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Mechanisms of Dendritic Maturation

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Abstract

The highly complex geometry of dendritic trees is crucial for neural signal integration and the proper wiring of neuronal circuits. The morphogenesis of dendritic trees is regulated by innate genetic factors, neuronal activity, and external molecular cues. How each of these factors contributes to dendritic maturation has been addressed in the developing nervous systems of animals ranging from insects to mammals. The results of such investigations have shown that the contribution of intrinsic and extrinsic factors and activity, however, appear to be weighted differentially in different types of neurons, in different brain areas, and especially in different species. Moreover, it appears that dozens of molecules have been found to regulate dendritic maturation, but it is almost certain that each molecule plays only a specific role in this formidable cooperative venture. This article reviews our current knowledge and understanding of the role of various factors in the establishment of the architecture of mature dendritic trees.

Index Entries: Dendrite; growth; CNS development; neuronal structure.

Introduction

The control of neuronal architecture is a central issue in studying brain development. More than 100 years ago, the Italian neuroanatomist Camillo Golgi first visualized the structure of single neurons with his newly discovered silver-staining method. A first observation was that each neuron consisted of a cell body from which arose a long process called the axon and a highly complex tree-like structure called the dendritic tree. About 15 years later, Ramon y Cajal, a Spanish neuroanatomist, implemented and improved Golgi's staining method to

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study neuronal architecture in great detail and suggested that neurons obey the "law of polarization" *(1).* Accordingly, dendrites were postulated to constitute the input site of the neuron, serving as collectors of synaptic input from other neurons and carriers of these synaptic potentials toward the cell body or directly to the axon. We now know that the highly complex shape of dendritic trees modulates the biophysical properties of neurons *(2,3),* and thus is crucial for neural signal integration properties and firing patterns *(4,5).* Consequently, neuronal properties underlying brain function are strongly dependent on the factors that control dendritic shape. In addition, it is clear that the maturation of dendritic architecture plays a crucial role in determining connectivity patterns in the adult brain. But how do the complex shapes of dendrites and the wiring of the brain develop, and what determines how dendrites, once mature, are maintained or altered in order to allow the animal to adapt to developmental or environmental changes? Recent studies indicate that similar signals seem to control dendritic shape during development and during experience-dependent plasticity. Because of the considerable amount of data on dendrites, this review focuses on the mechanisms controlling dendritic maturation. Other closely related subjects such as dendritic synaptogenesis, spine formation, and experience-dependent dendritic plasticity have been reviewed in depth elsewhere *(6–10).*

Dendritic Architecture

Seeing Dendrites

To study dendritic growth, one first needs a method to assess dendritic structure at different time intervals during neuronal development. Two major approaches have been used. In the first approach, individual neurons are labeled with a probe, and a sensitive imaging technique is applied to visualize the growth of dendrites, either in organotypic cultures of selected nervous tissues or *in situ.* One preparation that has yielded considerable information regarding dendritic growth using this first approach is the optical tectum in the albino *Xenopus* tadpole *(6).* Another preparation is the mammalian retina, which houses a wide variety of ganglionic neurons and is ideal for investigating dendritic development with live-cell-imaging approaches *(11).*

Despite the appeal of live imaging, many problems remain to be solved. First, during acquisition, the neurons experience photodamage and photobleaching. This problem can be reduced with two-photon imaging or by combining a highly sensitive camera with low levels of illumination. Second, the inability to perform histological manipulations significantly reduces the resolution of imaging live tissue as compared to fixed tissue. Third, the time window for the experiment is limited by the longevity of the preparation (which usually lasts from hours to a few days). Despite these limitations, sampling the same neuron "before and after" provides a direct assessment of the changes in the detailed architecture of neurons and avoids large-scale sampling and statistical analysis when examining neurons from different preparations. In addition, the limitations are beginning to be overcome with recent imaging techniques. For instance, Trachtenberg et al. *(12)* and Grutzendler et al. *(13)* have tracked the stability of structures as small as spines over intervals ranging from a few days to several months using two-photon microscopy with low-energy photons to excite green or yellow fluorescent protein-labeled cortical neurons.

In the second approach, neurons have been stained and sampled at high resolution under different experimental manipulations. Of special interest is the identified neuron in invertebrate nervous systems, which, when combined with quantitative morphometric analysis, has been used to follow the maturation of dendritic trees *(14–16).* The fact that some invertebrate neurons are individually identifiable, in that they show a characteristic dendritic architecture, sets the ground for quantitative analy-

sis of dendritic architecture at different developmental stages because a given identified neuron can be sampled in a large number of animals. The constant 3-D architecture of identified neurons is used as a template for examining the effects of various experimental manipulations on their geometry. Furthermore, modern genetic tools applied to *Drosophila* allow the visualization of identified neurons or classes of neurons. For example, the dendrites of a given class of neurons can be labeled with GFP using the UAS-Gal4 system and single neurons can be labeled with the MARCM technique. This may allow a systematic genetic approach to dendritic maturation.

Measuring Dendrites

Dendritic maturation proceeds by both elongation of dendritic segments and dendritic branching. Although dendritic trees mature by a net increase in the number of branches, live-cell imaging has revealed that dendritic maturation is a highly dynamic process of branch addition and retraction. The precise evaluation of the changes in dendritic architecture requires the combination of neuronal staining and measurements of dendritic geometry. Measures that are in use for quantifying these changes can be divided into those that describe the metric features of a dendritic tree and those describing the topological structure of a tree. It has been noted that metric measures and topology are different aspects of tree structure and should be analyzed separately *(17).*

A few examples of metric measures are total dendritic length, total dendritic surface area, dendritic segment length and diameter, and number of branches. However, because a single parameter cannot describe all geometric properties of a dendritic tree, it is necessary to use several parameters to describe neuronal architecture. For instance, if the first of two dendritic trees has fewer branches but longer segments than the second, both trees may have identical total dendritic surface area. Moreover, one can imagine two neurons with similar metric parameters but with different 3-D architectures. To understand specific factors that control the 3-D geometry of a neuron, it is necessary to establish and combine defined measures to assess the dendritic branching patterns.

Topological measurements are concerned with the pattern of branching. Two commonly used methods to examine changes in the branching pattern are Sholl analysis and branch order analysis. The analysis of the spatial distribution of dendritic branches is implemented in Sholl analysis *(18).* Briefly, this consists of counting the number of occurrences of branch points in the dendritic tree falling between concentric spheres separated by a fixed number of microns. Sholl analysis is by far the most commonly used method for spatial analysis of dendritic trees. In branch order analysis, the frequency distribution of the number of dendritic branches is plotted as a function of branch order. The segment of the dendritic tree arising from the cell body and extending to the first node is called a first-order branch. The daughter branches arising from the first node are second-order branches, and so on. The branches arising from a common first-order branch constitute a dendritic tree. Branch-order analysis is useful for evaluating the growth and pruning of trees. This analysis can be used to distinguish between dendritic branching by tip bifurcation (i.e., splitting of a growth cone), or by interstitial branching (i.e., splitting from a dendritic shaft) *(15,19).*

The spatial orientation and density of dendritic trees has also been studied by other methods, including the "principal axes," "circular orientation," and "Cartesian grid density" methods *(17).* Such methods usually result only in determining the general orientation of the dendritic tree in space, and important geometric information is lost. More recently, fractal analysis has been used to evaluate the complexity of neuronal dendritic arborizations *(20–22).* Fractal dimension is a measurement of complexity of form. Although the fractal dimension can be used to evaluate the complexity of a two- or three-dimensional

object, it is not very useful for determining the degree of resemblance between two dendritic trees. Determining the degree of resemblance between two dendritic trees can be approached using a distance measure based on the Hausdorff distance *(23).* The use of both metric and topological analysis of dendritic trees is particularly important when examining large and complex trees and especially when comparing values from different neurons sampled in different animals. Moreover, the choice of analyzing a specific metric parameter or the use of a given topological methodology will depend on the experimental question.

