Title

Molecular mechanisms regulating the spatial configuration of neurites

Author(s)

Koichi Hasegawa and Ken-ichiro Kuwako

Journal

Seminars in Cell & Developmental Biology Volume 129, Pages 103-114

Published

Available online 2 March 2022, Version of Record 4 August 2022.

URL

https://doi.org/10.1016/j.semcdb.2022.02.015

Molecular mechanisms regulating the spatial configuration of neurites

Koichi Hasegawa and Ken-ichiro Kuwako*

Department of Neural and Muscular Physiology, School of Medicine, Shimane University, 89-1 Enya-cho, Izumo-shi, Shimane 693-8501, Japan.
Email; khasega9@med.shimane-u.ac.jp (K. H.) and kuwako@med.shimane-u.ac.jp (K. K.)

Corresponding author*
Ken-ichiro Kuwako
Email address: kuwako@med.shimane-u.ac.jp

Footnote:
Arp2/3, actin-related protein 2/3; DAAM1, dishevelled associated activator of morphogenesis 1; DCC, deleted in colorectal cancer; GSK3β, glycogen synthase kinase 3β; Lhx2, LIM homeobox 2; LIMK, LIM kinase; mGluR5, metabotropic glutamate receptor 5; LKB1, liver kinase B1; MTSS1, metastasis suppressor 1; Robo, roundabout; Rac1, RAS-related C3 botulinus toxin substrate 1; SH2/SH3, src homology 2/src homology 3; SIK1/2, salt-inducible kinase 1/2; TAO2, thousand and one amino acid protein 2.

Abstract

Precise neural networks, composed of axons and dendrites, are the structural basis for information processing in the brain. Therefore, the correct formation of neurites is critical for accurate neural function. In particular, the three-dimensional structures of dendrites vary greatly among neuron types, and the unique shape of each dendrite is tightly linked to specific synaptic connections with innervating axons and is correlated with its information processing. Although many systems are involved in neurite formation, the developmental mechanisms that control the orientation, size, and arborization pattern of neurites definitively defines their three-dimensional structure in tissues. In this review, we summarize these regulatory mechanisms that establish proper spatial configurations of neurites, especially dendrites, in invertebrates and vertebrates.

Keywords

Dendrite, Spatial configuration, Orientation, Size, Arborization

1. Introduction

Neurons are extremely polarized cells harboring two structurally and functionally distinct types of neurites: axons and dendrites. Since the precise connection of axons and dendrites is essential for information transmission in the brain, the correct spatial configuration of neurites across large numbers of neurons is imperative to form functional neural circuits in a limited space [1]. Each neuron type has its own unique three-dimensional (3D) structure of neurites, which has important implications directly related to neural function. During neurogenesis, neural stem cells are not morphologically polarized, but once they differentiate into immature neurons, they give rise to axons and dendrites [2,3]. To establish precise neural circuits, the developing axons and dendrites undergo distinct steps, including elongation, branching, pathfinding, and synaptic connection [4–9], and the systems that regulate neurite growth orientation, growth size, and arborization patterns are particularly fundamental to determine the spatial configuration of neurites (Fig. 1A).

Properly regulated neurite orientation is a crucial to the exact flow of information in neurons. For example, cerebellar Purkinje cells (PCs) extend their dendrites exclusively toward the molecular layer, the most superficial layer of the cerebellar cortex, where dendrites receive the local synaptic inputs from the parallel and climbing fibers [10] (Fig. 1B). By contrast, PC axons project toward the granule cell layer, which is the vertically opposite of the molecular layer, and eventually innervate the deep cerebellar nucleus to output information [11] (Fig. 1B). This oriented growth of PC dendrites and axons is the basis for the appropriate information flow in the cerebellum. Size control of neurites, especially dendrites, is also important for neural function. During development, the dendrites of individual neurons cover a defined area to correctly assemble the receptive

field that acts as an antenna to receive axonal inputs. In *Drosophila* larvae, the dendrites of each dendritic arborization (da) sensory neurons grow until they reach an appropriate size to cover a given space [12,13]. Then, tiling neighboring dendrites generate receptive fields that cover the entire body wall, allowing the larva to receive external cues [14]. Together with dendrite size control, the precise branching pattern formation of dendrites is also important for the assembly of functional receptive fields. Most dendrites have the so-called self-avoidance system, which prevents their own neurites from crossing each other to efficiently fill the receptive field [15–17]. Starburst amacrine cells in the retina [18–20], PCs [20–22], and *Drosophila* da neurons [23–25], exhibit well-organized nonredundant dendritic arborization patterns. Thus, various neurons across species are equipped with mechanisms to control the spatial configuration of neurites.

In this review, we summarize the regulatory mechanisms controlling neurite orientation, size, and arborization patterns that are critical for establishing the spatial configuration of neurites. Since dendrites have many distinctive systems to form sophisticated 3D structures, this review mainly focuses on the molecular mechanisms of dendritic growth and branching.

2. Control of neurite orientation

2.1 Establishment of neurite orientation during development

Determining neurite growth orientation is the first important step for establishing the proper spatial configuration of neurites. At the early stage of neurogenesis, the emergence of immature axons and dendrites breaks the morphological symmetry of neurons [26]. As soon as an axon/dendrite polarity is established, axons project toward far-distant destinations, whereas dendrites branch out to fill a defined area that is usually biased in a

particular direction. For example, similar to the axon/dendrite orientation in PCs as described in section 1, the pyramidal neurons in the cerebral cortex also develop the orientated axons and dendrites. These pyramidal neurons initially form multiple short neurites, one of which rapidly elongates tangentially along the ventricle to form an axon [27,28]. Subsequently, these neurons transform into a bipolar shape and migrate radially away from the ventricle to form an L-shaped axon [27,28]. By contrast, the dendrites of pyramidal neurons extend toward both the cortical surface and ventricle to develop apical and basal dendrites, respectively, thereby ensuring apicobasal polarity [28] (Fig. 2A). As will be discussed in section 2.2.1, this oriented neurite growth is regulated by classical guidance systems. Spiny stellate neurons in the barrel structure of the mouse somatosensory cortex are another example of oriented dendrites. Whisker pads and barrels form a one-to-one topography [29,30], and the accurate formation of each barrel structure is essential to create a functional topographic map for tactile inputs. The mature barrel is composed of spiny stellate neurons and thalamocortical axons (TCAs) projecting from the ventral posterior medial nucleus of the thalamus [31,32] (Fig. 2B). Spiny stellate neurons align the barrel edge and extend dendrites specifically toward the inside of the barrel to synapse with innervating TCAs [32]. In the early postnatal period, spiny stellate neurons extend their dendrites toward random directions but by the end of the first postnatal week, all but the dendrites extending toward the center of the barrel are eliminated, creating an inside-oriented dendritic structure [33] (Fig. 2B). Therefore, in addition to guidance system specifying neurite growth directions, pruning mechanisms are also involved in the formation of oriented neurite structures.

2.2 Mechanisms establishing oriented dendrite structures

Various neurons have oriented neurites, and their control mechanisms are diverse. Neurons in both invertebrates and vertebrates have strikingly oriented dendrites whose structures are important for appropriate neuronal inputs. Likewise, all axons show oriented growth, which is navigated by axon guidance mechanisms to correctly arrive at their target cells to form specific synapses.

2.2.1 Guidance systems directing neurite orientation

Many sets of attractive/repulsive receptor-ligand systems including Netrin-DCC, Slit-Robo, Ephrin-Eph, Semaphorin-Plexin/Neuropilin, and Wnt-Frizzled control axonal pathfinding in various neurons such as spinal commissural neurons, thalamocortical neurons, and retinal ganglion cells (RGCs) [34,35]. Although dendrites are far shorter than axons, guidance molecules are also involved in the mechanisms that determine dendritic orientation.

