

ACTIVITY-DEPENDENT REGULATION OF DENDRITIC GROWTH AND PATTERNING

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One of the most remarkable features of the developing brain is its ability to undergo structural change in response to experience. Among the cellular elements that show this kind of plasticity are dendrites, which are the components that receive and process synaptic information. Recent observations indicate that calcium signalling in neurons can regulate dendritic growth and remodelling by several mechanisms, and these mechanisms are likely to be key mediators of structural plasticity in the developing brain.

PARALLEL FIBRES

The axons of cerebellar granule cells. Parallel fibres emerge from the molecular layer of the cerebellar cortex towards the periphery, where they extend branches perpendicular to the main axis of Purkinje neurons and form so-called *en passant* synapses with this cell type.

Dendrites allow neurons to integrate information from an array of synaptic inputs, and the specific branching pattern of dendrites limits the number and type of inputs that a neuron can receive. Dendritic morphology also influences how synaptic signals decay as they propagate towards the soma. Consequently, there is a well-defined relationship between the dendritic morphology of a neuron and its function^{1,2}. Because the spatial pattern of the dendritic arborization influences neuronal function, it is important to determine how dendrites acquire their characteristic size and morphology during development.

The dendritic tree grows by the addition and elongation of branches. Primary dendrites emerge from the cell body, and branch to form secondary and then tertiary dendrites. However, the branching patterns of neurons are not random; rather, they are unique to each cell type in the central nervous system (CNS). The precise branching pattern of a neuron is established not only by branch addition and maintenance, but also by branch retraction and elimination^{3,4}. Because this process of structural remodelling occurs in concurrence with growth, understanding dendritic development requires knowledge of the mechanisms that coordinate the overall growth and detailed patterning of the arborization.

Although several mechanisms influence dendritic development, signalling from afferents seems to be particularly important for dendritic growth^{5,6}. The timing of

afferent innervation and synapse formation coincides with the period of maximum growth and dendritic remodelling, and recent studies underscore the importance of afferent activity in regulating dendritic development. Furthermore, a common finding across systems is that neurotransmission, evoked either spontaneously or by sensory input, triggers changes in intracellular calcium levels that affect the dendritic cytoskeleton. The intracellular signalling pathways that depend on calcium regulation seem to converge to affect local and global development of the dendritic arborization. In this review, we highlight evidence that supports a role for afferent activity in dendritic development, and discuss the calcium-dependent signalling events that mediate this process.

Dendritic morphology and connectivity
In many parts of the CNS, dendritic arborizations are oriented in specific ways to make contact with relevant synaptic inputs. For example, in the cerebellum, the arborizations of Purkinje cells are asymmetrically organized, projecting towards the plane of PARALLEL FIBRES from which they receive their main input (FIG. 1a). Neurons in the neocortex also have polarized dendrites, forming apical and basal arborizations that receive different sets of connections (FIG. 1b). In the somatosensory cortex of rodents, the terminal arborizations of thalamocortical afferents that represent each vibrissa, or

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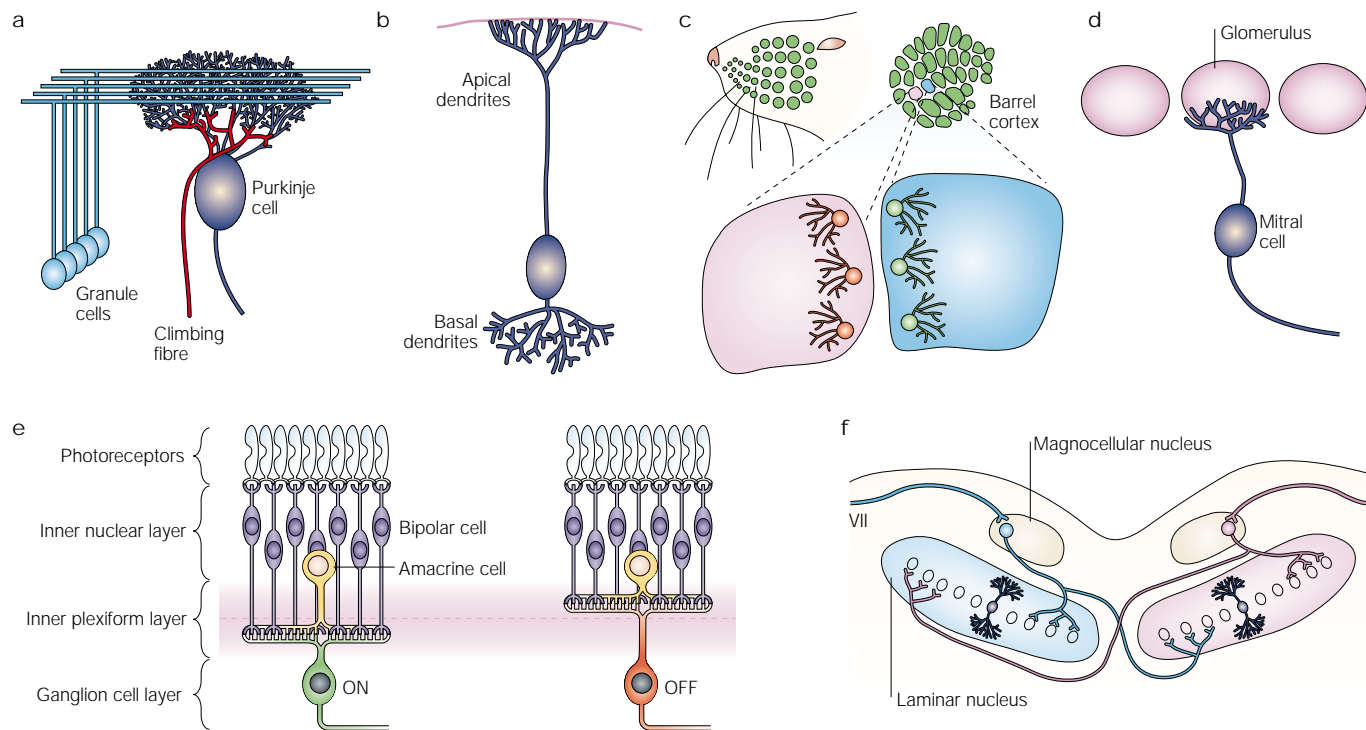


