages and is maintained without qualitative change in larvae.

Alteration in md neuron axon morphology by Robo family members

Our anatomical data revealed several features of da neuron axon projections that correlated with previously described dendritic classes, including mediolateral position, midline crossing, presence or absence of collateral branches, and relative position in the DV axis. Because of the role of Robo receptors in specifying
longitudinal position in the VNC (Simpson et al., 2000; Rajagopalan et al., 2000; Zlatic et al., 2003), we examined the possible functions of these genes in sensory axons. Loss of either Robo, Robo2 or Robo3 caused severe (Robo) or mild (Robo2 and 3) alteration in sensory axon morphology (Fig. 5A). Due to the widespread regulation of VNC patterning by Robo family members (Rajagopalan et al., 2000; Simpson et al., 2000) these defects could arise by autonomous or non-autonomous regulation of sensory arbor morphology. We found that misexpression of Robo3, but not Robo2, under the control of pppk-eGFP in the background to label class IV axons. Arrows indicate the class II axon. The pppk-eGFP scaffold is marked (IV). Notice that the class II axon terminates lateral to the class IV scaffold (arrowhead) and all other da axons (which are labeled by CD2). (E) Relative terminal positions of class II, III and IV neurons. The cell body position is indicated along with the class of the neuron. Genotypes: (B) hsFLP+; pppk-Gal4/ppk-Gal4; UAS>CD2>mCD8::GFP+; (C) hsFLP+; Gal4109(2)80; UAS>CD2>mCD8::GFP+. (D) hsFLP+; Gal4109(2)80; UAS>CD2>mCD8::GFPlppk-eGFP; (E) tubP-Gal80, hsFLP; FRT19Alyw FRT19A; Gal4109(2)80, UAS-mCD8::GFP+. Scale bars: 25 μm. D, dorsal; L, lateral; V, ventral.
A forward genetic screen to identify regulators of axon and dendrite morphology

We next took an unbiased forward genetic approach to identify genes that regulate the morphology of axons and dendrites, using the stereotyped pattern of class IV neurons as an assay. We screened 3299 EMS-induced lines marked with ppk-eGFP in late embryos and early first instar larvae. The wild-type pattern of class IV axons in embryos was a simple ladder-like scaffold of longitudinal and commissural branches (Fig. 6A). From the first-round of screening, we identified 68 lines with defects in this axon pattern that did not show pronounced changes in neuron number or obvious embryonic patterning defects, such as missing or fused segments. We recovered several mutants that showed dendrite-specific phenotypes (B.Y., W.B.G., S.Y., L.Y.J., Y-N.J., unpublished), and 25 mutants showing aberrant dendrite and axon morphology. Although we have not investigated the cell autonomy or non-autonomy of these phenotypes, the mutations recovered suggest that dendritic and axonal morphogenesis proceed by both common and distinct molecular mechanisms. Here we introduce a subset of the mutations that may be relevant to understanding mechanisms of sensory axon patterning.

We identified several mutant lines with disrupted class IV longitudinal or commissural projections (Fig. 6; Table 1). The defects could be separated into two groups: lines with a majority of projections missing (either longitudinal or commissural) (Fig. 6B- F), and lines with apparently intact, but mispositioned class IV axons (Fig. 6J-O). The first group included the transcription factor prospero (pros; four alleles), l(3)3456 (two alleles), l(3)3425 (three alleles) and l(3)7, l(3)12563 and l(3)12580 (one allele each). Two additional lines, l(3)2332 (Fig. 6E) and a mutant that we named boaum (bum; Fig. 6F,H) after the similarity between the dendrite phenotype and the shape of the Mexican desert tree, were the only lines with absent commissural branches. Based on complementation studies, neither was allelic to comm (data not shown). We examined bum further, and found that embryos showed severe dendritic growth defects (Fig. 6G-H). Fas II-labeled fascicles in the CNS were not grossly disrupted in bum mutants, although staining was unusually strong in cell bodies throughout the CNS (data not shown). Labeling of bum mutants with BP102 showed that an overall normal axon scaffold lost integrity in a posterior-to-anterior progression during embryonic development (Fig. 6I). bum therefore appears to affect multiple aspects of neuronal morphology, including midline crossing and mediolateral positioning of axons, and growth and arborization of dendrites. These phenotypes may be due to general defects in the maintenance of dendrite and axon morphology. Based on the anatomical data presented above (Figs 1-3), and preliminary analysis of mutant phenotypes, this first group of sensory axon phenotypes could arise by a failure of axon branching and growth, a failure of commissural and longitudinal branches to segregate, or a failure in axon or dendrite maintenance. Further studies of these mutants, including their autonomous or non-autonomous effects on axon and dendrite morphology, should contribute to our understanding of the molecular control of these events.

