Chapter 3

Evidence that Astroglia Influence Dendrite Morphogenesis and Synaptogenesis Independently in the Vertebrate Central Nervous System

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Additional information is available at the end of the chapter

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Abstract

In the absence of external spatial cues, dendritic arbors of neurons grown *in vitro* approximate those observed *in situ*. Absent, however, from these culture models is patterned orientation of dendritic trunks, and variation of branch geometry that provide identifiable characteristics of the cytoarchitecture of the intact brain. Although astroglia are present during key stages of dendritic development *in vivo*, little is known about whether local interactions with glia shape dendritic growth. Astroglial cells are good candidates for this kind of regulation because they can exert control over the formation of synapses, an event correlated with the maturational state of the dendrite. The present review highlights some key findings from vertebrate model systems offering evidence that astroglia can contribute to the shape, and growth, of the dendritic arbor. Drawing from our recent work using a co-culture system composed of neurons growing in differential contact with astroglia, we discuss findings that suggest: 1) growth of dendrites, and addition of synapses, can be independent; further, while astroglia promote synapse formation, they inhibit dendritic growth; 2) astroglia mediate dendrite growth through both paracrine, and contact-dependent mechanisms; and 3) astroglia appear to impose pattern by constraining the growth of dendrites within their zones of influence.

Keywords: dendrite morphogenesis, neuron-astroglia interactions, dendritic development, dendritic patterning, dendritic growth

1. Introduction

The size and shape of the dendritic arbor is a key factor in determining the connective potential of a neuron. While programs intrinsic to the neuron itself can instruct the general morphology of the dendritic arbor [1], it has long been recognized that the form dendrites take as they mature is under significant influence from extrinsic factors [2, 3]. The complexity of extrinsic influences, and the collective impact they have upon dendritic architecture, is evident when one compares the spatial patterning of dendritic arbors that have developed *in situ*, or within a tissue context, against those that form *in vitro*, largely deprived of patterned contact with other cells (e.g., see **Figure 1**). Although the importance of identifying and understanding how such extrinsic influences work has been recognized by neuroscientists for decades, relating specific influences to specific aspects of dendritic morphogenesis has proven challenging.