Figure 1 | Dendritic arborizations of mature central neurons have distinct morphologies. **a** | In the cerebellum, Purkinje cells have a highly branched distal arborization that receive inputs from parallel fibres of granule cells. Inputs from climbing fibres innervate only the proximal part of the arborization. **b** | Pyramidal cells of the neocortex have distinct apical and basal dendrites that receive connections from different sets of inputs. **c** | The somatosensory cortex of rodents is patterned into rows and columns of barrels. Inputs that represent a single vibrissa on the animal's snout terminate within a barrel. Dendrites of layer 4 cells at the barrel boundaries are directed towards the barrel centre. **d** | In the olfactory bulb, each glomerulus that receives converging inputs from olfactory sensory neurons with the same receptor type is contacted by the dendrites of a single mitral cell. **e** | In the vertebrate retina, retinal ganglion cells (RGCs) — the output neurons — have dendritic arborizations that stratify in distinct sublaminae within the inner plexiform layer (IPL). Shown here are two types of RGC with arborizations in the 'ON' and 'OFF' sublaminae of the IPL. They are contacted by amacrine and bipolar cells. **f** | In birds, the auditory signal from each ear is relayed by the VIII nerve to the ipsilateral magnocellular nucleus (NM). The dorsal dendrites of laminar nucleus (NL) neurons receive ipsilateral NM input, whereas the ventral dendrites are innervated by the contralateral NM. The projections from NM to NL are arranged in a tonotopic map. Adapted, with permission, from REF. 72 © 1981 John Wiley & Sons.

BARREL

A cylindrical column of neurons that is found in the rodent neocortex. Each barrel receives sensory input from a single whisker follicle, and the topographical organization of the barrels corresponds precisely to the arrangement of whisker follicles on the face.

GLOMERULUS

Axon terminals end in a variety of configurations within the neuropil. The most common is *en passant* or *de passage*, in which axons make simple synapses as they pass dendrites or cell bodies. By contrast, some axons end in — or produce strings of — enlargements that are often packed with synaptic vesicles. These glomerular-type endings synapse with large numbers of dendrites and other axons clustered around the glomerular ending.

whisker, are confined to separate **BARRELS** in layer 4 (REF. 7). Layer 4 neurons with cell bodies located at the barrel boundaries orient their dendrites towards the centre of the barrel⁸ (FIG. 1c). This displacement of the dendritic arborization seems to maximize contact with a preferred set of afferents. Likewise, in the olfactory bulb, the dendrites of mitral cells are highly oriented to contact a single knot of presynaptic elements, the **GLOMERULUS**⁹ (FIG. 1d). Each glomerulus receives converging input from olfactory sensory neurons that express the same receptor subtype.

The segregation of dendritic trees into separate regions of the neuropil is even more marked in the vertebrate retina (FIG. 1e). The dendritic arborizations of different morphological and functional classes of retinal ganglion cells (RGCs) occupy distinct sublayers of the **INNER PLEXIFORM LAYER**. The responses of these cells to light stimuli directly correspond to their dendritic stratification patterns¹⁰. In the avian auditory brain stem, target neurons spatially separate the inputs from two distinct sources by their dendritic patterning^{11,12}. The dendrites of neurons of the laminar nucleus are segregated into

dorsal and ventral dendritic 'tufts'. Each tuft is contacted exclusively by afferents from the ipsilateral or contralateral magnocellular nucleus, which in turn receives impulses from the corresponding cochlea (FIG. 1f).

What are the cellular and molecular mechanisms that cause dendrites to acquire unique morphologies? The fact that neurons of the same type bear striking similarities in their morphology, and that this is repeated from animal to animal, supports a role for genetic specification of dendritic morphology. This concept is borne out by the observation that retinal cells in culture have characteristic morphologies even when they are isolated from other cells^{13,14}. Similarly, pyramidal cells and stellate cells can be identified in cortical culture on the basis of their distinct morphologies¹⁵. However, a wealth of evidence indicates that the growth and detailed patterning of dendrites can be greatly influenced by environmental signals. These signals include retrograde feedback from distant targets^{16–20}, interactions with neighbouring cells of the same kind^{21,22}, and contact with presynaptic cells^{23,24}. The identity of several molecular signals that

mediate these effects has been determined. For example, semaphorin 3A (**Sema3A**), **Slit1**, **Notch** and brain-derived neurotrophic factor (**BDNF**) have been shown to regulate the growth and branching of cortical pyramidal neuron dendrites^{25–29}.

Afferent influences on dendritic development In many developing systems, there is a close correlation between the ingrowth of afferents and dendritic maturation. The precise relationship between afferent innervation and dendritic development has perhaps been most extensively and carefully examined in the developing cerebellum^{30–32}. The cerebellum has been a useful preparation for these studies, because it has a relatively simple architecture and the developmental history of the principal cell types is well documented. At the onset of differentiation, the Purkinje cells sit below the external granule layer (EGL), which contains the granule cell precursors. As the granule cells become postmitotic, they extend processes orthogonal to the plane of the Purkinje cell dendrites. The granule cell soma then migrates down through the Purkinje cell layer to take its position in the internal granule layer (IGL), leaving behind a bifurcated axon that forms the characteristic 'T' of the parallel fibres. As more and more granule cell neurons migrate away from the EGL, the zone occupied by the parallel fibres (the molecular layer) expands. The expansion of the molecular layer is accompanied by the growth of Purkinje cell dendrites into this zone. As parallel fibres provide the principal input to Purkinje cell dendrites, it seems that parallel fibre input might have an important role in regulating dendritic growth in Purkinje cells (FIG. 1a).

