

MOLECULAR CONTROL OF CORTICAL DENDRITE DEVELOPMENT

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■ **Abstract** Dendritic morphology has a profound impact on neuronal information processing. The overall extent and orientation of dendrites determines the kinds of input a neuron receives. Fine dendritic appendages called spines act as subcellular compartments devoted to processing synaptic information, and the dendritic branching pattern determines the efficacy with which synaptic information is transmitted to the soma. The acquisition of a mature dendritic morphology depends on the coordinated action of a number of different extracellular factors. Here we discuss this evidence in the context of dendritic development in the cerebral cortex. Soon after migrating to the cortical plate, neurons extend an apical dendrite directed toward the pial surface. The oriented growth of the apical dendrite is regulated by Sema3A, which acts as a dendritic chemoattractant. Subsequent dendritic development involves signaling by neurotrophic factors and Notch, which regulate dendritic growth and branching. During postnatal development the formation and stabilization of dendritic spines are regulated in part by patterns of synaptic activity. These observations suggest that extracellular signals play an important role in regulating every aspect of dendritic development and thereby exert a critical influence on cortical connectivity.

INTRODUCTION

The structural and molecular differences that characterize axons and dendrites form the basis of information flow in neuronal circuits. The presence of voltage-gated sodium channels allows axons to propagate action potentials to synaptic terminals, which leads to the release of neurotransmitter into the synaptic cleft. The postsynaptic elements at excitatory synapses in the central nervous system (CNS) are often dendritic spines. Dendritic spines contain neurotransmitter receptors, which mediate ligand-dependent influx of ions into the postsynaptic neuron. Although it was long considered that dendrites then passively transmit this information to the soma, recent evidence suggests that dendrites act as dynamic integrators of synaptic input (Hausser et al. 2000). The transmission of the synaptic signal to

the soma is greatly affected by the branching pattern of the dendritic tree, and the striking variations in dendritic morphology in the CNS therefore have an enormous consequence for neuronal information processing (Hausser et al. 2000).

In this review we focus on our current understanding of the mechanisms that regulate the principal features of dendritic morphology in the developing cortex. Most cortical neurons are pyramidal in morphology and are characterized by a prominent apical dendrite that allows these neurons to integrate information from superficial cortical layers. Perhaps the most distinguishing feature of dendrites is a highly branched morphology, with decreasing diameter at successive branch points, which gives it the appearance of a tree (dendron in Greek means tree). The specific branching pattern and the distribution of ion channels on dendrites influence how synaptic signals decay as they propagate toward the soma. The dendrites in most cortical neurons are studded with small protrusions called spines, which are the major sites of excitatory synaptic transmission. We begin this review with a consideration of how neurons acquire a polarized morphology and how the growth of the apical dendrite toward the pial surface is regulated. We then move to a discussion of the influence of extracellular signals in regulating dendritic branching patterns. Finally we will consider the mechanisms that regulate the formation of dendritic spines and close with a discussion of intracellular signaling pathways that mediate dendritic growth and remodeling. The main generalization that can be drawn from these studies is that a large variety of extracellular signals, which includes both proteins and neurotransmitters, plays a critical role in specifying the mature dendritic morphology. There has been a great deal of recent progress in identifying these extracellular signals, and the challenge ahead is to understand how the action of these signals is coordinated so that they induce the acquisition of stereotyped neuronal morphologies specialized to process particular kinds of information.

MORPHOLOGICAL FEATURES OF CORTICAL DENDRITES

The mammalian neocortex can be divided into six layers, which can be distinguished based on neuronal morphology and density. Much of our knowledge of dendritic architecture in the cortex is based on anatomical studies of the visual cortex (Gilbert 1983). Each cortical layer serves a specific function, illustrated by their afferent inputs and efferent projections. The superficial layers 2, 3, and 4 are mainly responsible for intracortical projections. On the other hand, the deeper layers 5 and 6 contain neurons that project subcortically. Cells in layer 5 project to the superior colliculus, pons, and spinal cord, while layer 6 neurons project to the claustrum and thalamus.

There is a well-defined relationship between the morphology of cortical neurons and their function. The main excitatory neuronal subtypes are the pyramidal cells and the spiny stellate cells (Figure 1). The other main cell type is the smooth stellate cell, which is thought to be inhibitory (Houser et al. 1983, Prieto et al. 1994). Pyramidal neurons are the dominant cell type in the neocortex. They typically have an apical dendrite that branches out in an apical tuft that terminates

in layer 1. In addition, pyramidal neurons contain several highly branched basal dendrites that emanate from the cell body. These neurons are mainly present in layers 2, 3, 5, and 6. Within a layer, pyramidal neurons can be morphologically classified based on their projection areas. In layer 6 for instance, neurons projecting to the lateral geniculate nucleus have an apical dendrite that terminates in layer 3, with side branches in layer 4 and 5, while claustrum-projecting neurons have an apical that extends to layer 1, with side branches only in layer 5 (Katz 1987). In contrast to pyramidal neurons, spiny stellate cells are exclusively found in layer 4. They usually have many spiny dendrites of similar length radiating from the cell body. Inhibitory smooth stellate cells have varying nonpyramidal morphologies and are present in all layers of the cortex.

During early development, just after they have reached the cortical plate, all excitatory cortical neurons share a common morphological phenotype. They contain a single apical dendrite that branches within layer 1 (Marin-Padilla 1992, Miller 1981). With time, basal dendrites appear and oblique side branches emerge from the apical shaft. Spines start to appear as the arborization of the apical and basal dendrites becomes more complex. When pyramidal neurons reach their mature morphology, they have a highly complex dendritic arbor and are covered with spines. Interestingly, early spiny stellate neurons in layer 4 also start out with a pyramidal morphology. These neurons, however, acquire a stellate morphology by retracting their apical dendrite at an early postnatal age (Vercelli et al. 1992). A similar phenomenon has also been described for certain layer 5 pyramidal neurons. Callosally projecting pyramidal neurons were found to specifically retract their apical dendrite to obtain their characteristically short pyramidal morphology (Koester & O'Leary 1992). This is in contrast to corticotectal-projecting layer 5 pyramidal neurons, which maintain their apical dendrite to layer 1. These results emphasize that early dendritic development of cortical neurons is mediated in part by an intrinsic growth program, but specific refinement occurs later on to generate class-specific dendritic morphologies.

NEURONAL POLARITY AND THE REGULATION OF APICAL DENDRITE ORIENTATION BY SEMAPHORIN SIGNALING

Most cortical neurons are generated from precursors proliferating in the germinal zones lining the ventricle (Figure 2A). Recent evidence suggests that radial glial cells are the main neural precursors in the developing cortex (Malatesta et al. 2000, Miyata et al. 2001, Noctor et al. 2001). These dividing precursors display a polarized localization of signaling molecules such as Notch, Numb, Numbl, and β -catenin (Chenn & McConnell 1995, Chenn et al. 1998, Zhong et al. 1997). Therefore, one model to explain the emergence of neuronal polarity postulates that postmitotic neurons leaving the ventricular zone are polarized due to asymmetric targeting of signaling molecules.

Dendritic differentiation, as determined by expression of dendrite-specific genes such as MAP-2, does not begin until the cells have completed their migration.