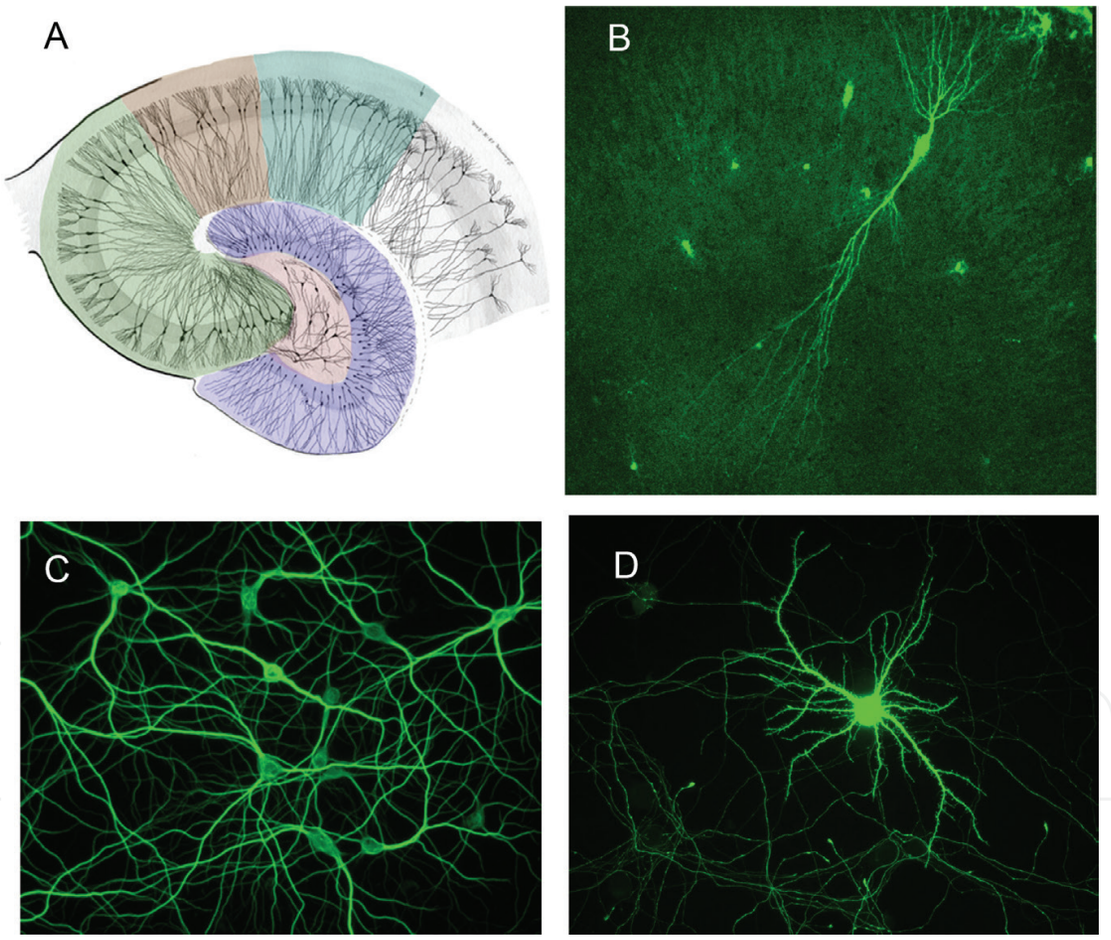

Figure 1. Comparing hippocampal pyramidal neurons grown under different conditions can help distinguish intrinsically determined features of the dendritic arbor from those under extracellular control. (A) Camera lucida drawing of the dendritic arbors of pyramidal neurons of the CA fields of the hippocampus and granule cells of the dentate gyrus, based on Golgi-Cox impregnation of an adult rat (modified from [4]). The dendritic arbor has pronounced apical and basilar domains that are physically segregated and oriented in opposite directions. (B) A hippocampal neuron labeled by biolistic transfection of eGFP in an organotypic slice culture from rat. (C) Dissociated hippocampal neurons growing in primary culture, immunostained with MAP2 to reveal the dendritic arbors of the neurons present in the field of view. (D) An individual cultured neuron, labeled by transfection with eGFP, from within a similarly dense field of neurons (unstained) as in (C). Based on the shape of the soma, and orientation of the primary dendrites, the major dendrite that points toward the upper left of the frame might be a candidate "apical" dendrite.

But while fundamental questions remain, new tools are being brought to bear in this area of active investigation, and a series of insights have unfolded over the last decade. For example, interactions within, and between, neurons are one important source of cues involved in ontogenesis of the dendritic arbor. The mechanism of "self-avoidance" between dendrites within a given arbor can help establish appropriate spacing of branches (for review, see [5]). Similarly, segregation of branch territories has also been recognized as important in understanding how the dendritic arbors of a single type, or class, of neuron within a brain region are arranged in a territorial configuration. Such an arrangement optimizes dendritic capture of incoming afferents and is now understood at a mechanistic level [6, 7]. It is hard to envision how these homotypic mechanisms contribute to the cases where branching pattern and density vary stereotypically along a single primary stalk of the arbor, however.

The hypothesis that astrocytes might also shape dendrites has received less attention. In 2010, Procko and Shaham proposed that glial cells might play such a role, although, at that time, direct evidence in vertebrate systems was lacking [8]. Mounting evidence, however, demonstrated that interactions between neurons and astroglia were crucial to other aspects of dendritic development [9–11]. Astroglia secrete factors that facilitate synapse formation, both in terms of the onset [12–16] and of rate [17, 18]. Because immature dendrites are not receptive to innervation [19], these synaptogenic effects could imply an astroglial contribution to dendrite maturation. In addition, astroglia produce factors that modulate synaptic efficacy [20] and regulate synapse pruning [21]. Moreover, a number of growth factors have been identified that selectively alter dendritic, but not axonal growth, of forebrain neurons, e.g., [22, 23] and these factors may be produced, or regulated by astroglia [24, 25]. Collectively, these findings point toward mechanisms whereby astroglia could influence the competence, or developmental state, of the dendrite. It is therefore becoming increasingly important to characterize these effects in more detail so as to determine the roles of astroglia as regulators of synapse formation versus sculptors of dendritic arbor size and shape.

