BioArchitecture 1:1, 2-4; January/February 2011; © 2011 Landes Bioscience

Function directs form of neuronal architecture

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Key words: neuron, development, cell adhesion molecules, brain, synapse, in vivo, dendrite, growth

Submitted: 11/30/10

Accepted: 12/06/10

DOI: 10.4161/bioa.1.1.14429

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The human brain is an architectural marvel. The structure of each brain neuron and the interconnected networks they create are critical to all brain functions, and structural abnormalities are directly associated with neurological disorders. Although the human genome encodes only a few tens of thousands of genes, the human brain is estimated to be composed of hundreds of billions of neurons connected by trillions of synapses. Each brain neuron has an elaborate morphology critical to its functions of collecting, processing and disseminating information. Brain neurons are typically polar cells, composed of a tree-like dendritic arbor that receives and integrates synaptic input from upstream sources, a cell body and an output axonal process and arbor that terminates locally or distantly to transmit signals to downstream targets. Although their morphologies follow neuronal-type basic patterns, each neuron is unique in the number and position of axonal and dendritic branches and the constellation of pre- and postsynaptic cells contacted. Deciphering how such complex cell morphologies and networks form is a leading question in developmental neuroscience, central to understanding the origins of numerous common neurodevelopmental disorders, such as autism spectrum disorders, schizophrenia and epilepsy. Recent research has begun to unravel this mystery, revealing that the structure of complex neural systems can emerge through self-organization following rules based on use-dependent testing of synapses, and synapse-mediated stabilization of dynamically growing neuronal processes.

An understanding of how brain neurons grow within native environments and the molecular mechanisms involved is rapidly emerging due to recent advances in technologies for imaging neuronal growth within intact developing brains. One powerful approach has been to take advantage of transparent, externally developing vertebrate embryos of albino *Xenopus laevis* and zebrafish, since they allow direct imaging within the intact organism during critical stages of brain circuit development, including neurogenesis, neuronal growth, synaptogenesis and functional circuit refinement. Individual brain neurons within these organisms can be fluorescently labelled using single-cell electroporation that can target transfection to individual cells while leaving the rest of the brain unaltered.[1,2] Further, the development of two-photon microscopy has allowed in vivo time-lapse imaging to capture high-resolution, 3D images of neurons deep within living brains with reduced phototoxic stress.[3] Finally, creation of sophisticated computer software to track and measure dendritic arbor growth in 3D over short and long intervals has allowed comprehensive quantification of dendritogenesis.[4,5] Together, these new methods of labelling, imaging and quantification represent an emerging field termed *dynamic morphometrics* that allows precise characterization of neuronal growth behavior.

Application of these technologies for time-lapse imaging using intervals of hours or days can capture the entire development of brain neuron dendritic arbors, and finds that arbors of *Xenopus laevis* tadpole tectal neurons elaborate and reach maturity over 4 to 5 days.[6] Directly following differentiation, tectal neurons are simple spheres and begin their growth program by extending an axonal process. While the axon seeks its targets

during the next 2 days, short dendritic processes, or filopodia, extend with little net growth of the dendritic arbor. Then, when axonal targets are reached, a burst of dendritic branch addition and elongation occurs over the next 2 days. Four to 5 days following differentiation, the dendritic abor matures, entering a stable stage of restricted growth with no further net addition of branch number or length.

Using new methods for rapid time-lapse imaging of growing neurons within the intact and awake developing brain has added a new layer of understanding of the processes underlying dendritogenesis. Such imaging has revealed a surprising amount of motility and turnover of dendritic processes over periods of minutes, which could not be predicted from long interval imaging.[4,5,7] Short dendritic filopodia and longer branches are added and extend, retract and are eliminated. Remarkably, almost all dendritic filopodia retract within minutes of initial extension. What is the purpose of such high turnover? While initially appearing as a waste of cellular resources, this behavior suggests a search routine with a high threshold for process stabilization and maintenance. A leading theory to explain this pattern of dynamic growth is the synaptotropic model of dendritogenesis, in which motile processes seek appropriate presynaptic partners and synapse formation confers morphological stabilization.[8] Strong support for this model comes from in vivo time-lapse imaging of dendritic growth in zebrafish tectal neurons expressing fluorescently tagged postsynaptic protein PSD-95, which demonstrates strong correlation between formation of PSD-95 puncta and stabilization of dendritic filopodia.[7]

An important implication of the synaptotropic model is that this mechanism allows function to drive form, since the appropriateness of new synapses to circuit function is constantly tested leading either to further synapse maturation and strengthening or synapse elimination.[9] Therefore, even though the vast majority of dendritic filopodia are rapidly retracted, they function to sufficiently search the local neuropil for optimal contacts. The number of filopodia added, their orientation and distance extended, determine the extracellular volume sampled and thus, the potential for identifying and connecting to the best presynaptic axons. Once synapses are formed and tested, filopodia are stabilized to prevent retraction and can then extend further to create persistent branches. Indeed, rapid time-lapse imaging over periods of hours finds that dendritic filopodia can stabilize, elongate and transition into branches that support further filopodial extension. Since each dendritic branch in the mature arbor makes a significant contribution to neuronal and network function, high filopodial turnover and appropriate extracellular searching ensures each mature branch formed is optimal. In this manner, brain neuronal dendritic arbors grow through use-tested self-organization to create functional structures and circuits.

