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Morphology of Dopaminergic Amacrine Cells in the Mouse Retina: Independence from Homotypic Interactions

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Abstract

To determine the role of homotypic interactions between neighboring dopaminergic amacrine (DA) cells upon dendritic morphogenesis, the morphology of single cells was examined relative to the positioning of all neighboring homotypic cells. For each labeled cell, the dendritic field was reconstructed, its Voronoi domain calculated, and the two were related. The dendritic fields of DA cells were observed to be large, sparse and highly irregular. Dendrites readily overlapped those of neighboring cells, showing no evidence for dendritic tiling nor inter-digitation consistent with homotypic repulsion or avoidance. Furthermore, a direct comparison of dendritic field area to the Voronoi domain area of the same cell showed no evidence for dendritic growth being constrained or biased by the local distribution of homotypic neighbors in wild-type retinas. A comparison of the processes of adjacent filled cells confirmed their immediate proximity to one another within the inner plexiform layer, indicating that they do not engage in mutual avoidance by coursing at different depths. Together, these results suggest that the morphogenesis of DA cells is independent of homotypic interactions. However, in the absence of the pro-apoptotic *Bax* gene, which yields a four-fold increase in DA cell number, a small but significant reduction in dendritic field size was obtained, though not so great as would be predicted by the increase in density. The present results are considered in light of recent studies on the role of cell adhesion molecules expressed by developing DA cells.

Keywords

Dscam; differentiation; cell adhesion; avoidance; dendritic field; overlap; Voronoi domain

Introduction

Dopaminergic amacrine (DA) cells are retinal interneurons that modulate a variety of key visual processes, most notably, those associated with light adaptation and the transition from scotopic to photopic visual function (Witkovsky, 2004). In the mouse retina, the DA cells are all positioned within the inner nuclear layer (INL), extending their dendritic and axonal processes primarily within the outermost stratum of the inner plexiform layer (IPL), in S1 (Ballesta et al., 1984). Dopamine receptors, by contrast, are widely distributed throughout the retina (Veruki and Wässle, 1996), and dopamine release, occurring through both synaptic as well as extra-synaptic exocytosis (Puopolo et al., 2001), is believed to exert its

pan-retinal modulatory actions through volume transmission (Witkovsky et al., 1993). The DA cells are one of the sparsest cell types within the retina, amounting to less than one-hundredth of 1% of all retinal neurons. Despite comprising such a tiny minority of all retinal cells, the total number of DA cells in the mouse retina is tightly regulated, exhibiting minimal variation within a given strain of mice despite large inter-strain differences (Whitney et al., 2009). These cells are distributed equally across the four quadrants of the mouse retina (Savy et al., 1999), but their local distribution has been variously described as “regularly ordered” (Wulle and Schnitzer, 1989), “irregular” (Gustincich et al., 1997), or “randomly distributed” (Versaux-Botteri et al., 1984). They have been shown to be modelled by distributions of randomly assigned cells that are precluded from being positioned in close proximity to neighboring like-type cells, capturing the essence of how these mosaics differ from truly random distributions of cells while appearing highly irregular (Raven et al., 2003; see also Mariani et al., 1984).

The morphology of these cells has not been studied extensively in the mouse retina. Most of these studies rely on immunostaining the retina to reveal the network of dopaminergic cells and their processes, where the full extent of the dendritic arbor, as well as the processes arising from neighboring cells, are often difficult to discern. Furthermore, many of these studies failed to discriminate between the lengthy axonal processes that traverse the retina and the local dendritic arbor. In general, those studies suggest that these cells give rise to a large if sparse and irregular dendritic arbor (Versaux-Botteri et al., 1984; Wulle and Schnitzer, 1989; Puopolo et al., 2001), which, in the rat retina, has been suggested to differentiate in relation to neighboring homotypic DA cells, in order to produce a “constant degree of overlap in the adult” (Savy et al., 1989). Two related studies in cat and monkey have considered the density of dendritic processes relative to the spatial distribution of cells by randomly positioning the dendritic arbor of reconstructed cells at each soma in the mosaic, conveying some appreciation of the relative density of those processes, but neither of those studies were able to address whether the dendritic morphology bore any spatial relationship to neighboring cells (Voigt and Wässle, 1987; Dacey, 1990).