The second broad category of mutations included those with more subtly mispositioned axons. We isolated several mutations that affect the integrity and coherence of sensory axon projections (Fig. 6J-O, Table 1). l(3)11534, l(3)1025, l(3)9573 and l(3)10363 (Fig. 6I-M) showed a grossly normal scaffold, but showed extensive defasciculation or disorganization of longitudinal projections. We mapped mutations in l(3)9573 to brahma (brm), a transcriptional activator related to yeast SNF2/SWI2 (Tamkun et al., 1992) and in l(3)10363 to protein tyrosine phosphatase 69D (Desai et al., 1996). The cell autonomy of these mutant phenotypes and other lines has yet to be fully characterized. We also isolated a complementation group consisting of four alleles that we named boa bab (boa) for the frequent inverted class IV axon thickeninges (Fig. 6N-O). Dendrites were not disrupted in boa mutants (Fig. 6P). Labeling of the boa axon scaffold with BP102 revealed weakly labeled longitudinal and commissural tracts, suggesting that this mutation may generally affect axon development (Fig. 6Q)

Given that class IV axons usually terminate in a tract next to class III and II axons, further mosaic studies of mutations in which class IV axons are severely unbundled or laterally displaced might provide insight into the development of axon patterning. We suspect that the high sensitivity of our screen will allow the identification and characterization of genes not previously implicated in neuronal morphogenesis.

**DISCUSSION**

**Representation of somatosensory axons in the CNS: relating dendrite morphology to sensory function**

Insect sensory systems, including visual, olfactory, gustatory and somatosensory, represent the external world as sensory maps in the CNS. The nature of the map varies with sensory system (Blagburn and Bacon, 2004). Visual systems retain a map of sensory position in their central axonal projections. The axons of olfactory receptor neurons project to similar positions in the brain if they express the same odorant receptor, and thus transform information about
stimulus quality into a spatial map (Gao et al., 2000b; Vosshall et al., 2000). Gustatory projections retain aspects of both stimulus location and quality in their map (Wang et al., 2004).

Much of our knowledge about somatotopic maps in insect mechanosensory systems derives from studies of bristle afferents with peripheral receptive fields that approximate a point source. *Drosophila* da neurons have largely overlapping peripheral sensory fields and may, as a group, respond to several distinct stimuli (Grueber et al., 2002). How is information from this predominant body wall sensory system represented in the CNS and what might this organization tell us about the possible functions of da neurons? We show that neurons with different dendritic branching morphologies target distinct regions of the CNS, supporting the existence of a modality map of da neuron axons. We further provide evidence for nested somatotopic mapping in class I and class IV da neurons. Individual class I neurons extend their dendritic and axonal arbors in the same preferred direction along the AP body axis (Fig. 1C). Class IV axons project across the midline according to the cell body position along the DV axis of the body wall, with dorsal and ventral cells, but not more lateral cells, crossing the midline. It is possible that class II and III neurons also project in a somatotopic pattern that was not uncovered by our analysis. Thus, da neuron connectivity appears to incorporate both class and position-specific components, with some apparent correlations with dendritic field orientation.

In the context of sensory processing, these data suggest distinct functions for different morphological classes of da neurons. The class II-IV neurons target a ventral region of the neuropil; thus information from these neurons might be processed similarly to ventral-projecting tactile sensory bristle neurons (Schrader and Merritt, 2000). Within this ventral region, the class-specific laminar pattern could allow differential connectivity with second-order interneurons. Additionally, class II neurons, with their collateral branches, might provide information to distinct central circuits. The class I neurons targeted a more dorsal region of the neuropil, which is generally a characteristic of proprioceptive afferents in insects (Merritt and Murphey, 1992; Murphey et al., 1989; Pfuger et al., 1988; Schrader and Merritt, 2000). Indeed, a class of da neurons in *Manduca* has been shown to target dorsal neuropil, and to respond to stretch of the cuticle (Grueber et al., 2001). Many insect proprioceptors, including chordotonal organs and the bipolar dendrite neurons, have dendrites oriented in a preferential direction relative to the body axis. Notably, the primary dendrites of each class I neuron are oriented dorsally and secondary dendrites are oriented anteriorly and posteriorly (Grueber et al., 2002). This arrangement could allow larvae to compare distension along major body axes. While the anatomical arrangement of their axons suggests distinct functions for different da neurons, dissecting these different functions will ultimately require behavioral and physiological studies (Ainsley et al., 2003; Liu et al., 2003; Tracey et al., 2003).

