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Calcium Signals Control Wnt-Dependent Dendrite Growth

Activity-dependent dendritic growth is dependent upon intracellular calcium signaling. Yet the specific mechanisms by which calcium signals lead to morphologic changes in dendrites are not well understood. A paper in this issue of *Neuron* by Wayman et al. describes a novel calcium-dependent signaling cascade linking neuronal activity and calcium influx to expression of Wnt-2, a member of a family of proteins that controls elaboration of dendrites.

Nowhere is the adage "form follows function" more appropriate than in the field of neurobiology. In order for an individual neuron to fulfill its specific function, it must develop a specialized morphology. The development of the dendritic arbor is especially important, as this is the primary site at which synaptic input upon a cell is integrated. Factors that influence dendritic morphology include intrinsic genetic cues, extrinsic cues including peptide growth factors such as neurotrophins, and synaptic input from other neurons. The dendritic tree is sculpted in a highly dynamic manner through processes of dendrite addition and elimination, as well as elongation and branching. The incredibly diverse array of dendritic structures seen across various classes of neurons is one indication of just how tightly linked neuronal form is to function.

Classic experiments where sensory stimulation is blocked demonstrate that neuronal activity is required for proper dendritic arbor formation. At the molecular level, NMDA receptors, voltage-gated calcium channels (VGCC), as well as calcium induced calcium release (CICR), have all been implicated in synaptic activity-dependent development and maintenance of dendritic arbors (Rajan and Cline, 1998; Redmond et al., 2002; Lohmann et al., 2002). While changes in intracellular calcium levels are critical for dendrite dynamics, the calcium-dependent signaling pathways and downstream genetic programs affecting dendritic morphology are not well characterized.

In this issue of *Neuron*, Wayman et al. (2006) provide compelling evidence that a previously undescribed calcium signaling pathway plays a major role in translating neuronal activity signals into changes in dendrite morphology. Calcium/calmodulin-dependent protein kinases (CaMKs) have been implicated in dendrite development, yet which of the many CaMK isoforms are involved has been difficult to discern. To circumvent the nonspecific and nonphysiological effects often seen with the use of pharmacological inhibitors or overexpression of dominant negative and wild-type CaMKs, the authors employ siRNAs to silence specific CaMK isoforms. Using this approach, Wayman et al. (2006) unveil a provocative mechanism whereby synaptic activity results in activation of a CaMKIγ signaling pathway and transcription of Wnt-2, a member of a protein family that regulates dendritic elaboration.

The authors' previous work revealed crosstalk between CaMK and Erk signaling pathways affecting axon extension in hippocampal neurons (Wayman et al., 2004; Schmitt et al., 2004). In the current study, this group examines whether these activity-dependent signaling pathways also converge to direct development of the dendritic arbor. Indeed, as described for axon extension, crosstalk between the CaMKK/CaMKIγ and Erk signaling pathways underlies the dendrite growth response to membrane depolarization.

The authors then go on to identify downstream gene targets of the CaMKK/CaMKIγ/Erk pathway involved in dendritic growth. They look at activation of the transcription factor CREB, a well known target of calcium and MAPK signaling pathways previously implicated in the control of dendritic arborization. Using their siRNA approach the authors demonstrate that CaMKIγ activates CREB-mediated transcription and that this activation is required for activity-induced dendritic growth.

To identify CREB targets that control dendrite growth, the authors use a SACO screen, a powerful approach used previously to identify CREB target genes (Impey et al., 2004). They found that the Wnt-2 gene is bound by CREB and transcriptionally activated in response to membrane depolarization and calcium influx. Moreover, transcriptional activation of Wnt-2 is mediated through the aforementioned CaMKK/CaMKIγ/Erk pathway. This is an important finding because recent studies have implicated Wnt proteins as critical for neurite outgrowth (Yu and Malenka, 2003; Rosso et al., 2004). Thus, activity-induced dendritic growth could be due, at least in part, to CaMKK/CaMKIγ/Erk-dependent expression and secretion of Wnt proteins. Consistent with this hypothesis, Wayman et al. (2006) demonstrate that blocking the ability of Wnts to interact with their receptor Frizzled blocks the stimulatory effects of constitutively active forms of CaMKI, MEK, and CREB on dendritic outgrowth.

This CaMKIγ-Wnt connection is a particularly exciting aspect of the current study in that it provides additional evidence for the role of the Wnt family in dendrite development and maintenance. Wnt-Frizzled signaling can occur through at least three different pathways: the canonical or Wnt/β-catenin pathway, the planar cell polarity pathway (PCP), and the Wnt/calcium pathway (for review see Ciani and Salinas [2005]). Yu and Malenka (2003) showed that Wnts are involved in activity-dependent dendritic growth through a transcription independent, adhesion-mediated branch of the canonical β-catenin pathway. In addition, noncanonical Wnt signaling can affect dendritic outgrowth through activation of Rac and JNK and downstream effects on the actin cytoskeleton (Rosso et al., 2004). The current study complements these previous observations and explains how synaptic activity initiates the process through the transcriptional upregulation of Wnt-2 expression.