Semaphorin 3A (Sema3A) regulates axon guidance through chemorepulsive effects [36,37]. Sema3A induces growth cone collapse via its receptors Plexin/Neuropilin-1 (Nrp1) in dorsal root ganglion cells [38,39], and Sema3A-Nrp1 interaction instructs the patterning of axonal projections in the cerebral cortex [40]. Contrary to its repulsive effects on axons, Sema3A orchestrates the apicobasal dendritic orientation of cortical pyramidal neurons through chemoattractive effects (Fig. 2A). Sema3A derived from the cortical surface (marginal zone) attracts apical dendrites of pyramidal neurons toward the pia via Nrp1/Plexin A receptor complexes [41,42]. Layer V pyramidal neurons in the cerebral cortex of Sema3A-deficient mice show an aberrant orientation of dendritic projections including the horizontally directed dendrites, upside-down apical/basal dendrites, and dendrites without polarity [42]. Pathways regulating apical/basal dendrite

orientation downstream of Sema3A have also been identified. Since the Src family kinase Fyn, which associates with Plexin A2 and activates cyclin-dependent kinase (Cdk)5 pathways, plays a pivotal role in Sema3A signaling, the phenotypes of dendritic orientation in Fyn-deficient cortical neurons are quite reminiscent of those in Sema3A-deficient neurons [42]. Furthermore, distinct pathways downstream of Sema3A signaling seem to specifically determine apical or basal dendritic orientation. The deletion of p35, a neuron-specific Cdk5 activator, causes severe defects in the apical dendritic orientation of pyramidal neurons [43], suggesting that the Cdk5 pathway mainly controls the orientation of apical dendrites. The synaptic vesicle-binding protein synapsin III, a Cdk5 substrate, mediates Sema3A-Cdk5 pathway-dependent control of dendritic orientation because inhibition of synapsin III phosphorylation results in misoriented dendrites including completely upside-down apical and basal dendrites [44]. By contrast, the deletion of GSK3β, a serine/threonine kinase involved in Sema3A signaling, specifically impairs the orientation of basal dendrites [45]. The c-Jun N-terminal kinase pathway, which is activated by the serine/threonine kinase TAO2 that associates with Nrp1, is also required for the proper orientation of basal but not apical dendrites [46]. In addition to Sema3A, other semaphorins are involved in the apicobasal dendritic orientation of cortical neurons. Sema4D-Plexin B1 signaling inactivates the Ras-related signal transducer M-Ras to promote actin depolymerization that leads to inhibition of dendrite elongation [47]. In the cerebral cortex, the forced expression of a dominant negative Plexin B1 or constitutively activate M-Ras, increase dendritic branching and disorganize the apicobasal orientation of pyramidal neurons [47].

In *Drosophila* larvae, da sensory neurons also exhibit distinctive oriented dendrite structures. The da neurons extend their dendrites in a 2D plane at the basal surface of the

epithelium to cover the entire body wall [48]. The class I da neuron ddaE develops comb-like dendritic arborizations, with their primary dendrites extending along the dorsal-ventral axis and secondary dendrites along the anterior-posterior axis (Fig. 2C). The growth orientation of secondary dendrites is guided by gradients of cell adhesion molecules. The molecular gradient of Ten-m, a homophilic cell adhesion molecule of the teneurin family, along the anterior-posterior axis in the epidermis confines the growth of secondary dendrites of ddaE neurons [49]. High expression of Ten-m in ddaE neurons and the anterior-high/posterior-low expression of Ten-m in the dermis ensure the posterior-oriented comb-like growth of secondary dendrites.

Thus, guidance systems, including axon guidance and cell adhesion molecules, play important roles not only in axonal pathfinding but also in the regulation of dendrite orientation.

2.2.2 Pruning systems controlling neurite orientation

In addition to mechanisms determining neurite growth directions, neurite elimination, so-called pruning, also shapes neurite orientation. As described in section 2.1, spiny stellate neurons in the somatosensory cortex represent a pruning-based assembly of oriented dendrites [32] (Fig. 2B). In mice lacking the NR1 and NR2B subunits of the glutamatergic *N*-methyl-D-aspartate receptor (NMDAR) or the metabotropic glutamate receptor (mGluR)5, the dendritic asymmetry of spiny stellate neurons is abolished, causing loss of the barrel structure [50–52]. This strongly indicates that TCA input-dependent neural activity mediates dendrite pruning in spiny stellate neurons. Although the molecular basis for this selective dendrite pruning within a single neuron remains unknown, a Hebbian mechanism has been proposed [51]. At an early developmental stage,

randomly directed dendrites of spiny stellate neurons initially receive TCA inputs representing multiple whiskers. Should TCAs representing one whisker outnumber other TCA inputs representing different whiskers, the spiny stellate neuron may generate action potentials according to the firing pattern of the dominant TCAs. Over time, synchronous firing may strengthen these synapses and promote local growth of the dendrites that receive inputs from dominant TCAs, thereby becoming inside-oriented dendrites, whereas asynchronous firing may destabilize synapses and prune the dendrites representing other whiskers [51].

Another molecule, Broad complex, Tramtrack, and Bric-à-brac/Poxvirus and zinc finger (BTB/POZ) domain-containing 3 (Btbd3), has been identified as a regulator for dendrite pruning of spiny stellate neurons [53]. Deletion of Btbd3 abolishes the dendritic asymmetry of spiny stellate neurons and disrupts the well-defined barrel structure [53]. Btbd3 is considered a putative transcription factor and normally localizes in the nucleus. However, in mice with cortex-specific NR1 deficiency that show strongly suppressed neural activity, Btbd3 fails to localize to the nucleus and resides in the cytoplasm of spiny stellate neurons [53]. This indicates that increased neural activity during the early postnatal week triggers the nuclear translocation of Btbd3 to regulate target gene transcription, resulting in dendrite pruning of spiny stellate neurons. The loss of dendritic asymmetry in these neurons in mice lacking the LIM homeodomain transcription factor Lhx2, which positively regulates Btbd3 expression, also supports the importance of Btbd3 in barrel formation [54]. Furthermore, Sema7A-deficient mice lose the polarized dendrite structure of spiny stellate neurons, suggesting that a specific receptor-ligand system is crucial for dendrite pruning of this neuron [55]. Indeed, recent studies have demonstrated that Sema7A and its receptor Plexin C1 regulate the dendrite pruning of mitral cells

[56,57]. Further studies will be necessary to identify the molecular link between neural activity-dependent transcriptional regulation by Btbd3 and cell-surface signaling via Sema7A in dendrite pruning of spiny stellate neurons.

These neuronal activity-dependent and receptor-ligand systems identified in spiny stellate neurons also play important roles in the pruning of the oriented dendrites in other neurons. Mature mitral cells in the adult olfactory bulb project a single primary dendrite into only one glomerulus, in which the dendrite arborizes to synapse with olfactory neuron axons [58] (Fig. 2D). In the early postnatal period, this one-to-one dendrite projection is not established; multiple dendrites of a single mitral cell extend to multiple glomeruli. By postnatal day 6, only a single primary dendrite remains attached to a single glomerulus, and all other connections are lost [59]. In developing mitral cells, bone morphogenetic protein receptor (BMPR)2, a BMP receptor serving as a transmembrane serine/threonine kinase, stabilizes dendrites by promoting actin polymerization in a ligand BMP2/4-dependent and neuronal activity-dependent manner [60]. In dendrites with low neuronal activity, BMPR2 intracellularly sequesters LIMK, a major kinase involved in actin polymerization of mitral cells, resulting in actin depolymerization-induced dendrite pruning. Conversely, a ligand binding to BMPR2 releases LIMK to promote actin polymerization-mediated dendrite stabilization [60]. Thus, developing mitral cells either stabilize or prune dendrites depending on whether the BMP receptor-ligand system and neural activity act in concert. However, the mechanism that selects only one primary dendrite per mitral cell has not yet been elucidated.

3. Mechanism controlling neurite size

Correctly controlling the size of neurites, especially dendrites, is crucial for building

proper receptive fields and neural connections [61]. Dendrite sizes vary among neurons, and even the same neuron type may have different sizes depending on which target cells it connects to. In this section, we focus on three important mechanisms for neurite size control: competition-based, space filling-based, and targeting-based mechanisms.

Competition-based dendrite scaling depends on the relative acquisition level of external growth-promoting signals among neighboring neurons. In the cerebellum, the levels of the neurotrophin-3 (NT-3) –tropomyosin-related kinase C (TrkC) and cerebellin-1 (Cbln1) –glutamate delta2 (GluD2) signaling determine the dendrite size of PCs [62,63]. Space filling-based neurite scaling is essentially mediated by local repulsive signals from neighboring neurites. The repulsion of dendritic branches via cell-surface molecules, such as Slit-Robo, stops dendritic overgrowth [64]. This mechanism, so-called tiling, allows space to be efficiently covered by a population of neurons to form a functional receptive field. Targeting-based neurite scaling is tightly coupled with synaptic connections with target cells. In the retina, specific sets of cell adhesion molecules like down syndrome cell adhesion molecule (Dscam; see also section 4.2.1) and Sidekick are exclusively expressed in certain retinal neurons to connect each other in defined layers [65]. Axons or dendrites that have been able to connect with the appropriate target cells through specific cell adhesion molecules will stop growing at the right place, thereby determining neurite size.

3.1 Competition-based size control of PC dendrites

PCs develop a highly elaborate single dendrite that spans the entire molecular layer of the cerebellar cortex (Fig. 1B). Parallel fibers of granule cells (GCs) orthogonally penetrate PC dendritic arbors, a fan-like monoplanar structure, and form numerous GC-

PC synapses on a single PC [66].