Following migration, pyramidal neurons extend an axon toward the ventricle and an apical dendrite toward the pial surface (Figure 2B). Is this directed growth of axons and dendrites simply a consequence of the intrinsic polarity of neurons evident during radial migration, or are there extrinsic cues that direct the growth of axon and dendrites in appropriate directions? To test the role of the local cortical environment in directing the growth of nascent axons and dendrites, an *in vitro* assay was developed in which dissociated neurons from a donor cortex were plated onto cortical slices and cultured organotypically (Polleux et al. 1998). Only two to three hours after plating, the vast majority of neurons extended an axon directed toward the ventricle (Figure 2C). This demonstrates the existence of extracellular cues that are sufficient to induce the directed outgrowth of the axon toward the ventricle (Polleux et al. 1998). This ventrally oriented outgrowth is due to the chemorepulsive action of a diffusible semaphorin (Sema3A) expressed in a gradient with highest levels near the marginal zone.

Cortical neurons in the slice overlay assay do not begin to extend a well-differentiated dendrite until a day or two after the axon has emerged. Strikingly, the dendrites of neurons plated on cortical slices behave just like the endogenous pyramidal neurons and extend an apical dendrite toward the pial surface. Cell biological experiments indicate that the oriented growth of dendrites toward the pia is regulated independently from the axons and is due to the action of a chemoattractant present near the marginal zone. Remarkably, the dendritic chemoattractant is Sema3A, the factor that repels cortical axons (Polleux et al. 2000). These experiments indicate that the differential response of axons and dendrites to the same chemotropic cue leads to the specification of the basic pyramidal morphology that characterizes most cortical projection neurons (Figure 2D).

What are the mechanisms that might lead to the generation of opposite responses in two compartments of the same neuron? The work of Mu-Ming Poo and his colleagues had previously shown that elevation of the intracellular level of cGMP is sufficient to convert Sema3A from a chemorepellant to a chemoattractant in *Xenopus* spinal cord axons (Song et al. 1998). The ability of cGMP to switch chemotropic responses appears to be involved in the differential response of axons and dendrites to Sema3A. The enzyme that regulates cGMP production, soluble guanylate cyclase (sGC), is localized asymmetrically in immature cortical neurons and is preferentially targeted to the emerging apical dendrite (Figure 2E) (Polleux et al. 2000). Pharmacological inhibition of sGC activity or one of its downstream targets, cGMP-dependent protein kinase, abolishes the ability of Sema3A to attract apical dendrites (Polleux et al. 2000). Thus the basis of the differential response of axons and dendrites to Sema3A appears to be asymmetric targeting of a signaling molecule to the emerging dendrite.

These findings show that the asymmetric targeting of signaling molecules can serve as a mechanism for generating a polarized neuronal response to an extracellular signal, as had been shown previously in amoeba and leukocytes (Parent & Devreotes 1999) and in budding yeast (Pruyne & Bretscher 2000). During chemotaxis, for example, chemoattractant receptors and G proteins are not clustered at the leading edge of the chemotaxing cells (Servant et al. 1999, Xiao et al. 1997). Instead

specific intracellular effectors such as cytosolic regulator of adenylate cyclase (CRAC) are rapidly redistributed to the leading edge of chemotaxing cells through specific targeting to the membrane via pleckstrin homology domains (Parent et al. 1998). Interestingly, this is similar to the cortical neuron response to Sema3A, during which the semaphorin receptor Neuropilin-1 is present both on the axon and the developing apical dendrite (Polleux et al. 2000), but a polarized response is generated due to asymmetric localization of the downstream effector molecule sGC.

Until recently, most of our knowledge concerning the emergence of neuronal polarity was based on studies using the classic cell culture system developed by Banker and colleagues (Bradke & Dotti 2000, Craig & Banker 1994). This assay takes advantage of the fact that dissociated hippocampal neurons represent a morphologically homogenous population of pyramidal neurons and rapidly become polarized *in vitro* following a reproducible and well-characterized program (Dotti et al. 1988). Four stages have been distinguished: At stage 1 the cells are round following dissociation and immediately after plating; at stage 2, shortly after plating, hippocampal neurons extend 4–5 neurites; and within 24 h after plating, one of the neurites starts to elongate to become the axon (stage 3). At this stage if the axon is cut, then other neurites can still be converted into axons instead of becoming dendrites (Dotti & Banker 1987). At stage 4, dendrites acquire their morphological and structural features and lose their plasticity to form a new axon when it is sectioned. This model has been used extensively to explore the factors controlling both the emergence of neuronal polarity and the polarized sorting of axonal and dendritic cues (Bradke & Dotti 2000).

Using this assay, Dotti and collaborators have proposed a model in which a stochastic process marks one neurite to become the axon. The chosen neurite then inhibits other neurites from growing and expressing axon-specific features (Bradke & Dotti 1997, 2000). The same group has shown that local perfusion of the actin-depolymerizing agent, cytochalasin D, onto a randomly chosen growth cone of a stage 2 neuron induces it to grow as an axon, which suggests that actin depolymerization is sufficient for axon specification (Bradke & Dotti 1999). Observations from the slice overlay assay described above do not fully support this model of axon specification through neurite selection. Time-lapse imaging experiments indicate that only one process initially emerges from cells plated onto slices, and that process goes on to become an axon (F. Polleux & A. Ghosh, unpublished observation). Instead the emergence of the axon at a specific pole of the cell and a later emergence of the apical dendrite appear to be regulated by extracellular cues present in the local environment of cortical neurons (Polleux et al. 1998).

REGULATION OF DENDRITIC GROWTH AND BRANCHING BY NEUROTROPHIC FACTORS

Studies over the last few years provide compelling evidence that neurotrophic factors play an important role in regulating dendritic growth and branching in cortical neurons. Many of these studies have focused on the role of neurotrophins, which

consist of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4 (Huang & Reichardt 2001). These factors exert their effects through the Trk family of tyrosine kinase receptors. NGF binds to TrkA, BDNF and NT-4 bind to TrkB, and NT-3 preferentially binds to TrkC. Experiments in which the effects of neurotrophins on dendritic growth control have been examined in slice cultures indicate that in general, neurotrophins increase the dendritic complexity of pyramidal neurons by increasing total dendritic length, the number of branchpoints, and/or the number of primary dendrites (Baker et al. 1998, McAllister et al. 1995, Niblock et al. 2000). The response is rapid and an increase in dendritic complexity is readily apparent within 24 h of neurotrophin exposure. There is a clear specificity in the short-term response of pyramidal neurons of different cortical layers to each of the neurotrophins. For instance, NT-3 strongly increases dendritic complexity in layer 4 neurons but has no apparent effect on layer 5 neurons. In addition, basal dendrites in specific layers respond most strongly to single neurotrophins, whereas apical dendritic growth is increased by a wider array of neurotrophins. Live imaging of layer 2/3 neurons expressing BDNF shows a high level of dendrite dynamics. Both dendritic branches and spines are rapidly lost and gained in BDNF transfected neurons (Baker et al. 1998, McAllister et al. 1995, Niblock et al. 2000). BDNF overexpression favors addition of primary dendrites and proximal branches at the expense of more distal segments. Similarly, overexpression of TrkB in layer 6 pyramidal neurons results in a predominance of short proximal basal dendrites (Yacoubian & Lo 2000).