A role for parallel fibre input in regulating the development of Purkinje cell dendrites is supported by experiments in which this input is disrupted, often owing to granule cell abnormalities. X-irradiation can be used to eliminate the majority of granule neurons in newborn mice; as a result of this treatment, these animals have very few parallel fibres. Although Purkinje cell survival is not affected in these mice, their dendrites do not develop normally³³. Moreover, Purkinje cell dendrites do not extend properly in the cerebella of **WEAVER** and **REELER** mutants, in which granule cell development is severely compromised³¹.

The relationship between afferent innervation and dendritic development is conserved in many other regions of the developing nervous system. For example, the most active phase of dendritic growth in the rat cerebral cortex takes place between the first and third postnatal weeks, which closely parallels the onset of sensory input to the cortex^{34,35}. In the *Xenopus* tectum, the onset of dendritic development closely parallels the formation of the first synaptic inputs³⁶. The dendritic arborization expands rapidly when the dendritic tree is relatively simple, and the rate of growth slows down as the dendritic tree becomes increasingly complex and when synaptogenesis draws to a close³. Collectively, these observations across different systems indicate that afferent inputs are likely to have a considerable influence on the dendritic development of their postsynaptic targets.

Sensory stimulation and dendritic growth. Afferents are likely to influence the development of postsynaptic dendrites by both activity-independent and activity-dependent mechanisms. The presence of these dual mechanisms might have long obscured the role of neuronal activity in this process. For example, it was reported that visual deprivation does not significantly affect dendritic growth in cortical layer 4 stellate cells³⁷ or in layer 3 pyramidal cells³⁸. By contrast, in the rat visual cortex, the orientation of layer 4 dendrites is altered by **DARK REARING**, and exposure to a patterned visual stimulus affects the orientation of layer 3 and 4 dendrites in the cat^{39,40}. Similarly, monocular deprivation alters dendrite development in the lateral geniculate nucleus and the visual cortex^{41,42}. A role for neuronal activity in the development of cortical neurons is also supported by the observation that exposure to enriched environments or training on a motor-learning task increases dendritic growth and branching in cortical pyramidal neurons^{43–46}. Neuronal activity has also been implicated in the control of dendritic development in auditory brainstem neurons, cerebellar Purkinje cells, spinal motor neurons and ciliary ganglia^{30,32,47–50}. A recent series of experiments indicates that motor learning, but not simply motor activity, induces dendritic growth in cerebellar stellate cells⁵¹. So, the pattern of activity, and not just the overall level of activity, might be the key determinant of activity-dependent dendritic growth.

Much of our understanding of the relationship between synaptic input and dendritic development has come from studies in the *Xenopus* retinotectal system, in which time-lapse imaging has allowed detailed investigations of dendritic development *in vivo*. Tectal neurons receive synaptic input when they have relatively simple dendritic trees. The subsequent growth of the dendritic arborization is characterized by a high level of dynamic rearrangement of dendritic branches^{36,52}. A similar rearrangement of dendritic branches during development has been reported in the zebrafish tectum⁵³. The early synaptic currents in tectal neurons are mediated principally by **NMDA** (*N*-methyl-D-aspartate)-type glutamate receptors, and pharmacological antagonism of NMDA receptors markedly reduces dendritic growth rates in the *Xenopus* tectum⁵⁴. So, glutamatergic transmission has an important regulatory role in this system.

Although these studies indicate that synaptic input regulates dendritic growth, until recently, relatively little was known about the acute relationship between sensory input and dendritic growth. In a recent study, Sin *et al.*⁵⁵ examined the effects of visual stimulation on dendritic growth, and they provided compelling evidence for fairly rapid regulation of dendritic growth by sensory input. When tadpoles were exposed to light for four hours after they had been in darkness for four hours, there was a marked increase in dendritic growth rates and a concomitant increase in total dendritic length. Previous studies had shown that the growth of the arborization is affected by both the extension of existing dendritic branches and the

INNER PLEXIFORM LAYER

The retinal layer that is formed by synaptic contacts between the bipolar, the amacrine and the ganglion cells.

WEAVER

This mouse strain is characterized by cerebellar abnormalities and ataxia, which are associated with a mutation in an inwardly rectifying potassium channel.

REELER

A mutant mouse that is characterized by tremors, dystonia and ataxia. These phenotypes are associated with mutations in a protein known as *reelin*.

DARK REARING

An experimental condition in which an animal is reared in total darkness so that only endogenous activity is present in the developing visual system.

CLIMBING FIBRES
Cerebellar afferents that arise from the inferior olivary nucleus, each of which forms multiple synapses with a single Purkinje cell.

balance between addition and retraction of dendritic segments. Time-lapse imaging of dendritic dynamics revealed that much of the light-induced growth was due to the growth of new dendritic branches and to increased stability of existing branches. By contrast, when the animals were first exposed to light for four hours and then moved to the dark for four hours, the growth rates did not decrease. So, visual stimulation activates a process that seems to act over a prolonged period of time to influence dendritic growth. These observations highlight the importance of normal visual experience in regulating dendritic growth.

To evaluate the role of synaptic transmission in vision-induced increases in dendritic growth rates, Sin and colleagues examined the effects of inhibiting glutamatergic transmission⁵⁵. They showed that the light-induced increase in dendritic growth was suppressed by both the NMDA receptor antagonist AP5 (D-2-amino-5-phosphonopentanoate) and the AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione). AP5 blocked the increase in branch additions and enhanced branch retractions. So, a glutamate-receptor-mediated signalling mechanism mediates dendritic growth in response to visual stimulation.

Apart from the frog tectum, NMDA-receptor-mediated signalling is likely to be widely involved in activity-dependent dendritic growth, as it has previously been shown that NMDA receptor antagonists suppress dendritic growth in spinal motor neurons⁵⁰ and in the supraoptic nucleus⁵⁶. Moreover, Chevalyere and colleagues⁵⁶ reported that an NMDA-induced increase in dendritic branches could be suppressed by

blockers of voltage-gated calcium channels (VGCCs) or inhibitors of intracellular calcium mobilization, indicating that NMDA receptor activation recruits other calcium signalling pathways to regulate dendritic growth.