The molecular mechanisms mediating dendritogenesis are emerging and have provided further support for the synaptotropic model, since discrete components of synapse formation and maturation have now been linked to specific aspects of dynamic growth behavior. The first step in synapse formation involves interactions between cell adhesion molecules (CAMs) on the surface of axonal and dendritic processes.[10-12] Trans-synaptic CAM binding confers initial recognition of appropriate partners and creates adhesion junctions that provide structural stabilization. Subsequently, CAM binding initiates nascent synapse development by acting as nucleators to attract additional synaptic proteins to both pre- and postsynaptic sites. While CAMs have been extensively studied for their roles in synaptogenesis, little is known of their contribution to dendritic arbor growth. Recent in vivo two-photon rapid time-lapse imaging of growing neurons in *Xenopus laevis* tadpole optic tectum finds that the pre- and postsynaptic CAM ligand pair, neurexin (NRX) and neuroligin1 (NLG1), directly influence dendritic filopodial dynamic behavior with lasting effects on arbor structure.[5] Blocking trans-synaptic NRX-NLG1 interactions by applying extracellular soluble recombinant NRX (NRX-fc) prevents normal dendritic filopodial stabilization, while increasing NRX-NLG1 interactions by overexpression of NLG1 hyper-stabilizes filopodia. Trans-synaptic NRX-NLG interactions induce both pre- and post-synaptic differentiation through triggering bi-directional recruitment of synaptic proteins through intracellular C-terminal PDZ (PSD-95/Dlg/ZO1) binding motifs.[13] Expression of NLG1-ΔC, a NLG1 mutant lacking this PDZ domain and able to bind NRX but unable to recruit PSD-95, prevents filopodial stabilization. However, NLG1-ΔC does induce a transient increase in filolopodial lifetimes that is blocked by soluble NRX-fc, demonstrating that cell adhesion complexes transiently stabilize the membrane to limit process elimination, but do not contribute to cytoskeletol changes underlying filopodial motility needed to confer persistent stabilization.

Following formation of CAM adhesions, lasting structural stabilization requires synapse maturation. NRX-NLG1 interactions contribute further synapse formation by recruiting and assembling protein complexes, including receptors, scaffolding proteins and signalling molecules to contact sites.[14] Immature glutamatergic synapses may be "silent" synapses, characterized electrophysiologically by the presence of NMDA receptors (NMDARs), but not AMPA receptors (AMPARs). Maturation to 'AMPAfied' synapses is mediated by activation of NMDA receptors, which triggers downstream signalling cascades promoting AMPAR insertion into synapses.[15-17] Interestingly, it was found that overexpression of NLG1 is insufficient to stabilize dendritic filopodia when NMDARs are blocked pharmacologically. This demonstrates that although NRX-NLG1 complexes can cluster PSD-95, which binds to other cytoskeletal scaffold proteins, this interaction is insufficient to promote transition of motile filopodia into persistently stabilized dendritic branches, and that glutamatergic synaptic transmission is required.[5] These findings are supported by previous work showing inhibition of NMDARs or AMPARs decreases tectal neuron dendritic arbor growth.[18-21]

Interestingly, activity-dependent dendritogenesis appears to share molecular mechanisms with synapse plasticity underlying learning and memory in the mature brain. Activation of NMDARs allows calcium entry at postsynaptic sites, which in turn actives CaMKII, a critical component

for early-phase long-term potentiation (LTP) at mature synapses.[23-25] Blocking endogenous CaMKII activity either pharmacologically or by inhibitory peptides induces excessive dendritic arbor growth.[26] In addition, a recent discovered constitutively active version of PKC, protein kinase Mζ (PKMz), implicated in late-phase LTP,[27] also regulates developmental dendritogenesis.[4] Inhibiting PKMz activity by delivering a PKMz inhibitor peptide promotes dendrite outgrowth. Strikingly, overexpression of CaMKII or PKMz reduces thresholds for synapse maturation and decreases growth plasticity by inducing morphological hyper-stabilizing.[4,26] A relationship between mechanisms underlying long-term depression (LTD) in mature brain and developmental dendrite growth has also been established. Inhibition of calcineurin (CaN), a calcium/calmodulin-dependent serine/threonine phosphatase required for expression of NMDAR-dependent LTD, results in increased dendritic arbor complexity.[28]

Results from these studies have revealed the need to reinterpret the synaptotropic dendritogenesis model. A simple interpretation of the model predicts that manipulations that increase synapse formation and maturation would promote formation of larger dendritic arbors, while treatments that reduce synapse maturation would result in smaller arbors. Strikingly, multiple manipulations affecting different components of the molecular pathways underlying synaptogenesis find the opposite results. Thus, a modified version of the synaptotropic model has emerged in which graded levels of synaptic maturation produce corresponding levels of stabilization. In this model, upon initial contact CAMs provide activity-independent membrane-based cell-cell adhesions that provide sufficient tension to prevent filopodia from rapidly retracting, yet do not alter cytoskeletal motility. Subsequent CAM-initiated coalescence of synaptic proteins at nascent synapses, along with activity-dependent synapse formation, results in immature synapses sufficient to stabilize the filopodial cytoskeleton to prevent retraction, yet also allowing continued extension from the synaptic site. Activity-dependent synapse maturation mediated by CaMKII and PKMz produces strong, AMPAfied synapses that further stabilize dendritic structures, preventing further process elongation. Newly differentiated neurons lacking CaMKII demonstrate high dendritic process turnover, without branch stabilization and elongation, since activity-dependent synapse formation is restricted. Upon CaMKII expression, synapses can be formed to provide structural stabilization and elongation. In mature neurons, with strong glutamatergic synapses and large AMPA currents, further dendritic growth is inhibited produce the mature stable arbor morphology. This graded synaptotropic model explains the maturational progression of brain neuron dynamic growth behavior.

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