Recently, osteogenic protein-1 (OP-1) (aka BMP7), which is a member of the transforming growth factor-beta superfamily, was shown to increase total dendritic growth and branching from dissociated embryonic cortical neurons (Le Roux et al. 1999). Furthermore, insulin-like growth factor-1 (IGF-1) has been shown to affect dendrite growth and branching of postnatal layer 2 cortical neurons (Niblock et al. 2000). In contrast to neurotrophins, IGF affected both basal and apical dendritic growth and remodeling, illustrating that the final dendritic complexity of pyramidal neurons is likely to be influenced by the action of multiple neurotrophic factors.

How do neurotrophic factors mediate the morphological changes linked with dendritic remodeling? The observed short-term dynamics indicate a rapid modulation of cytoskeletal elements by neurotrophic factor signaling. Of the major signaling pathways activated by Trk receptors and most other tyrosine kinase receptors, the MAP kinase and PI-3Kinase pathways have been implicated in neurite formation in both neuronal cell lines and primary neurons (Posern et al. 2000, Wu et al. 2001). It is likely that these signaling pathways influence neuronal morphology by regulating the activity of the Rho family GTPases, which mediate actin cytoskeleton dynamics and are known to induce rapid dendritic remodeling. Experiments in neuronal cell lines show that NGF can activate the small GTPase Rac1 in a PI-3kinase-dependent manner, and this activation is necessary for neurite elaboration (Kita et al. 1998, Posern et al. 2000, Yasui et al. 2001). It will be of interest to determine if this pathway is involved in neurotrophin-induced dendritic morphogenesis of cortical neurons.

Part of the neurotrophic factor effect on dendritic morphogenesis may also include the control of expression of structural proteins because long-term exposure to neurotrophins leads to net dendritic growth. It was recently reported that BDNF can upregulate local protein synthesis in dendrites within hours (Aakalu et al. 2001). In addition, specific mRNAs for several cytoskeletal proteins are present in dendrites (Kuhl & Skehel 1998). This raises the interesting possibility that local synthesis of structural components is involved in neurotrophic factor control of dendritic growth.

REGULATION OF DENDRITIC GROWTH AND BRANCHING BY NOTCH SIGNALING

The diversity of signals that can influence dendritic morphology is underscored by a series of recent studies on the role of mammalian Notch proteins in regulating dendritic growth and branching. Originally identified in *Drosophila*, Notch is a type I cell-surface protein, approximately 300 kDa in size, which functions as a receptor (Artavanis-Tsakonas et al. 1995, 1999; Weinmaster 1997). Proteolytic processing of full-length Notch generates two fragments that associate at the plasma membrane to form a receptor complex. The mechanism of Notch receptor activation involves cleavage and nuclear translocation of the intracellular domain of the receptor (Weinmaster 2000). The intracellular domain of Notch enters the nucleus and binds the transcription factor Suppressor of Hairless [Su(H)], activating gene transcription. Mammalian homologs of Notch (Notch1-4), the Notch ligands Delta (Delta1-3) and Serrate (Jagged1, Jagged2), the transcription factors Su(H) (CBF1/RBP-Jk), and E(Spl) (Hes1-5) have been isolated (Weinmaster 2000). Several of these genes are expressed in the developing brain and spinal cord and are likely to control various aspects of neural development (Bettenhausen et al. 1995, de la Pompa et al. 1997, Del Amo et al. 1992, Dunwoodie et al. 1997, Furukawa et al. 1992, Lardelli et al. 1994, Lindsell et al. 1996, Nakamura et al. 2000, Ohtsuka et al. 1999, Reaume et al. 1992).

The possibility that Notch might play a role in regulating dendritic patterning was suggested by immunocytochemical localization studies that showed that mammalian Notch1 is expressed by both dividing cells in the ventricular zone and postmitotic neurons in the cortical plate (Redmond et al. 2000, Sestan et al. 1999). Several observations suggest that Notch signaling mediates contact-dependent inhibition of neurite outgrowth. For example, in postmitotic neurons there is an inverse correlation between Notch1 expression and total neurite length, and overexpression of a constitutively active Notch1 construct leads to a reduction in the total neurite length (Sestan et al. 1999). Cocultures of cortical neurons with Delta- or Jagged-expressing cell lines, or addition of soluble ligands leads to a decrease in total neurite length, suggesting that Delta or Jagged are the relevant Notch1 ligands (Sestan et al. 1999). Also, overexpression of Numb and Numbl-like, intracellular modulators that inhibit Notch activation via Su(H)/CBF1, leads to an increase in

total neurite length (Sestan et al. 1999). Berezovska and coworkers (1999) have also found that expression of constitutively active Notch1 in hippocampal neurons leads to an inhibition of neurite outgrowth. A study examining Notch function in neuroblastoma cells came to a similar conclusion regarding the effects of Notch signaling on neurite length (Franklin et al. 1999). Together these observations indicate that Notch signaling has an inhibitory effect on process outgrowth.

Recent experiments (Redmond et al. 2000) indicate that, in addition to restricting length, Notch signaling in cortical neurons has a major influence on dendritic branching. The effects of Notch1 signaling on dendrite morphology were examined by measuring several parameters of dendrite complexity, including process and branch point number, dendrite length, and branching index. Inhibition of Notch1 signaling by overexpression of a dominant negative Notch1 construct or with antisense oligonucleotide treatment leads to a decrease in dendritic branching in neurons. In addition, overexpression of a constitutively active Notch1 construct decreases average dendrite length but increases the branching index, resulting in an overall increase in dendritic complexity. Taken together these experiments reveal a positive role for Notch in dendrite branching and a negative role in dendrite and total neurite length.

It is not yet known whether Notch regulation of dendritic development involves the same effectors that regulate cell fate decisions, or if a different set of effector proteins mediates dendritic development. Some of the Notch effects on dendrites may be mediated by molecules previously implicated in cell fate decisions because Hes-1, Neurogenin-1, and MASH-1 are all involved in neuronal differentiation and neurite outgrowth (Castella et al. 1999, Olson et al. 1998, Yavari et al. 1998). An alternate mechanism for the effects of Notch signaling on process outgrowth has recently been proposed by Giniger (Giniger 1998). In a series of genetic studies in *Drosophila*, Notch and the tyrosine kinase Abl were shown to interact synergistically in producing axonal defects. In addition, both Notch and Abl are present in the axon, and Notch can biochemically interact with the Abl-interacting protein Disabled. Since Abl and Disabled are thought to control the axonal cytoskeleton, they could provide a link between Notch activation and cytoskeleton changes, thus mediating the effects of Notch signaling on axonal morphology. It will be interesting to determine if Abl and Disabled are present in dendrites and whether they play a role in mediating the effects of Notch on dendritic patterning.

REGULATION OF DENDRITIC SPINE DEVELOPMENT

Normal Development of Dendritic Spines: Golgi Studies

The final step in the acquisition of a mature dendritic morphology is the development of spines. Historically, spines have been categorized on the basis of morphology as thin spines (with thin necks and bulbous heads), mushroom spines (with broader heads), and stubby spines (with no necks). These small dendritic protrusions harbor the vast majority of excitatory synapses and contain receptors

and other proteins necessary for synaptic transmission (Kennedy 2000). The function of spines may be to modulate the synaptic response in a compartment separate from the dendritic shaft.