Is Development of Dendritic Architecture Under Strict Genetic Control?

The strikingly similar morphology of vertebrate neurons of the same type (e.g., pyramidal cells or Purkinje cells), and the ubiquitous morphological similarity of identified invertebrate neurons from animal to animal suggest a significant contribution of genetic control for specification of dendritic architecture. Among the best-studied examples of identified neurons are the large interneurons of insects, such as the abdominal giant interneurons (GIs) of crickets and cockroaches *(23,24),* the giant interneurons of the vertical system (VS) in the Drosophila lobula plate of the optic lobe *(25),* and motoneurons in the holometabolous insects, *Manduca sexta (26–29)* and *Drosophila melanogaster (30).* The giant interneurons serve as integrators of sensory information from wide arrays of sensory receptors and have highly complex dendritic trees. Yet, because their dendritic trees are highly stereotyped from animal to animal, it is assumed that internal genetic programs largely determine dendritic morphogenesis *(16,23,25).* Furthermore, when examining pairs of homologous neurons, a given identified neuron is more similar to its contralateral mirror image in the same animal than it is to its homolog from different animals, as shown for the visual neurons of grasshoppers *(31)* and the giant interneurons of the cockroach *(23).* Even for identified invertebrate neurons with fairly stereotyped architecture, the dendritic branching pattern shows some variability, indicating that dendritic architecture is not under strict genetic control. Although the locations of the primary dendrites occur with little variability, the fine pattern of higher-order distal dendritic branching is variable *(14,23,31).* Finally, for insect identified motoneurons and Kenyon cells, morphological uniqueness is lost in primary cell culture *(32,33).*

In the vertebrate CNS, several kinds of neurons exhibit features of their cell-type-specific stereotypical dendritic morphologies even when isolated in culture (retinal cells [*34,35*]; pyramidal and stellate cells [*36,37*]). However, the principal radial orientation of stellate cell dendrites is defined in vitro by a cell-intrinsic program, whereas the expanse and complexity of their dendrites is modulated by the presence of granule cells *(38).* Similarly, cultured Purkinje cells exhibit some aspects of their typical morphology when completely isolated, but other aspects are realized only during normal development in the cerebellum *(39).* In summary, it has become clear for both invertebrate and vertebrate neurons that intrinsic programs control certain aspects of dendritic differentiation, but mature dendritic structure is further regulated by extrinsic signals.

Activity-Dependent Maturation of Dendritic Architecture

A possible candidate for extrinsic signals is neural activity. Although many studies have focused on the role of activity in sculpting axonal shape *(40),* there is increasing evidence from multiple systems that activity also affects dendritic architecture. However, the degree to which activity shapes developing dendrites seems to vary considerably among different species and among various brain areas. This section will assess the main findings on the role of neuronal activity in the maturation of

dendritic trees and its function in developing neural circuits. The effects of activity on dendritic spine formation and synaptogenesis will not be addressed, but have been reviewed in detail elsewhere *(7,8,10).*

During neural circuit development and dendritic maturation, two general forms of activity have to be distinguished: first, experience-dependent activity that is mediated by the firing of afferent neurons, and second, spontaneous activity in developing circuits that is independent of sensory input *(41)* or motor output. Both types of activity can affect dendritic structure. During embryonic development, patterned spontaneous activity can occur in immature networks long before the onset of sensory input, and has been described in different species and in a large variety of circuits (reviewed in ref. *42*). Examples are the developing vertebrate retina (reviewed in refs. *10,43*), the chick spinal cord *(44,45),* the hippocampus *(46),* and the cochlear nucleus *(47).* In general, spontaneous activity in such developing circuits is characterized by bursts of action potentials that occur as correlated firing of large populations of neurons. Such waves of activity might spread between different brain regions, as in the developing vertebrate visual system *(48),* or may remain localized to specific domains and specific developmental periods *(49).* In many systems, the latter depends on electrical coupling via gap junctions and thus might account for aspects of dendritic maturation that are insensitive to action-potential block by TTX injections.

In contrast, experience-dependent activity during development consists of spike trains in afferent neurons that cause excitation of either ionotropic or metabotropic receptors on the dendrites of postsynaptic neurons *(50,51).* In many developing systems, the arrival of afferents correlates in time with dendritic maturation. This relationship has been documented in very diverse circuits ranging from the development of escape circuits in insects, such as crickets and cockroaches, where the structure of the dendritic field of giant interneurons is influenced by the growth of afferent hair cell axons *(15,52),* to the development of the vertebrate cerebellum *(53–55),* and is seen in many other regions of the developing brain. A first step to investigate the role of afferent input for dendritic maturation is to ablate the presynaptic neurons. However, these experiments do not distinguish between activity-dependent mechanisms and other contact-dependent interactions between pre- and postsynaptic cells.

A classical way to distinguish these possibilities is either to reduce afferent input during development without removing the afferent cells, or to increase afferent activity. To exclude the possibility of nonspecific overall effects, such as, for instance, experience-dependent alterations in hormonal state, these experiments require internal controls. Such internal controls are usually achieved by manipulating only one side of a bilaterally symmetrical animal. For example, in the vertebrate visual system monocular deprivation decreases dendritic length of neurons in the cat lateral geniculate nucleus *(56,57)* and in the rat visual cortex *(58).* Similarly, in the insect *Drosophila melanogaster,* monocular deprivation decreases lamina volume *(59).* Rearing in different light regimes causes changes in neural circuitry, which, in turn, affect visual orientation behavior of Drosophila *(60)* as well as of the fly *Boettcherisca (61,62).* In contrast, Drosophila dark rearing has no effect on the dendritic structure of motion-sensing lobula plate neurons *(16).* Similarly, in the vertebrate visual system, the maturation of specific neurons does not always depend on afferent activity, as visual deprivation does not affect dendritic growth of cortical layer 4 stellate cells *(63)* or of layer 3 pyramidal cells in the visual cortex *(64).*

In a reverse set of experiments, activity is not interrupted, but is increased either by raising animals in enriched environments or by specifically delivering precisely controlled sensory stimulation during early development. For the example of the visual system, it has recently been shown by in vivo time-lapse imaging of optic tectal cells that enhanced visual activity driven by light promotes dendritic growth *(65).*

In conclusion, afferent activity shapes postsynaptic dendrites in a variety of systems, but not all types of neurons seem to be affected *(66,67).* Additional comparative studies will be necessary to understand the observed differences in the role of afferent activity for dendritric maturation in different systems. Furthermore, it will be important to elucidate whether the same mechanisms underlie experience-dependent dendritic maturation in different types of neurons, or whether multiple cellular mechanisms exist to translate changes in afferent activity during development into changes in dendritic architecture.

Moving from the level of sensory experience to the level of neural circuitry, the role of electrical activity in dendritic growth has been investigated by pharmacological and genetic manipulations. Surprisingly, a considerable number of studies in which TTX was injected to prevent neuronal activity have shown that dendritic growth is independent of sodium conductances *(68–70).* Although TTX blocks all sodium-carried action potentials, presynaptic neurons might still release neurotransmitter spontaneously and affect postsynaptic cells. In fact, in some systems, it has been found that glutamate release does indeed affect dendritic growth in the presence of TTX *(71–73).* Furthermore, calcium influx-mediated depolarizations can occur in a stage-specific manner during development without sodium-mediated action potentials in both vertebrate *(74)* and invertebrate systems *(27,28).*

The role of synaptic transmitter release in the assembly of neuronal circuits during embryonic development has been analyzed in two genetic model systems. During *Drosophila* embryonic development, the basic circuitry for peristaltic crawling develops in the complete absence of sensory input. This was demonstrated by targeted expression of tetanus toxin light chain (TeTxLC) in the neurons of the peripheral nervous system during embryogenesis, thus blocking neurotransmitter release from these cells. However, the actual patterns of locomotion at later developmental stages are partially impaired, suggesting that initial dendritic growth and synapse formation are independent of sensory activity; however, fine tuning of dendritic shape and synaptic connectivity may depend on sensory activity *(75).* Furthermore, after targeting TeTxLC to the *Drosophila* central nervous system with a pan-neuronal GAL4 driver, motoneurons develop with normal morphology and retain their capacity to form synapses in the absence of synaptic activity *(76),* at least when monitored at embryonic stages. However, it should be noted that genetic expression of tetanus toxin does not completely abolish spontaneous transmitter release, but reduces it by 50–75% *(77).*