Part of the significance of the Wayman et al. (2006) study is that it both challenges and builds upon the

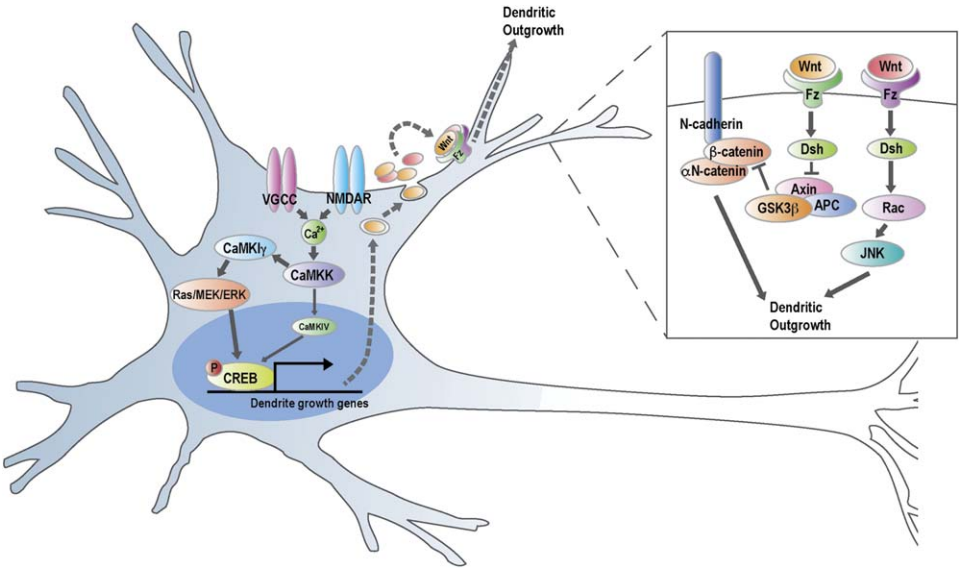

Figure 1. Calcium/Calmodulin-Dependent Protein Kinase (CaMK) Pathways Implicated in Control of Dendritic Growth

The CaMKIγ pathway specifically regulates expression of Wnt-2. Members of the Wnt family affect dendritic outgrowth, possibly through both canonical and noncanonical pathways.

current models in the field. While this study identifies the NMDAR as the source of calcium influx leading to dendritic outgrowth, previous work has implicated L-type VGCCs in this process (Redmond et al., 2002). The reason for this discrepancy is unclear, as both studies use KCl to simulate neuronal depolarization. Yet differences in the mode of calcium entry could explain why different groups observe distinct requirements for the various CaMK signaling pathways. For instance, Wayman et al. (2006) provide a wealth of evidence for the importance of CaMKIγ activity in stimulus-induced dendritic arborization and suggest that CaMKIV plays less of a role. This assessment is largely due to the lack of an effect of a nucleus-specific dominant-negative CaMKIV construct on inhibition of dendritic outgrowth. The authors argue that previous studies that report an effect of dominant negative CaMKIV constructs neglected to limit its expression to the nucleus, where CaMKIV is normally found, so any inhibitory effects could be artifactual, possibly through sequestering CaMKK and inhibition of other CaMK isoforms. Although this is a provocative argument, it should be noted that CaMKIV knockout mice do exhibit dendritic arbor defects in Purkinje cells of the cerebellum and a deficiency in phosphorylation of CREB in multiple populations of neurons (Ho et al., 2000; Ribar et al., 2000). In addition, previous work indicates that activation of CaMKIV leads to upregulation of BDNF, itself known to enhance dendritic outgrowth (Shieh et al., 1998; McAllister et al., 1995). These findings taken together suggest that both CaMKI and CaMKIV are involved in the process of activity-induced CREB activation and dendritic growth, although their relative contributions may depend upon neuronal type and the nature of the stimulus (see Figure 1).

In addition to members of the Wnt family, there are many different secreted factors shown to affect dendritic morphology and maintenance. In an in vivo setting, distinct patterns of electrical activity impinging upon a cell could determine which of these factors are produced and released to sculpt the dendritic arbor of a single postsynaptic neuron. Expanding upon this idea, patterns of synaptic and intrinsic activity could serve as key determinants in defining morphological differences in dendritic arbors between very different types of neurons. Perhaps patterns of synaptic and spontaneous activity in the cerebellum differ enough from those occurring in the neocortex to affect the expression of distinct extrinsic dendritogenic factors, thereby creating the differences between the dendritic tree of the Purkinje cell versus that of the cortical pyramidal cell.

Additional research will provide even more clues as to what properties define the signals, both intrinsic and extrinsic, required for proper dendritic growth and patterning. Ultimately, understanding the underlying basis for induction of dendritic morphology will bring us another step closer to understanding the intricacies of neuronal development and function.