The formation of spines appears to be a developmentally regulated program, which may be influenced by activity. Miller (1981) performed a systematic Golgi survey of dendritic development in early postnatal rat visual cortex. He examined the density of spines on the apical dendrites of both layer 2/3 and layer 5 pyramidal cells, which mature similarly despite the three-day difference in their birthdates. During the first week after birth, there are very few spines present along the apical shaft. However, at the end of the first week, the number of spines increases dramatically, especially between postnatal day 6 (P6) and P9. A second increase in spine density occurs between P12 and 15. The appearance of spines at P6–9 and P12–15 correlates with the arrival of geniculate axons in the cortical plate and eye opening, respectively, which suggests that activity may regulate some aspects of spine development.

Spine density continues to increase through the first postnatal month, after which it declines slightly. Initially, spines are distributed relatively evenly along the apical dendrite, excluding only the area immediately adjacent to the soma. However, by P15, spine density increases with distance from the cell body. This trend is observed along the first 125 μm , after which the density remains fairly stable or declines slightly.

Studies in other animal models and cortical areas have reached similar conclusions about the dynamics of spine development yet reveal important morphological differences between neurons in different layers and areas. For example, work done in primates allows more precise laminar localization of Golgi-impregnated neurons. In the visual cortex of *Macaca nemestrina* monkeys (pig-tailed macaques), Boothe et al. (1979) studied the development of pyramidal neurons in layers 3B and 6 and spiny stellate neurons in layers 4C α and 4C β . During the first eight weeks of life, corresponding to the critical period for development of normal binocular vision, the density of spines increases greatly for both populations of pyramidal neurons, after which it declines. Like the rat neurons described by Miller (1981), these pyramidal neurons show a higher density of spines in the proximal apical dendrite. In contrast, the layer 4 spiny neurons have a constant density of spines throughout the length of their dendrites. In addition, the layer 4 cells demonstrated a much more gradual rise in the density of spines during the critical period. These differences between the two populations of cells presumably reflect differences in the nature of their synaptic input and response to activity during the critical period.

Petit et al. (1988) carried out a similar study, but in the rat sensorimotor cortex. These investigators found an increase in spine density until P30 on the apical dendrites of layer 5 pyramidal neurons but no period of accelerated spine formation. This may reflect an intrinsic difference between the wiring of visual and somatosensory cortices. Interestingly, when they examined the terminal dendritic branches of the apical dendrites, they found that the spine density continued to increase until adulthood.

Most studies on dendritic spines have focused on one region of the cortex at a time, such as visual or somatosensory cortex. Jacobs et al. (2001) arranged eight Brodmann's areas into a functional hierarchy based on the complexity of neural processing occurring in that area. For example, primary motor cortex was considered to be a region of low integration, while the supplementary motor cortex was designated a region of high integration. Next, they compared the dendritic complexity and spine density of Golgi-stained human tissue from the different Brodmann's areas. Although there were significant interindividual and interarea differences, areas of high integration consistently had longer basal dendrites and larger numbers of dendritic spines. This study indicates that an increased density of spines is associated with increased complexity of synaptic integration.

Normal Development of Dendritic Spines: Time-Lapse Studies

How do dendritic spines form? Early studies combined Golgi staining with electron microscopy (EM), revealing the detailed ultrastructure of dendrites and spines once a neuron had been identified. Miller & Peters (1981) studied the development of layer 5 pyramidal neurons in the rat visual cortex with such an approach. During the first postnatal week, before a significant number of spines had formed, dendrites had a large number of filopodia-like processes and stubby spines. The filopodia were long and thin, lacking a bulbous head. Each appeared to be directly apposed to an axon, and while no synapses were present, both the axonal and filopodial membranes were thickened, suggesting that a synapse was forming. Stubby spines, on the other hand, were associated with synapses, but smaller and symmetric ones. However, more protuberant stubby spines formed the asymmetric junctions associated with excitatory synapses, which suggested that stubby spines might grow into taller spines as the synapse matured. As the number of mature spines increased, the number of filopodia and stubby spines decreased, which suggested that this transition is linked to the development of spines.

Time-lapse experiments in which DiI-labeled hippocampal neurons in slices were imaged (Dailey & Smith 1996) revealed that dendritic filopodia are highly dynamic projections, rapidly changing length and shape but lasting only short periods of time. These filopodia would either disappear or become more stable protospines or spines. This suggested a model in which highly protrusive filopodia explore the local environment, make contact with an axon, and guide it back to the dendrite to form a spine. However, it was not clear from these time-lapse studies whether the filopodia directly transition into spines or whether a synapse must first form directly on the shaft. An EM study (Fiala et al. 1998) found that 70% of the synapses in the CA1 region of the hippocampus were present on the dendritic shaft or at the base of a filopodia, which suggests that filopodia are involved in inducing shaft synapses.

A recent paper (Marrs et al. 2001) appears to help resolve this issue of spine development. Particle-mediated gene delivery was used to transfect early postnatal hippocampal slices with a cDNA construct encoding PSD-95 [a component of

the postsynaptic density (PSD)] tagged with enhanced green fluorescent protein (GFP). This protein localized to PSDs, which could then be visualized. PSDs were found to be highly dynamic and were able to appear, move, and disappear in a matter of minutes. These PSD95-GFP clusters largely co-localized with synapsin-I, a marker of presynaptic terminals, and were found in mature spines. Transient filopodia were observed; often they would regress spontaneously, but in those cases where a PSD95-GFP cluster developed, the structure would stabilize into a protospine or spine. This suggests that the cluster formed because of a synaptic contact and that this contact was responsible for transforming the filopodia into a spine. Additionally, some spines were formed directly by extension from the shaft, which suggests that shaft synapses can directly form spines.

Dendritic Spines and Activity-Dependent Plasticity

The imaging studies described above were performed in the hippocampus rather than in the neocortex, but presumably the mechanisms of spine formation are the same. Spines and excitatory synapses in the hippocampus have been extensively studied in order to understand how the structure of the brain may change in response to experience. The hippocampus is known to be essential for learning and memory, and synapses in the hippocampus can change the strength of their activation in response to activity, a phenomenon known as long-term potentiation (LTP).

Dendritic spines in the hippocampus undergo morphological changes in response to activity. This area has been extensively reviewed recently (Yuste & Bonhoeffer 2001), but to summarize, there is evidence that synaptic activity results in the enlargement of spines and the shortening of spine necks. These changes reflect the number of presynaptic-docked vesicles and postsynaptic receptors and may affect the efficiency with which an excitatory postsynaptic potential is transmitted to the dendritic shaft or that calcium is extruded. Additionally, increases in activity result in the formation of new spines.

What is the evidence for activity-dependent changes in cortical spines? The majority of reports have been Golgi-staining studies following various deprivation paradigms. For example, Valverde (1967) raised mice in the dark for the first three postnatal weeks and then examined the apical dendrites of layer 5 pyramidal cells where the dendrites traverse through layer 4. He found a significant reduction in the density of spines in the visual cortex. Spine density in the temporal region was unaffected, which leads to the conclusion that the decrease in spine density was due to the reduction in visual input.