NT-3 and its receptor TrkC control the size of PC dendritic arbors via competition-based mechanisms [62] (Fig. 3A). The GC-derived NT-3 binds to TrkC on PC dendrites. A mosaic analysis demonstrates that sparse deletion but not global deletion of TrkC in developing PCs significantly stunts their dendritic arbors. This TrkC-dependent dendrite growth in PCs is mediated by GC-derived NT-3 because additional NT-3 deletion ameliorates dendrite size defects caused by sparse TrkC deletion. Therefore, developing PCs compete for NT-3, and their dendrite sizes are determined by the relative levels of NT-3-TrkC signaling [62]. Moreover, the synapse organizers GluD2 and its presynaptic ligand Cbln1 regulate arbor sizes of PC dendrites through similar competition-based mechanisms described for NT-3-TrkC signaling [63] (Fig. 3A). During development, PCs express GluD2 on their dendrites, while GCs secrete Cbln1. Cbln1 also binds neurexin, a presynaptic receptor on parallel fibers; thus, GluD2, Cbln1, and neurexin form a tripartite synaptic complex that promotes the formation of GC-PC synapses [67]. Sparse but not global deletion of GluD2 decreases or increases the ramification of PC dendrites in the deep or superficial molecular layer, respectively [63] (Fig. 3A). By contrast, of GluD2 overexpression in developing PCs causes dendrite overelaboration in the deep molecular layer [63] (Fig. 3A). Additional Cbln1 deletion rescues the ramification phenotype induced by sparse GluD2 deletion, indicating that the relative levels of Cbln1-GluD2 signaling define PC arbor sizes in the deeper/superficial molecular layers [63]. Thus, competition for forming synapses with GCs determines the size of dendritic arbors in PCs.

Collectively, receptor-ligand systems in PCs demonstrate that competition for external signals among a neuronal population is a crucial mechanism for determining dendrite size.

3.2 Space filling-based size control of sensory neuron dendrites in *Drosophila*

The basis of tiling is neighboring cell-dependent scaling of dendrites, and excessive dendrite extension is inhibited by repulsive interactions with neighboring dendrites via cell-surface molecules on dendrites [17]. An appropriate repulsive signal stops the dendrite from growing, but if this signal is removed, the dendrite will continue to extend even after contact with neighboring dendrites, resulting in increased size. This regulatory mechanism defines the size of dendrites and allows neurons to align in a tiling pattern without overlapping (Fig. 3B). Several cell-surface molecules involved in tiling, such as Dscam, Slit-Robo, and Semaphorin-Plexin, have been identified in *Drosophila* larval sensory neurons and retinal amacrine cells [68]. In *Drosophila* larval class IV da neurons, the deletion of Robo or Slit, a repulsive receptor and its ligand, increases dendrite size, whereas Robo overexpression decreases it [64], indicating that the level of Slit-Robo repulsive signaling scales dendritic arbors (Fig. 3B).

In class IV da neurons, additional transcription factors and kinases have been identified to be involved in tiling-based control of dendrite sizes. Deletion of the transcription factors Cut and Knot, or the tumor suppressor kinase Hippo results in a decreased dendrite size, whereas overexpression of these genes increases the size [69–71]. The up- and downstream signals of this phenomenon are not well understood, but some repulsive cell-surface molecules may be involved.

3.3 Laminar-specific neurite size control in the retina

Layer-specific synaptic connections in the retina are closely related to arborization sizes of neurites. In the retina, the visual input gathered by photoreceptors is transmitted to RGCs via bipolar cells. Processes of the interneurons, including bipolar and amacrine

cells, form synapses on RGC dendrites in the inner plexiform layer (IPL) which is further divided into five major sublaminae, S1 to S5 (Fig. 3C). In the chick, subsets of interneurons and RGCs expressing the same cell adhesion molecules, such as Dscam, DscamL, Sidekick1, Sidekick2, and contactin, specifically form synapses via their homophilic binding in one or a few of these sublaminae [65,72] (Fig. 3D). Ectopic expression or deletion of these cell adhesion molecules in RGCs impairs their laminar-specific targeting of dendrites, eventually altering dendrite size. For example, Dscam-expressing RGCs normally project their dendrites into sublamina 5, but the deletion of Dscam in those RGCs results in overshooting of their dendrites far beyond this sublamina [65] (Fig. 3D). A similar homophilic cell adhesion system organizes the laminar targeting of bipolar cells and defines their arbor sizes in the mouse retina. Cadherin 8 and Cadherin 9, the type II cadherins, are selectively expressed in sublamina 2-projecting OFF bipolar cells (Type2 OFF) and sublamina 5-projecting ON bipolar cells (Type5 ON), respectively, to form layer-specific synaptic connections with direction-selective ganglion cells [73]. Altered expression levels of Cadherin 8 and Cadherin 9 in Type2 OFF or Type5 ON bipolar cells result in changes of their axonal arbor sizes that are associated with the mistargeting of axons [73]. Furthermore, a repulsion-based system instructs the neurite sizes of retinal neurons. The repulsive molecule Sema6A and its receptors Plexin A2 and Plexin A4 play important roles in the laminar targeting of mouse retinal neurons [74,75]. Sema6A is expressed in RGCs and amacrine cells that project to the ON layer (sublaminae S3-S5 of the IPL), whereas Plexin A2 and Plexin A4 are expressed in bipolar and amacrine cells that project to the OFF layer (sublaminae S1 and S2 of the IPL) (Fig. 3E). Based on this complementary expression pattern, Sema6A, for example, in dendrites of RGCs and amacrine cells, which terminate in the ON layer, strongly repels Plexin A2-

expressing dendrites of amacrine cells and excludes them from the ON layer, keeping them in the OFF layer [74] (Fig. 3E). The loss of Sema6A, Plexin A2, and Plexin A4 in retinal neurons causes overshooting of neurites beyond the destined ON/OFF layer [74,75] (Fig. 3E).

Taken together, cell-surface molecules-mediated laminar targeting defines axon/dendrite sizes in the retina.

4. Control of arborization patterns via self-avoidance mechanisms

4.1 Self-avoidance in invertebrate and vertebrate neurons

To receive synaptic inputs and process information accurately and efficiently, complex dendritic branches of each neuron must fill the space in an orderly fashion without colliding. Along with the tiling mechanism involving neighboring cells, self-avoidance is one of the crucial systems for establishing space-filling dendritic arborization in which sister branches of the same neuron avoid crossing each other and bundling, thereby maximally and evenly covering a defined space with dendrites [14] (Fig. 4A). This phenomenon was first observed in mechanosensory neurons of the leech *Haementeria ghilianii* [76]. Subsequently, numerous genetic studies in invertebrate neurons, such as *Drosophila* da sensory neurons [23–25] and *Caenorhabditis elegans* PVD nociceptive sensory neurons [77], have elucidated the molecular machinery of dendrite self-avoidance. Recent studies have identified molecules involved in dendrite self-avoidance in vertebrate neurons, such as mouse RGCs, amacrine cells, and PCs [18,19,21,22]. Therefore, self-avoidance is a highly conserved physiological mechanism to establish an appropriate spatial configuration of dendrites.

4.2 Repulsion-based regulators of dendrite self-avoidance

Various molecules that regulate dendrite self-avoidance have been identified in invertebrates and vertebrates (Table 1). Among them, cell-surface molecules are of great importance because local repulsion of neurites via specific cell-surface molecules is a pivotal common mechanism to prevent the intermingling of neurites from the same neuron [17]. This repulsion-based mechanism allows dendrites to spread out as much as possible while minimizing overlap. To specifically repel their own neurites, neurons need to discriminate "self" from "non-self" neurites, and the fascinating mechanisms underlying this discrimination have been demonstrated for two different molecules, Dscam and clustered protocadherins (Pcdhs) [20,23–25]. By matching isoforms on the neurite surface, the diverse extracellular domains of Dscam1 and Pcdhs recognize "self" or "non-self" via homophilic binding of the same isoform and cause repulsion of self-neurite [78].

4.2.1 Dscams

Dscam is an evolutionarily conserved cell adhesion molecule with homophilic binding ability [79,80]. *Drosophila* Dscam1, which was originally identified as a binding protein for the SH2/SH3 adaptor protein Dock [80], first demonstrated the importance of self-avoidance mechanisms via homophilic binding of cell-surface molecules [23–25]. Suppression of Dscam1 expression in *Drosophila* da sensory neurons or mushroom body neurons results in the clumping of dendrites from the same neuron [23–25,81] (Fig. 4B). Dscam1 is a single-pass transmembrane protein that contains 10 immunoglobulin (Ig) domains and 6 fibronectin repeats in the extracellular region [80]. Intriguingly, selective splicing of the *Dscam1* gene can give rise to 19,008 and 2 isoforms in the extracellular

and transmembrane regions, respectively. Therefore, a *Dscam1* gene can theoretically produce 38,016 isoforms [80] (Fig. 4C). Individual neurons express only one random Dscam1 isoform in neurites, and the characteristics of homophilic binding between identical isoforms cause the repulsive effect [82]. In the extracellular region of Dscam1, three domains, Ig2, Ig3, and Ig7, undergo selective splicing, and a strong homophilic binding occurs only between isoforms that completely match these three domains [82] (Fig. 4C). This system matching a huge number of Dscam1 isoforms contributes to the discrimination between "self" and "non-self", thereby repelling only dendrites of the same neuron [83]. By contrast, there are two mouse *Dscam* genes, *Dscam* and *Dscaml1*, both of which do not show the same splicing diversity as *Dscam1* in *Drosophila* [19]. Thus, murine DSCAM and DSCAML1 may not be involved in the discrimination between "self" and "non-self". Nevertheless, loss-of-function experiments demonstrate that DSCAM and DSCAML1 play critical roles in dendrite self-avoidance in RGCs, amacrine cells, and bipolar cells [19]. DSCAM and DSCAML1 prevent excessive neurite contacts by inhibiting cell adhesion through other molecules, such as cadherins, rather than directly producing repulsive effects [84].