In this regard, data from two human neurodevelopmental disorders, Rett Syndrome and Fragile X mental retardation, implicate astroglia as a regulatory influence on the growth of dendrites [26, 27]. In Rett Syndrome, single-gene mutations in the X-linked transcription factor methyl-CpG-binding protein 2 (*MeCP2*) are associated with infant death in males, while females begin to display signs of mental retardation, autism, and epilepsy between 6 and 18 months of age [28], coincident with the time when dendritic outgrowth is most robust. Mutations in the fragile X mental retardation 1 gene (*Fmr1*) cause similar cognitive and behavioral impairments, and individuals with Fragile X have abnormal dendrites [29]. Isolating how interactions between different cell types bearing the gene mutations could produce defects in dendritic development *in vivo* is difficult, such that *in vitro* models can be the best option for screening for effects of specific interaction between identified cell types. Accordingly, when wild-type neurons were co-cultured with astroglial cells bearing mutations in *MeCP2*, or *Fmr1*, they showed altered dendritic development. These are effects that would have been difficult to detect and attribute directly to astroglia, using *in situ* analyses of tissue from the transgenic animals. It is noteworthy that much of what we know about the development of dendrites has, in fact, been learned using *in vitro* models (for example, see [30–33]). The power of these models is that they permit direct microscopic observation and enable manipulation of the extent to which neurons can interact dynamically with astrocytes as they form dendrites.

2. Dendritic arbors of isolated neurons grown *in vitro* exhibit features that are intrinsically determined and lack those features patterned by extrinsic influences

The first microscopic views of the intact hippocampus, impregnated with Golgi stain, illustrated the extent to which dendritic arborization is patterned (**Figure 1A**). This distinctly polarized arbor, with zonal variation in branching pattern, also forms in organotypic slice cultures, a method that preserves some populations of afferents, astroglia, and microglia [34] as dendritic outgrowth and maturation takes place [35]. In contrast, dissociated cultures of hippocampal neurons isolated from embryonic rat brain remove spatial cues that come from organized inputs and contain predominantly neurons with an excitatory phenotype. These cells generate MAP2 positive dendritic arbors that proceed to form post-synaptic specializations expected of pyramidal cells *in vivo* [31]. Thus, an advantage of this *in vitro* model is that the developmental trajectory parallels development in the intact neuropil [23, 30]. And because they grow at low density while flat on a coverslip, benchmarks of morphological maturation can be readily observed and quantified. For example, processes become tapered and generate spines (see **Figure 1C** and **D**). Despite the physical isolation of these neurons, the dendritic arbors that form sometimes have a prominent dendrite that is somewhat thicker and distinct from the other dendrites that form off of the cell body, suggesting a rudimentary form of an apical dendrite. By comparing dendritic architecture of hippocampal pyramidal neurons from the intact brain, slice cultures, and dissociated neurons, we can separate basic features of the dendritic arbor that are expressed robustly across this range of extracellular contexts and therefore likely intrinsically determined from those features that require extracellular influences to be expressed.

3. Both the presence of astroglia and factors derived from astroglia alter the spatial patterning of dendritic arbors grown *in vitro*

3.1. Effects produced by secreted, soluble factors present in media conditioned by astroglia

In vitro approaches to studying neuron development were transformed when Gary Banker reported a new method that allowed dissociated embryonic hippocampal neurons from the rat to be grown on glass using defined serum-free medium [36]. There was one telling technical detail, however: long-term survival of neurons required astroglial cells to be present in the culture, although not in direct physical contact with neurons. In fact, these cultures were typically prepared with the astroglial cells grown as a separate monolayer culture on the bottom of the culture dish, while the neuronal cultures were grown on glass coverslips that were several millimeters away. These observations revealed that astroglia secreted trophic factors upon which the viability of neurons depended. Other data suggested that astroglia enabled more than just survival. Sympathetic ganglion cells, for example, formed axons readily *in vitro*, but

dendrites could only be produced in the presence of glia [37]. The dendrite-specific factor necessary for this polarized outgrowth was later identified as BMP-7 [38].