Riccio & Matthews (1985) further examined this phenomenon by injecting tetrodotoxin into one eye to abolish action potentials, thereby completely silencing the input from the injected eye for three weeks. Spine densities in layer 5 pyramidal cells in sham-injected animals and internal controls (corresponding to the uninjected eye) are very similar. However, the tetrodotoxin treatment results in a 26% reduction in spines. Changes in spine density, however, have not been seen in every study. Veas et al. (1998) studied the somatosensory cortex of rats following

whisker plucking between one and two months of age. The spines of spiny stellate neurons in layer 4 were examined following serial EM reconstruction. Although there were no differences in spine density, spines contralateral to the deprived side tended to have decreased volume and surface area of the spine head and increased length of the spine neck.

In a recent technological advance, it has become possible to study the effect of experience-dependent plasticity *in vivo* (Lendvai et al. 2000). The barrel cortices of P8–18 postnatal rats were infected with Sindbis virus containing the gene for eGFP, and labeled layer 2/3 neurons were imaged *in vivo* with time-lapse two-photon laser scanning microscopy. Dendritic protrusions were captured every 10 min and quantified as the length of individual protrusions as a function of time. Their imaging demonstrated that both filopodia and spines are highly motile *in vivo*. Since filopodial motility had previously been linked to changes in synapse formation, Lendvai et al. deprived the barrel cortex of activity by trimming the rat's whiskers one to three days before imaging. During a brief period, P11–13, spine motility was reduced by the deprivation. This time period corresponds to a period of intense synaptogenesis, when the rats first start using their whiskers to explore their environment. However, deprivation did not affect spine density, length, or morphology, in contrast to some of the other anatomical studies.

Mechanisms of Spine Motility

What is responsible for the rapid motility of dendritic spines and filopodia? A study in the hippocampus (Fischer et al. 1998) demonstrated that the motility is actin based. Cultured hippocampal cells were transfected with GFP-tagged actin, which accumulates in the actin-rich spines. Time-lapse imaging of the GFP fluorescence demonstrated that spines can be very motile, changing their shape on a timescale of seconds or less. This phenomenon is actin dependent because drugs interfering with actin polymerization rapidly abolish the spine motility.

These findings were extended to cortical neurons in slice culture by Dunaevsky et al. (2001) using particle-mediated transfection of GFP in cultured mouse brain slices. Time-lapse imaging revealed these spines to be highly motile. Furthermore, this motility was developmentally regulated. When slices cultured at the day of birth were examined at one week, 74% of spines were motile, compared with 50% of the spines in slices that had been cultured for three weeks. Similar findings were reported by Lendvai et al., who found that protrusive spine motility in the somatosensory cortex decreases with age.

There is also evidence for the regulation of spine formation and spine motility by the neurotrophin BDNF. In addition to affecting dendritic morphology as described earlier, particle-mediated gene transfer of BDNF into ferret slices affected spines (Horch et al. 1999). Over a 16-h period, there was a 2.5-fold reduction in spine density. Of the spines that remained, a higher percentage had an increased turnover rate. This is an exciting finding because it provides a mechanism by which local activity-dependent activation of BDNF could cause local destabilization of spines, thus facilitating synaptic remodeling. While a number of perturbations have

now been shown to influence spine formation or spine motility, the consequences of these treatments on synaptic function are not known. Combining morphological analysis of spines with single-cell electrophysiological recordings should provide important insight into the relationship between dendritic morphology and synaptic function.

Dendritic Spines and Mental Retardation

The proper development of dendritic spines is essential for normal cognitive development. Many abnormalities associated with mental retardation (MR) have been identified. For example, Purpura (1975) recognized that many individuals with nonsyndromic MR (MR not associated with a known genetic defect) have dendritic spines that are abnormally sparse, long, and thin, reminiscent of the filopodia seen during early spine development. Improper spine (and synapse) formation may therefore be the anatomical cause of some forms of MR.

Some genetic disorders associated with MR also demonstrate defects in dendritic spines, including Down syndrome, Rett syndrome, and Fragile-X (FraX) syndrome [reviewed extensively by (Kaufmann & Moser 2000)]. Mouse models of MR syndromes may help elucidate some of the mechanisms of normal and abnormal development of dendritic spines. For example, Comery et al. (1997) studied the dendritic spines of mice with a targeted deletion in FMR1, the FraX gene, at four months of age. Dendritic spines in knockout mice were longer and spine density was higher than in wild-type controls, consistent with a role for the FMR1 protein in spine maturation and pruning.

More recent work (Nimchinsky et al. 2001) has extended the analysis of the FMR1 mice to earlier developmental stages. Layer 5 barrel cortex neurons were imaged with two-photon microscopy following infection with eGFP-Sindbis virus. At one week after birth, dendritic spines were longer and more dense in knockout animals. However, these differences decreased or disappeared after the second postnatal week. This study suggests that FMR1 is required at two different stages of spine formation or function because of this spine normalization after the first week.

It is clear that similar studies in the future will provide great insight into the molecular mechanisms of dendritic spine formation. In addition to mouse models of MR, the roles of other genes could be studied after their introduction via viral vectors to determine what effects their products might have on spine formation, stability, or motility.

LOOKING INSIDE DENDRITES: mRNAs, PROTEINS, AND CONTROL OF THE DENDRITIC CYTOSKELETAL

Dendritic Targeting of mRNAs and Proteins

Ultrastructurally, dendrites contain almost all of the organelles present in the soma, especially mRNAs and free or membrane-bound ribosomes, which suggests an efficient translational activity (Craig & Banker 1994). Beyond the axonal hillock,

only few ribosomes and mRNAs can be found (Craig & Banker 1994, but see also Bassell et al. 1998). This property of local protein synthesis is believed to be important in mediating long-term adaptive responses in neurons, but the mechanisms by which mRNAs are targeted to dendrites and how their translation within dendrites is regulated are not well understood.

The specialized function of dendrites requires selective trafficking of proteins to the dendritic compartment. Over the past two decades, the mechanisms leading to the polarized sorting of membrane proteins have been extensively studied in the epithelial Madin-Darby canine kidney (MDCK) cells, a useful experimental model in which specific proteins are selectively sorted either to the apical or basolateral domains. Dotti & Simons (1990) have proposed that neurons and epithelial cells might use similar cellular mechanisms to generate a polarized distribution of membrane proteins. This was based on a study showing that hippocampal neurons infected with vesicular stomatitis virus or influenza virus display a targeted localization of some viral proteins such as G proteins to the somatodendritic domains. These proteins are normally found in the basolateral domain of MDCK cells. On the other hand, influenza hemagglutinin, which is targeted to the apical domain of MDCK cells, was targeted to the axon of hippocampal neurons. Subsequent studies have shown that mutation of sequences required for basolateral localization in MDCK cells also disrupt dendritic targeting, which suggests the involvement of common sorting and targeting mechanisms (Jareb & Banker 1998). However, the generality of this analogy is not clear since for some proteins, such as transferrin receptor, dendritic targeting is mediated by a signal distinct from that mediating basolateral targeting (Haass et al. 1995, Tienari et al. 1996, Wozniak & Limbird 1998).