Similar results were obtained with Munc18–1 mutant mice embryos, which show a lack of neurotransmitter secretion from synaptic vesicles throughout development. The initial circuitry assembly seems unaffected *(78),* although detailed quantitative analysis of dendritic structure has not yet been conducted. Later on during development, Munc18-1 mutants show abnormal rates of apoptotic neurons, resulting in degeneration of neural circuitry *(78).* One possible explanation for these findings is that dendrite maturation does not depend on synaptic activity, but maintenance of the mature dendritic tree does. However, as in the Drosophila system, it remains unclear whether normal synaptic activity might be crucially important for the maturation of dendritic shape during later stages. A reasonable explanation might be that early phases of dendritic growth follow an activity-independent program, but that synaptic activity becomes increasingly important for dendritic maturation during refinement of the circuitry. Such a differentiation into two separate modes of dendritic growth has also been suggested for motoneuron dendritic remodeling during insect metamorphosis *(14).* In the sphinx moth *Manduca sexta,* metric analysis of the dendritic tree of an identified motoneuron at consecutive developmental stages revealed a first phase of overall growth of all dendrites independent of their branch order, which is likely to be controlled by steroid hormones.

This was followed by a second phase of growth restricted to high-order branches, which might be dependent on afferent input and, specifically, on activity-dependent calcium influx (*14;* Duch, unpublished).

In contrast, compelling evidence for the role of synaptic transmission in dendritic growth during early phases of dendritic maturation comes from the *Xenopus* retinotectal system *(6).* Tectal neurons receive synaptic input during early stages, when their dendritic trees are rather immature. During this initial developmental time frame, synaptic currents are predominantly mediated by NMDA receptors (NMDARs), whereas the contribution of AMPA receptors (AMPARs) to synaptic currents increases at later stages of development *(79,80).* Blocking NMDARs at early stages significantly reduces dendritic branching dynamics, and, as a consequence, total dendritic length *(81).* Furthermore, visual stimulation increases dendritic growth of tectal neurons in an NMDAR-dependent manner *(65).* Strikingly, during later stages, synaptic transmission actively prevents further growth by stabilizing dendritic branches in an AMPAR-dependent manner. At these stages, blocking AMPARs results in the loss of dendrites *(81).* Therefore, release of the same transmitter can have either a motility- and growth-promoting effect or a motility-inhibiting and stabilizing effect, depending on the receptor distribution on the dendritic tree *(6).*

Differential regulation of glutamate receptor expression on developing dendrites has also been described in the cortex, as newly formed thalamocortical synapses are mediated by NMDARs, but more synapses acquire AMPARs as the dendritic tree matures *(82).* NMDAR-dependent dendritic growth has also been demonstrated during early postnatal life in spinal cord motoneurons. Similar to the retinotectal system, NMDAR activity does not affect dendritic maturation of spinal motoneurons during later stages *(83).* Finally, in NMDAR1 mutant mice the dendritic development of cortical neurons seems impaired *(84),* and NMDAR blockade inhibits dendritic growth in the supraoptic nucleus *(85).*

In summary, the data suggest that, at least in some vertebrate systems, the role of synaptic activity in dendritic maturation appears to be twofold. During the first phase, NMDAR-dependent activity promotes growth and sprouting, and during the second phase, NMDAR-independent but AMPAR-dependent activity stabilizes dendrites *(6).* However, to date it remains unclear whether similar activity-dependent processes also take place in invertebrates. There, the main transmitter within the CNS is not glutamate but acetylcholine (ACh). Thus, dendritic maturation regulated by synaptic currents would have to be mediated by AChRs.

NMDA-induced increases in dendritic branching are inhibited by blockers of voltage dependent calcium channels (VDCCs) and by blockers of calcium release from the endoplasmic reticulum (ER) *(85).* Therefore, it appears likely that NMDAR activation recruits intracellular calcium signaling pathways to regulate dendritic growth *(67).* In principle, such calcium signaling pathways could be recruited by AChRs, too, as suggested for dendritic stabilization in chick retinal neurons by local increases in dendritic calcium concentrations *(86).*

Molecular Mechanisms of Activity-Dependent Dendritic Maturation

Increasing evidence makes calcium a key candidate for translating neuronal activity into changes in dendritic architecture. Both intrinsic and synaptic activity can lead to increases in intracellular dendritic calcium concentrations. First, NMDARs and nAChRs are permeable to calcium. Second, dendritic calcium influx through VDCCs can be induced by action potentials and by synaptic depolarizations in vertebrate *(87)* and in invertebrate neurons *(28).* Both mechanisms can trigger further calcium-induced calcium release (CICR). Third, calcium can be released from the ER in response to IP3 binding to ryanodine receptors *(88).*

Spontaneous spiking activity in developing neurons will most likely induce calcium elevations in their somata and possibly also in large parts of their dendrites, depending on the ion channel distribution. In contrast, synaptic activity will most likely only induce local increases in dendritic calcium levels. In fact, dendritic calcium elevations can even be restricted to individual dendritic spines *(89).* Somatic or global calcium elevations might have very different effects as compared with local elevations. Therefore, intrinsic spiking and synaptic activity might recruit different cellular mechanisms affecting dendritic structure.

Alterations in somatic calcium concentration can affect gene transcription *(90,91).* Transcription-dependent induction of a developmental program for dendritic growth has recently been suggested in cortical-slice culture *(92).* There, the effect of VDCC-dependent control of dendritic growth was dependent on CaM kinase IV. This effect might be under transcriptional control for two reasons. First, CaM kinase IV is localized in the nucleus, and second, VDCC- and CaM kinase IV-dependent dendritic growth is suppressed in the absence of the cyclic-AMP-responsive-element binding protein, CREB *(92).* Therefore, VDCC-dependent control of dendritic growth in cortical slice culture seems to depend on transcription factors like CREB. However, the downstream genes in this pathway still need to be identified. One good candidate might be BDNF, as it is among the multiple target genes of CREB, and it is known to exert effects on dendritic structure in vertebrate neurons *(71,93).* In summary, activity-dependent calcium influx through VDCCs might affect dendritic growth by altering gene expression. It would be interesting to test whether activity-dependent elevations in somatic calcium concentrations affect transcriptional programs for dendritic growth in other systems, too.

For the remodeling of motoneurons during insect metamorphosis it has been shown that specific phases of dendritic growth correlate in time with the transient occurrence of calcium action potentials during development *(27).* Such calcium spikes significantly elevate the somatic calcium concentrations via influx through VDCCs in these neurons *(28),* although the somata of insect motoneurons are usually not equipped with voltage-dependent conductances. However, a role for transcriptional regulation of dendritic growth remains to be investigated in invertebrate neurons.

In contrast to a global effect of activity-dependent control of gene transcription, which may, in turn, alter overall dendritic growth, more precise actions on dendritic shape are mediated by local increases in dendritic calcium concentrations, which are induced by synaptic activity. As mentioned previously, NMDAR-dependent increases in dendritic motility and growth are dependent on elevations in dendritic calcium concentrations *(85).* But how are dendritic calcium elevations translated into structural changes of the cytoskeleton? Good candidates for this mediation are the RHO GTPases Rac, RhoA, and Cdc42, as they are key players in shaping dendritic structure. Constitutively active or suppressed Rac and RhoA significantly affect dendritic shape in cultured neurons *(94)* and in vivo *(95,96).* Furthermore, Rac and Rho are regulated by neuronal activity in a glutamate receptor-dependent manner *(97).* In *Xenopus* optic tectal cells, NMDAR-dependent dendritic growth requires calcium influx through VDCC, decreased RhoA activity, and increased Rac and Cdc42 activity *(65).* However, it still remains to be clarified how, exactly, neurotransmission regulates RHO GTPases, and how these in turn affect the cytoskeleton.

