Contact Is Repulsive, but Please Note the "Enclosed"

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Previous models of neuronal dendrite arborization suggested that contact-dependent self-avoidance between dendrite branches prevents self-crossings within the arbor. Two papers in *Neuron* show how integrin-mediated adhesion to the extracellular matrix restricts dendrites to a two-dimensional space to optimize this mechanism (Han et al., 2012; Kim et al., 2012).

The size and shape of a neuron's dendritic arbor are major determinants of its receptive field. To maximize coverage while minimizing redundancy, neurons of a specific type frequently "tile," meaning that each dendritic arbor occupies a given territory with little or no overlap with the dendrites of neighboring neurons of the same type, an example of "heteroneuronal" self-avoidance. Within this territory, coverage is further optimized in an individual dendritic arbor by minimizing self-crossings, points at which two branches of the same arbor overlap. This requires self-avoidance between the branches of a single neuron, an example of "isoneuronal" self-avoidance. Although the mechanisms by which dendritic arbors achieve their anatomy remain incompletely understood, *Drosophila* have devised a clever mechanism for arborization through isoneuronal self-avoidance. This mechanism relies on self-recognition conferred by the highly alternatively spliced *Dscam1* gene (Hattori et al., 2008). *Drosophila* Dscam1 is a transmembrane adhesion molecule with 19,008 possible alternative extracellular domains that bind homophilically with isoform specificity. Each neuron expresses a small, stochastic subset of these isoforms and therefore is able to recognize itself but is invisible to its neighbors. Homophilic binding of Dscam1 confers the repulsive signal that mediates isoneuronal self-avoidance and prevents dendrite self-crossings (Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007).

This system is elegant, but can a repulsion-based mechanism alone account for the patterning of dendritic arbors? Furthermore, Dscam1-mediated repulsion requires contact between two outgrowing processes, but this could result in dendrites spreading in three dimensions, whereas many neurons have essentially flat arbors. Two papers in the latest issue of *Neuron* address these issues by demonstrating that integrin-dependent adhesion of developing dendrites to the extracellular matrix (ECM) keeps these processes in an essentially 2D state and thus facilitates contact-dependent repulsion and self-avoidance. When this interaction with the ECM is disrupted, more dendrites become "enclosed" within the overlying epidermal cells, which enwrap portions of the dendrites. This increases the apparent number of self-crossings, but these are "noncontacting" crossings, in which the epidermal cell intervenes between the overlapping dendrites (Figure 1).

Both studies examine the *Drosophila* dendrite arborization (da) neurons of the larval body wall. Kim and colleagues in the Grueber lab use electron microscopy to show that dendrites of da neurons typically grow in contact with the ECM on the basal side of the body wall epithelium, although some dendrites are enclosed within the epidermal cells and are not in contact with the ECM. The investigators also identify the Coracle protein as a marker of enclosed dendrites, allowing them to quantify the extent of dendrite enclosure. Mutation or knockdown of αPS1 or βPS integrin result in more enclosed dendrite segments and therefore fewer dendrites in contact with the ECM. Importantly, the loss of integrin function also leads to more dendritic self-crossings, but these crossings are "noncontacting," where typically one enclosed dendrite passes over an ECM-bound dendrite without touching it, thus circumventing the contact-dependent, Dscam1-mediated self-avoidance. Conversely, overexpression of integrins leads to more dendrites adhering to the ECM. Analysis of MARCM clones shows that the integrins function cell autonomously in da neurons to promote ECM adhesion and minimize dendrite enclosure.

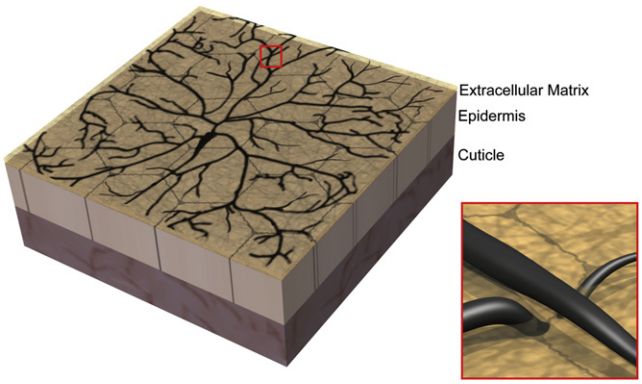

Figure 1. Enclosed Dendrites
The dendrites of *Drosophila* da neurons bind laminin in the extracellular matrix through cell-autonomous integrin-mediated adhesion. This interaction creates a two-dimensional environment that optimizes contact-dependent self-avoidance to prevent self-crossings in the branches of the arbor. Impaired integrin function increases the number of enclosed dendrites, which pass through epidermal cells and cross other branches without direct contact (inset).