Recently, an elegant study performed by Stowell & Craig (1999) has provided evidence that specific sequences in the C-terminal of the cytoplasmic tail of metabotropic glutamate receptors (mGluR) are sufficient for their appropriate targeting to the somatodendritic or axonal domain. The authors took advantage of the fact that mGluR2 is targeted to dendrites and excluded from axons, whereas mGluR7 is targeted both to axons and dendrites. Using viral-mediated expression of chimeric or deleted proteins in hippocampal neurons, they showed that axonal exclusion of mGluR2 versus axon-specific targeting of mGluR7 is mediated by a 60-amino acid-long C-terminal cytoplasmic region. Addition of the mGluR7 C-terminal sequence to mGluR2 or to an unrelated somatodendritic protein was sufficient to induce axonal targeting. The mechanism by which this sequence regulates protein targeting is not yet known.

Rho GTPases and the Regulation of Actin Dynamics

Actin and microtubules are the cytoskeletal components that provide the structural basis for dendrites. Microtubules form the core of dendritic shafts, while actin is located at the rim and at the tips of dendrites. Actin components of the neuronal cytoskeleton drive exploratory activity, while microtubules stabilize newly formed processes. Regulation and restructuring of both actin and microtubule components therefore forms the basis for dendritic growth and remodeling.

The Rho family of small GTPases has lately received a lot of attention for its role in mediating changes in dendritic shape. Rho family GTPases are regulators of actin dynamics and act as molecular switches. They cycle between an active GTP-bound state and an inactive GDP-bound state. In their GTP-bound state they are able to bind and activate downstream effector proteins. The transition from inactive to active state is mediated by guanosine nucleotide exchange factors (GEFs), and the return to the inactive GDP-bound state is catalyzed by GTPase activating proteins (GAPs). The Rho family of GTPases consists of ten members, and the best-studied members are RhoA, Rac1, and Cdc42. Recent studies have demonstrated the central role for these proteins in mediating dendrite growth and remodeling (Lee et al. 2000, Li et al. 2000, Nakayama et al. 2000, Ruchhoeft et al. 1999, Threadgill et al. 1997).

Experiments in vertebrates and invertebrates indicate that RhoA influences the growth of dendritic arbors. Expression of a constitutively active form of RhoA in fly mushroom body neurons (Lee et al. 2000), *Xenopus* and chick retinal ganglion cells (Ruchhoeft et al. 1999, Wong et al. 2000), *Xenopus* tectal neurons (Li et al. 2000), and hippocampal neurons (Nakayama et al. 2000) generally leads to a dramatic decrease in dendritic growth. Active RhoA not only prevents the formation of new dendrites but also seems to induce retraction of existing branches. Conversely, blocking RhoA function in these systems promotes growth of dendritic segments. This is nicely demonstrated in *Drosophila*, in which selective removal of RhoA in individual fly mushroom body neurons leads to overextension of dendrites into areas normally not occupied by these neurons (Lee et al. 2000). However, in most other systems, blocking RhoA function results in only a mild phenotypic defect. Perhaps the RhoA pathway is normally inactive to allow dendrite extension and is only activated locally when dendrite arbor growth should be restricted.

Rac1, and to a lesser extent Cdc42, appear to control dendritic branching and remodeling. The most striking phenotype of Rac1 activation in several systems is the selective increase in dendrite branch additions and retractions (Li et al. 2000, Wong et al. 2000). This restructuring induced by Rac1 is rapid, and while Rac1 has been reported to increase branching complexity, the overall dendritic morphology is not greatly affected (Li et al. 2000, Nakayama et al. 2000, Ruchhoeft et al. 1999, Threadgill et al. 1997, Wong et al. 2000).

Rho proteins also exert considerable influence on dendritic spines. Transfection of Rac1 and RhoA constructs into cultured mouse cortical slices perturbs spine formation (Tashiro et al. 2000). Constitutively active Rac1 promotes spine formation, while constitutively active RhoA reduces the number of spines and the length of spine necks. Similar observations have been made in Purkinje cells and hippocampal neurons (Luo et al. 1997, Nakayama et al. 2000, Tashiro et al. 2000). Spines are actin-based structures, which explains the strong influence of Rac1 on spine morphology.

The regulators of the RhoGTPases, GEFs, and GAPs are also likely to be important for spine formation. Consistent with that possibility, Kalirin-7, a RhoGEF with exchange activity for Rac1, was recently found to be targeted to postsynaptic densities in spines through its association with PSD-95 (Penzes et al. 2001). In

cultured cortical neurons, transfection of Kalirin-7 resulted in the production of spine-like protrusions of various morphology, reminiscent of Rac activation. Targeting of the GEF to the PSD may be a mechanism that controls spine formation and morphology.

The effects of RhoA on dendritic morphology appear to be mediated by the Rho-associated kinase (ROCK). Blocking ROCK activation prevents RhoA-induced dendritic simplification of hippocampal neurons, whereas expression of activated ROCK mimics the effect of RhoA (Nakayama et al. 2000). ROCK has been shown to activate actomyosin-based contractility and to suppress microtubule assembly in neuroblastoma cells (Hirose et al. 1998), indicating a possible mechanism by which ROCK mediates dendritic retraction. Less is known about downstream effectors mediating the effects of Rac1 and Cdc42 on dendrite remodeling. Given the similarity of the morphological changes induced by activated Rac1 and Cdc42, it is likely that they signal through a common effector protein. A well-known effector that can be activated by both Rac1 and Cdc42 is the serine/threonine kinase PAK1. PAK1 activation has been shown to induce neurite formation in PC12 cells (Daniels et al. 1998). Future experiments should clarify the role of PAK1 and other downstream effectors on Rho family GTPase-induced morphological changes.

Considering the large body of work that shows the role for Rho family GTPases in dendritic patterning, it is surprising how little is known about the mechanisms by which extracellular signals regulate Rho GTPase activity. According to estimates based on the human genome sequence, there are about fifty GEFs, the activators of Rho family GTPases, and more than fifty GAPs, which inactivate Rho family GTPases. It is likely that many of these are expressed in neurons. This high number suggests that multiple factors influence Rho GTPase activity with a significant level of spatial and temporal specificity. Experiments in *Xenopus* tectal neurons indicate that modulation of dendritic development by NMDA receptor activation is influenced by RhoA (Li et al. 2000) and provides one of the few examples for which Rho GTPase function has been linked to dendritic remodeling induced by an extracellular signal. It is likely that dendritic remodeling induced by other extracellular factors will also be mediated at least in part by Rho family GTPases, and exploring the link between specific extracellular signals and the activation of particular GEFs and GAPs will likely be an active area of investigation in the coming years.

Microtubules and Dendritic Stability

While actin plays a critical role in regulating cytoskeletal dynamics, microtubules provide structural integrity to dendrites. Stabilization of newly formed dendritic branches and consolidation of the dendritic tree therefore requires microtubule invasion. Microtubules are oriented strictly with their plus-end distal to the cell body throughout the axon (Baas et al. 1989, Heidemann et al. 1981). In contrast, microtubules in the proximal and middle regions of dendrites are nonuniformly oriented (Baas et al. 1988, 1989). Given that the polarity of microtubules is relevant to both the dynamics and transport properties, these distinct patterns could provide

the basis for some of the functional and morphological differences characterizing axons and dendrites (Black & Baas 1989).