Mouse DA cells were recently shown to express the Down syndrome cell adhesion molecule (Dscam). Mice possessing a loss-of-function mutation in *Dscam* exhibit abnormalities in DA cell spacing and differentiation, leading to the suggestion that Dscam “mediates isoneuronal and heteroneuronal self-avoidance” (Fuerst et al., 2008). Indeed, such avoidance would be a mechanism for ensuring a uniform distribution of dendritic processes across the retinal surface. In *Drosophila*, sensory cells express *Dscam*, and a multiplicity of alternative splice variants exists rendering each cell distinct from one another, thereby engaging in self-avoidance but failing to avoid the processes of adjacent like-type cells. If, however, every cell is forced to express the same isoform, then such avoidance is observed between the processes of neighboring cells, and the population achieves a uniform tiling of the body wall (Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007). The mouse lacks multiple splice variants of *Dscam*, and so might be expected to mediate such interneuronal avoidance or repulsion and thereby contribute to the uniformity of coverage amongst DA cells across the retina. The present investigation has examined the dendritic morphology of single DA cells with this issue in mind. Specifically, we have examined the relationship between the dendritic organization of single, labeled DA cells relative to their immediate neighbors in the mouse retina, to examine whether DA cells with a functional *Dscam* gene exhibit any evidence consistent with this interpretation of *Dscam* function. A preliminary report of these results was presented at the Society for Neuroscience meeting in 2008.

Materials and Methods

Transgenic *TH-RFP* mice, in which the red fluorescent protein DsRed2–1 is driven by a 4.5 kb fragment of the rat tyrosine hydroxylase (*TH*) promoter (Zhang et al., 2004), and *Bax* knockout mice (*Bax*^{−/−}) were obtained from Douglas McMahon at Vanderbilt University and Jackson Labs (Bar Harbor, ME), respectively. The *TH-RFP* and *Bax*^{−/−} mice had each been backcrossed with C57BL/6/J mice for at least 6 or 10 generations, respectively, and were bred in the Animal Resource Center at the University of California at Santa Barbara (UCSB). All experiments were conducted under authorization by the Institutional Animal Care and Use Committee at UCSB and in accord with the NIH Guide for the Care and Use of Laboratory Animals.

Single-cell Injection and Immunofluorescence

Mice were euthanized with sodium pentobarbital (120 mg/kg, i.p.), and eyecups were immersion-fixed in 4% paraformaldehyde in 0.1M sodium phosphate buffer (pH 7.2 at 20°C) for 30 minutes. Retinas were dissected into wholemounts and transferred to a fixed stage Nikon Eclipse E600 epifluorescence microscope. Single DA cells expressing RFP were visualized with a 60X water dipping objective and impaled with a micropipette filled with 0.5% DiI dissolved in 100% ethanol. Positive current of approximately 100nA was passed for roughly 10 seconds until a visible bolus of DiI was deposited at the site of the micropipette tip. Because of the hydrophobic nature of DiI, little diffusion of the dye is detected at the time of the injection; rather, by waiting 24 hours, this lipophilic dye diffuses throughout the plasma membrane, providing excellent labelling of single cells.