4.2.2 Clustered protocadherins

Since mouse DSCAMs lack isoform diversity, other molecules may be responsible for discriminating between "self" and "non-self" dendrites in mammals. Clustered Pcdhs, which have many isoforms, became candidate molecules for a role similar to that of *Drosophila* Dscam1. In mice, three Pcdh clusters, Pcdhα, Pcdhβ, and Pcdhγ, produce via promoter selection 14, 22, and 22 isoforms, respectively, resulting in 58 isoforms [85] (Fig. 4D). Dendrites of the same neuron express the same Pcdhγ isoform repertoire,

whereas different neurons have different repertoires [86, 87]. The six extracellular domains of the same Pcdhγ isoforms specifically form a zipper-like structure between the dendrites of the same neuron, resulting in a local repulsive effect [86, 87]. Mice lacking the entire Pcdhγ cluster exhibit severe abnormalities in dendrite self-avoidance in starburst amacrine cells and PCs [20] (Fig. 4E). Furthermore, deletion of both Pcdhα and Pcdhγ clusters show more severe abnormalities in dendrite self-avoidance in those neurons than Pcdhγ alone deletion, indicating that Pcdhs mediate dendrite self-avoidance in a cooperative manner [88].

4.2.3 Other regulators of self-avoidance

In addition to Dscams and Pcdhs, various molecules have been demonstrated to regulate dendrite self-avoidance. Many other cell-surface systems control dendrite self-avoidance, especially in invertebrates. In *C. elegans*, the ternary interaction of epidermal SAX-7 and MNR-1 with DMA-1 causes dendritic branching and self-avoidance in PVD nociceptive neurons [89,90]. Likewise, the binding of the secreted ligand UNC-6/netrin to its cell-surface receptors UNC-40 and UNC-5 activates dendrite self-avoidance in PVD nociceptive neurons [91]. Furthermore, the transmembrane protein MIG-14 regulates self-avoidance via homophilic binding in PVD nociceptive neurons [92], indicating that homophilic interaction of cell-surface molecules is also crucial to self-avoidance in *C. elegans*. In *Drosophila*, the seven-pass transmembrane cadherin Flamingo binds to the LIM domain protein Espinas to activate dendrite self-avoidance in da sensory neurons [93]. The conserved Ig superfamily member Turtle is also involved in self-avoidance in da neurons [94]. Interestingly, the intracellular region of Turtle is dispensable to dendritic branching, suggesting that Turtle regulates self-avoidance as a ligand or co-receptor for

yet unidentified molecules [94]. Moreover, integrins and Plexin B in da neurons control dendrite self-avoidance via binding to epithelium-derived laminin and Sema2b, respectively [95,96]. Similar to the Semaphorin-Plexin system in *Drosophila*, Sema6A and Plexin A2/A4 play essential roles in dendrite self-avoidance in retinal starburst amacrine cells of mice [75,97]. Slit2-Robo2 is also a repulsive ligand-receptor complex that prevents self-crossing of highly elaborate PC dendrites [21] (Fig. 4D).

Other molecules regulating signal transduction and cytoskeletal arrangement have been implicated in self-avoidance mechanisms. Downstream of the UNC-6–UNC-40–UNC-5 cell-surface complex, intracellular UNC-34, WSP-1, UNC-73, MIG-10, and the Arp2/3 complex promote dendrite self-avoidance via the dendrite retraction in *C. elegans* PVD nociceptive neurons [98]. In *Drosophila*, the target of rapamycin complex 2 interacts with Plexin B to regulate dendrite self-avoidance [96]. In PCs, the LKB1-SIK1/2 kinase pathway controls the dendritic localization of Robo2 to prevent self-crossing and clumping of dendrites [22], highlighting the importance of cellular delivery systems for specific cell-surface molecules involved in self-avoidance (Fig. 4D). Moreover, a recent study demonstrated that the inverse-BAR protein MTSS1 orchestrates via binding to the actin nucleator formin DAAM1 the contact-dependent retraction of dendritic protrusions leading to self-avoidance in PCs [99].

In conclusion, various regulatory systems based on the interactions of cell-surface molecules ensure neuronal self-avoidance which is essential for precise neural connectivity and information processing.

5. Conclusions and future directions

In this review, we highlight the mechanisms that control neurite orientation, size, and

arborization patterns and that are essential for establishing the spatial configuration of neurites, especially dendrites. These regulatory mechanisms allow dendrites to build functional receptive fields that are crucial for accurate synaptic input. Cell-surface molecules play particularly important roles in these mechanisms. For example, interactions between neurites via homophilic cell adhesion molecules and repulsive receptor-ligands, such as Dscam, Slit-Robo, and Semaphorin-Plexin, are all involved in the control of dendrite orientation, size, and self-avoidance. Additionally, glutamate receptors like NMDARs and mGluR5 are involved in neural activity-dependent pruning that determines dendrite orientation, and the neurotorophin NT-3 and synapse organizer Cbln1 control competitive dendrite scaling. These highly elaborate systems establish the functional spatial configuration of neurites.

However, some important phenomena regarding the spatial configuration of neurites remain to be fully elucidated. For example, PCs generate characteristic monoplanar dendrites presumably to receive efficient input from parallel fibers and to appropriately integrate neural information in the cerebellum [66] (Fig. 1B). Although the cytoskeletal protein βIII-spectrin is involved in the formation of the monoplanar PC dendrites, the underlying mechanism remains unknown [100,101]. In the formation of the 2D dendrite structure of *Drosophila* sensory neurons, which is similar to that of PC dendrites, the interaction between integrins on the dendrites and laminin in the extracellular matrix restricts the growth orientation of dendrites in the 2D space beneath the epidermis [48]. This repulsive integrin-laminin signal regulates dendrite patterning, as in other systems that control neurite structures. Therefore, a universal system across species via cell-surface molecules may also be involved in the formation of monoplanar PC dendrites. Future studies elucidating such unidentified mechanisms will facilitate our understanding

of the assembly of elaborate neural circuits.

Acknowledgement

We are grateful to all the present members of the Kuwako laboratory for discussions of this manuscript. This work was supported by grants from the JSPS KAKENHI (21K20692) and the Takeda Science Foundation to K.H., as well as grants from the JSPS KAKENHI (19K22476 and 20H03352), Daiichi Sankyo Foundation of Life Science, The Sumitomo Foundation, Life Science Foundation of Japan, The Mother and Child Health Foundation to K.K.

Competing interest declaration

The authors have no competing interests to declare.

References

[1] L. Luo, Architectures of neuronal circuits, Science. 373 (2021). https://doi.org/10.1126/science.abg7285.

[2] S. Tahirovic, F. Bradke, Neuronal polarity., Cold Spring Harbor Perspectives in Biology. 1 (2009). https://doi.org/10.1101/cshperspect.a001644.

[3] J. Arikkath, Mechanisms of axon polarization in pyramidal neurons, Molecular and Cellular Neuroscience. 107 (2020) 103522. https://doi.org/10.1016/j.mcn.2020.103522.

[4] L. Luo, Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity, Annual Review of Cell and Developmental Biology. 18 (2002) 601–635. https://doi.org/10.1146/annurev.cellbio.18.031802.150501.

[5] J.L. Goldberg, Intrinsic neuronal regulation of axon and dendrite growth, Current Opinion in Neurobiology. 14 (2004) 551–557. https://doi.org/10.1016/j.conb.2004.08.012.

[6] T.W. Yu, C.I. Bargmann, Dynamic regulation of axon guidance, Nature Neuroscience. 4 (2001) 1169–1176. https://doi.org/10.1038/nn748.

[7] M. O'Donnell, R.K. Chance, G.J. Bashaw, Axon growth and guidance: Receptor regulation and signal transduction, Annual Review of Neuroscience. 32 (2009) 383–412. https://doi.org/10.1146/annurev.neuro.051508.135614.

[8] J.R. Sanes, M. Yamagata, Many paths to synaptic specificity, Annual Review of Cell and Developmental Biology. 25 (2009) 161–195. https://doi.org/10.1146/annurev.cellbio.24.110707.175402.

[9] J.R. Sanes, S.L. Zipursky, Synaptic Specificity, Recognition Molecules, and Assembly of Neural Circuits, Cell. 181 (2020) 536–556. https://doi.org/10.1016/j.cell.2020.04.008.

[10] M.E. van der Heijden, R. V. Sillitoe, Interactions Between Purkinje Cells and Granule Cells Coordinate the Development of Functional Cerebellar Circuits, Neuroscience. 462 (2021) 4–21. https://doi.org/10.1016/j.neuroscience.2020.06.010.

[11] B.R. Sastry, W. Morishita, S. Yip, T. Shew, GABA-ergic transmission in deep cerebellar nuclei, Progress in Neurobiology. 53 (1997) 259–271. https://doi.org/10.1016/s0301-0082(97)00033-6.