Further evidence of the importance of developmental cross-talk between astroglia and dendritic morphogenesis emerged. Astroglia native to the cortex promoted dendrite formation of cortical neurons more effectively than astroglia from other regions of the brain [39–42]. These studies supported the hypothesis that astroglia could influence dendritic growth in a brain-region specific manner. Taken together, these findings suggest that the developmental interactions between astroglia and the forming dendritic arbor might be multiple and significant.

It was in this context that we sought to observe dendritogenesis *in vitro*, while controlling the extent to which developing neurons were exposed to astroglial cells. As a first step, cohorts of neurons were grown for several days under two conditions: in medium that had been conditioned by brief exposure to astroglia (24 h or less) versus in co-culture with a feeder layer of astroglia continuously present but with neurons isolated from physical contact. Neurons grown in conditioned medium formed dendritic arbors but did not form synaptic contacts. Conversely, sibling neurons grown with astroglia continuously present (yet not in direct contact) formed dendrites that displayed presynaptic contacts (**Figure 2**) [43]. Given previous reports that astroglia produced factors essential for synapse formation in retinal ganglion cells (reviewed in [44]) and in hippocampal neurons [17], the failure of synapses to form in glial-deprived cultures was not surprising.

What was unexpected, however, was that the dendritic arbors that formed in the glial-deprived neuron cultures were more extensive than those of neurons grown in an astroglial co-culture,

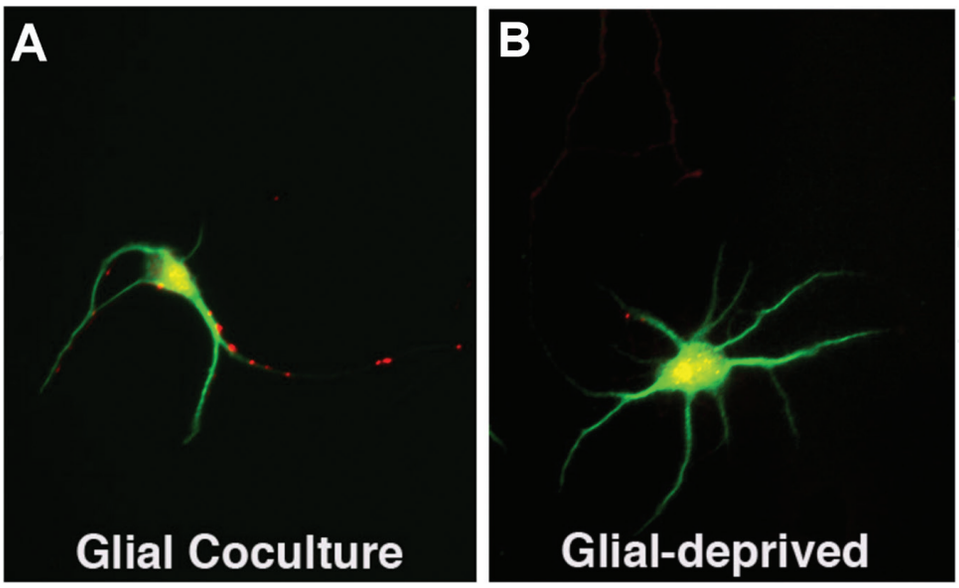

Figure 2. Dendritic arbors of neurons that are glial-deprived are more extensive but have few presynaptic contacts (B), compared to those co-cultured with astroglia (A). MAP2-stained dendrites are green, Synapsin1 puncta, representing presynaptic contacts, are red. Modified from Withers et al. [43].

with significantly more primary and higher-order branches [43]. These findings revealed that astroglia exert two effects on dendritic development that seem paradoxical. On the one hand, astroglia were permissive to synapse formation, and on the other hand, their presence limited dendritic outgrowth. A similar inhibitory effect by astroglia has been reported to occur in brain stem neurons *in vitro* [45] and the enabling effects of astroglia on synapses formation have been characterized in detail (see references above).