After several isolated cells had been injected, retinas were subsequently stained for the remaining population of DA cells, using sheep polyclonal antibodies to tyrosine hydroxylase (TH; Millipore AB1542; Bedford, MA). The immunogen used in the generation of this affinity-purified polyclonal antibody was native TH from rat pheochromocytoma, and its specificity had been tested through immunoblotting, revealing the ~60 kDa TH protein in PC12 cells (Haycock and Waymire, 1982). Retinal wholemounts stained with this antibody revealed comparable distributions and densities of DA cells in the mouse retina as has been shown by others (Versaux-Botteri et al., 1984; Wulle and Schnitzer, 1989; Masland et al., 1993; Whitney et al., 2009). Retinas were immersed in 5% normal donkey serum in phosphate buffered saline (PBS) for 3 hours, rinsed with PBS, then incubated for 5 days with primary antibodies (1:10,000) in PBS. Retinas were rinsed again, then soaked overnight with donkey anti-sheep secondary IgG conjugated to Cy2 (1:200; Jackson ImmunoResearch Labs, 713–225–147; West Grove, PA) in PBS. All steps were done at 4°C with agitation and without detergent to preserve the integrity of DiI-filled cells.

For some preparations, adjacent pairs of cells were labelled, using the above protocol for DiI and employing a second pipette filled with 5% Lucifer Yellow. Negative current of roughly 5nA was passed for several minutes until the entire dendritic arbor filled with Lucifer Yellow. Pairs of DA cells were imaged on an Olympus Fluoview 1000 laser scanning confocal microscope using a 25x water immersion objective, and image stacks with captured at 0.5 μ m intervals along the z-axis. Maximum projection images were reconstructed from these stacks using MetaMorph software (MDS Analytical Technologies; Sunnyvale, CA). To achieve high-resolution images of entire DA cells, between 15 and 25 high-magnification image stacks were acquired for each cell and their maximum projection reconstructions were aligned using the photomerge function in Photoshop CS3 (Adobe Systems Incorporated; San Jose, CA).

Morphometric and Mosaic Analyses

Stained retinas were mounted on slides in GEL/MOUNT (Biomedica Corporation; Foster City, CA) and examined using an Olympus BH2 fluorescence microscope equipped with a Sony video camera. The dendrites of filled DA cells, identified as the thick planar processes arising from the soma that reached a discrete endpoint, were digitally traced using a computer running Bioquant Nova Topographer software (BIOQUANT Image Analysis Corporation; Nashville, TN); additionally, the location of each DA cell in the surrounding population was identified and recorded.

Several features of DA cell morphology were measured for each traced cell. The number of primary dendrites was counted, as was the total number of higher order branch points. The size of the dendritic field was determined as the area of a convex polygon that encompasses the entirety of the dendritic arbor, while the linear distances of all dendrites were summed to calculate the total dendritic length. The Voronoi domain of each of these cells was determined based upon the spatial relationship to neighboring cells, derived from the X and Y coordinates for each cell upon the retina, using customized software (Raven and Reese, 2002).

Statistical Analysis

Students' t-tests were performed for all comparisons with a p-value < 0.01 determining significance. Pearson's correlation coefficients were measured to examine the relationship between dendritic field size and Voronoi domain area. All data are represented as means $\pm$ standard errors.

Results

The morphological properties of single DA cells

The somata of DA cells give rise to 2–3 long primary dendrites that yield infrequent higher-order branches, producing a sparse wide-field dendritic arbor with an average diameter of 495 μm (figure 1a–e). In addition to the dendritic arbor, an axon typically emerges from the soma or primary dendrite near the soma (arrowheads in figure 1), itself branching infrequently and coursing extensively, well beyond the dendritic field itself (arrows in figure 1). The axon is readily distinguished by virtue of its thinner course, its lengthy profile, and by a progressive diminution of labeling with distance; by contrast, the dendrites are typically thicker and consequently more heavily labeled, and terminate more abruptly than do the axons. The dendritic fields of the DA cells were notably irregular in their branching pattern, often elongated (figure 1b, e) or asymmetric (figure 1c). The scarcity of branches within the convex polygon defined by the entire dendritic field was striking, being conspicuously less dense than those described for the cat or monkey retina (Voigt and Wässle, 1987; Dacey, 1990). Each of the cells in figure 1 has been framed to include the entirety of the dendritic field; many of their axon branches extend well beyond the dendritic field (arrows), though could not always be traced to an unambiguous end-point.