The findings of Han et al. (2012), working with Lily and Yuh-Nung Jan, are very consistent with those of Kim et al. (2012), but they address two additional points. First, the data indicate that laminin in the ECM is the major integrin ligand. Second, the results clarify an important aspect of cell-autonomous signaling in self-avoidance. Han et al. determine that proteins previously thought to be part of the self-avoidance/repulsion signaling mechanism are, in fact, part of the integrin-dependent cell adhesion mechanism that keeps dendrites in contact with the ECM. Intracellular signaling factors including Hippo, Tricornered, Furry, and components of the TORC2 complex are important in preventing isoneuronal self-crossings and also in heteroneuronal tiling of da dendrites (Jan and Jan, 2010). However, with the new appreciation of the three-dimensionality of enclosed dendrites, Han et al. show that mutations impacting this system cause noncontacting crossings. The overexpression of integrins can rescue this self-crossing phenotype by promoting dendrite contact with the ECM. This result indicates that integrins function downstream or in parallel to this pathway and that Dscam1-mediated self-avoidance is preserved in their absence. It is also noteworthy that integrin overexpression rescues the tiling defects of these mutants. Given that tiling does not depend on Dscam1, these findings suggest that territories of individual arbors may be constrained by interactions with the ECM.

Therefore, both papers conclude that Dscam1-dependent, contact-mediated self-avoidance is a critical part of dendrite arborization but that this mechanism benefits from an essentially 2D environment that is generated by adhesion of dendrites to the ECM. Enclosure of dendrites in the epidermal cells creates a 3D environment in which noncontacting self-crossings are possible. The mechanism(s) of enclosure remains to be determined and may simply be a default of not attaching to the ECM, but the very reproducible percentage of dendrite length that is enclosed suggests that this is a nonrandom process. The physiological function of enclosure is also speculative at this point, though it is possible that enclosure may provide a physical anchor for da neurons necessary for their function as mechanotransducers.

Many neuronal cell types are likely to undergo similar processes, in principle if not in identical molecular terms. Self-avoidance and tiling have been observed from leeches to mammals. Two examples of particularly interesting dendritic architecture in vertebrates are cerebellar Purkinje cells and neurons in the retina. In both cases, the dendrites are confined to a nearly 2D plane and may therefore be able to minimize self-crossings through a contact-dependent self-avoidance mechanism. Purkinje cells have a fan-like dendritic arbor, and retinal neurons tend to stratify their processes in very specific laminae of the synaptic inner plexiform layer. However, in the retina most cell types do not truly tile, but instead overlap with their homotypic neighbors, so a mechanism of isoneuronal self-avoidance must also allow heteroneuronal tolerance. Kim et al. (2012) discuss the concept of balanced adhesion, in which adhesive and repulsive forces cooperate and counterbalance one another. This idea is consistent with the fasciculation of dendrites and clumps of cell bodies among cells of the same type observed in the retina with the loss of mouse *Dscam* (which is not alternatively spliced), which suggest an adhesive mechanism that is now unopposed by self-avoidance (Fuerst et al., 2008). However, the retinal inner plexiform layer does not have a pronounced laminin-rich ECM, suggesting that although self-avoidance and balanced adhesion may be in play, other molecular interactions may be used. In addition to balancing adhesion, other work from the Grueber lab has also determined that Dscam1 counteracts netrin-dependent attraction of outgrowing processes (Matthews and Grueber, 2011). The extent to which this function of Dscam1 may share signaling pathways with self-avoidance to oppose adhesion will be interesting to examine. Mechanisms by which contact-dependent signaling can be bypassed by preventing contacts from occurring will also be important in considering how three-dimensional dendritic arbors form. Since cell morphology ultimately has such a large impact on the functional anatomy of the nervous system, further defining how systems such as integrin-mediated adhesion integrates with Dscam-mediated repulsion is essential for understanding how neuronal circuits develop.

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