Afferent activity and dendritic patterning. As well as promoting the net growth of the arborization, afferent activity might also shape dendritic branching patterns. For example, only dendritic segments that are contacted by the appropriate inputs are maintained⁵⁷. This is exemplified by mitral cells, which have multiple primary dendrites that branch and contact adjacent glomeruli during development (FIG. 2). Association with a single glomerulus occurs by the retraction of most dendrites and the maintenance of a single dendritic branch⁹. Dendritic remodelling seems to be a simple way for mitral cells to attain their desired connectivity without the need to reorganize the structure of the glomeruli on which many sensory axons converge. Of course, synapse elimination can, and does, occur with the withdrawal of axons⁵⁸.

A direct demonstration of the importance of dendritic remodelling in specifying connectivity patterns comes from observations in the retina. Early in development, RGC dendrites are not confined to specific laminae in the inner plexiform layer (FIG. 2). Chalupa and colleagues⁵⁹ showed recently that when the dendritic arborizations are not yet stratified, RGCs receive converging inputs from two distinct types of excitatory interneuron — the 'ON' and 'OFF' bipolar cells. These bipolar cells respond with depolarization (ON) or hyperpolarization (OFF) to increases in illumination. After the dendrites become stratified, ganglion cells retain only connections with ON or OFF bipolar cells (FIG. 1e). If dendritic stratification is pharmacologically prevented *in vivo*^{60,61}, ganglion cells fail to lose one set of connections⁶². So, the connectivity of ganglion cells is fine-tuned by selective dendritic maintenance, elaboration and elimination. Although this might not be the only mechanism that shapes connectivity patterns in the CNS (for example, CLIMBING FIBRES are eliminated on Purkinje cells without an apparent loss of dendrites), determining the mechanisms that stabilize dendrites remains crucial to our knowledge of how circuits form appropriately during development.

To determine whether neurotransmission is the important factor, pharmacological and surgical methods have been used to silence neurons, while leaving afferents intact. Complete blockade of activity produces disparate results, largely because it is difficult to separate changes in growth from those of dendritic maintenance. In some cases, loss of activity has no overt effect on the dendritic tree^{63,64}, although it should be noted that the outcome of activity blockade might depend on the developmental stage^{54,65}. Experiments in which an imbalance of input activity is created demonstrate more clearly the importance of neurotransmission. For example, monaural deprivation results in a shortening of laminar nucleus dendrites that correspond to the plugged ear^{66,67}.

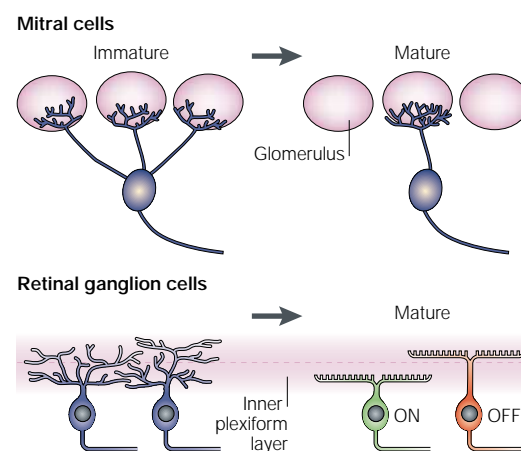


Figure 2 | Two examples of central neurons that undergo dendritic remodelling to refine their connectivity. Mitral cells lose all but one dendritic branch to maintain contact with a single glomerulus as they mature. The dendrites of retinal ganglion cells (RGCs) are initially diffusely distributed in the synaptic plexus — the inner plexiform layer. With maturation, RGCs lose dendrites and restrict their arborization to either the 'ON' or the 'OFF' sublamina, where they receive inputs from functionally distinct classes of retinal interneurons.

In some instances, dendrites are found to reorganize so that they preferentially contact active inputs. For example, in the cat visual cortex, monocular deprivation results in spiny stellate cells in layer 4 redirecting dendrites towards neighbouring OCULAR DOMINANCE COLUMNS that represent the open eye⁶⁸. Interestingly, arborizations might also be normally positioned or repositioned after a manipulation to avoid contact with afferents that have action potential activities that are not temporally synchronized. For example, in the barrel cortex, removal of one row of whiskers results in the formation of a 'giant barrel'. In contrast to the highly oriented arborizations of normal animals, layer 4 cells in the centre of the expanded barrel develop symmetrical arborizations^{69,70}. When a third eye is transplanted into frog embryos, and the axons of the implanted eye are forced to innervate a tectum that is normally innervated by only one eye, the projections of the two eyes separate into ocular dominance stripes. Unlike those in normal tadpoles, many tectal neurons in three-eyed frogs develop dendritic trees that are biased to one or other ocular stripe⁷¹. Rearrangement of dendritic fields in response to asynchronous activity of the inputs might also explain why mitral cells normally withdraw dendrites from all but one glomerulus.

Although afferent 'activity', or even its temporal patterns, can shape dendritic arborizations, it is not well understood how neurotransmission can locally regulate dendritic structure, allowing or causing retraction of some processes, but promoting stability and even growth of others. Blocking presynaptic activity from magnocellular nucleus neurons by removing the cochlea does not produce as pronounced a reduction in dendritic length in laminar nucleus neurons as is observed after deafferentation⁷². It is possible that, whereas deafferentation eliminates all action-potential-mediated neurotransmission⁷³, subthreshold release of transmitter might still occur after cochlea removal. In fact, local responses to subthreshold activity would be an ideal way to regulate dendritic structure in an activity-dependent manner. It is more difficult to imagine how global activation of the soma and dendritic tree would locally stabilize some dendrites, and not others.