Thrombospondin (TSP) is the synaptogenic factor that is produced by astroglia and promotes the formation of presynaptic contacts onto dendrites both *in vitro* and *in vivo* [14]. Thus, in the glial-deprived paradigm, a straightforward prediction was that if TSP was added, the neurons growing under glial deprivation would form presynaptic contacts. They did. A second prediction could also be made: if TSP mediated the astroglial restriction of dendrite outgrowth as well, then those same neurons would be expected to have arbors that would be reduced in size compared with glial-deprived neurons not exposed to TSP. Instead, glial-deprived neurons + TSP still had dendritic arbors that were significantly larger than those growing in the presence of astroglia, and after 48 h of exposure, they were even greater than those growing under glial deprivation without TSP. A simple interpretation of these data is that the astroglia effects on dendritic growth are separate from the effects produced by TSP. The selective effects of TSP seem to suggest a mechanistic dissociation between the inhibition of dendritic growth and the formation of synapses.

3.2. Effects mediated by local contact between astroglia and neurons

Co-plating neurons and astroglia on the same coverslip offers opportunities for local interaction between the two cell types that could involve signals both soluble and contact-dependent. In our work, we have observed that neurons in full contact with astroglia had dendritic arbors with reduced size compared with neurons that did not contact astroglia at all. These effects could be mediated by the same mechanism as described earlier, but given that the neuron has

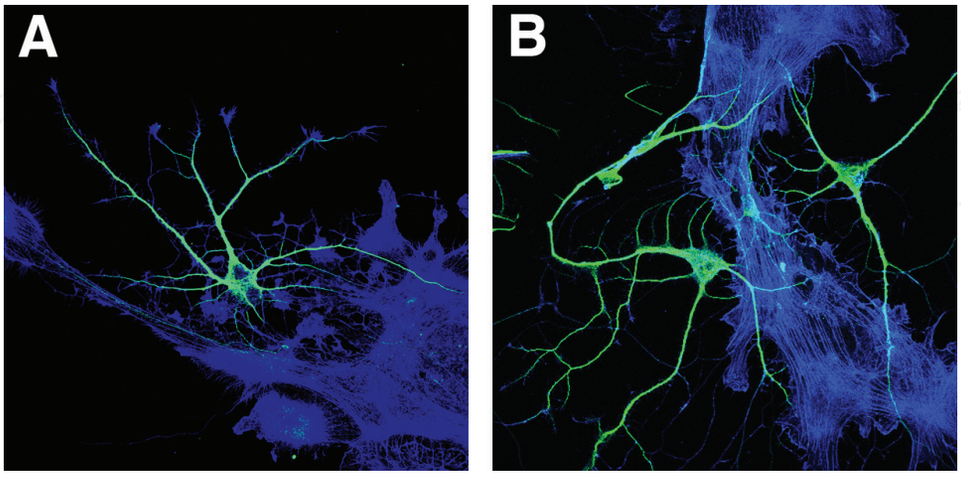

Figure 3. Asymmetry of dendritic arbors in partial contact with astroglia (A and B). The dendritic arbors are revealed by MAP2 immunostaining (green). Polymerized actin, stained with fluorescently conjugated phalloidin (blue), highlights astroglia, as well as growth cones at the tips of dendrites.

grown while adhering to an astroglial island, it seems very likely that the signal(s) originated from the astroglial cell on which it resided. The interesting case comes when physical contact is limited, when a neuron straddles an astroglial cell, such that part of the growing arbor touches and part does not (**Figure 3**). When in partial contact, the dendritic arbor forms asymmetrically, with the most extensive arborization not in direct contact. One interpretation of this biased growth is that it is the product of an interplay between the action of soluble factors produced by astroglia and a separate inhibition of growth when dendrites are in direct contact with the surface of astroglial cells.

4. If astrocytes sculpt dendrites *in vitro*, might they also influence dendrite arbor shape *in vivo*?

In vivo, the onset of astrogliogenesis occurs before robust dendritic outgrowth begins and immediately precedes peak synaptogenesis in the hippocampus [46–51]. For humans and nonhuman primates, the dendritic arbors of forebrain neurons take years to reach their full extent [52]. Dendritic development in rats is similarly protracted, with the elaboration of branches and the addition of synaptic contacts upon them occurring over weeks [53, 54]. This timing makes astroglia good candidates for secreting signals and providing physical cues to guide dendrite growth. Clues to the role astroglia might play *in vivo* could come from analyzing their spatial relationships with dendrites in mature tissue (see **Figures 4** and **5**) and the temporal sequence by which these relationships arise during development.