The relationship between DA cell morphology and homotypic neighbors

To study the relationship between dendritic morphology and homotypic neighbors, we reconstructed the Voronoi domain of single DA cells by labeling the entire population within the local vicinity and determining the Voronoi tessellation using customized software for this purpose (Raven and Reese, 2002). The Voronoi domain (defined by the bisectors of each segment interconnecting a given cell with all of its immediate Delaunay neighbors) describes the territory in the plane of the retina closer to a given cell than to any of its neighboring cells, and as such, conveys information about the spatial distance to each

neighboring cell, about asymmetries in the positioning of neighboring cells, and about the local density of this cell-type at the cell itself, being the reciprocal of this area.

Figure 2 shows the dendritic arbors of six wild-type DA cells in relation to the mosaic of all surrounding DA cells, along with the associated Voronoi domain of each filled cell (lightly shaded). For each cell, the Voronoi domain appears substantially smaller than the dendritic arbor. By estimating the size of the dendritic arbor using the convex polygon approach, a direct comparison can be made with the area of the Voronoi domain of each cell. Figure 3a shows, for a sample of 21 wild-type cells, that dendritic field area is, in fact, nearly eight times larger than the territory of its Voronoi domain. Consistent with other studies on the dendritic morphology of DA amacrine cells in other mammals, the dendritic arbors of DA cells in the mouse retina extend too far to achieve a “tiling” of the retinal surface produced by contact inhibition at their dendritic tips. Note as well that the dendritic fields often extend well beyond the positioning of neighboring somata; indeed, the average diameter of the DA dendritic field (495 μm) is more than five times the average nearest neighbor distance (92.7 μm), and consequently, these cells cannot be simply interleaving their dendrites to achieve an average dendritic field area in excess of the Voronoi domain area but without producing dendritic crossings (e.g. Fuerst and Burgess, 2009).

Even if DA cells do not exhibit contact-inhibition to constrain dendritic growth, one might still envision that homotypic interactions between neighboring cells would constrain dendritic growth in a density-dependent manner. Specifically, dendritic field area might still vary in size with local homotypic density, suggesting the presence of a homotypically derived factor that compromises further growth. Figure 3b shows, however, for these same 21 cells, that their field size is unrelated to Voronoi domain area, which is, of course, the reciprocal of local DA cell density. Indeed, while dendritic field area increased slightly with eccentricity ($r^2 = 0.344$), the size of Voronoi domains did not ($r^2 = 0.002$). DA cells do not apparently modulate their overall dendritic growth in relation to local homotypic density.

The DA mosaic is nearly random, but for the presence of a large exclusion zone surrounding single DA cells (Raven et al., 2003). Because this large exclusion zone does not approximate the size required to render such a sparse population to be hexagonally packed, many individual cells have their surrounding neighbors at variable distances from the cell (Raven et al., 2003). As has been shown above (figures 1 and 2), individual DA cells have dendritic morphologies that are conspicuously variable with respect to their geometry, some being relatively asymmetric, others showing prominent elongation in one axis. To examine the relationship between this dendritic geometry and the positioning of immediate neighbors, we determined the long axis of each dendritic field and then measured the angular difference to the long axis of the Voronoi domain for this same population of 21 cells. Figure 3c shows that these two measures appear to be entirely unrelated; DA cells show no tendency to orient their elongated dendritic arbors along the axis defining the farthest homotypic neighbors. This same conclusion is borne out by inspection of single dendritic fields shown in figure 2: there appears to be no greater tendency for dendrites to emerge from, or arborize within, the territory on the side of a soma where immediate neighbors are farthest removed.