Calcium signalling and dendritic development
The intracellular signalling pathways that mediate the effects of afferent activity on dendritic growth and patterning are not completely understood, but calcium seems to be a key component. Pathways that involve changes in intracellular calcium have long been known to influence neuronal differentiation, as well as axon outgrowth and pathfinding⁷⁴. The role of calcium signalling pathways in dendritic spine development and synaptic plasticity has been investigated extensively^{75–77}. Recent studies indicate a key role for calcium signalling in dendritic growth and patterning. Calcium influx through VGCCs activates a transcriptional programme that regulates overall dendritic growth⁷⁸. At the same time, calcium-induced calcium release (CICR) in

individual dendrites seems to regulate dendritic branch stability⁷⁹.

Transcriptional control of dendritic growth. Calcium influx through VGCCs affects dendritic growth in cortical slice cultures⁸⁰. In a recent study, Redmond *et al.*⁷⁸ examined mechanisms of calcium-induced dendritic growth in cortical neurons, and found that depolarization-induced calcium influx through VGCCs was sufficient to induce a programme of dendritic growth. The effect of VGCC activation on dendritic growth was suppressed by pharmacological inhibitors of calcium/calmodulin-dependent protein kinases (CaM kinases), and mimicked by transfection of an activated form of CaM kinase IV (CaMKIV), but not CaMKII. These observations indicate that the effects of VGCC activation on dendrites might be mediated by CaMKIV. Consistent with this possibility, the authors found that CaMKIV is developmentally regulated⁷⁸. CaMKIV expression reaches maximal levels during the second and third postnatal week, which coincides with the period of maximal dendritic growth³⁵. Interestingly, overexpression of wild-type CaMKIV did not by itself induce dendritic growth, but greatly potentiated the effects of calcium influx on the growth of dendrites.

The effects of CaMKIV on dendritic development are likely to be transcriptionally mediated, as CaMKIV is localized exclusively to the nucleus. The best-characterized nuclear target of CaMKIV is the transcription factor cyclic-AMP-responsive-element-binding protein (CREB). Redmond *et al.*⁷⁸ showed that dendritic growth that is induced by VGCC and CaMKIV activation is suppressed by DOMINANT NEGATIVE CREB. So, calcium-induced dendritic growth in cortical neurons seems to be mediated by CaMKIV and CREB-dependent transcription (FIG. 3a).

The mechanism by which CREB-mediated transcription regulates dendritic growth is not well understood, but it is interesting to note that one of the targets of CREB is the neurotrophin BDNF^{81,82}. BDNF is known to regulate dendritic growth in cortical and cerebellar neurons^{29,80,83–85}, and it is tempting to speculate that the effects of CREB on dendritic development are mediated by BDNF. These observations indicate that calcium influx through VGCCs influences dendritic growth, at least in part by a transcription-dependent mechanism.

Although the Redmond study emphasizes the CaMKIV–CREB pathway, other signalling mechanisms are also likely to contribute to calcium-dependent dendritic growth. For example, in a recent study, Vaillant *et al.*⁸⁶ reported that calcium influx induces dendritic formation in sympathetic neurons through a CaMKII and mitogen-activated protein kinase (MAPK)-dependent mechanism.

Local calcium signalling and dendritic patterning. Calcium levels can be altered locally in dendrites in several ways (FIG. 3b). First, NMDA receptors and neuronal nicotinic cholinergic receptors (nAChRs) are

OCULAR DOMINANCE COLUMNS

In the mature primary visual cortex of mammals, most neurons respond predominantly to visual inputs from one eye or the other. Cells that respond to a given eye are arranged in stripes — the ocular dominance columns — that alternate with stripes of neurons that respond to the other eye.

DOMINANT NEGATIVE

Describes a mutant molecule that can form a heteromeric complex with the normal molecule, knocking out the activity of the entire complex.

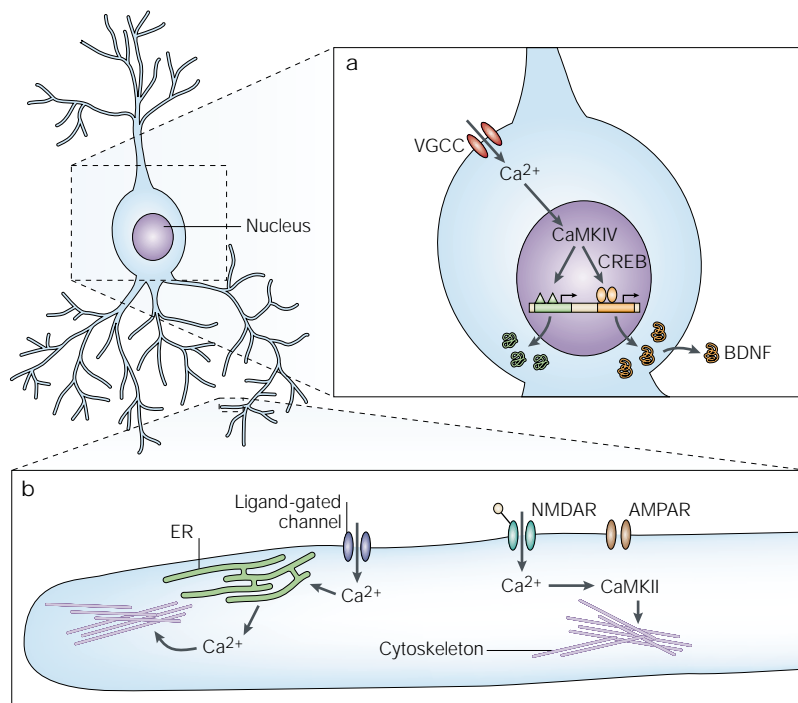


Figure 3 | Schematic of mechanisms that might mediate calcium-dependent dendritic growth. a | In cortical neurons, calcium influx through voltage-sensitive calcium channels (VGCCs) leads to the activation of calcium/calmodulin-dependent protein kinase IV (CaMKIV). CaMKIV activates cyclic-AMP-responsive-element-binding protein (CREB) and other transcription factors, which regulate the expression of proteins that regulate dendritic growth. **b** | Calcium signalling pathways in dendrites that regulate branch stability. AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; BDNF, brain-derived neurotrophic factor; ER, endoplasmic reticulum; NMDAR, *N*-methyl-D-aspartate receptor.

permeable to calcium. Second, the opening of VGCCs in response to membrane depolarization leads to an influx of calcium from the extracellular environment. Third, calcium is released from intracellular stores by the binding of inositol-1,4,5-trisphosphate (Ins(1,4,5)P₃) and ryanodine, or by CICR from the endoplasmic reticulum. The endoplasmic reticulum is present in dendritic shafts and spines, and it is strategically positioned adjacent to the plasmalemma to sense local changes in the environment^{87–89}.