The effects of physical contact between a dendritic branch and astroglia *in vitro* provide an example of how functional domains within the dendritic field might be organized, at least in part, based on cross-talk between a specific dendritic branch and a neighboring glial cell. In support of this hypothesis, within intact neuropil, individual astroglia are arranged in non-overlapping territories that occupy a fraction of the dendritic arbor of an individual principal neuron [56]. Stains that identify dendrites and astroglia in tissue show their interwoven relationship (see **Figure 4A**, **C**, and **D**). Further, the spatial domain of a single astrocyte contacts synapses of multiple neurons [57], with fine processes extending dynamically to make physical contact at individual synapses [58]. There is an extensive literature documenting astroglial-synapse interactions that is well beyond the scope of this chapter, see [59]. These data fit well with the growing recognition that astroglia may contribute to the construction and function of cortical circuits and maps, physically defining and coordinating synaptic territories [60–62]. Further, dendrite-astroglia interactions during development could help to scale the growth of a synaptic network to match the available nutritional network [63], similar to the mechanisms involved in building the retina [64]. Control over dendrite arbor shape could be an important part of these mechanisms.

The arbors of pyramidal neurons in hippocampal subfield CA1 offer a useful model because this population of cells has elaborate arbors, yet the arrangement of arbor branches repeats with striking regularity (see **Figure 4B**). The story of how this pattern of arborization arises