In addition to promoting dendritic motility, synaptic input also functions to stabilize dendrites, as shown for AMPAR-dependent stabilization of the dendrites of *Xenopus* optic tectal neurons *(6)* and for AChR-dependent dendritic stabilization of retinal neurons *(86).* Chick retinal neurons show two distinct types of periodic calcium elevations: global elevations that are inhibited by blocking retinal waves with TTX application, and local calcium elevations that are resistant to TTX application and are

most likely caused by cholinergic transmission from bipolar and amacrine cells. Blockade of local, but not global, calcium elevations causes rapid retraction of dendrites. Furthermore, local uncaging of calcium can prevent dendritic collapse. Local uncaging of calcium in selected dendrites causes CICR from internal stores. Therefore, synaptic transmission may locally increase calcium levels by CICR and stabilize dendrites *(86).*

An additional mechanism for dendrite stabilization upon synaptic activity has been demonstrated in the *Xenopus* retinotectal system *(6).* There, AMPAR-dependent calcium elevations cause dendrite stabilization of tectal neurons. Blocking and overexpression experiments have clearly demonstrated that CaM kinase II is necessary and sufficient to mediate this effect *(98,99).* Neuronal CaM kinase II activity can be regulated by changing intracellular calcium concentrations, and thus may resemble a precise readout of activity. In fact, in cultured dorsal root ganglion, CaM kinase II activity is regulated differentially by calcium spikes resulting from different action potential frequencies, ranging from 0.1 to 1 Hz *(100).* As CaM kinase II is localized predominantly postsynaptically, it is a good candidate for local readout of synaptically increased dendritic calcium levels. Correspondingly, disruption of dendritic translation of CaM kinase II impairs stabilization of synaptic plasticity in adult mice *(101).* During development, CaM kinase II activity might translate synaptic activity received in different dendrites into precise local alterations in dendritic shape.

In a recent study on cultured sympathetic neurons, it was demonstrated that neural activity leads to stimulation of both CaM kinase II and MEK-ERK pathways, and that inhibition of either one of these pathways is sufficient to inhibit dendrite stabilization via MAP-2 microtubule interactions *(102).* These data further indicate that activity regulates dendritic stability via CaM kinase II and MEK-ERK, both of which may regulate MAP-2-microtubule interactions. Further interactions and cross-talk among these pathways seem likely, as, for instance, CaM kinase II may also regulate RHO GTPases, such as Rac1 *(103).*

Recent data on the mechanisms underlying motoneuron remodeling during *Manduca* metamorphosis also suggest calcium- and CaM kinase II-dependent mechanisms. In this system, rapid growth-cone-dependent dendritic sprouting is followed by a phase of dendritic stabilization and further sprouting of high-order dendrites only *(14).* The switch between phases correlates in time with developmental changes in calcium channel expression *(27,28).* Furthermore, developmental changes in calcium influx result in altered CaM kinase II activity in these motoneurons (Duch, unpublished). Preliminary results indicate that blockade of either VDCCs or of CaM kinase II during these stages might affect dendritic structure (Duch, unpublished). However, the role of CaM kinase II and downstream signals for dendritic maturation in invertebrates remains to be further investigated.

Hormonal Control of Dendritic Maturation

Studies in several different systems have shown that dendritic shape can be influenced by thyroid and steroid hormones and by glucocorticoids *(104–109).* For example, in developing rats, altered thyroid levels affect dendritic branching of Purkinje and pyramidal cells *(104,110).* High levels of glucocorticoids have been demonstrated to decrease dendritic length and the complexity of pyramidal cells in the adult hippocampus *(111),* thus being considered a stress effect on dendritic structure *(107,108).* Examples of steroid hormone effects on dendritic maturation are estradiol-dependent dendritic outgrowth in hypothalamic slice cultures *(112),* steroid-dependent maturation of the song-control system in birds *(113),* and ecdysteroid-dependent dendritic growth of insect motoneurons during metamorphosis *(106,114,115).* This review is not intended to discuss the actions of these different hormones on the dendrites of neurons in the various

systems mentioned above, but is rather aimed at briefly discussing whether hormonal effects might act in concert with activity-dependent dendritic growth.

The first hurdle to overcome in interpreting the results of hormonal manipulation on dendritic structure is to determine whether the effects are direct or indirect. One way to test for direct hormone action is to isolate the neurons of interest in cell culture and apply hormones in vitro. Manduca thoracic motoneurons placed in culture respond to ecdysteroids with an increase in dendritic branching complexity, similar to the events observed during normal pupal development in vivo *(32,106).* Likewise, exposure of dissociated rat hippocampal pyramidal neurons to estradiol causes a twofold increase in spine density *(116).* Although these studies provide strong evidence that neurons may be direct targets of steroid action, in both culture systems, insect motoneurons and rat pyramidal neurons, additional cell types are also present. This leaves open the possibility that the effects are mediated via alterations in other cells.

It remains unclear whether hormone-induced alterations in dendritic architecture are mediated by hormone-specific cellular mechanisms, or whether hormonal effects converge with the previously discussed activity-dependent mechanisms. In dissociated Manduca motoneurons it has been clearly demonstrated that ecdysteroid application promotes dendritic outgrowth and growth-cone complexity *(117),* which is correlated with increased calcium current amplitude *(118).* Similarly, in dissociated rat hippocampal neurons it has been demonstrated that estradiol-induced spine formation is accompanied by increased activity in pyramidal neurons *(119).* Likewise, there is an increase in the cellular calcium response to glutamate following estradiol application. Taken together, these results suggest that excitation leads to an increase in free intracellular calcium concentration and CREB phosphorylation, which, in turn, leads to activation of the nucleus to produce more spine-associated proteins *(119).* Therefore, hormone effects seem to act in concert with activity-dependent mechanisms to regulate dendritic shape.

Steroid hormone signaling and the pathways activated by diffusible signals might also converge to affect dendritic structure. Colocalization of estrogen and neurotrophin receptors (tyrosine receptor kinases, trks) has been demonstrated in a variety of neurons. This colocalization leads to cross-talk between the signaling pathways, in particular at the level of the mitogen-activated protein (MAP) kinase cascade. Estradiol causes estrogen–receptor- and trk-independent activation of the MAP kinases ERK1 and ERK2 (extracellular signal-regulated kinases), suggesting nongenomic actions of estradiol *(112).* Accordingly, estradiol application leads to ERK phosphorylation even in ER-alpha knockout mice. As suggested for estradiol, neurotrophin-mediated activation of trk directly activates the RAS-MEK-ERK pathway, which may affect dendritic structure by regulating MAP-2-microtubule interactions *(103).* At this level the pathways of several signals affecting dendritic structure may converge, as MAP-2-microtubule interactions might also be regulated by neuronal activity via CaM kinase II (see above, *103*). These data support the view that estradiol-induced alterations in dendritic structure might be mediated not only by transcriptional events via the estrogen receptor, but also by rapid, nongenomic events.

Clear evidence for direct transcriptional regulation of dendritic shape comes from a recent study on motoneuron dendritic growth during *Drosophila* metamorphosis. Normal dendritic growth, as occurs in identified motoneurons during wild-type pupal development *(30),* is impaired in mutants for a specific ecdysteroid early response gene, the *broad complex (BRC).* In *BRC* mutants the extent of dendritic arbors of the motoneurons is markedly reduced as compared with stage-matched wild-type controls. Replacement with a wild-type transgene rescues the wild-type dendritic phenotype *(120).* This clearly demonstrates that steroid hormones can affect dendritic maturation via the action of their nuclear receptor on the tran-

scriptional machinery. However, whether these are direct effects or whether dendritic shape might be affected as a consequence of altered cell-cell interactions remains to be investigated. Furthermore, it will probably prove useful to identify additional downstream target genes of transcriptional regulation during hormone-dependent dendritic maturation.