A rise in calcium levels in the dendritic arborization can be confined to structures as small as dendritic spines⁹⁰. Although many studies have pursued an understanding of the role of local calcium elevations in spine function and synaptic plasticity^{1,75–77,91}, fewer have addressed how calcium levels regulate dendritic patterning during development. This might be because it has only recently been possible to assess the dynamics of dendritic restructuring in live imaging studies^{92–95}. Furthermore, to temporally relate structural changes to calcium levels in the dendrite is a challenging task.

Recently, calcium imaging of hippocampal neurons in culture raised the tantalizing possibility that localized increases in intracellular calcium levels could affect dendritic structure. Neonatal hippocampal CA1 neurons show spontaneous elevations in intracellular calcium that spread through short lengths of dendrites,

occurring most frequently at branch points⁹⁶. This rise in calcium levels is due to the release of calcium from Ins(1,4,5)P₃- and ryanodine-sensitive stores. A function for these spontaneous ‘elementary’ calcium events has not been determined for hippocampal neurons, but a recent study of the developing retina supports a role for local calcium events in stabilizing dendrites.

By loading cells with calcium indicator dyes using a BALLISTIC METHOD of transfection, Lohmann *et al.*⁹⁷ recorded spontaneous elevations in intracellular calcium levels in developing RGCs during the period in which dendrites of these neurons have not yet stratified⁷⁹ (FIGS 3b and 4a,b). As in hippocampal neurons, two distinct types of calcium event are apparent — global increases in calcium levels in the cell body and across the arborization, and local increases that are restricted to short lengths of dendrites (FIG. 4c,d). The local flashes represent CICR events, whereas global activation of the arborization is associated with membrane depolarization and action potential activity. Remarkably, the inhibition of local, but not global, events results in a rapid retraction of dendrites, occurring within several minutes when stores are blocked pharmacologically. To show that CICR is a mechanism by which dendritic stability can be regulated locally, the authors attempted to ‘rescue’ dendrites from retracting. The retraction of dendrites was first induced by placing the retina in zero calcium conditions. Then, calcium was focally released within a small part of the arborization by the photic release of CAGED CALCIUM. Uncaging caused CICR and prevented the stimulated dendritic terminals from shrinking. By contrast, uncaging in the presence of intracellular-store blockers did not stop retraction, even though a significant calcium rise occurred on uncaging. Together, these observations point to the importance of CICR in maintaining dendrites, and further emphasize that the pathway by which calcium levels are altered determines the action of this ion.

Some insight into how the local flashes are evoked comes from time-lapse observations of contact formation between a presynaptic amacrine cell and a ganglion cell⁷⁹. On initial contact, no calcium elevation is seen in the ganglion cell dendrite, but local flashes appear about an hour later. In hippocampal cultures, molecules that are localized to synaptic sites have been observed to congregate within 30 min of initial contact⁹⁸. So, it is possible that the local calcium-release events reflect sites of new but maturing synapses. Whether this mechanism is involved in selectively maintaining the dendrites of ganglion cells in the appropriate sublamina of the inner plexiform layer remains to be examined.

CICR is unlikely to be the only mechanism that neurons use to locally regulate dendritic stability. The influx of calcium through NMDA receptors is necessary to promote dendritic growth in developing tectal neurons⁵⁴. However, with age, dendrites become stabilized by the activation of both NMDA and AMPA receptors, the latter receptor type being recruited during maturation. Calcium influx on depolarization activates

BALLISTIC METHOD
A transfection method in which the gene of interest is used to coat gold particles, which are then ‘fired’ into the biological sample using an air gun.

CAGED CALCIUM
In general terms, a caged molecule is a labile derivative of a biologically active molecule that will break down after appropriate (commonly luminous) stimulation to yield the bioactive compound.