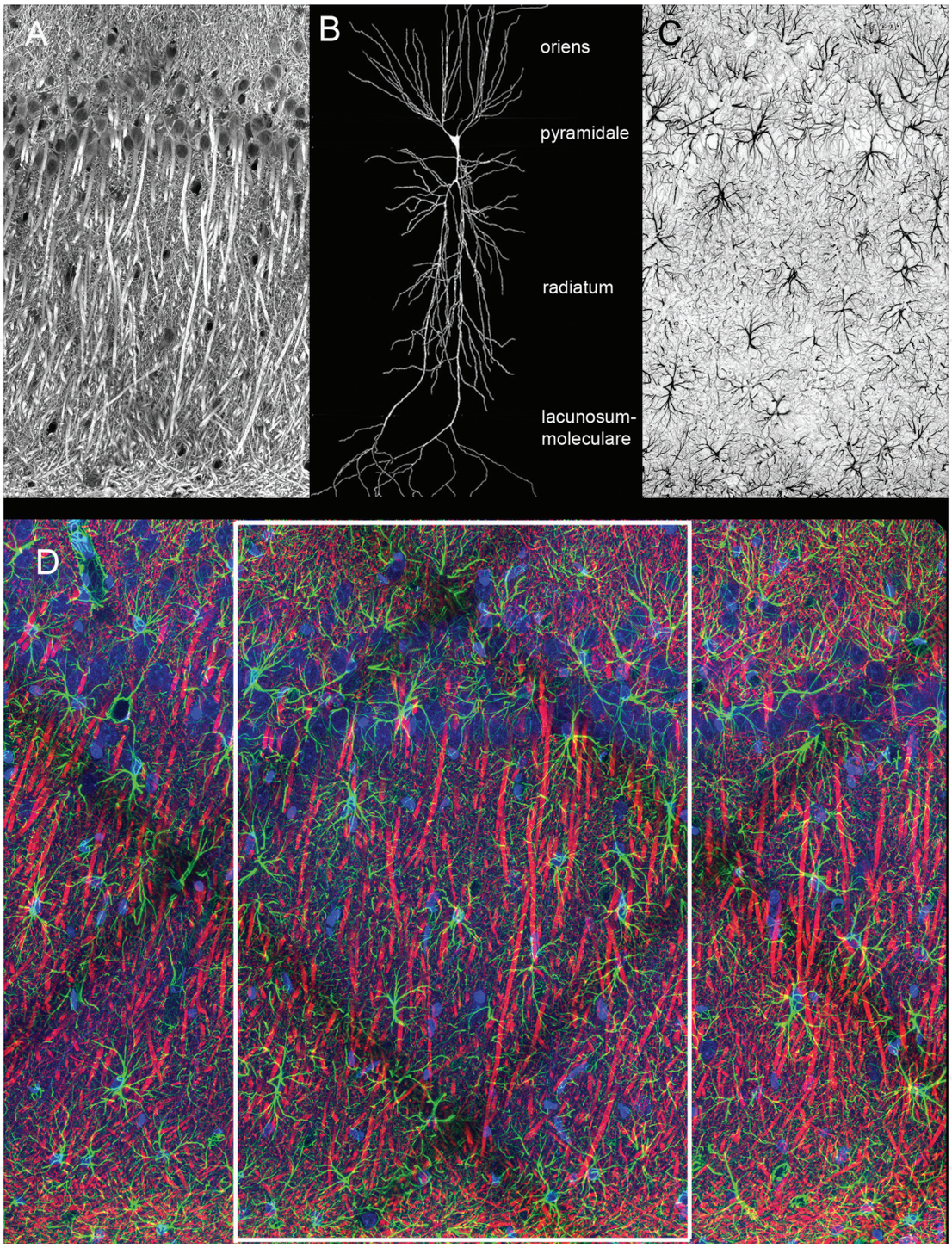

Figure 4. Cytoarchitecture of the CA1 field of the hippocampal formation from rat brain illustrates laminar variation in dendritic branching and astrocyte morphology. (A) Immunostaining for the dendritic marker MAP2. (B) The dendritic arbor of a typical CA1 (modified from [55]). (C) Immunostaining for the astrocytic marker GFAP from the same region as (A). (D) The colorized overlay, with dendrites in red, astroglia in green, and nuclei in blue. The white box surrounds the field shown in panels and (C). The field within which dendrites develop is tessellated with astroglia: the size of individual astrocytic territories is appropriate to exert local influences on a sub-laminar scale, and the astrocytes themselves appear to show cytoarchitectural variations across laminae.

in development is summarized nicely by Pokorny and Yamamoto [35]. In that report, dendrite branching and elongation, as measured in Golgi-impregnated pyramidal cells, was not synchronous but rather followed a distinct sequence. For example, the apical dendrite

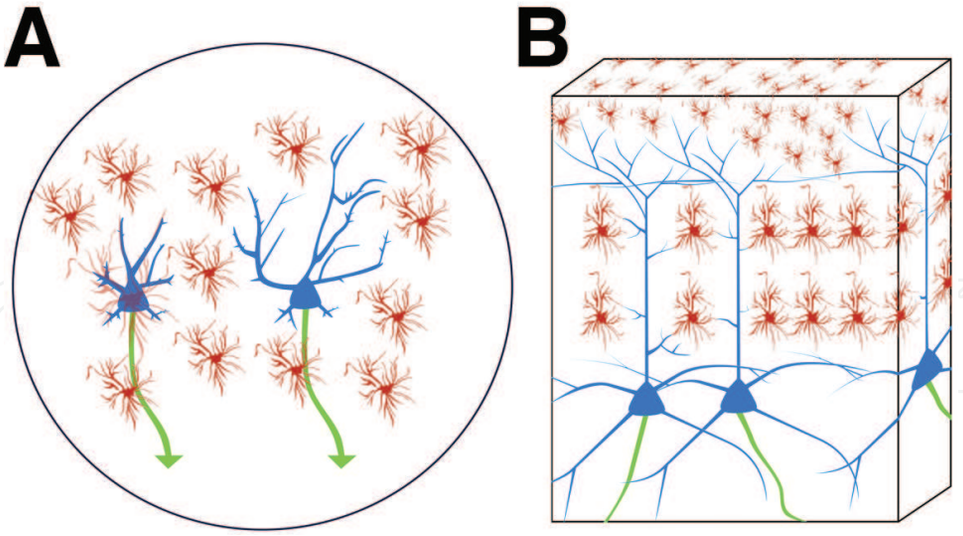

Figure 5. Illustration of how differential contact between astroglia and neurons can contribute to patterns of dendritic arborization. (A) Dendritic arbors of neurons grown *in vitro* are inhibited by contact with astroglia. (B) Dendritic arbors of CA1 hippocampal neurons show varied branching across laminae, coincident with changes in the distribution, and size, of astroglia. Astroglia, red; dendrites, blue; axons, green.