[12] W.B. Grueber, L.Y. Jan, Y.N. Jan, Tiling of the *Drosophila* epidermis by multidendritic sensory neurons, Development. 129 (2002) 2867–2878. https://doi.org/10.1242/dev.129.12.2867.

[13] L. Kilo, T. Stürner, G. Tavosanis, A.B. Ziegler, *Drosophila* dendritic arborisation neurons: Fantastic actin dynamics and where to find them, Cells. 10 (2021). https://doi.org/10.3390/cells10102777.

[14] Y.N. Jan, L.Y. Jan, Branching out: Mechanisms of dendritic arborization, Nature Reviews Neuroscience. 11 (2010) 316–328. https://doi.org/10.1038/nrn2836.

[15] A.P. Kramer, J.Y. Kuwada, Formation of the receptive fields of leech mechanosensory neurons during embryonic development, The Journal of Neuroscience. 3 (1983) 2474–2486. https://doi.org/10.1523/JNEUROSCI.03-12-02474.1983.

[16] A.P. Kramer, G.S. Stent, Developmental arborization of sensory neurons in the leech *Haementeria ghilianii*. II. Experimentally induced variations in the branching pattern, The Journal of Neuroscience. 5 (1985) 768–775. https://doi.org/10.1523/JNEUROSCI.05-03-00768.1985.

[17] W.B. Grueber, A. Sagasti, Self-avoidance and tiling: Mechanisms of dendrite and axon spacing., Cold Spring Harbor Perspectives in Biology. 2 (2010). https://doi.org/10.1101/cshperspect.a001750.

[18] P.G. Fuerst, A. Koizumi, R.H. Masland, R.W. Burgess, Neurite arborization and

mosaic spacing in the mouse retina require DSCAM, Nature. 451 (2008) 470–474. https://doi.org/10.1038/nature06514.

[19] P.G. Fuerst, F. Bruce, M. Tian, W. Wei, J. Elstrott, M.B. Feller, L. Erskine, J.H. Singer, R.W. Burgess, DSCAM and DSCAML1 function in self-avoidance in multiple cell types in the developing mouse retina, Neuron. 64 (2009) 484–497. https://doi.org/10.1016/j.neuron.2009.09.027.

[20] J.L. Lefebvre, D. Kostadinov, W. v. Chen, T. Maniatis, J.R. Sanes, Protocadherins mediate dendritic self-avoidance in the mammalian nervous system, Nature. 488 (2012) 517–521. https://doi.org/10.1038/nature11305.

[21] D.A. Gibson, S. Tymanskyj, R.C. Yuan, H.C. Leung, J.L. Lefebvre, J.R. Sanes, A. Chédotal, L. Ma, Dendrite self-avoidance requires cell-autonomous slit/robo signaling in cerebellar purkinje cells, Neuron. 81 (2014) 1040–1056. https://doi.org/10.1016/j.neuron.2014.01.009.

[22] K. Kuwako, H. Okano, The LKB1-SIK Pathway Controls Dendrite Self-Avoidance in Purkinje Cells, Cell Reports. 24 (2018) 2808-2818.e4. https://doi.org/10.1016/j.celrep.2018.08.029.

[23] P. Soba, S. Zhu, K. Emoto, S. Younger, S.J. Yang, H.H. Yu, T. Lee, L.Y. Jan, Y.N. Jan, *Drosophila* sensory neurons require Dscam for dendritic self-avoidance and proper dendritic field organization, Neuron. 54 (2007) 403–416. https://doi.org/10.1016/j.neuron.2007.03.029.

[24] M.E. Hughes, R. Bortnick, A. Tsubouchi, P. Bäumer, M. Kondo, T. Uemura, D. Schmucker, Homophilic Dscam interactions control complex dendrite morphogenesis, Neuron. 54 (2007) 417–427. https://doi.org/10.1016/j.neuron.2007.04.013.

[25] B.J. Matthews, M.E. Kim, J.J. Flanagan, D. Hattori, J.C. Clemens, S.L. Zipursky, W.B. Grueber, Dendrite self-avoidance is controlled by Dscam, Cell. 129 (2007) 593–604. https://doi.org/10.1016/j.cell.2007.04.013.

[26] J. Hakanen, N. Ruiz-Reig, F. Tissir, Linking cell polarity to cortical development and malformations, Frontiers in Cellular Neuroscience. 13 (2019). https://doi.org/10.3389/fncel.2019.00244.

[27] Y. Hatanaka, K. Yamauchi, Excitatory cortical neurons with multipolar shape establish neuronal polarity by forming a tangentially oriented axon in the intermediate zone, Cerebral Cortex. 23 (2013) 105–113. https://doi.org/10.1093/cercor/bhr383.

[28] A. Sakakibara, Y. Hatanaka, Neuronal polarization in the developing cerebral cortex, Frontiers in Neuroscience. 9 (2015).

https://doi.org/10.3389/fnins.2015.00116.

[29] C.C.H. Petersen, The functional organization of the barrel cortex., Neuron. 56 (2007) 339–355. https://doi.org/10.1016/j.neuron.2007.09.017.

[30] J.F. Staiger, C.C.H. Petersen, Neuronal circuits in barrel cortex for whisker sensory perception, Physiological Reviews. 101 (2021) 353-415. https://doi.org/10.1152/physrev.00019.2019.

[31] A. Agmon, L.T. Yang, D.K. O'dowd, E.G. Jones, Organized growth of thalamocortical axons from the deep tier of terminations into layer IV of developing mouse barrel cortex, The Journal of Neuroscience. 73 (1993) 53655362. https://doi.org/10.1523/JNEUROSCI.13-12-05365.1993.

[32] A. Datwani, T. Iwasato, S. Itohara, R.S. Erzurumlu, NMDA receptor-dependent pattern transfer from afferents to postsynaptic cells and dendritic differentiation in the barrel cortex, Molecular and Cellular Neuroscience. 21 (2002) 477–492. https://doi.org/10.1006/mcne.2002.1195.

[33] S. Nakazawa, H. Mizuno, T. Iwasato, Differential dynamics of cortical neuron dendritic trees revealed by long-term in vivo imaging in neonates, Nature Communications. 9 (2018). https://doi.org/10.1038/s41467-018-05563-0.

[34] I. Dudanova, R. Klein, Integration of guidance cues: Parallel signaling and crosstalk, Trends in Neurosciences. 36 (2013) 295–304. https://doi.org/10.1016/j.tins.2013.01.007

[35] R. Klein, R.J. Pasterkamp, Recent advances in inter-cellular interactions during neural circuit assembly, Current Opinion in Neurobiology. 69 (2021) 25-32. https://doi.org/10.1016/j.conb.2020.12.004.

[36] Y. Luo, D. Raible, J.A. Raper, Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones, Cell. 75 (1993) 217–227. https://doi.org/10.1016/0092-8674(93)80064-l.

[37] E.K. Messersmith, E.D. Leonardo, C.J. Shatz, M. Tessier-Lavigne, C.S. Goodman, A.L. Kolodkin, Semaphorin III can function as a selective chemorepellent to pattern sensory projections in the spinal cord, Neuron. 14 (1995) 949–959. https://doi.org/10.1016/0896-6273(95)90333-x.

[38] T. Takahashi, A. Fournier, F. Nakamura, L.-H. Wang, Y. Murakami, R.G. Kalb, H. Fujisawa, S.M. Strittmatter, Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors, Cell. 99 (1999) 59–69. https://doi.org/10.1016/s0092-8674(00)80062-8.

[39] L. Tamagnone, S. Artigiani, H. Chen, Z. He, G. Ming, H. Song, A. Chedotal, M.L. Winberg, C.S. Goodman, M. Poo, M. Tessier-Lavigne, P.M. Comoglio, Plexins

are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates, Cell. 99 (1999) 71–80. https://doi.org/10.1016/s0092-8674(00)80063-x.

[40] F. Polleux, R.J. Giger, D.D. Ginty, A.L. Kolodkin, A. Ghosh, Patterning of cortical efferent projections by semaphorin-neuropilin interactions, Science. 282 (1998) 1904–1906. https://doi.org/10.1126/science.282.5395.1904.

[41] F. Polleux, T. Morrow, A. Ghosh, Semaphorin 3A is a chemoattractant for cortical apical dendrites, Nature. 404 (2000) 567–573. https://doi.org/10.1038/35007001.

[42] Y. Sasaki, C. Cheng, Y. Uchida, O. Nakajima, T. Ohshima, T. Yagi, M. Taniguchi, T. Nakayama, R. Kishida, Y. Kudo, S. Ohno, F. Nakamura, Y. Goshima, Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex, Neuron. 35 (2002) 907–920. https://doi.org/10.1016/s0896-6273(02)00857-7.

[43] T. Chae, Y.T. Kwon, R. Bronson, P. Dikkes, E. Li, L.H. Tsai, Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality, Neuron. 18 (1997) 29–42. https://doi.org/10.1016/s0896-6273(01)80044-1.