Emerging dendritic branches are first invaded by plus-end-distal microtubules, followed by the gradual addition of minus-end-directed microtubules. Active transport of microtubules may be essential for establishing the microtubule array in developing dendrites (Baas 1999). Several reports have shown that proteins involved in microtubule transport are essential for dendritic development. For instance, mutations in the minus-end-directed motor protein dynein or its associated protein Lis1 inhibit dendritic growth and branching in neurons of the *Drosophila* mushroom body (Liu et al. 2000). Interestingly, Lis1 haploinsufficiency in humans causes lissencephaly or "smooth brain." Although this defect is caused mainly by defects in cortical neuronal migration, reduced function of Lis1 in mice also affects the morphology of early postmitotic cortical neurons (Cahana et al. 2001). Another microtubule motor protein involved in dendritogenesis is CHO1/MKLP1, which is believed to transport minus-end-distal microtubules into dendrites. Antisense depletion of this motor protein in early sympathetic cultures prevents formation of dendrites (Sharp et al. 1997) and alters the microtubule composition of dendrites in older cultures (Yu et al. 2000).

Little is known about how extracellular signals can influence microtubule dynamics or how interactions between the actin and microtubule cytoskeleton are orchestrated. The importance of coordinated interaction of actin and microtubule structures in dendritogenesis is illustrated by the gene *Kakapo*. The *Kakapo* gene, and its vertebrate homolog *MACF*, encode a cross-linking factor that contains a domain that binds to actin and a domain that binds to and stabilizes microtubules (Leung et al. 1999). *Drosophila* CNS neurons carrying a mutation in the *Kakapo* gene display a reduction in dendritic branching and sprouting (Gao et al. 1999, Prokop et al. 1998), showing that interactions between the actin and microtubule cytoskeleton are essential for dendritic arbor development. Exploring the link between extracellular control of the actin cytoskeleton and the subsequent mobilization of microtubules should provide us with a greater understanding of the molecular mechanisms that specify neuronal morphology.

CONCLUSIONS

Research on dendritic development and dendritic function has progressed rapidly in the past few years. There is now compelling evidence that dendritic development is a highly dynamic process and that extracellular signals play an important role in specifying the final dendritic morphology of neurons (Figure 3). The orientation of dendritic growth appears to be regulated by gradients of local environmental cues, and the findings with semaphorins indicate that a common set of extracellular signals regulate axonal and dendritic development. Interestingly, guidance cues have distinct effects on axonal and dendritic development, and this differential response is mediated in part by asymmetric distribution of intracellular signaling molecules to the two compartments. The growth and branching of

dendrites is regulated by a number of factors including trophic factors, Notch, and neuronal activity. An area of particularly rapid advance has been the study of dendritic filopodia and spines. Observations on dendritic filopodial and spine dynamics suggest that highly motile filopodia play an active role in searching out synaptic contacts and that synaptic activity can influence the final morphology of dendritic spines. What is wonderful is that all of this new knowledge about dendritic growth control has come at a time when investigations of the consequences of dendritic morphology on neuronal information processing are in bloom. The emerging view is that dendrites are not just passive conveyors of synaptic input but that they process the incoming information based on the physical and electrophysiological characteristics of the dendritic tree. The ability to manipulate dendritic morphology by molecular perturbation provides a golden opportunity to address long-standing questions regarding the relationship between dendritic form and function.

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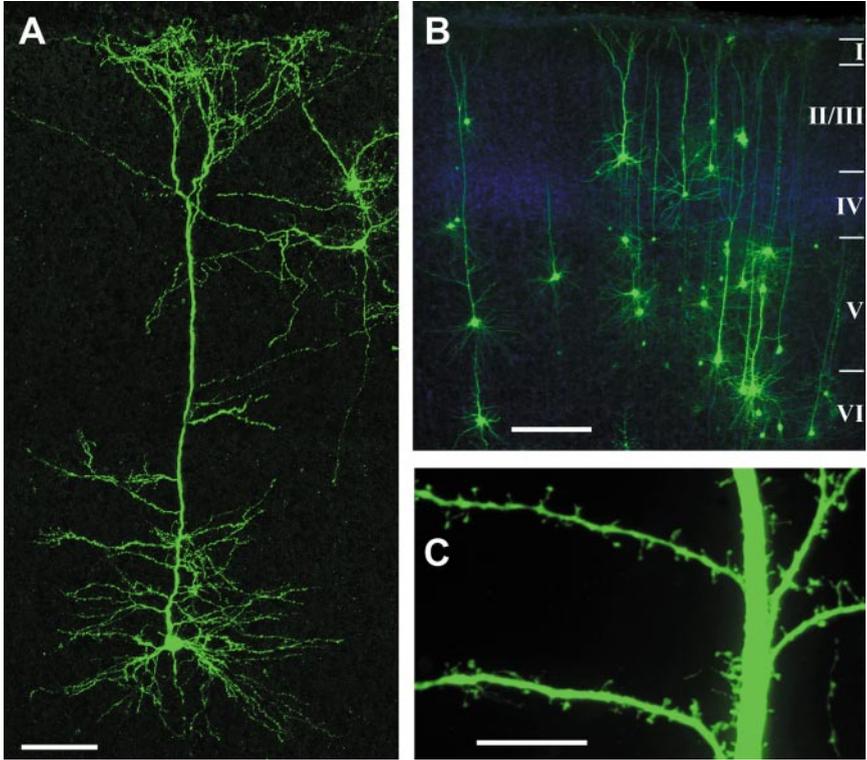
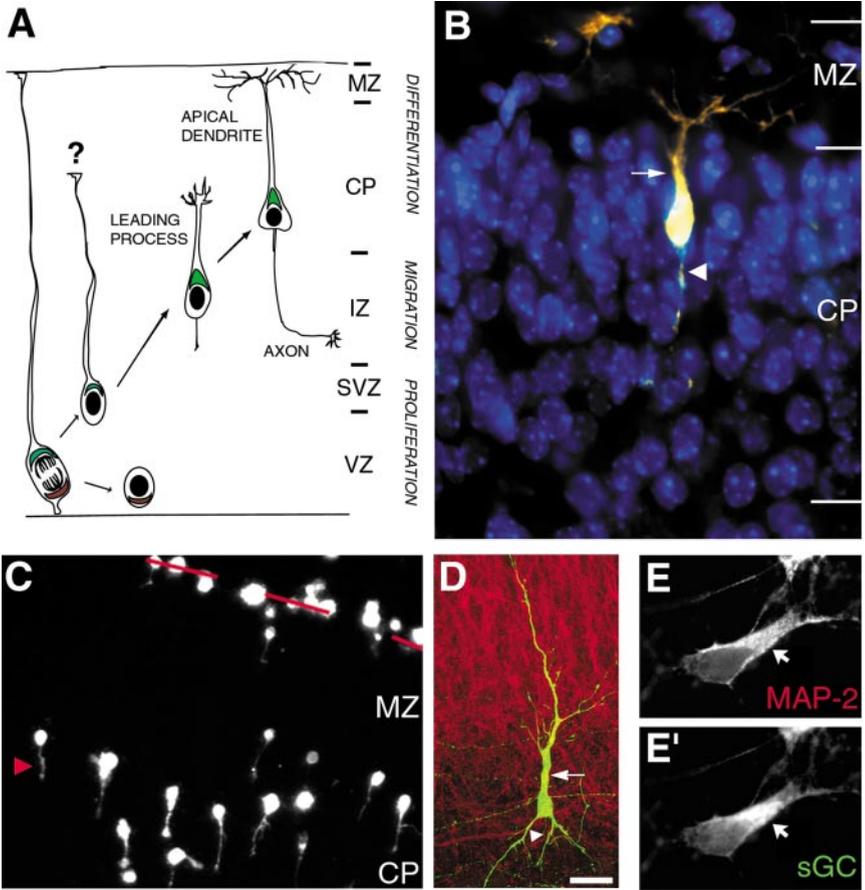