Further evidence suggesting that like-type dendrites do not constrain dendritic growth comes from the observation that the dendritic arbors of single DA cells in figure 2 (e.g. panels a, c, f) frequently cross over one another, rather than running parallel to, or away from, neighboring processes from the same cell. Indeed, such a mechanism may contribute to the dendritic differentiation of starburst amacrine cells, wherein dendrites branch as a function of increasing distance from the soma but rarely cross one another (Sugimura et al., 2007). DA cells, by contrast, have dendritic fields that are exceedingly sparse (figures 1 and 2), lacking the symmetry characteristic of starburst amacrine cells in the mouse retina (Farajian

et al., 2004; Keeley et al., 2007), as in other species (Tauchi and Masland, 1984; Voigt, 1986). Still it remains a possibility that the processes of single DA cells avoid proximity by coursing at different depths within the IPL. To examine this, we injected pairs of DA cells using Lucifer Yellow and DiI, and reconstructed their dendritic arbors relative to one another. Figure 4a–c shows the labeled processes of three such pairs of cells, confirming that immediate neighbors have widely overlapping dendritic fields. More critically, figure 4d–f show single 0.5 μ m optical sections from each of these Z-stack reconstructions, illustrating, within the limits of this approach, that the processes from adjacent cells are in close proximity, perhaps even in contact. These cross-over points show no obvious diversion of trajectory or other interruptions in their course. Comparable results were obtained for 27 cross-over points examined from a total of 7 pairs of cells—their processes do not avoid one another by diverting course through the depth of the IPL, be they dendrites crossing dendrites (figure 4d, e) or a dendrite crossing an axon (figure 4f). Rather, they are contained within the same single stratum. These results would suggest that the processes of DA cells are largely indifferent to the presence of those from homotypic neighbors.

DA cell morphology and relationship to homotypic neighbors in the *Bax*^{−/−} retina

We have recently shown that the number of DA cells shows a four-fold increase in *Bax*-knockout mice (Whitney et al., 2009), consistent with large increases in this same population shown in previous studies using *Bcl2*-overexpressing transgenic mice (Strettoi and Volpini, 2002a). We consequently asked whether such a large increase in DA cell density would alter the dendritic organization of single DA cells. Figure 5 shows five examples of the dendritic morphology of single DA cells in the *Bax*^{−/−} retina. The general characteristics of these cells are entirely unchanged, and subjectively, it is impossible to discriminate the neurons in *Bax*^{−/−} retinas from those in littermate *Bax*^{+/+} retinas shown in figure 1.

Figure 6 shows the dendritic fields and Voronoi domains of three individual DA cells from *Bax*^{−/−} retinas relative to their surrounding mosaic, and, for comparison, three fields from single cells from littermate control mice (*Bax*^{+/+}). Qualitatively, these reconstructed dendritic arbors in the *Bax*^{−/−} retina look like typical DA cells, despite the obvious increase in the local density of neighboring cells. The number of primary dendrites emerging from the soma was unchanged (figure 7a), and the number of higher order branches was also comparable between the groups (figure 7b). Curiously, however, the average size of the dendritic fields in the *Bax*^{−/−} retinas ($n = 22$) was significantly smaller than was that from the *Bax*^{+/+} retinas ($n = 15$; figure 7c). This difference in field area was accompanied by a 20% reduction in total dendritic length (figure 7d). The decrease in dendritic field area was, however, only a 28% reduction in size, whereas, for a subset of these cells in which their Voronoi domains were determined, the four-fold increase in local density was confirmed (figure 7e). Figure 7f plots the ratio of the Voronoi domain area to the dendritic field area, for this same subset of cells in the two groups ($n = 16$ *Bax*^{−/−} cells, and $n = 7$ *Bax*^{+/+} cells). If DA cells scaled their dendritic areas in proportion to the increase in DA cell density, the two should be the same, yet the ratio is nearly four times larger in the *Bax*^{+/+} retinas.

Discussion

The present study has demonstrated that DA cells in the wild-type retina would appear to differentiate their dendritic fields without influence from homotypic neighbors. This has some bearing upon recent claims that DA cells express a cell-surface molecule, Dscam, that mediates homotypic repulsion or avoidance (Fuerst et al., 2008). We also found, however, that dendritic outgrowth and field extent were significantly reduced in the presence of a four-fold increase in homotypic density in the *Bax*^{−/−} retina, although not to the extent predicted by the increase in homotypic density. We will consider both of these issues in

detail below, but before doing so, will address the relevance of the present findings to previously published work.