Other Diffusible Factors and Control of Dendritic Maturation

Several diffusible signals affect dendritic maturation. In the vertebrate cortex, neurotrophins, which include at least four related proteins (NGF: nerve growth factor; BNDF: brain-derived neurotrophic factor; and NT-3 and NT-4: neurotrophin 3 and 4), regulate the dendritic growth of pyramidal neurons. The underlying cellular and molecular mechanisms have recently been reviewed *(66,103).* Briefly, the starting point of these investigations was the discovery that NGF is critical for dendritic growth and maintenance of sympathetic neuron dendrites *(121).* This observation was later substantiated in cortical neurons as well *(93).* The effect of neurotrophins on pyramidal neurons from different layers shows a different pattern for basal and apical dendrites *(66).* Thus, neurotrophins are not simply inducing overall dendritic growth but affect distinct regions of the dendritic arborizations. Furthermore, Horch and Katz *(122)* have recently shown that BDNF released locally from dendrites exerts direct action on branching of the dendrites of neighboring neurons in a distance-dependent manner.

Activity-dependent dendritic growth is enhanced by coincident exposure to neurotrophins; both neuronal activity and neurotrophins converge to activate CaM kinase II and the MEK-ERK pathway (see section on activity-dependent maturation of dendritic architecture). Thus, neurotrophins may act in concert with neuronal activity, as their effect on growth is increased in active neurons and reduced in inactive neurons *(66).* However, it was recently shown that the role of BDNF might be restricted to the maintenance of dendrite architecture rather than growth, thereby stabilizing connectivity during synaptic maturation *(123).*

Another example of a factor influencing dendritic maturation is semaphorin 3A (Sema 3A), a member of a large family of semaphorins with repulsive and attractive functions on dendritic growth. Sema 3A acts both as a chemoattractant on apical dendrites of pyramidal cortical neurons and as a chemorepellant on axonal growth *(66).* Two additional families of chemoattracting proteins are Netrins and Slits. Exposure of cortical neurons to Slit1 increases dendritic growth and branching, and addition of Robo-Fc fusion protein, which inhibits interaction between Slit 1 and its receptor, inhibits dendritic growth *(124).*

Dendritic Tiling Limits Overgrowth of Dendrites

In the developing nervous system, as the dendrites of individual neurons grow, the spacing between the dendritic fields of neighboring neurons must be adjusted to reach optimal occupation of nervous territory. There will always be a trade-off among the number of synapses, conduction delay, and computation time. Moreover, because of space constraints, the size of dendritic and axonal arborizations, which take up a large fraction of brain volume, is optimized as a result of evolutionary and developmental constraints *(125).* In fact, wiring of neuronal tissues as diverse as the retina, cerebellum, olfactory bulb, and neocortex obeys a simple mathematical rule of wiring optimization *(125).* Optimization of all parameters requires precise regulation of dendritic shape and dendritic contacts.

The two possible cellular mechanisms that have been proposed for restricting dendrites to grow within their own specific territories are diffusible factors, such as growth factors, and specific signaling mediated by direct contact between dendrites. In the developing rat retina,

it has been suggested that "contact-inhibition" between retinal ganglion neurons regulates the spread of their dendritic arbors as the retina expands. For instance, lowering of retinal ganglion neuron density by target ablation causes an expansion and shift of the dendritic fields of neighboring ganglion cells toward the depleted area *(11).* This suggests that dendritic growth is regulated in part by interactions with neighboring dendrites. However, since removal of neurons leaves their presynaptic partners without a target, the observed excess growth of neighboring ganglion cells could, in part, be due to excess innervation by presynaptic partners to accommodate for additional inputs.

In a recent study, Gao et al. *(126)* analyzed factors that affect the morphology of sensory multiple-dendrite neurons, called DA neurons, of Drosophila larvae. Specific groups of neurons meet with their contralateral homologs in the midline, and once there, appear to repel each other. Using photoablation, evidence was provided for dendritic competition between dendritic trees of neurons and their contralateral homologs from different segments. A G-protein-coupled receptor called flamingo seems to be required for this competitive interaction between dendrites *(127).* In this system, excess dendritic growth cannot be attributed to changes in presynaptic innervation, because DA neurons are primary sensory neurons. Yet, the dendrites of distinct classes of *Drosophila* neurons within a segment show different capabilities for dendritic competition *(128).* Within a given segment, while the dendritic arborizations of class IV neurons show strong repulsive interactions, those of class I and II neurons do not. Since flamingo is expressed in all DA neurons, there must be additional factors involved in mediating these cell-cell interactions.

Dendro-dendritic competition was also analyzed in another system, the cercal circuitry of the cockroach *(129).* Thirty days after a single identified postsynaptic neuron was photoablated, the synaptic connectivity was reorganized in a manner that was consistent with functional plasticity. However, the removal of a post-synaptic neuron in this circuit had no effect on the morphology of either presynaptic or intact postsynaptic neurons *(15).* Thus, reorganization of circuitry can occur in the absence of macroscopic changes in the neuronal architecture.

Future Prospects

To further increase our understanding of the molecular and cellular mechanisms of dendritic development, the following issues/questions should be resolved:

1. How do intracellular cytoskeletal proteins and signaling cascades regulate the number, length, and diameter of dendritic trees, the decision whether to branch or not, and if branching, toward which direction?

   In this respect, 3-D quantitative metric analysis might help to reveal subtle differences in the cellular effect signals for dendritic growth. This would allow one to look at the effect of known and yet to be identified extrinsic and intrinsic factors on specific aspects of dendritic growth. Furthermore, it is clear that activity-dependent processes act on dendritic architecture. In order to understand the cellular consequences of these activity-dependent changes, we need to continue investigation of the cellular and molecular mechanisms of synapse formation using real-time functional imaging of labeled pre- and postsynaptic components.

2. How is the size of a dendritic field specified?

   The establishment of connectivity with synaptic partners is presumably important in determining the size of a dendritic field. However, dendritic tiling and intrinsic regulation of the extent of growth for a given neuronal type almost certainly play a role as well. One of the challenges will be to examine the contribution of each alone, perhaps with the design and application of techniques for *in situ* localization of specific proteins during branching dynamics. Today, it appears that dozens of molecules have been found to regulate dendritic maturation, but it is almost certain that each molecule plays only a specific role in this formidable cooperative venture. One of the greatest challenges of developmental neurobiology will be to specify the unique role of each of these molecules and how,

in concert, these orchestrate the maturation of complex three-dimensional trees.

3. What are the universal mechanisms underlying the maturation of dendritic architecture?

   To address this issue, comparative studies will be inevitable, because the role of given signals appears to be weighted differentially in different types of neurons, in different brain areas, and especially in different species. Thus, future studies should compare dendritic growth between different species and brain areas, despite the striking advantages of some systems for live cell imaging and elegant manipulation experiments. Although dendrites of invertebrate neurons have some unique structural and functional features, the mechanisms uncovered in invertebrates are likely to also be applicable to mammalian systems. The future for dendrite studies is based on the pursuit of comparative studies using invertebrate and vertebrate neurons and circuits. Intrinsic and extrinsic signaling pathways, with their receptor targets on the dendrites, appear to involve molecules with highly conserved structure, which is paralleled by conserved roles in dendritic growth. Hence, the discovery of clear organizational roles for chemically related factors in invertebrate animals might shed light on patterns in the evolution of the orchestration of dendritic architecture function in our own brain.