extended nearly to its mature length by postnatal day (P)10, but the lateral branches along the apical shaft had only extended a minor fraction of their mature length. There was also a temporal separation when these lateral branches formed. The number of lateral branches that arose from the apical shaft within the proximal stratum radiatum peaked at P15, whereas more distally, the number continued to be added out to P48. These zones within stratum radiatum correspond to afferent inputs from associative and commissural fibers (proximal stratum radiatum) and Schaffer collaterals (distal stratum radiatum). Branching within stratum lacunosum-moleculare, originating from the most distal portions of the apical dendrite, did not peak until after P48 and appeared to be more pronounced in the preterminal branches. The availability of afferents, which enter during embryonic development (for a review, see [65]), could be an important source of cues for dendritic development.

During the time frame when CA1 pyramidal cells are growing dendrites, astroglial cells in this region go through a number of transitions in number, and structure, that could be meaningful for establishing arbor pattern. Though relatively sparse before P10, astrocytes are present at the time when the apical dendrite is forming, and during the first 2 weeks of postnatal development, astroglia extend long filopodia-like processes [66]. An intriguing possibility is that during early stages of dendritic branch formation, the long filopodial extensions on glia serve a function related to branch formation or guidance, analogous to the guidance processes extended by radial glial cells. By the time astroglia begin to extend elaborate spongiform processes more characteristic of mature astroglia, the architecture of the arbor has been established, although branch growth continues beyond P30, when astrocytes have established nonoverlapping territories characteristic of mature neuropil [66].

Striking changes in the shape or spatial orientation of astroglia also accompany the most active periods of dendritic branch formation and growth. Astroglia are initially spherical but take on a polarized shape with development [66, 67]. In the stratum radiatum, this shape change

is oriented perpendicularly to the cell body layer, stratum pyramidale. In the stratum lacunosum-moleculare, the astroglia are elongated parallel to the cell body layer [67]. Coincidentally, this is the zone of the apical dendritic arbor that shows the most prominent lateral spread.

In vitro, local encounters between growing dendrites and astroglial cells can exert significant biases in the spatial patterning of the arbor. We have observed long filopodial-like processes extending from astroglia that resemble those reported in developing tissue *in vivo* (see the earlier section). These extensions could provide a mechanism for spatial capture of dendrites [43]. Time-lapse recordings of living cells have shown that, although slow growing, dendritic branches are dynamic structures that extend and retract growth cones and various forms of filopodia [68–70] (Withers and Wallace, unpublished observations). Cycles of extension and retraction create the opportunity for multiple physical or molecular interactions between these two cell types, analogous to neuron-astroglia interactions that occur during neuron migration [71, 72]. Collectively, such interactions could determine the trajectory of dendritic branch growth in three-dimensional space.

Comparison of dendritic arbors of neurons and arrangement of astrocytic processes in neuropil suggests that the structures of these two cell types co-vary in a nonrandom manner (see **Figure 4**). Such a view, however, only begins to represent more nuanced phenotypic heterogeneity of astroglia based on patterns of gene expression that are of emerging importance in current research, for review, see [73]. Likewise, these kinds of analyses only begin to disclose the developmental shifts in phenotype of astrocytes across lamina that may accompany distinct stages of dendritic branch formation. Such shifts appear to occur. As early as P8, GFAP-positive astroglia are densely arranged in the stratum lacunosum-moleculare, while remaining comparatively sparse in the stratum radiatum [67]. Additionally, two different transporters for glutamate show a different time course of expression and distinctive localization in different populations of astroglia in the developing hippocampus [48]. While such complexities are far from resolved, there is enough data available, we argue, to make the case that (1) patterning of dendritic branches is subject to the influence of astroglia and that (2) this relatively neglected developmental effect is distinct from the actively studied influences on synapse formation. The purpose of this chapter was to build on the hypothesis proposed by Procko and Shaham [8] by adding supporting evidence based on direct analysis of dendritic arbor formation in principle neurons of the central nervous system. Both the documented impact of astrocytes on dendritic arbor formation *in vitro*, and the fact that astrocytes are present but distributed differentially, during the extended period of dendritic outgrowth *in vivo*, support the argument that astrocytes could be a key part of the network of extrinsic influences that locally refines dendritic arbor geometry during development.

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