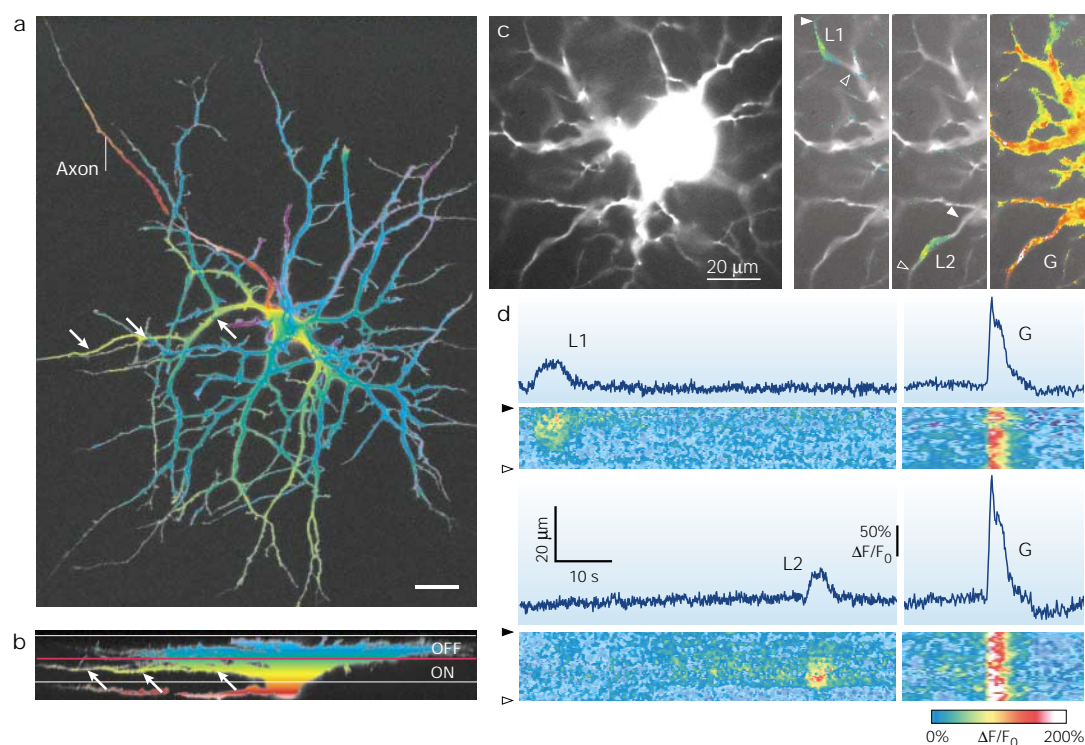


Figure 4 | **Local elevations in intracellular calcium might contribute to dendritic remodelling *in vivo*.** **a** | Arborization of an immature ferret retinal ganglion cell (RGC) reconstructed by TWO-PHOTON MICROSCOPY. Pseudocolour scale indicates relative depth in the inner plexiform layer, which is divided into two functionally distinct sublaminae — ‘ON’ and ‘OFF’. Note that although most of the arborization is located in the OFF sublamina, a major dendritic branch (arrows) resides in the ON sublamina. With maturation, this misplaced branch will be eliminated. **b** | Ninety-degree rotation of the image stack, providing a side view of the arborization. **c** | Embryonic chick RGC labelled with a calcium indicator, Oregon green 488 BAPTA-1, by ballistic delivery. Spontaneous increases in intracellular calcium, reported by a relative change in baseline fluorescence ($\Delta F/F_0$), are colour coded in **c** and **d**. Spatially localized flashes (L1, L2) occurred at different time points. Global (G) increases across the arborization were also observed. **d** | $\Delta F/F_0$ -versus-time rasters showing the spatial and temporal distribution of calcium levels within the two dendritic segments in **c** (arrowheads). Parts **a** and **b** reproduced, with permission, from REF. 114 © 2001 John Wiley & Sons; parts **c** and **d** reproduced, with permission, from *Nature* (REF. 79) © 2002 Macmillan Magazines Ltd.

RHO GTPASES

A family of proteins that are related to the product of the Ras oncogene and are involved in controlling the polymerization of actin.

TWO-PHOTON MICROSCOPY

A form of microscopy in which a fluorochrome that would normally be excited by a single photon is stimulated quasi-simultaneously by two photons of lower energy. Under these conditions, fluorescence increases as a function of the square of the light intensity, and decreases approximately as the square of the distance from the focus. Because of this behaviour, only fluorochrome molecules near the plane of focus are excited, greatly reducing light scattering and photodamage of the sample.

CaM kinases (particularly CaMKII), which act on the cytoskeleton to stabilize dendrites⁹⁹ (FIG. 3b). In RGCs, blocking nAChR activation reduces CICR and causes dendrites to retract⁷⁹. This occurs during the period in which cholinergic synapses are formed, and before bipolar glutamatergic synapses emerge in the inner retina. So, it is possible that, later in development, glutamatergic transmission onto RGCs, as in tectal cells, activates pathways that regulate dendritic stability. However, it is equally possible that cholinergic and glutamatergic inputs activate different downstream mechanisms to maintain the dendritic surface that they contact. In this way, it might be possible to separate the ‘signals’ from different types of input that are maturing. Alternatively, different cell types could use different calcium signalling mechanisms to effect a local change in dendritic structure.

Calcium regulation of the dendritic cytoskeleton. How does the regulation of calcium levels affect the dendritic cytoskeleton in ways that stabilize dendrites locally? Much is now known about how axonal growth cones extend and retract, but only recently has there been a

focus on dendritic movements. Axonal and dendritic movements share some molecular mechanisms that affect the cytoskeleton. Notably, the RHO GTPASES¹⁰⁰ have a prominent role in dynamically affecting both axonal and dendritic structure. When transfected into central neurons, constitutively active or dominant-negative Rac and RhoA alter the patterning of their dendrites *in vivo*^{101,102} and *in vitro*^{15,94,103,104}. Moreover, the effects of Rac and Rho on dendritic morphology are regulated by neural activity¹⁰⁵. The robust and universal effects of the small GTPases on dendritic architecture make it likely that changes in intracellular calcium levels act upstream of these molecules. However, so far, it is not known how this occurs.

Calcium might also affect dendritic stability by directly activating proteins that regulate the polymerization or depolymerization of actin and microtubules. One potential candidate is the actin-binding protein gelsolin. Gelsolin severs actin filaments and caps their barbed ends in a calcium-dependent manner¹⁰⁶. Recent studies of hippocampal neurons cultured from gelsolin-knockout mice indicate that this protein has a fundamental role in regulating neuronal