Rebecca S. Alvania,[1] Xi Chen,[1] and David D. Ginty[1]
[1] Department of Neuroscience
Howard Hughes Medical Institute
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

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A Choreography of Nicotinic Receptors Directs the Dopamine Neuron Routine

Modulation of the mesocorticolimbic dopamine system by nicotinic acetylcholine receptors (nAChRs) is thought to play an important role in both health and addiction. However, a clear understanding of how these receptors regulate in vivo firing activity has been elusive. In this issue of *Neuron*, Mameli-Engvall and colleagues report an impressive and thought-provoking series of in vivo experiments combining single-unit recordings from dopamine neurons with nAChR subunit deletions and region-specific lentiviral subunit re-expression.

Ventral tegmental area (VTA) dopamine neurons play a crucial role in reward processing (Schultz, 2002). They are a common target for drugs of abuse and are thought to play a central role in the development of addiction (Nestler, 2005). Nicotine activates the dopamine system through multiple effects at a rich diversity of nicotinic acetylcholine receptors (nAChRs). Recent studies have begun to reveal how nAChRs modulate synapses and membrane excitability in the VTA. For example, nicotine can have direct excitatory effects on dopamine neurons through β2-subunit-containing receptors and increases glutamate release onto dopamine neurons through α7-subunit-containing nAChRs. In addition, β2-nAChRs on GABAergic interneurons rapidly desensitize in response to nicotine, which reduces a tonic excitatory action of acetylcholine on these neurons, thereby reducing their inhibitory input onto dopamine neurons (Mansvelder and McGehee, 2002; Pidoplichko et al., 2004). Our understanding, however, of how this functional diversity is related to the regulation of dopamine neuron firing in vivo has been relatively limited.

Dopamine neurons are spontaneously active and switch between a single-action-potential pattern that can be regular or irregular and a bursting pattern where they fire two to three (sometimes more) action potentials in rapid bursts. Bursts are thought to be the means through which dopamine neurons signal reward prediction error (Schultz, 2002). Bursts are thought to crucially influence extracellular dopamine levels through the balance of release and uptake combined with short-term plasticity. It is known that nicotine can increase firing rate and bursting in dopamine neurons. Pharmacological studies in vivo have begun to suggest that bursting activity and firing rate might be differentially governed by discrete nAChR subpopulations: α7-subunit-containing receptors have been suggested to promote nicotine-induced bursting, whereas non-α7-subunit-containing receptors promote an increase in firing rate (Schilstrom et al., 2003). These studies, however, must be viewed cautiously because it is difficult to be sure of the exact subunit specificity of the agonists and antagonists used. In addition, besides the action of nicotine, little is known about how endogenous acetylcholine (ACh) regulates in vivo dopamine neuron activity through nAChRs.

In this issue of *Neuron*, Mameli-Engvall et al. (2006) report a series of intriguing studies recording from dopamine neurons, in vivo, in anesthetized mice with different nAChR subunit deletions. As a first step, they characterized firing rate and pattern in wild-type mice and show that firing activity can be classed as either low-firing/low-bursting (LFLB), low-firing/high-bursting (LFHB), high-firing/low-bursting (HFLB) or high-firing/high-bursting (HFHB). The merits of this scheme become more obvious when firing activity is observed in the knockout mice. In mice with a deletion for the β2 subunit, dopamine neurons fall almost entirely into the LFLB category. In mice with a deletion for the α7 subunit, dopamine neurons are either LFLB or HFHB. This is a somewhat surprising result. What does it mean? Mameli-Engvall et al. suggest that ACh acting at β2-subunit-containing receptors plays an obligatory role in switching dopamine neurons into any state of increased excitability (high-firing and/or high-bursting). They suggest that the role of the α7 subunit is more subtle: it helps sculpt this increased excitability into the more complex states of either LFHB or HFLB. This is a somewhat speculative suggestion, but it is an interesting and provocative notion that also provides some purpose for the subunit diversity.

Although these results suggest that the β2 subunit plays an obligatory role in switching dopamine neurons to states of increased firing and/or bursting, they do not tell us much about the neuroanatomical location of the crucial receptors. Next, therefore, Mameli-Engvall et al. show that selective lentiviral re-expression of this subunit in the VTA restores all four modes of firing. It seems, therefore, that the effects of nicotine on both firing rate and bursting require β2-containing nAChRs in the VTA. This is consistent with earlier studies showing that β2-containing nAChRs in the VTA are required for nicotine reinforcement (Picciotto et al., 1998; Maskos et al., 2005). However, β2-containing nAChRs are expressed in both dopaminergic and GABAergic neurons in the VTA. Indeed, the direct cholinergic innervation of dopaminergic cells may be relatively limited, which suggests that the effects at GABAergic neurons are likely to be important (Garzon et al., 1999). Further studies are therefore required to tease out the contribution of β2 receptors in each cell type.

So far this is quite a bit to digest, but there's more. They go on to examine the effects of nicotine on dopamine