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References

1. Ramon y Cajal (1897) Las leyes de la morfologia y dinamismo de las celulas nerviosas. *Revista Trim. Microgr.* **1.**
2 Rall W., Burke R.E., Holmes W.R., Jack J.J., Redman S.J., and Segev I. (1992) Matching dendritic neuron models to experimental data. *Physiol. Rev.* **72,** S159–186.
3 Koch C. and Segev I. (2000) The role of single neurons in information processing. *Nat. Neurosci.* **3,** 1171–1177.
4 Mainen Z.F. and Sejnowski T.J. (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. *Nature* **382,** 363–366.
5 Vetter P., Roth A., and Häusser M. (2001) Propagation of action potentials in dendrites depends on dendritic morphology. *J. Neurophysiol.* **85,** 926–937.
6 Cline H.T. (2001) Dendritic arbor development and synaptogenesis. *Curr. Opin. Neurobiol.* **11,** 118–126.
7 Cohen-Cory S. (2002) The developing synapse: Construction and modulation of synaptic structures and circuits. *Science* **298,** 770–776.
8 Garner C.C., Zhai R.G., Gundelfinger E.D., and Ziv N.E. (2002) Molecular mechanisms of CNS synaptogenesis. *Trends Neurosci.* **25,** 243–251.
9 Segal M. and Andersen P. (2000) Dendritic spines shaped by synaptic activity. *Curr. Opin. Neurobiol.* **10,** 582–586.
10 Wong W.T. and Wong R.O.L. (2000) Rapid dendritic movements during synapse formation and rearrangement. *Curr. Opin. Neurobiol.* **10,** 118–124.
11 Sernagor E., Eglen S.J., and Wong R.O. (2001) Development of retinal ganglion cell structure and function. *Prog. Retin. Eye Res.* **20,** 139–174.
12 Trachtenberg J.T., Chen B.E., Knott G.W., Feng G., Sanes J.R., Welker E., et al. (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **420,** 788–794.
13 Grutzendler J., Kasthuri N., and Gan W.B. (2002) Long-term dendritic spine stability in the adult cortex. *Nature* **420,** 812–816.
14 Libersat F. and Duch C. (2002) Morphometric analysis of dendritic remodeling in an identified motoneuron during postembryonic development. *J. Comp. Neurol.* **450,** 153–166.
15 Mizrahi A. and Libersat F. (2002) Afferent input regulates the formation of distal dendritic branches. *J. Comp. Neurol.* **452,** 1–10.
16 Scott E.K., Reuter J.E., and Luo L. (2003) Dendritic development of *Drosophila* high order visual system neurons is independent of sensory experience. *BMC Neurosci.* **4,** 1–6.
17 Uylings H.B.M., Ruiz-Marcos A., and van Pelt J. (1986) The metric analysis of three-dimensional dendritic tree patterns: A methodological review. *J. Neurosci. Meth.* **18,** 127–151.

18. Sholl D.A. (1953) Dendritic organization in the neurons of the visual cortex and motor cortices of the cat. *J. Anat. (Lond)* **87,** 387–406.
19 Acebes A. and Ferrus A. (2000) Cellular and molecular features of axon collaterals and dendrites. *Trends Neurosci.* **23,** 557–565.
20 Smith T.G.J., Marks W.B., Lange G.D., Sheriff W.H.J., and Neale E.A. (1989) A fractal analysis of cell images. *J. Neurosci. Meth.* **27,** 173–180.
21 Caserta F., Eldred W.D., Fernandez E., Hausman R.E., Stanford L.R., Bulderev S.V., et al. (1995) Determination of fractal dimension of physiologically characterized neurons in two and three dimensions. *J. Neurosci. Meth.* **56,** 133–144.
22 Smith T.G.J., Lange G.D., and Marks W.B. (1996) Fractal methods and results in cellular morphology—dimensions, lacunarity and multifractals. *J. Neurosci. Meth.* **69,** 123–136.
23 Mizrahi A., Ben-Ner E., Glusman G., Katz M., Kedem K., and Libersat F. (2000) Comparative analysis of dendritic architecture of identified neurons using the Hausdorff distance metric. *J. Comp. Neurol.* **422,** 415–428.
24 Mendenhall B. and Murphey R.K. (1974) The morphology of cricket giant interneurons. *J. Neurobiol.* **5,** 565–580.
25 Scott E.K., Raabe T., and Luo L. (2002) Structure of the vertical and horizontal system neurons of the lobula plate in *Drosophila. J. Comp. Neurol.* **454,** 470–481.
26. Weeks J.C. and Truman J.W. (1986) Steroid control of neuron and muscle development during the metamorphosis of an insect. *J. Neurobiol.* **3,** 249–267.
27 Duch C. and Levine R.B. (2000) Remodeling of membrane properties and dendritic architecture accompany the postembryonic conversion of a "slow" into a "fast" motoneuron. *J. Neurosci.* **20,** 6950–6961.
28 Duch C. and Levine R.B. (2002) Changes of calcium signaling during postembryonic dendritic growth in *Manduca sexta. J. Neurophysiol.* **87,** 1415–1425.
29 Consoulas C., Duch C., Bayline R.J., and Levine R.B. (2000) Behavioral transformations during metamorphosis: Remodeling of neural and motor systems. *Brain Res. Bull.* **5,** 571–583.
30 Consoulas C., Restifo L.L., and Levine R.B. (2002) Dendritic remodeling and growth of motoneurons during metamorphosis of *Drosophila melanogaster. J. Neurosci.* **22,** 4906–4917.
31 Goodman C.S. (1978) Isogenic grasshoppers: Genetic variability in the morphology of identified neurons. *J. Comp. Neurol.* **182,** 681–705.
32 Prugh J., Della Croce K., and Levine R.B. (1992) Effects of the steroid hormone, 20-hydroxyecdysone, on the growth of neurites by identified insect motoneurons in vitro. *Dev. Biol.* **154,** 331–347.
33 Kraft R., Levine R.B., and Restifo L.L. (1998) The steroid hormone 20-hydroxyecdysone enhances neurite growth of *Drosophila* mushroom body neurons isolated during metamorphosis. *J. Neurosci.* **18,** 8886–8899.
34. Montague P.R. and Friedlander M.J. (1989) Expression of an intrinsic growth strategy by mammalian retinal neurons. *PNAS* **18,** 7223–7227.
35. Montague P.R. and Friedlander M.J. (1991) Morphogenesis and territorial coverage by isolated mammalian retinal ganglion cells. *J. Neurosci.* **5,** 1440–1457.
36 Banker G.A. and Cowan W.M. (1979) Further observations on hippocampal neurons in dispersed cell culture. *J. Comp. Neurol.* **187,** 469–493.
37 Threadgill R., Bobb K., and Gosh A. (1997) Regulation of dendritic growth and remodeling by Rho, Rac, and Cdc42. *Neuron* **19,** 625–634.
38 Spatkowski G. and Schilling K. (2003) Postnatal dendritic morphogenesis of cerebellar basket and stellate cells in vitro. *J. Neurosci. Res.* **72,** 317–326.
39 Baptista C.A., Hatten M.E., Blazeski R., and Mason C.A. (1994) Cell-cell interactions influence survival and differentiation of purified Purkinje cells in vitro. *Neuron* **12,** 243–260.
40. Spitzer N.C. (2002) Activity-dependent neuronal differentiation prior to synapse formation: The functions of calcium transients. *J. Physiol. Paris* **96,** 73–80.
41 Weliky M. and Katz L.C. (1999) Correlational structure of spontaneous neuronal activity in the developing lateral geniculate nucleus in vivo. *Science* **285,** 599–604.
42 Feller M.B. (1999) Spontaneous correlated activity in developing neural circuits. *Neuron* **22,** 653–656.
43 Yuste R. (1997) Introduction: Spontaneous activity in the developing central nervous system. *Semin. Cell Dev. Biol.* **8,** 1–4.
44. O'Donavan M. and Chub N. (1997) Population behavior and self-organization in the genesis of spontaneous rhythmic activity by developing spinal networks. *Semin. Cell Dev. Biol.* **8,** 21–28.
45 Milner L.D. and Landmesser L.T. (1999) Cholinergic and GABAergic inputs drive pat-