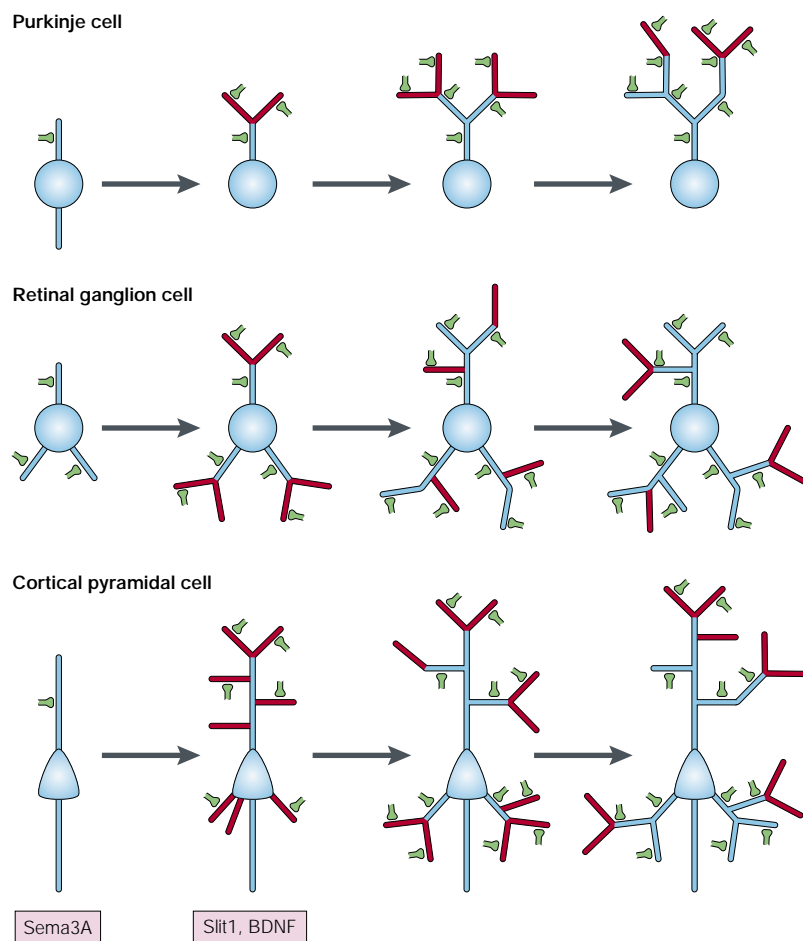


Figure 5 | Representation of how calcium signals might regulate dendritic growth and patterning during development. These stick diagrams of dendrites show the development of three cell types. The initial orientation of cortical apical dendrites is regulated by semaphorin 3A (Sema3A). In each case, the overall growth of the dendritic tree is regulated by calcium-dependent transcription. New dendritic branches are added, reflecting the instability of dendrites, and might be promoted by extracellular signals such as Slit1 and brain-derived neurotrophic factor (BDNF). If a new dendritic segment receives synaptic input, it can be stabilized by a mechanism that involves the local release of calcium from intracellular stores. In the absence of synaptic input, the dendritic branch withdraws. By such a mechanism, the pattern of synaptic input could have a significant influence on the final morphology of the dendritic tree.

FILOPODIA

Long, thin protrusions that are present at the periphery of migrating cells and growth cones. They are largely composed of F-actin bundles.

structure. Unlike wild-type neurons, the neurite shafts of *gelsolin*^{-/-} growth cones bear numerous FILOPODIA because of a reduced rate of filopodial retraction¹⁰⁷. Gelsolin is therefore needed to destabilize the actin-filled processes. By contrast, low-frequency stimulation of hippocampal neurons revealed that calcium influx through NMDA receptor channels leads to the stabilization of actin in dendritic spines, an effect that is much reduced in neurons from *gelsolin*^{-/-} mice¹⁰⁸. Whether activated gelsolin serves to stabilize or destabilize developing neurites is likely to depend on the local intracellular calcium concentration¹⁰⁹. So, gelsolin triggered by the local flashes might act to stabilize RGC dendrites. The presence of gelsolin in ganglion cells¹¹⁰ supports this possibility.

Other proteins that could be involved are rod-like proteins that are linked to microtubules and actin,

and have calcium-binding motifs¹¹¹. However, their potential function in dendrites is unclear. Finally, modulation of kinase activity might also be an important event in linking calcium-dependent events to regulation of the cytoskeleton. Of particular importance are CaMKII (REF. 99) and MAPKs¹¹², which influence dendritic structure in an activity-dependent manner. Clearly, much work is needed to identify the pathways that link changes in intracellular calcium levels to the dynamic and often rapid reorganization of dendrites.

Summary

Recent progress indicates that dendritic development is regulated by both activity-dependent and activity-independent cues. Studies in the developing cortex have shown that, in mammals, the influence of activity-independent cues might dominate during the embryonic period, and activity-dependent mechanisms might be more important after birth, during the period of synaptogenesis. For example, shortly after cortical neurons reach the cortical plate, they extend an apical dendrite towards the pial surface. The oriented growth of the apical dendrite is regulated by Sema3A, which acts as a chemoattractant for the growing apical process²⁵. The subsequent growth and branching of cortical dendrites is regulated by neurotrophins, as well as by the chemotropic protein Slit1 (REFS 5,26). It is likely that such molecular interactions have an important role in specifying the basic morphology of cortical and other neurons.

As neurons begin to receive innervation, the control of their dendritic growth and further patterning seems to be regulated by calcium-dependent signalling events. Whereas calcium-dependent transcription regulates the overall extent and direction of dendritic growth, local calcium signalling might independently control the stability of dendritic segments during the period of synapse formation and refinement (FIG. 5). The multiple and concurrent roles of calcium not only determine the final morphology of the neuron, but are also likely to have a crucial role in the specification of neuronal circuits.

What are the immediate challenges in our quest to understand dendritic growth and patterning? So far, how calcium signalling influences dendritic development has been assessed largely by examining the behaviour of the postsynaptic cell. To better understand how synaptic inputs influence dendritic development, it will be necessary in the future to simultaneously follow the dynamic interactions of pre- and postsynaptic processes. The rapid progress in cell-labelling techniques — in particular, the use of genetic methods to cause axons and dendrites, or component proteins of the synapse, to express different coloured fluorescent proteins *in vivo*¹¹³ — promises to help us reach this goal. Also, the ability to observe development *in vivo* and to manipulate the neuronal environment more specifically will be invaluable. Finally, comparison of the development of a variety of neuronal cell types will be important in helping us to identify common and cell-type-specific mechanisms that affect dendritic patterning in the CNS.

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