**Mechanisms of class-specific axon and dendrite patterning**

A notable feature of the mapping of da sensory afferents is their predominant organization into class-correlated mediolateral layers (Figs 2, 3), with class IV neurons in a medial layer, class III neurons intermediate, and class II neurons most lateral. These layers do not correspond to the medial, intermediate and lateral fascicles that have their position specified by a Robo combinatorial code (Rajagopalan et al., 2000; Simpson et al., 2000); thus novel molecular cues may...
contribute to this laminar organization. Indeed, it has been postulated that the Robo code provides information about the broad zone that a growth cone targets, while a complementary fasciculation code fine-tunes pathway choice within that zone (Rajagopalan et al., 2000). It is conceivable that Robo proteins could participate in specifying the lateral position of collateral branches, as Robo3 overexpression in individual neurons induced ectopic branching from the axon shaft. Although we did not detect cell-type-specific expression of Robo3 in neurons that normally show such branching, it is notable that Robo3 has been implicated in cell-type-specific patterning decisions of PNS axons (Zlatic et al., 2003), and that Slit2 has been proposed as a positive regulator of collateral branching of dorsal root ganglion sensory axons (Wang et al., 1999).

The mechanisms for targeting of somatosensory afferents should act to properly position axons of different classes relative to one another. Several alternative mechanisms could contribute to this positioning. One potentially important component of axon sorting could involve interactions between homotypic or heterotypic axons. Heterotypic axons could repel each other to sort to discrete bundles. Likewise, homotypic axons could adhere to one another to ensure that they terminate together. Olfactory receptor neuron axons engage in extensive hierarchical interactions to establish precise targeting in olfactory glomeruli (Komiyama et al., 2004), and dendrites of da neurons engage in class-selective interactions during development to ensure their proper spacing (Grueber et al., 2003b; Sugimura et al., 2003). It is therefore conceivable that intraclass and interclass interactions could participate in the sorting of somatosensory axons. Axons from different classes could also project to specific layers that are prepatterned by the processes of target interneurons or by other axons. Finally, the projection of da axons to different layers could conceivably reflect a temporal order of sensory axon arrival in the CNS, similar to the three-way correlation between mediolateral axon position, physiological function and time of differentiation among Drosophila wing campaniform sensilla (Palka et al., 1986). Among the ventral cluster da neurons, a group with differentiation that has been examined in the greatest detail, the class II neurons appear to be the first-born, followed by class III neurons and class IV neurons (Orgogozo et al., 2001). These data suggest a possible correlation.
between birth order and axon position in the neuropil, although additional early markers of da neurons and further high-resolution imaging studies are required to further test this scenario.

The molecular basis for insect sensory neuron differentiation, as well as anatomical studies of somatosensory axon mapping and VNC circuitry, have been subjects of considerable study (Blagburn and Bacon, 2004), and principles are emerging that link the two areas into molecular models of connectivity and synaptic specificity (Merritt et al., 1993; Merritt and Whitington, 1995; Nottebohm et al., 1992; Zlatic et al., 2003). Among embryonic sensory neurons in Drosophila, there is a three-way correlation between soma position, proneural transcription factor expression and axon projection pattern, suggesting that these transcription factors may endow aspects of modality-specific axonal projections (Merritt and Whitington, 1995).

Table 1. Dendrite and axon phenotypes of EMS-induced third-chromosome mutation, as assessed in ppk-eGFP embryos

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<th>Gene/mutation</th>
<th>Phenotype</th>
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<td>l(3)1025</td>
<td>None</td>
<td>Defascilication</td>
<td>61A;66D10</td>
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<td>ptp69D</td>
<td>B</td>
<td>Defascilication</td>
<td>69D6;69E1</td>
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<td>l(3)9374</td>
<td>B</td>
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<td>daily</td>
<td>G</td>
<td>Disorganization</td>
<td>66E1-3</td>
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<td>brahma</td>
<td>B</td>
<td>Disorganization</td>
<td>72C1</td>
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<td>l(3)1872 (fng)**</td>
<td>G</td>
<td>Disrupted longitudinals*</td>
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<td>Scm</td>
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<td>Disrupted longitudinals*</td>
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<td>R, B</td>
<td>Missing longitudinals</td>
<td>72D1; 86D3</td>
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<td>G</td>
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</table>

B, branching; G, growth; R, routing; T, tiling.

*Occasional loss of longitudinal and commissural branches.

**Mutation detected in fringe but, by complementation analysis, may not account for the complete phenotype.

We thank Atsushi Miyawaki for Kaede plasmid; Barry Dickson, Kei Ito, Graeme Mardon, Veronica Rodrigues, Kristin Scott, Jessica Treisman, David Van Vactor, Marta Zlatic and the Bloomington Stock Center for fly stocks; Zuoren Wang for helpful advice on using the FLP-out system; Fabrice Roeigers for assistance in making the UAS-Kaede lines; and members of the Jan lab for comments.

References