terned spontaneous motoneuron activity before target contact. *J. Neurosci.* **19,** 3007–3022.
46. Garaschuk O., Hanse E., and Konnerth A. (1998) Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J. Physiol. (Lond.)* **50,** 219–236.
47 Rubel E.W. and Fritsch B. (2002) Auditory system development: Primary auditory neurons and their targets. *Annu. Rev. Neurosci.* **25,** 51–101.
48 Feller M.B., Wellis D.P., Stellwagen D., Werblin F.S., and Shatz C.J. (1996) Requirement for cholinergic synaptic transmission in the propagation of spontaneous retinal waves. *Science* **272,** 1182–1187.
49. Yuste R., Nelson D.A., Rubin W.W., and Katz L.C. (1995) Neuronal domains in developing neocortex: Mechanisms of coactivation. *Neuron* **11,** 118–126.
50 Zhang L.I., Tao H.W., and Poo M. (2000) Visual input induces long-term potentiation of developing retinotectal synapses. *Nat. Neurosci.* **3,** 708–715.
51 Volman S.F. and Camhi J.F. (1988) The role of afferent activity in behavioral and neuronal plasticity in an insect. *J. Comp. Physiol. A* **162,** 781–791.
52 Murphey R.K., Medenhall B., Palka J., and Edwards J.S. (1975) Deafferentation slows growths of specific dendrites of identified giant interneurons. *J. Comp. Neurol.* **159,** 407–418.
53 Rakic P. and Sidman R.L. (1973) Organization of cerebellar cortex secondary to deficit of granule cells in weaver mutant mice. *J. Comp. Neurol.* **152,** 133–161.
54. Rakic P. (1975) Role of cell interactions in development of dendritic patterns. *Adv. Neurol.* **12,** 117–134.
55. Mason C.A., Morrison M.E., Ward M.S., Zhand Q., and Bird D.H. (1997) Axon-target interactions in the developing cerebellum. *Perspect. Dev. Neurobiol.* **5,** 69–82.
56 Wiesel T.N. and Hubel D.H. (1963) Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. *J. Neurophysiol.* **26,** 978–993.
57 Friedlander M.J., Stanford L.R., and Sherman S.M. (1982) Effects of monocular deprivation on the structure/function relationship of individual neurons in the cat's lateral geniculate nucleus. *J. Neurosci.* **2,** 321–330.
58 Borges S. and Berry M. (1978) The effects of dark rearing on the development of the visual cortex of rat. *J. Comp. Neurol.* **180,** 277–300.
59 Barth M., Hirsch H.V., Meinertzhagen I.A., and Heisenberg M. (1997) Experience-dependent developmental plasticity in the optic lobe of *Drosophila melanogaster. J. Neurosci.* **17,** 1493–1504.
60. Hirsch H.V.B., Potter D., Zawierucha D., Choudhri T., Glasser A., Murphey R.K., et al. (1990) Rearing in darkness changes visually-guided choice behavior in *Drosophila. Visual Neurosci.* **5,** 281–289.
61. Mimura K. (1986) Development of visual pattern discrimination in the fly depends on light experience. *Science* **232,** 83–85.
62. Mimura K. (1987) Persistence and extinction of the effect of visual pattern deprivation in the fly. *J. Exp. Biol.* **46,** 155–162.
63. Lud J.S., Holbach S.M., and Chung W.W. (1991) Postnatal development of the thalamic recipient neurons in the monkey striate cortex. II. Influence of afferent driving on spine acquisition and dendritic growth of layer 4C spiny stellate neurons. *J. Comp. Neurol.* **30,** 29–140.
64 Tieman S.B., Zec N., and Tieman D.G. (1995) Dark rearing fails to affect the basal dendritic fields of layer 3 pyramidal cells in the kitten visual cortex. *Brain Res. Dev. Brain Res.* **84,** 39–45.
65 Sin W.C., Haas K., Ruthazer E.S., and Cline H.T. (2002) Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. *Nature* **419,** 475–480.
66 McAllister A.K. (2000) Cellular and molecular mechanisms of dendrite growth. *Cereb. Cortex* **10,** 963–973.
67 Wong R.O.L. and Gosh A. (2002) Activity-dependent regulation of dendritic growth and patterning. *Nature Rev. Neurosci.* **3,** 803–812.
68 Goodman L.A. and Model P.G. (1990) Eliminating afferent impulse activity does not alter the dendritic branching of the amphibian Mauthner cell. *J. Neurobiol.* **21,** 283–294.
69 Kossel A.H., Williams C.V., Schweizer M., and Kater S.B. (1997) Afferent innervation influences the development of dendritic branches and spines via both activity-dependent and non-activity-dependent mechanisms. *J. Neurosci.* **17,** 6314–6324.
70 Frotscher M., Drakew A., and Heimrich B. (2000) Role of afferent innervation and neuronal activity in dendritic development and spine maturation of fascia dentata granule cells. *Cereb. Cortex* **10,** 946–951.
71 Jin X., Hu H., Mathers P.H., and Agmon A. (2003) Brain-derived neurotrophic factor mediates activity-dependent dendritic growth

in nonpyramidal neocortical interneurons in developing organotypic cultures. *J. Neurosci.* **23,** 5662–5673.
72 McAllister A.K., Katz L.C., and Lo D.C. (1996) Neurotrophin regulation of cortical dendritic growth requires activity. *Neuron* **17,** 1057–1064.
73 McKinney R.A., Capogna M., Durr R., Gahwiler B.H., and Thompson S.M. (1999) Miniature synaptic events maintain dendritic spines via AMPA receptor activation. *Nat. Neurosci.* **2,** 44–49.
74 Spitzer N.C., Kingston P.A., Manning T.J., and Conklin M.W. (2002) Outside and in: Development of neuronal excitability. *Curr. Opin. Neurobiol.* **12,** 315–323.
75 Suster M.L. and Bate M. (2002) Embryonic assembly of a central pattern generator without sensory input. *Nature* **416,** 174–178.
76 Baines R.A., Seugnet L., Thompson A., Salvaterra P.M., and Bate M. (2001) Regulation of synaptic connectivity: Levels of Fasciclin II influence synaptic growth in the *Drosophila* CNS. *J. Neurosci.* **22,** 6587–6595.
77 Sweeney S.T., Broadie K., Keane J., Niemann H., and O'Kane J. (1995) Targeted expression of tetanus toxin light chain in *Drosophila* specifically eliminates synaptic transmission and causes behavioral defects. *Neuron* **14,** 341–351.
78 Verhage M., Maia A.S., Plomp J.J., Brussaard A.B., Heeroma J.H., Vermeer H., et al. (2000) Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science* **287,** 864–869.
79 Wu G.Y., Malinow R., and Cline H.T. (1996) Maturation of central glutamatergic synapses. *Science* **174,** 972–976.
80. Cantallops I., Haas H., and Cline H.T. (2000) CPG15 expression enhances presynaptic axon growth and retinotectal synapse maturation. *Nat. Neurosci.* **3,** 498–503.
81 Rajan I. and Cline H.A.T. (1998) Glutamate receptor activity is required for normal development of tectal cell dendrites in vivo. *J. Neurosci.* **18,** 7836–7846.
82 Isaac J.T., Crair M.C., Nicoll R.A., and Malenka R.C. (1997) Silent synapses during development of thalamocortical inputs. *Neuron* **18,** 269–280.
83 Kalb R.G. (1994) Regulation of motor neuron dendrite growth by NMDA receptor activation. *Development* **120,** 3063–3071.
84 Iwasato T., Datwani A., Wolf A.M., Nishiyama H., Taguchi Y., Tonegawa S., et al. (2000) Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature* **406,** 726–731.
85 Chevaleyre V., Moos F.C., and Desarmenien M.G. (2000) Interplay between presynaptic and postsynaptic activities is required for dendritic plasticity and synaptogenesis in the supraoptic nucleus. *J. Neurosci.* **22,** 265–273.
86 Lohman C., Myhr K.L., and Wong R.O.L. (2002) Transmitter-evoked local calcium release stabilizes developing dendrites. *Nature* **418,** 177–181.
87 Tank D.W., Sugimori M., Connor J.A., and Llinas R. (1988) Spatially resolved calcium dynamics in mammalian Purkinje cells. *Science* **242,** 773–777.
88 Berridge M.J. (1998) Neuronal calcium signaling. *Neuron* **21,** 13–26.
89 Yuste R., Mejewska A., and Holthoff K. (2000) From form to function: Calcium compartmentalization in dendritic spines. *Nature Neurosci.* **3,** 653–659.
90 Hardingham G.E., Chawla S., Johnon C.M., and Bading H. (1997) Distinct functions of nuclear and cytoplasmatic calcium in the control of gene expression. *Nature* **385,** 260–265.
91 West A.E., Griffith E.C., and Greenberg M.E. (2002) Regulation of transcription factors by neuronal activity. *Nat. Rev. Neurosci.* **3,** 921–931.
92 Redmond L., Kashani A., and Gosh A. (2002) Calcium regulation of dendritic growth via CaM kinase IV and Creb-mediated transcription. *Neuron* **34,** 999–1010.
93 McAllister A.K., Lo D.C., and Katz L.C. (1995) Neurotrophin regulation of dendritic growth in developing visual cortex. *Neuron* **15,** 791–803.
94 Nakayama A.Y., Harris M.B., and Luo L. (2000) Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. *J. Neurosci.* **20,** 5329–5338.
95 Ruchhoeft M.L., Ohnuma S., McNeill L., Holt C.E., and Harris W.A. (1999) The neuronal architecture of *Xenopus* retinal ganglion cells is sculpted by rho-family GTPases in vivo. *J. Neurosci.* **19,** 8454–8463.
96 Li Z., Van Aelst L., and Cline H.T. (2000) Rho GTPases regulate distinct aspects of dendritic growth in *Xenopus* central neurons in vivo. *Nat. Neurosci.* **3,** 217–225.
97 Li Z., Aisenman C.D., and Cline H.T. (2002) Regulation of Rac and Rho GTPases by