Figure 1 Morphological features of cortical dendrites as revealed by biolistic transfection of GFP into rat cortical slices. (A) Example of a layer 5 pyramidal neuron at P10. The salient features of pyramidal neuron morphology include an apical dendrite that extends toward the pial surface and terminates in an apical dendritic tuft, numerous side branches that emerge from the apical dendrite, and several basal dendrites that emerge from the soma. Scale bar, 100 μm . (B) Low magnification image of cortical neurons transfected at P12. Note that the majority of neurons have a pyramidal morphology and that the extent of the apical dendrites varies between deep-layer neurons. Scale bar, 250 μm . (C) High magnification image of apical dendrite and side branches in a cortical neuron transfected at P18. Note that the dendrites are studded with dendritic filopodia and spines, which are sites of excitatory synaptic transmission. Scale bar, 10 μm . (Images courtesy of Paul Dijkhuizen, Daniele Peters, and Vivian Fenstermaker.)



See legend on next page.

Figure 2 (See figure on previous page) Emergence of axonal and dendritic polarity of cortical neurons in vivo and in vitro. (A) Summary of the different steps in cortical neuron differentiation. Recent results suggest that radial glial cells are the main neuronal precursors in the ventricular zone (VZ; *left hand side*). These precursors display asymmetric localization of signaling components such as Notch (*green crescent*) and Numb (*brown crescent*). The precursors divide asymmetrically to give rise to two daughter cells that inherit distinct amounts of the cues located at the two poles of the cell. Once in the cortical plate (CP), neurons start differentiating by extending an axon toward the intermediate zone (IZ) and an apical dendrite toward the pial surface. (B) Morphology of immature pyramidal neurons in the cortex. This cell was labeled retrogradely by the fluorescent carbocyanine (DiI, *yellow*) injected in the intermediate zone at E18 in the mouse. This labeling reveals the developing apical dendrite growing dorsally (*arrow*) and the axon growing ventrally (*arrowhead*). The cytoarchitecture of the cortex is revealed by a nuclear counterstaining with bisbenzimidazole (*blue*). (C) In vitro study of initial steps of neuronal polarization using the slice overlay assay (Polleux et al. 1998). Three hours after plating, 80% of E18 dissociated cortical neurons differentiating in the CP emit only one neurite (the future axon) growing ventrally toward the IZ (*arrowhead*), thereby mimicking the in vivo situation. This demonstrates the presence of extracellular cues in the CP that are able to direct axon growth in cortical neurons. The *red dotted line* indicates the pial surface. (D) In vitro study of factors controlling the polarized outgrowth of apical dendrites in the cortex using the slice overlay assay (Polleux et al. 2000). Four days after plating on cortical slices, GFP-expressing neurons (*green*) growing in the CP have a well-differentiated apical dendrite (*arrow*) directed toward the pial surface and an axon (*arrow*) directed toward the IZ. The red counterstaining is obtained using MAP2 immunofluorescence. (E–E') Asymmetric localization of soluble guanylate cyclase (sGC) in cortical pyramidal neurons. E18 rat cortical neurons were cultured in vitro for two days and double immunofluorescent staining was performed against MAP2 (E; a dendritic marker) and sGC (E'). Value of the scale bar indicated in D: 15 microns for A; 20 microns for C; 30 microns for D; 10 microns for E and E'.

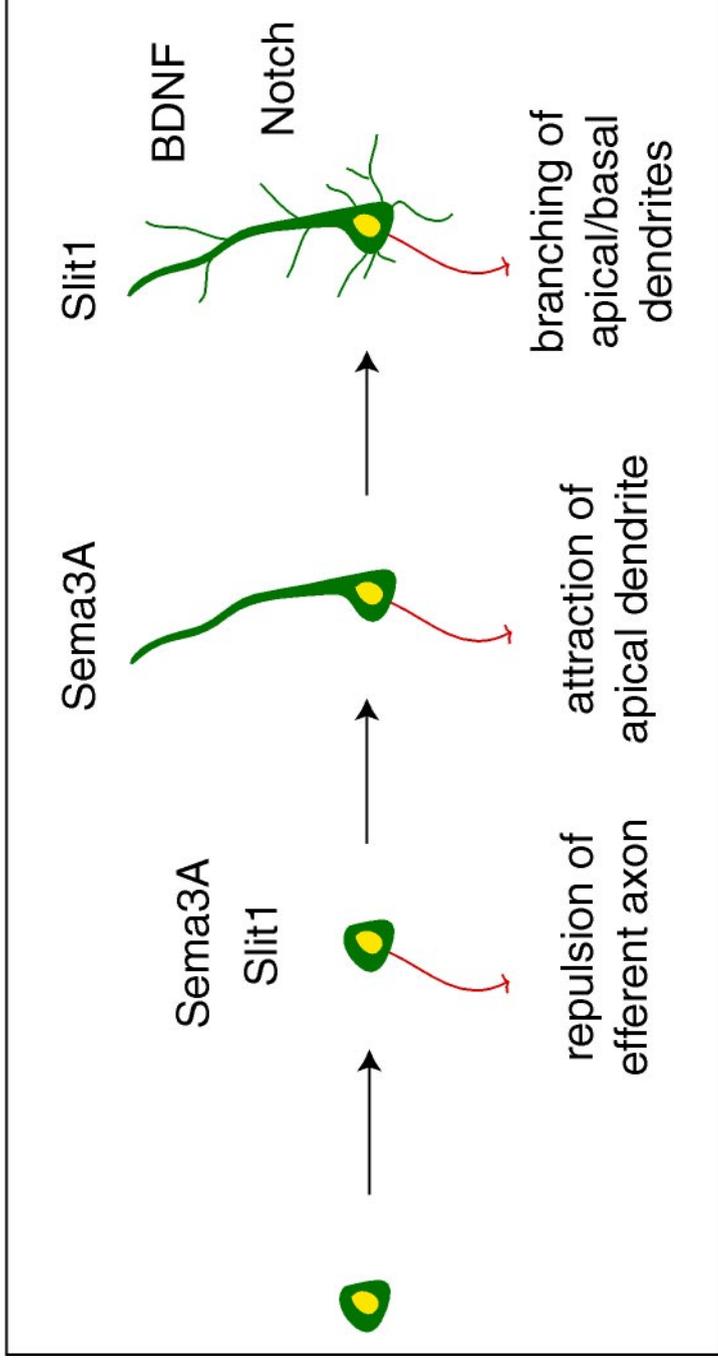


Figure 3 A model of how sequential action of extracellular factors might specify cortical neuron morphology. A newly postmitotic neuron arrives at the cortical plate, where it encounters a gradient of Sema3A (Polleux et al. 1998), which directs the growth of the axon toward the white matter. The same gradient of Sema3A attracts the apical dendrite of the neuron toward the pial surface (Polleux et al. 2000). Other factors, such as BDNF and Notch, control the subsequent growth and branching of dendrites.