[44] L.E. Perlini, J. Szczurkowska, B.A. Ballif, A. Piccini, S. Sacchetti, S. Giovedí, F. Benfenati, L. Cancedda, Synapsin III acts downstream of semaphorin 3A/CDK5 signaling to regulate radial migration and orientation of pyramidal neurons in vivo, Cell Reports. 11 (2015) 234–248. https://doi.org/10.1016/j.celrep.2015.03.022.

[45] M. Morgan-Smith, Y. Wu, X. Zhu, J. Pringle, W.D. Snider, GSK-3 signaling in developing cortical neurons is essential for radial migration and dendritic orientation, eLife. 3 (2014) e02663. https://doi.org/10.7554/eLife.02663.

[46] F.C. de Anda, A.L. Rosario, O. Durak, T. Tran, J. Gräff, K. Meletis, D. Rei, T. Soda, R. Madabhushi, D.D. Ginty, A.L. Kolodkin, L.H. Tsai, Autism spectrum disorder susceptibility gene TAO2 affects basal dendrite formation in the neocortex, Nature Neuroscience. 15 (2012) 1022–1031. https://doi.org/10.1038/nn.3141.

[47] G.I. Tasaka, M. Negishi, I. Oinuma, Semaphorin 4D/Plexin-B1-mediated M-Ras GAP activity regulates actin-based dendrite remodeling through Lamellipodin, Journal of Neuroscience. 32 (2012) 8293–8305. https://doi.org/10.1523/JNEUROSCI.0799-12.2012.

[48] C. Han, D. Wang, P. Soba, S. Zhu, X. Lin, L.Y. Jan, Y.N. Jan, Integrins Regulate Repulsion-Mediated Dendritic Patterning of *Drosophila* Sensory Neurons by Restricting Dendrites in a 2D Space, Neuron. 73 (2012) 64–78.

https://doi.org/10.1016/j.neuron.2011.10.036.

[49] Y. Hattori, T. Usui, D. Satoh, S. Moriyama, K. Shimono, T. Itoh, K. Shirahige, T. Uemura, Sensory-neuron subtype-specific transcriptional programs controlling dendrite morphogenesis: Genome-wide analysis of abrupt and knot/collier, Developmental Cell. 27 (2013) 530–544. https://doi.org/10.1016/j.devcel.2013.10.024.

[50] T. Iwasato, A. Datwani, A.M. Wolf, H. Nishiyama, Y. Taguchi, S. Tonegawa, T. Knopfel, R.S. Reha S. Erzurumlu, S. Itohara, Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex, Nature. 406 (2000) 726–731. https://doi.org/10.1038/35021059.

[51] J.S. Espinosa, D.G. Wheeler, R.W. Tsien, L. Luo, Uncoupling dendrite growth and patterning: single-cell knockout analysis of NMDA receptor 2B, Neuron. 62 (2009) 205–217. https://doi.org/10.1016/j.neuron.2009.03.006.

[52] C.J. Ballester-Rosado, M.J. Albright, C.S. Wu, C.C. Liao, J. Zhu, J. Xu, L.J. Lee, H.C. Lu, mGluR5 in cortical excitatory neurons exerts both cell-autonomous and -nonautonomous influences on cortical somatosensory circuit formation, Journal of Neuroscience. 30 (2010) 16896–16909. https://doi.org/10.1523/JNEUROSCI.2462-10.2010.

[53] A. Matsui, M. Tran, C.A. Yoshida, S.S. Kikuchi, T. Michael, M. U, M. Ogawa, T. Shimogori, BTBD3 controls dendrite orientation toward active axons in mammalian neocortex, Science. 342 (2013) 1111–1114. https://doi.org/10.1126/science.1244505.

[54] C.F. Wang, H.W. Hsing, Z.H. Zhuang, M.H. Wen, W.J. Chang, C.G. Briz, M. Nieto, B.C. Shyu, S.J. Chou, Lhx2 Expression in Postmitotic Cortical Neurons Initiates Assembly of the Thalamocortical Somatosensory Circuit, Cell Reports. 18 (2017) 849–856. https://doi.org/10.1016/j.celrep.2017.01.001.

[55] I. Carcea, S.B. Patil, A.J. Robison, R. Mesias, M.M. Huntsman, R.C. Froemke, J.D. Buxbaum, G.W. Huntley, D.L. Benson, Maturation of cortical circuits requires Semaphorin 7A, Proceedings of the National Academy of Sciences of the United States of America. 111 (2014) 13978–13983. https://doi.org/10.1073/pnas.1408680111.

[56] N. Inoue, H. Nishizumi, H. Naritsuka, H. Kiyonari, H. Sakano, Sema7A/PlxnCl signaling triggers activity-dependent olfactory synapse formation, Nature Communications. 9 (2018). https://doi.org/10.1038/s41467-018-04239-z.

[57] N. Inoue, H. Nishizumi, R. Ooyama, K. Mogi, K. Nishimori, T. Kikusui, H. Sakano, The olfactory critical period is determined by activity-dependent

Sema7A/PlxnC1 signaling within glomeruli, eLife. 10 (2021). https://doi.org/10.7554/eLife.65078.

[58] K. Mori, H. Sakano, How is the olfactory map formed and interpreted in the mammalian brain?, Annual Review of Neuroscience. 34 (2011) 467–499. https://doi.org/10.1146/annurev-neuro-112210-112917.

[59] D.M. Lin, F. Wang, G. Lowe, G.H. Gold, R. Axel, J. Ngai, L. Brunet, Formation of precise connections in the olfactory bulb occurs in the absence of odorant-evoked neuronal activity, Neuron. 26 (2000) 69–80. https://doi.org/10.1016/s0896-6273(00)81139-3.

[60] S. Aihara, S. Fujimoto, R. Sakaguchi, T. Imai, BMPR-2 gates activity-dependent stabilization of primary dendrites during mitral cell remodeling, Cell Reports. 35 (2021). https://doi.org/10.1016/j.celrep.2021.109276.

[61] J.L. Lefebvre, J.R. Sanes, J.N. Kay, Development of dendritic form and function, Annual Review of Cell and Developmental Biology. 31 (2015) 741–777. https://doi.org/10.1146/annurev-cellbio-100913-013020.

[62] William Joo, Simon Hippenmeyer, Liqun Luo, Dendrite morphogenesis depends on relative levels of NT-3/TrkC signaling, Science. 346 (2014) 626–629. https://doi.org/10.1126/science.1256717.

[63] Y.H. Takeo, S.A. Shuster, L. Jiang, M.C. Hu, D.J. Luginbuhl, T. Rülicke, X. Contreras, S. Hippenmeyer, M.J. Wagner, S. Ganguli, L. Luo, GluD2- and Cbln1-mediated competitive interactions shape the dendritic arbors of cerebellar Purkinje cells, Neuron. 109 (2021) 629-644.e8. https://doi.org/10.1016/j.neuron.2020.11.028.

[64] S. Dimitrova, A. Reissaus, G. Tavosanis, Slit and Robo regulate dendrite branching and elongation of space-filling neurons in *Drosophila*, Developmental Biology. 324 (2008) 18–30. https://doi.org/10.1016/j.ydbio.2008.08.028.

[65] M. Yamagata, J.R. Sanes, Dscam and Sidekick proteins direct lamina-specific synaptic connections in vertebrate retina, Nature. 451 (2008) 465–469. https://doi.org/10.1038/nature06469.

[66] C. Sotelo, I. Dusart, Intrinsic versus extrinsic determinants during the development of Purkinje cell dendrites, Neuroscience. 162 (2009) 589–600. https://doi.org/10.1016/j.neuroscience.2008.12.035.

[67] M. Yuzaki, The C1q complement family of synaptic organizers: not just complementary, Current Opinion in Neurobiology. 45 (2017) 9–15. https://doi.org/10.1016/j.conb.2017.02.002.

[68] T.Y. Lin, P.J. Chen, H.H. Yu, C.P. Hsu, C.H. Lee, Extrinsic Factors Regulating

Dendritic Patterning, Frontiers in Cellular Neuroscience. 14 (2021). https://doi.org/10.3389/fncel.2020.622808.

[69] W.B. Grueber, L.Y. Jan, Y. Nung Jan, Different levels of the homeodomain protein cut regulate distinct dendrite branching patterns of *Drosophila* multidendritic neurons, Cell. 112 (2003) 805–818. https://doi.org/10.1016/s0092-8674(03)00160-0.

[70] K. Emoto, J.Z. Parrish, L.Y. Jan, Y.N. Jan, The tumour suppressor Hippo acts with the NDR kinases in dendritic tiling and maintenance, Nature. 443 (2006) 210–213. https://doi.org/10.1038/nature05090.

[71] S. Jinushi-Nakao, R. Arvind, R. Amikura, E. Kinameri, A.W. Liu, A.W. Moore, Knot/Collier and cut control different aspects of dendrite cytoskeleton and synergize to define final arbor shape, Neuron. 56 (2007) 963–978. https://doi.org/10.1016/j.neuron.2007.10.031.

[72] M. Yamagata, J.R. Sanes, Expanding the Ig superfamily code for laminar specificity in retina: Expression and role of contactins, Journal of Neuroscience. 32 (2012) 14402–14414. https://doi.org/10.1523/JNEUROSCI.3193-12.2012.