crosstalk and neuronal activity in vivo. *Neuron* **33,** 741–750.
98 Wu G.Y. and Cline H.T. (1998) Stabilization of dendritic arbor structure in vivo by CaM KII. *Science* **279,** 222–226.
99. Zhou D.J. and Cline H.T. (1999) Coordinated regulation of retinal axon and tectal cell growth by endogenous CaM KII in vivo. *J. Neurosci.* **19,** 8909–8918.
100 Eshete F. and Fields R.D. (2001) Spike frequency decoding and automonous activation of calcium calmodulin dependent protein kinase II in dorsal root ganglion neurons. *J. Neurosci.* **21,** 6694–6705.
101 Miller S., Yasuda M., Coats M.E., Jones Y., Martone M.E., and Mayford M. (2002) Disruption of dendritic translation of CaMKIIa impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* **36,** 507–519.
102 Vaillant A.R., Zanassi P., Walsh G.S., Aumont A., Alonso A., and Miller F.D. (2002) Signaling mechanisms underlying reversible, activity-dependent dendrite formation. *Neuron* **34,** 985–998.
103 Miller F. and Kaplan D.R. (2003) Signaling mechanisms underlying dendrite formation. *Curr. Opin. Neurobiol.* **13,** 391–398.
104. Gould E., Woolley C.S., and McEwen B.S. (1991) The hippocampal formation: Morphological changes induced by thyroid, gonadal and adrenal hormones. *Psychoneuroendocrinol.* **16,** 67–84.
105 Kawata M. (1995) Roles of steroid hormones and their receptors in structural organization in the nervous system. *Neurosci. Res.* **24,** 1–46.
106 Levine R.B. and Weeks J.C. (1996) Cell culture approaches to understanding the actions of steroid hormones on the insect nervous system. *Dev. Neurosci.* **18,** 73–86.
107 Sapolsky R.M. (1996) Why stress is bad for your brain. *Science* **273,** 749–750.
108 Sapolsky R.M. (1996) Stress, glucocorticoids, and damage to the nervous system: The current state of confusion. *Stress* **1,** 1–19.
109. Mussa G.C., Mussa F., Bretto R., Zambelli M.C., and Silvestro L. (2001) Influence of thyroid in nervous system growth. *Minerva Pediatr.* **53,** 325–353.
110 Nicholson J.L. and Altman J. (1972) Synaptogenesis in the rat cerebellum: Effects of early hypo- and hyperthyroidism. *Science* **176,** 530–532.
111 Woolley C.S., Gould E., and McEwen B.S. (1990) Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res.* **531,** 225–231.
112 Toran-Allerand C.D., Singh M., and Setalo G. Jr. (1999) Novel mechanisms of estrogen action in the brain: New players in an old story. *Front. Neuroendocrinol.* **20,** 97–121.
113 Bottjer S.W. and Arnold A.P. (1997) Developmental plasticity in neural circuits for a learned behavior. *Annu. Rev. Neurosci.* **20,** 459–481.
114 Truman J.W. and Reiss S.E. (1988) Hormonal regulation of the shape of identified motoneurons in the moth, *Manduca sexta. J. Neurosci.* **8,** 765–775.
115 Weeks J.C. and Levine R.B. (1990) Postembryonic neuronal plasticity and its hormonal control during insect metamorphosis. *Annu. Rev. Neurosci.* **13,** 569–592.
116 Murphy D.D. and Segal M. (1996) Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. *J. Neurosci.* **16,** 4059–4068.
117 Matheson S.F. and Levine R.B. (1999) Steroid hormone enhancement of neurite outgrowth in identified insect motor neurons involves specific effects on growth cone form and function. *J. Neurobiol.* **38,** 27–45.
118 Grunewald B. and Levine R.B. (1998) Ecdysteroid control of ionic current development in *Manduca sexta* motoneurons. *J. Neurobiol.* **37,** 211–223.
119 Segal M. and Murphy D.D. (2001) Estradiol induces formation of dendritic spines in hippocampal neurons: Functional correlates. *Hormones and Behavior* **40,** 156–159.
120. Consoulas C. (2003) A steroid-regulated gene is required for dendritic growth of motoneurons during metamorphosis of *Drosophila melanogaster.* In *Proceedings of the 29th Göttingen Neurobiology Conference,* Elsner N. and Zimmermann H., eds., Thieme, Stuttgart, New York, abstr 88.
121 Purves D., Snider W.D., and Voyvodic T. (1988) Trophic regulation of nerve cell morphology and innervation in the autonomic system. *Nature* **336,** 123–128.
122 Horch H.W. and Katz L.C. (2002) BDNF release from single cells elicits local dendritic growth in nearby neurons. *Nat. Neurosci.* **5,** 1177–1184.
123 Gorski J.A., Zeiler S.R., Tamowski S., and Jones K.R. (2003) Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. *J. Neurosci.* **23,** 6856–6865.

124 Whitford K.L., Marillat V., Stein E., Goodman C.S., Tessier-Lavigne M., Chedotal A., et al. (2002) Regulation of cortical dendrite development by Slit-Robo interactions. *Neuron* **33,** 47–61.

125 Chklovskii D.B. (2000) Optimal sizes of dendritic and axonal arbors in a topographic projection. *J. Neurophysiol.* **83,** 2113–2119.

126 Gao F.B., Kohwi M., Brenman J.E., Jan L.Y., and Jan Y.N. (2000) Control of dendritic field formation in *Drosophila:* The roles of flamingo and competition between homologous neurons. *Neuron* **28,** 91–101.

127 Gao F.B. and Bogert B.A. (2003) Genetic control of dendritic morphogenesis in *Drosophila. Trends Neurosci.* **26,** 262–268.

128 Grueber W.B., Ye B., Moore A.W., Jan L.Y., and Jan Y.N. (2003) Dendrites of distinct classes of *Drosophila* sensory neurons show different capacities for homotypic repulsion. *Curr. Biol.* **13,** 618–626.

129. Mizrahi A. and Libersat F. (2001) Synaptic reorganization induced by selective photoablation of an identified neuron. *J. Neurosci.* **21,** 9280–9290.