Relation to previous studies

The DA amacrine cell mosaic is readily labeled with antibodies to TH, and has consequently been described in many different species. The features that have been most frequently described include their scarce nature, their irregular distribution, and their stratification in the IPL. Occasionally, their morphology has been described where strong immunolabeling is obtained, but rarely does this approach reveal the entirety of the dendritic arbor nor the distinction between the axonal arbor from that associated with the dendrites, both being distributed within the same sublamina, S1, of the IPL (Peichl, 1991; Müller and Peichl, 1991; Wulle and Schnitzer, 1989; Wang et al., 1990). Occasionally, single cell injections have been employed to study the morphology of DA cells (Voigt and Wässle, 1987; Dacey, 1990), though these studies vary with respect to the level of morphometric detail provided. No study that we know of, however, has attempted to provide any degree of quantitative analysis of the relationship between the dendritic morphology of these cells and the presence of their homotypic neighbors. Consistent with all other studies, the present study shows directly that DA cells do not tile the retina by producing dendritic fields that approximate their Voronoi domains. Further, it makes clear that the dendrites of neighboring cells are simply too large to interleave their dendrites and thereby avoid homotypic crossing. While this might be assumed given the reconstructed dendrites of DA cells in species such as the cat (Voigt and Wässle, 1987), it is not always so apparent for other species such as the tree shrew or ferret, despite the presence of occasional cross-overs shown in the literature (Keyser et al., 1987; Müller and Peichl, 1991). Further, DA cells in the mouse retina would appear to be far more sparse and irregular in their dendritic morphology than are those in other species like the cat (Voigt and Wässle, 1987), further warranting this closer consideration of their relationship to one another. The present study, being the only study to examine the proximity of processes as they cross over one another, has shown that these cells do not avoid one another by coursing at differing depths within the IPL. The general conclusion of these analyses, therefore, is that the morphogenesis of DA dendritic fields is largely indifferent to homotypic interactions. Before turning our attention to the relevance of these results for interpreting the role of *Dscam* in DA cell development, we will next consider the significance of the change in the dendritic field area of DA cells in *Bax*^{-/-} retinas.

DA cell morphology in the *Bax*^{-/-} retina

The reduction in the dendritic field size of DA cells in the *Bax*^{-/-} retina may very well reflect an increase in homotypic interactions that constrains dendritic outgrowth. By this view, the lack of such evidence in the wild-type retina may indicate that the interactions present in the *Bax*^{-/-} retina do not play a role during normal development, suggesting that the surplus DA cells in the *Bax*^{-/-} retina, or their growth behavior, are not quite normal. In the *Bcl2*-overexpressing transgenic mouse retina, where the population of DA cells undergoes a nine-fold increase (Strettoi and Volpini, 2002a), those cells no longer exhibit the presence of an exclusion zone between homotypic neighbors, such that pairs of cells in immediate somal contact are as frequent as random distributions would predict (Raven et al., 2003). Perhaps such closer somal proximity impacts dendritic outgrowth, at least for such closer neighbors, and their reduced growth contributes sufficiently to reduce the mean field size for the population. Alternatively, DA cells in the wild-type retina may simply fail to generate a sufficient effect for detection at the lower densities present using our assays.

Yet another interpretation, however, is that the change in field area is brought about by some other *heterotypic* interaction in the *Bax*^{-/-} retina. For instance, the number of retinal

ganglion cells undergoes a two-fold increase in the *Bax*^{-/-} retina (Ogilvie et al., 1998), and the INL also shows a significant increase in thickness indicative of increased numbers of bipolar and amacrine cells (Péquignot et al., 2003). As one example, a class of intrinsically photosensitive (melanopsin-positive) retinal ganglion cell arborizes in the S1 stratum of the IPL, being synaptically connected with the DA cells (Viney et al., 2007; Vugler et al., 2007). Interestingly, this cell type undergoes a two-fold increase in number in the *Bax*^{-/-} retina as well (Lee and Reese, unpub. obs.). Any of these cell types that have increased in number might provide a signal that contributes to the reduced dendritic growth of DA cells.