[73] X. Duan, A. Krishnaswamy, I. de La Huerta, J.R. Sanes, Type II cadherins guide assembly of a direction-selective retinal circuit, Cell. 158 (2014) 793–807. https://doi.org/10.1016/j.cell.2014.06.047.

[74] R.L. Matsuoka, K.T. Nguyen-Ba-Charvet, A. Parray, T.C. Badea, A. Chédotal, A.L. Kolodkin, Transmembrane semaphorin signalling controls laminar stratification in the mammalian retina, Nature. 470 (2011) 259–264. https://doi.org/10.1038/nature09675.

[75] L.O. Sun, Z. Jiang, M. Rivlin-Etzion, R. Hand, C.M. Brady, R.L. Matsuoka, K.W. Yau, M.B. Feller, A.L. Kolodkin, On and off retinal circuit assembly by divergent molecular mechanisms, Science. 342 (2013). https://doi.org/10.1126/science.1241974.

[76] J.G. Nicholls, D.A. Baylor, Specific modalities and receptive fields of sensory neurons in CNS of the leech, Journal of Neurophysiology. 12 (1968) 740–756. https://doi.org/10.1152/jn.1968.31.5.740.

[77] C.J. Smith, J.D. Watson, W.C. Spencer, T. O'Brien, B. Cha, A. Albeg, M. Treinin, D.M. Miller, Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in *C. elegans*, Developmental Biology. 345 (2010) 18–33. https://doi.org/10.1016/j.ydbio.2010.05.502.

[78] S.L. Zipursky, W.B. Grueber, The molecular basis of self-avoidance, Annual

Review of Neuroscience. 36 (2013) 547–568. https://doi.org/10.1146/annurev-neuro-062111-150414.

[79] K.L. Agarwala, S. Nakamura, Y. Tsutsumi, K. Yamakawa, Down syndrome cell adhesion molecule DSCAM mediates homophilic intercellular adhesion, Molecular Brain Research. 79 (2000) 118–126. https://doi.org/10.1016/s0169-328x(00)00108-x.

[80] D. Schmucker, J.C. Clemens, H. Shu, C.A. Worby, J. Xiao, M. Muda, J.E. Dixon, S.L. Zipursky, *Drosophila* Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity, Cell. 101 (2000) 671–684. https://doi.org/10.1016/s0092-8674(00)80878-8.

[81] X.-L. Zhan, J.C. Clemens, G. Neves, D. Hattori, J.J. Flanagan, T. Hummel, M.L. Vasconcelos, A. Chess, S.L. Zipursky, Analysis of Dscam diversity in regulating axon guidance in *Drosophila* mushroom bodies, Neuron. 43 (2004) 673–686. https://doi.org/10.1016/j.neuron.2004.07.020.

[82] W.M. Wojtowicz, W. Wu, I. Andre, B. Qian, D. Baker, S.L. Zipursky, A vast repertoire of Dscam binding specificities arises from modular interactions of variable Ig domains, Cell. 130 (2007) 1134–1145. https://doi.org/10.1016/j.cell.2007.08.026.

[83] D. Hattori, Y. Chen, B.J. Matthews, L. Salwinski, C. Sabatti, W.B. Grueber, S.L. Zipursky, Robust discrimination between self and non-self neurites requires thousands of Dscam1 isoforms, Nature. 461 (2009) 644-648. https://doi.org/10.1038/nature08431.

[84] A.M. Garrett, A. Khalil, D.O. Walton, R.W. Burgess, DSCAM promotes self-avoidance in the developing mouse retina by masking the functions of cadherin superfamily members, Proceedings of the National Academy of Sciences of the United States of America. 115 (2018) E10216–E10224. https://doi.org/10.1073/pnas.1809430115.

[85] B. Tasic, C.E. Nabholz, K.K. Baldwin, Y. Kim, E.H. Rueckert, S.A. Ribich, P. Cramer, Q. Wu, R. Axel, T. Maniatis, Promoter choice determines splice site selection in protocadherin alpha and gamma pre-mRNA splicing, Molecular Cell. 10 (2002) 21–33. https://doi.org/10.1016/s1097-2765(02)00578-6.

[86] R. Rubinstein, C.A. Thu, K.M. Goodman, H.N. Wolcott, F. Bahna, S Mannepalli, G. Ahlsen, M. Chevee, A. Halim, H. Clausen, T. Maniatis, L Shapiro, B Honig, Molecular logic of neuronal self-recognition through Protocadherin domain interactions, Cell. 163 (2015) 629-642. http://dx.doi.org/10.1016/j.cell.2015.09.026.

[87] J. Brasch, K.M. Goodman, A.J. Noble, M. Rapp, S. Mannepalli, F. Bahna, V.P. Dandey, T Bepler, B. Berger, T. Maniatis, C.S. Potter, B. Carragher, B. Honig, L Shapiro, Visualization of clustered protocadherin neuronal self-recognition complexes, Nature. 569 (2019) 280-283. https://doi.org/10.1038/s41586-019-1089-3.

[88] S. Ing-Esteves, D. Kostadinov, J Marocha, A.D. Sing, K.S. Joseph, M.A. Laboulaye, J.R. Sanes, J.L. Lefebvre, Combinatorial effects of alpha- and gamma-Protocadherins on neuronal survival and dendritic self-avoidance, The Journal of Neuroscience. 38 (2018) 2713-2729. https://doi.org/10.1523/JNEUROSCI.3035-17.2018.

[89] X. Dong, O.W. Liu, A.S. Howell, K. Shen, An extracellular adhesion molecule complex patterns dendritic branching and morphogenesis, Cell. 155 (2013) 296. https://doi.org/10.1016/j.cell.2013.08.059.

[90] Y. Salzberg, C.A. Díaz-Balzac, N.J. Ramirez-Suarez, M. Attreed, E. Tecle, M. Desbois, Z. Kaprielian, H.E. Bülow, Skin-derived cues control arborization of sensory dendrites in Caenorhabditis elegans, Cell. 155 (2013) 308. https://doi.org/10.1016/j.cell.2013.08.058.

[91] C.J. Smith, J.D. Watson, M.K. Vanhoven, D.A. Colón-Ramos, D.M. Miller, Netrin (UNC-6) mediates dendritic self-avoidance, Nature Neuroscience. 15 (2012) 731–737. https://doi.org/10.1038/nn.3065.

[92] C.P. Liao, H. Li, H.H. Lee, C.T. Chien, C.L. Pan, Cell-Autonomous Regulation of Dendrite Self-Avoidance by the Wnt Secretory Factor MIG-14/Wntless, Neuron. 98 (2018) 320-334.e6. https://doi.org/10.1016/j.neuron.2018.03.031.

[93] D. Matsubara, S.Y. Horiuchi, K. Shimono, T. Usui, T. Uemura, The seven-pass transmembrane cadherin Flamingo controls dendritic self-avoidance via its binding to a LIM domain protein, Espinas, in *Drosophila* sensory neurons, Genes and Development. 25 (2011) 1982–1996. https://doi.org/10.1101/gad.16531611.

[94] H. Long, Y. Ou, Y. Rao, D.J. van Meyel, Dendrite branching and self-avoidance are controlled by Turtle, a conserved IgSF protein in *Drosophila*, Development. 136 (2009) 3475–3484. https://doi.org/10.1242/dev.040220.

[95] M.E. Kim, B.R. Shrestha, R. Blazeski, C.A. Mason, W.B. Grueber, Integrins establish dendrite-substrate relationships that promote dendritic self-avoidance and patterning in *drosophila* sensory neurons, Neuron. 73 (2012) 79–91. https://doi.org/10.1016/j.neuron.2011.10.033.

[96] S. Meltzer, S. Yadav, J. Lee, P. Soba, S.H. Younger, P. Jin, W. Zhang, J. Parrish, L.Y. Jan, Y.N. Jan, Epidermis-Derived Semaphorin Promotes Dendrite Self-

Avoidance by Regulating Dendrite-Substrate Adhesion in *Drosophila* Sensory Neurons, Neuron. 89 (2016) 741–755. https://doi.org/10.1016/j.neuron.2016.01.020.

[97] R.L. Matsuoka, Z. Jiang, I.S. Samuels, K.T. Nguyen-Ba-Charvet, L.O. Sun, N.S. Peachey, A. Chédotal, K.W. Yau, A.L. Kolodkin, Guidance-cue control of horizontal cell morphology, lamination, and synapse formation in the mammalian outer retina, Journal of Neuroscience. 32 (2012) 6859–6868. https://doi.org/10.1523/JNEUROSCI.0267-12.2012.

[98] L. Sundararajan, C.J. Smith, J.D. Watson, B.A. Millis, M.J. Tyska, D.M. Miller, Actin assembly and non-muscle myosin activity drive dendrite retraction in an UNC-6/ netrin dependent self-avoidance response, PLoS Genetics. 15 (2019). https://doi.org/10.1371/journal.pgen.1008228.

