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Circuit Wiring: Neurite Speed Dating versus Stable Synaptic Matchmaking

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Understanding the mechanisms establishing the complex but precise pattern of connectivity characterizing neural circuits remains an immense challenge. In a recent issue of *Neuron*, Mao and colleagues (2018) provide new insights by showing that the activation kinetics of EphB2, a transmembrane receptor tyrosine kinase, control whether dendritic filopodia makes a synapse with candidate axons.

Over the past 30 years, the field of developmental neuroscience has gone from a mostly cellular description of the mechanisms patterning neuronal connectivity by pioneers such as Cajal or Sperry to the identification of some of the key molecules underlying the guidance of axons and dendrites, their local branching pattern (Sanes and Zipursky, 2010), and—even more recently—the molecular mechanisms underlying synaptic specificity (de Wit and Ghosh, 2016), i.e., the ability of specific subsets of axons and dendrites to form synapses in a cell-type-specific and/or subcellular-specific way (Sanes and Zipursky, 2010). The model that emerges from this impressive body of work suggests that the adult pattern of connectivity characterizing a given functional circuit emerges through a series of steps progressively "simplifying" the complexity of the wiring diagram. First, axons, and to some extent their target dendrites, are guided by specific cues present at intermediate guideposts and/or within their final target. Second, once they reach their target structure(s), which still represents a vast potential "postsynaptic space," local branching of axons significantly reduces the number of potential postsynaptic dendrites with which these axons can form synapses. However, it has become apparent that the mere proximity of axons and dendrites is not sufficient to explain the specificity of synaptic connections. A major step toward answering this problem came from the identification of a large number of synaptic adhesion molecules that can (1) serve as *trans*-synaptic bridges between the correct pre- and postsynaptic partners while (2) directly or indirectly recruiting pre- and postsynaptic organizing molecules such as neurotransmitter receptors and the neurotransmitter release machinery (de Wit and Ghosh, 2016).

However, our understanding of the molecular mechanisms allowing the key transition between axon and dendrite guidance/branching and synaptogenesis is still fragmentary. One key limitation here is to improve our ability to image and ultimately understand the molecular mechanisms differentiating unfruitful contacts between axon and dendrites and contacts that lead to the formation of functional synapses.

To tackle this issue, in a recent issue of *Neuron*, Mao and colleagues (2018) conducted technically challenging experiments to image the localization and activity of the kinase EphB2 at developing dendritic filopodia as they scan their environment for potential axonal partners. EphrinBs and their EphB receptor tyrosine kinases (RTKs), including EphB2, are multifaceted, bi-directionally signaling transmembrane proteins known to control not only axon guidance and local axon branching (Kania and Klein, 2016) but also dendritic filopodia motility and stabilization of nascent spines by *trans*-synaptic interactions with ephrinB ligands (Kayser et al., 2008). This *trans*-synaptic interaction leads to presynaptic differentiation (Kayser et al., 2006), and postsynaptically, EphB2 also binds to and regulates

the clustering of NMDA clustering (Nolt et al., 2011).

The authors started by showing that EphB2 localizes to dendritic filopodia in close apposition to presynaptic release sites, overlapping with the presence of ephrinB2. The finding that EphB2 is present at highly motile, as well as stable, filopodia prompted the authors to further investigate how EphB2 receptor activation might regulate the different behaviors of dendritic filopodia leading to either stable connections or retractions. One hypothesis was that the transition between transient neuritic contacts to successful synapse formation was not simply due to EphB-ephrinB interaction but was a result of differences in the temporal dynamics of dendritic EphB2 RTK activation. In order to investigate this, the authors developed a phosphorylation-sensitive fluorescent kinase sensor (GPhos) and showed that this tool is a reliable, internally normalized and specific ratiometric reporter of EphB RTK activation. The authors show that the GPhos-EphB sensor at the tips of dendritic filopodia reliably reports the activation of EphB2 by its axonal ligand ephrinB1. The authors observe that GPhosEphB signals persist at elevated levels only when apposed axonal ephrinB1 signals are stable but decline to baseline rapidly when ephrinB1 puncta continue to move along the axon. By characterizing the kinetics of the GPhosEphB signal in relation to dendritic filopodia behavior, the authors report that, whereas a fast rise and transient activation of EphB2 leads to retraction of a filopodium, a slow and persistent activation stabilizes an axo-dendritic contact and leads to synapse formation. In order to further prove that rapid and transient activation of EphB2 can induce filopodial retraction, the authors employ a photoactivatable EphB2 that can be activated in seconds in a ligand-independent manner. Indeed, photostimulation of EphB2-cryptochrome enhances filopodia retraction.

It will be interesting to further understand what causes the differences in EphB2 activation kinetics in a physiological context, where unknown regulators may impose spatial ephrinB1 dynamics along the axon or where the presence of dendritic signaling modulators could regulate the temporal dynamics of EphB2 signaling in dendritic filopodia. In this context, it has been shown that presynaptic sites form at predefined locations along the axon (Sabo et al., 2006), even in the absence of dendritic contacts, suggesting that successful transitions between transient contacts to stable synapses might happen preferentially at sites along the axon where the neurotransmitter release machinery is pre-assembled and easily recruited. Whether this is the case in all contexts is controversial. Historically, it was proposed that synapse formation is at least partially activity dependent. Most strikingly, in cortical and hippocampal pyramidal neurons, which form excitatory glutamatergic synapses almost exclusively on small filopodial protrusions called dendritic spines, artificial release of the neurotransmitter glutamate using two-photon uncaging is sufficient to induce rapid filiopodial protrusion and formation of a functional synapse (Kwon and Sabatini, 2011). However, recent experiments aimed at abrogating neurotransmitter release throughout the development of the mouse cortex *in vivo* show strikingly limited effects on dendritic spine formation and synaptogenesis, suggesting that excitatory synapses can initially form in the absence of presynaptic neurotransmitter vesicle fusion (Sando et al., 2017; Sigler et al., 2017). These surprising results strongly suggest that the initial steps of synapse formation might be mediated by matching adhesion molecules expressed pre- and postsynaptically (de Wit and Ghosh, 2016). It will be interesting to further understand the mechanisms by which synaptogenic molecules such as Neurexin-Neuroligins and LRRTM recruit, coordinate, and organize the presynaptic active zone and the postsynaptic specialization.

Importantly, recent technological advances such as CRISPR-Cas9 genomic editing methods allow us to directly tag endogenous proteins with fluorescent tags, which will help us overcome the current limitations of immunohistochemistry or potential overexpression artifacts by deciphering the subcellular dynamics of synaptic proteins at single-cell resolution *in vivo*. The possibility of studying the cellular and molecular logic orchestrating synapse formation and maturation at single-cell resolution in intact animal models *in vivo* will undoubtedly unravel new and fundamental principles of circuit wiring.

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