For a cell-type that is near-randomly distributed across the retina, that differentiates such irregular dendritic arbors, and that participates in the non-image-forming functions of the retina, receiving innervation from intrinsically photosensitive retinal ganglion cells (Zhang et al., 2008) and employing volume transmission of dopamine secreted both synaptically and extrasynaptically (Witkovsky et al., 1993; Puopolo et al., 2001), we had expected that the dendritic fields of DA cells would be indifferent to their homotypic neighbors. The dendritic morphology of the DA cell would appear to be too variable to suggest that they follow a strict cell-intrinsic instruction to achieve their mature morphology, but of the potential environmental signals modulating their growth, those associated with homotypic neighbors do not appear to play a role, at least in the presence of normal densities. Exactly what a “normal” density is remains unclear, as different strains of mice exhibit a four-fold variation in the size of the DA cell population that cannot be explained by differences in the overall size of the retina (Whitney et al., 2009). While we have not studied the morphology of DA cells outside of the C57BL/6J mouse retina, we are inclined to interpret the change in size in the *Bax*^{-/-} retina as indicative of heterotypic regulation, but the source of this signal remains to be determined.

The role of *Dscam* in DA cell morphogenesis

The *Dscam*-mutant mouse retina presents a curious phenotype with respect to this DA cell-type: first, the processes of these cells tend to fasciculate with one another, a feature that is not present in the wild-type retina; second, the DA somata exhibit a tendency to cluster; and third, these retinas contain a near-doubling in the number of DA cells (Fuerst et al., 2008). The third feature remains an enigma, but the first two features were interpreted in light of the recent exciting analysis of the role of *Dscam* in *Drosophila* sensory neurons, amply reviewed in recent years (Hummel, 2007; Parrish et al., 2007; Schmucker, 2007; Millard and Zipursky, 2008). By virtue of thousands of alternatively spliced isoforms, every sensory cell expressing *Dscam* avoids the dendrites of only those emanating from the same cell. When multiple cells are engineered to express the same isoform, their adjacent dendritic fields show homotypic, not just self-, avoidance, yielding a tiled pattern to their adjacent dendritic arbors (Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007). The mouse, by contrast, shows no evidence for multiple isoforms of *Dscam*, leading to the expectation that a single commonly expressed *Dscam* on the surface of all DA cells might mediate self- as well as homo-typic avoidance (or “isoneuronal” and “heteroneuronal” avoidance, respectively). The presence of clustered cells and fasciculated dendrites would at first sight appear to be consistent with this expectation (Fuerst et al., 2008).

A closer examination of the wild-type DA cell, however, made available by labeling single cells, has herein revealed that the presence of a functional *Dscam* gene does not render a dendritic tree that either self-avoids or avoids the processes of neighboring cells, and nor does it appear to bias growth in relation to the density of such similarly expressing neighbors. Rather, these results suggest that, in the mouse, at least, *Dscam* masks the presence of an adhesive interaction between like-type cells, perhaps by complexing with some other cell adhesion molecule that neutralizes an otherwise homophilic interaction. Recent studies in mouse have shown that *Dscam* may act as a heterophilic receptor for the

netrin-1 ligand, and form receptor complexes with another netrin receptor, Dcc (Ly et al., 2008). Such results indicate a potentially greater complexity to the role of *Dscam*; for the moment, a simple interpretation for the effect of *Dscam* upon DA dendritic morphogenesis is that it may interfere with some other homophilic adhesion, and that the mutant mouse unmasks this interaction. A fuller consideration of this revised view of the function of *Dscam* in DA cells has recently been published (Fuerst and Burgess, 2009).