[99] K. Kawabata Galbraith, K. Fujishima, H. Mizuno, S.J. Lee, T. Uemura, K. Sakimura, M. Mishina, N. Watanabe, M. Kengaku, MTSS1 Regulation of Actin-Nucleating Formin DAAM1 in Dendritic Filopodia Determines Final Dendritic Configuration of Purkinje Cells, Cell Reports. 24 (2018) 95-106.e9. https://doi.org/10.1016/j.celrep.2018.06.013.

[100] Y. Gao, E.M. Perkins, Y.L. Clarkson, S. Tobia, A.R. Lyndon, M. Jackson, J.D. Rothstein, β-III spectrin is critical for development of Purkinje cell dendritic tree and spine morphogenesis, Journal of Neuroscience. 31 (2011) 16581–16590. https://doi.org/10.1523/JNEUROSCI.3332-11.2011.

[101] K. Fujishima, J. Kurisu, M. Yamada, M. Kengaku, βIII spectrin controls the planarity of Purkinje cell dendrites by modulating perpendicular axon-dendrite interactions, Development. 147 (2020). https://doi.org/10.1242/dev.194530.

FIGURE LEGENDS

Fig. 1. The spatial configuration of dendrites.

(A) To establish neural circuits that accurately perform neural functions, each neuron must generate its own functional 3D shape according to the developmental program. During neural development, growing axons are navigated to their predetermined targets usually over long distances. Dendrites, which are much shorter than axons, establish structures that are unique to each neuron type and directly relate to neural function. The

spatial configuration of dendrites is determined by various mechanisms, but three in particular; (1) growth orientation, (2) growth size, and (3) 3D arborization pattern. The growth orientation of dendrites is crucial to ensure appropriate inputs. The control of dendrite size is important for establishing a receptive field that is collaboratively established with neighboring cells. The 3D arborization patterns vary greatly among neurons but are important for efficient input and information processing. (B) Dendrites of cerebellar PCs have a distinctive 3D structure. The image shows a 3D reconstruction of PCs at postnatal day 21 that were labeled with green fluorescence protein. PCs project a single intricately branching dendrite toward the ML from the PCL, whereas they project axons into the GCL. PC dendrites span the entire ML to establish the receptive fields (upper panel). The dendritic arbor of PC is strictly regulated to form a fan-like monoplanar structure in which sister branches of the same cells avoid crossing each other (bottom panels). ML: molecular layer, PCL: purkinje cell layer, and GCL: granule cell layer.

Fig. 2. Oriented dendrite structures and their regulatory systems.

(A) Pyramidal neurons in the cerebral cortex have apical and basal dendrites. Sema3A-Nrp1/Plexin A2 signaling orchestrates the oriented growth of dendrites, thereby ensuring apicobasal polarity. The downstream mechanisms of apical and basal dendrite orientation via Sema3A-Nrp1/Plexin A2 signaling are shown in the box. L1–L6: layers 1–6, WM: white matter. MZ: marginal zone, CP: cortical plate, IZ: intermediate zone, SVZ: subventricular zone, VZ: ventricular zone, Sema3A: Semaphorin 3A, Nrp1: Neuropilin-1, Cdk5: cyclin dependent kinase 5, TAO2: thousand and one amino acid protein 2, JNK: c-Jun N-terminal kinase, GSK3β: glycogen synthase kinase 3β. (B) Remodeling of

dendrite orientation in spiny stellate neurons. During the first postnatal week, spiny stellate neurons in the barrel cortex establish the inside-oriented dendritic arborizations to synapse with innervating TCAs via dendrite pruning. Deletions of the glutamate receptors including NR1, NR2B, and mGluR5 abolish the dendrite pruning of spiny stellate neurons, indicating neural activity mediates this process probably through Btbd3. Neural activity-dependent nuclear translocation of Btbd3 is required for dendrite pruning. Sema7A-Plexin C1 signaling is also involved in this pruning. TCA: thalamocortical axon, N: nucleus, Btbd3: Broad complex, Tramtrack, and Bric-à-brac/Poxvirus and zinc finger (BTB/POZ) domain-containing 3, NMDAR1: *N*-methyl-D-aspartate receptor 1. (C) The *Drosophila* class I da neuron ddaE forms a comb-like dendritic structure in which primary (red) and secondary (blue) dendrites extend along the dorsal direction and the posterior direction, respectively. The homophilic cell adhesion molecule Ten-m mediates the interaction between the epidermis and the secondary dendrites to restrict the growth direction of these dendrites. A: anterior, P: posterior, D: dorsal, and V: ventral. (D) Remodeling of dendrite orientation in mitral cells. During the first postnatal week, mitral cells in the olfactory bulb establish a one-to-one dendritic projection pattern to the glomerulus via pruning mechanisms. BMP signaling and neural activity regulate dendrite pruning and stabilization of mitral cells via LIMK-dependent actin polymerization. BMP: bone morphogenetic protein, BMPR2: BMP receptor 2, LIMK: LIM kinase.

Fig. 3. Mechanisms controlling neurite size.

(A) Competition-based size control of PC dendrites. Relative acquisition levels of the NT-3-TrkC and Cbln1-GluD2 signals among neighboring neurons determines the sizes of PC dendrites. Parallel fibers secrete NT-3 and Cbln1 that bind to TrkC and GluD2,

respectively, on PC (boxes). GluD2, Cbln1, and Nrx1 form a tripartite synaptic complex. A PC with reduced NT-3-TrkC signaling decreases in dendrite size (red cell in bottom left scheme). A PC with reduced Cbln1-GluD2 signaling decreases or increases the dendritic arbor size in the deep or superficial ML, respectively (green cell in bottom right scheme). Enhanced Cbln1-GluD2 signaling causes dendrite overelaboration in the deep ML (yellow cell in bottom right scheme). PC: Purkinje cell, GC: granule cell, NT-3: neurotrophin-3, TrkC: tropomyosin-related kinase C, Cbln1: cerebellin-1, GluD2: glutamate delta2, Nrx1: Neurexin 1, ML: molecular layer. (B) Space filling-based scaling of dendrites. In *Drosophila* da neurons, repulsive interaction with neighboring dendrites via cell-surface molecules Slit and Robo defines dendritic arbor sizes (box). This system is a basis for tiling in which neurons can align orderly and efficiently to cover a field with dendrites. Enhanced Slit-Robo signaling in class IV da neurons decreases dendrite size, whereas the loss of this signal increases dendrite size. (C) Layer-specific synaptic connections in the retina. Processes of bipolar cells and amacrine cells form synapses on RGC dendrites in the IPL which is subdivided into five layers (OFF layer: layer 1 and 2, ON layer: layer 3–5). ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer, and RGC: retinal ganglion cell. (D) In the chick retina, homophilic cell adhesion molecules, such as Dscam, DscamL, Sidekick1 (Sdk1), and Sidekick2 (Sdk2), mediate specific synaptic connections of RGCs with bipolar and amacrine cells in certain sublaminae. Therefore, the mechanisms for laminar-specific targeting define neurite arbor sizes in retinal neurons. For example, the loss of Dscam expression in Dscam (+) RGCs that normally extends dendrites into IPL lamina 5 causes the overshoot of dendrites into the upper layer. (E) Sema6A is expressed in RGCs that project to the ON layer, whereas Plexin A2 and Plexin

A4 are expressed in amacrine cells that project to the OFF layer. Sema6A repels Plexin A2/A4-expressing dendrites of amacrine cells and excludes them from the ON layer. The loss of Sema6A in RGCs causes an overshoot of Plexin A2/A4-expressing neurites beyond the OFF layer. Sema6A: semaphorin 6A.

Fig. 4. Self-avoidance mechanisms establish functional dendritic arbors.

(A) Cell-surface molecules mediate dendrite self-avoidance via local repulsion of sister branches. This mechanism allows dendrites to cover a wider area more efficiently with less overlap. (B) In a *Dscam1* mutant, dendritic self-avoidance of *Drosophila* da neurons is unsuccessful, and the dendrites of the mutant become entangled (arrows). (C) *Drosophila Dscam1* generates by selective splicing 38,016 isoforms (19,008 in the extracellular region and 2 in the transmembrane region). Exon 4, exon 6, and exon 9 encode the immunoglobulin 2 (Ig)2, Ig3, and Ig7 domain, respectively, and exon 17 encodes the transmembrane domain (TMD). Numbers in the parentheses below the exons indicate the numbers of isoforms. Although a huge number of isoforms are theoretically generated, an individual neuron expresses only one random isoform, and only identical isoforms bind to each other. (D) Mouse clustered protocadherin (Pcdh) consists of *Pcdhα*, *Pcdhβ* and *Pcdhγ* forming a gene cluster, and there are a total of 58 protocadherin molecules with different extracellular regions. Isoforms of clustered protocadherins are produced by different promoter selection. Only identical isoforms bind to each other. Numbers in the parentheses indicate the numbers of isoforms. ECD: extracellular domain, ICD: intracellular domain. (E) Defects in dendrite self-avoidance in mouse amacrine cells and PCs. The loss of Pcdhγ causes dendrite clumping (arrows) in amacrine cells and dendrite self-crossings in PCs. Loss of Slit2-Robo2 signaling or its regulator LKB1 also

increases of dendrite self-crossings in PCs. LKB1: liver kinase B1.