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Figure 1.

Examples of five DiI-labeled DA cells from wild-type adult retinas (being *Bax*^{+/+} littermate control mice). Two to three thick primary dendrites emerge from each soma giving rise to long, sparsely branched dendrites that define a large, irregular dendritic field. In contrast, one to two thin axons arise from the soma or the primary dendrites (arrowheads), spanning long distances before the intensity of labeling becomes indiscernible. Arrows indicate axons that extend beyond the field of view. Scale bar = 100 μ m.

Figure 2.

Reconstructions of six DiI-labeled dendritic fields of individual DA cells from adult C57BL/6J mice (shown in black), along with the position of every other TH-immunopositive cell in the immediate vicinity (indicated in black dots). The presence of those neighboring cells was used to determine the Voronoi domain of the labeled cell (shaded in grey). The Voronoi domain encloses all points in the plane of the retina that are closer to a given cell than to any of its neighboring cells. DA cells clearly grow beyond the limits of their Voronoi domains, seemingly indifferent to the location of nearby neighbors. Scale bar = 100 μ m.

Figure 3.

Relationship between the area of the Voronoi domain (VD) relative to the area of the dendritic field (DF). a: The area of the DF ($194,795 \pm 9,841 \mu\text{m}^2$) is about eight times larger than the VD ($27,161 \pm 2,275 \mu\text{m}^2$), for a sample of 21 cells. b: The areal size of the DF is not significantly correlated with the areal size of the VD. c: The long axis of the DF is unrelated to the long axis of the VD. These results show that the spatial arrangement of DA cell dendritic fields is independent of homotypic neighbors.

Figure 4.

Dendritic overlap between neighboring labeled DA cells. a–c: Three examples of pairs of cells labeled with DiI (magenta) versus Lucifer Yellow (green). Each image is a Z-stack reconstruction collected through the IPL and INL, spanning an average distance of 20 μ m. Scale bar = 100 μ m. d–f: Single optical sections from each pair, 0.5 μ m in thickness, showing the coincidence of labeled processes from the two cells, indicating that DA cells do not alter the course of their dendrites either radially or tangentially to avoid neighboring processes. Scale bar = 20 μ m.

Figure 5. Examples of five DiI-labeled DA cells from *Bax*^{-/-} retinas (compare with those in figure 1, which are *Bax*^{+/+} littermate controls). DA cells in these retinas display a similar morphology to those from wildtype retinas. Conventions as in figure 1. Scale bar = 100μm.

Figure 6.

Examples of the dendritic fields and Voronoi domains of single DA cells in *Bax*^{+/+} (a–c) and *Bax*^{–/–} (d–f) retinas relative to the mosaic of neighboring TH-immunopositive cells. The dendritic fields of single cells from *Bax*^{+/+} littermate control retinas are comparable to those of the previously examined wild-type retinas; additionally, the fields of single cells from *Bax*^{–/–} retinas look in all respects normal, despite a marked increase in the number of neighboring DA cells, suggesting these cells do not scale their dendritic fields in proportion to the density of homotypic neighbors. Scale bars = 100μm.

Figure 7.

Morphometric results for the dendritic fields of single DA cells in *Bax*^{+/+} and *Bax*^{-/-} retinas. a: The number of primary dendrites emerging from the soma was not altered. b: The number of higher order branches emerging from those primary dendrites also was unaltered. c: The dendritic field area was, by contrast, smaller by about 28% in the *Bax*^{-/-} retina. d: Total dendritic length was also significantly reduced. e: The VD areas for DA cells in the *Bax*^{-/-} retina were necessarily reduced due to the increase in DA cell density, by about 77% compared to those in the *Bax*^{+/+} retina. f: The ratio of VD area to DF area was significantly reduced in the *Bax*^{-/-} retina, indicating that while these dendritic fields are smaller, they have not reduced their field extent in proportion to the increase in homotypic density.