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Glutamate-Dependent Stabilization of Presynaptic Terminals

Dissecting the mechanisms underlying synapse formation and elimination is fundamental to understand how the nervous system is constructed and subsequently modified. Two studies by Tashiro et al. and by Hashimoto and Kano in this issue of *Neuron* provide new insights into the roles of neurotransmitter glutamate release in regulating the motility of hippocampal mossy fiber filopodia and synaptic competition among climbing fibers.

During the development of the nervous system, axonal growth cones migrate over long distances to make contact with their postsynaptic partners. In this initial phase of synaptogenesis, both pre- and postsynaptic structures are highly motile, presumably to facilitate this matching process. Many of these early-formed synaptic connections, however, are eventually eliminated in order to establish precisely connected neuronal circuitry. A large body of evidence indicates that neuronal activity is essential for regulating pre- and postsynaptic motility during synapse formation and elimination (Yuste and Bonhoeffer, 2001). Although neurotransmitters, as key mediators of neuronal activity, play important roles in neuronal outgrowth and plasticity (Lipton and Kater, 1989), the precise nature and time scale of their effects on the formation and refinement of synaptic connections remain unclear.

In this issue of *Neuron*, Tashiro et al. (2003) describe the role of synaptic activity in regulating the motility of hippocampal mossy fiber filopodia in brain slices. While previous studies have mainly focused on the formation and plasticity of postsynaptic spines, little is known about the behavior of presynaptic axonal terminals and the regulation of such behavior during synaptogenesis. By imaging over time green fluorescent protein-transfected hippocampal mossy fibers with two-photon microscopy, Tashiro et al. demonstrate that axonal filopodia, initially highly dynamic in young brain slices, become significantly less motile in more mature slices. Combining time-lapse fluorescence imaging, electron microscopy, and immunocytochemistry approaches, the authors further reveal that the stabilization of filopodia correlates with a decrease in the extracellular space and the formation of contacts with postsynaptic interneurons. Importantly, the regulation of filopodia motility is modulated by glutamate activation of kainate receptors in a bidirectional manner: low kainate concentration (1 μ M) in slices results in increased filopodia motility while high concentration (10 μ M) leads to filo-

podia stabilization. Furthermore, direct electrical stimulation of slices also leads to up- or downregulation of filopodia motility through kainate receptor-mediated mechanisms. As mossy fiber terminals contain kainate receptors, these results provide strong evidence suggesting that glutamate activation of these receptors on presynaptic terminals plays an important role in regulating filopodia motility.

This finding by Tashiro et al. of a bidirectional regulation of filopodia motility through kainate receptor activation provides interesting insights into the role of neuronal activity in the formation of neuronal circuitry. Their work suggests that the environmental milieu could alter the level of neuronal activity, thus influencing the effects of activity-dependent regulation of filopodia motility. For example, in young slices, the larger extracellular space could significantly dilute glutamate released from presynaptic terminals. These low glutamate concentrations would then increase filopodia motility, allowing filopodia to explore the surrounding environment and find their synaptic partners. As animals age and the extracellular space decreases, the resulting increase in extracellular glutamate concentration could then stabilize filopodia, allowing them to make long-term connections. In addition, various neurotransmitters have been shown to either up- or downregulate neurite outgrowth and spine dynamics in other systems (for review, see Lipton and Kater, 1989; Segal and Andersen, 2000). For example, the motility of axonal filopodia in hippocampal cultures is inhibited by glutamate through AMPA/Kainate receptors (Chang and De Camilli, 2001). Individual spines in hippocampal cultures can undergo either elongation or shrinkage, depending on the duration of glutamate application (Korkotian and Segal, 1999). These studies suggest that bidirectional regulation of filopodia motility via neurotransmitters could be a common mechanism in a variety of systems.

The mechanisms underlying the bidirectional regulation of filopodia motility remain unclear. Tashiro et al. showed that the increased filopodia motility is blocked by Ni^{2+} while the reduced motility is blocked by TTX and Pertussis toxin, suggesting that different regulatory mechanisms within mossy fiber filopodia underlie kainate receptor-dependent filopodia motility. However, signaling mechanisms are likely to be complex because CA3 pyramidal neurons postsynaptic to mossy terminals also contain kainate receptors, and thus receptor activation could regulate motility indirectly, e.g., by factors released from other cell types within the brain slices. Direct examination of filopodial response to glutamate in a simpler system such as dentate granule cell cultures could be helpful in addressing this issue. Finally, changes in intracellular calcium levels, which have been implicated in neurite outgrowth, growth cone guidance, and the dynamics of pre- and postsynaptic structures (Lipton and Kater, 1989; Gomez and Spitzer, 2000; Segal and Andersen, 2000), could serve as a common mechanism that underlies the diverse effects of neurotransmitters on pre- and postsynaptic motility.

As key mediators of neuronal activity at synapses, it is probably not surprising that neurotransmitters also play important roles in the subsequent strengthening and weakening of existing synapses. In another elegant study in this issue of *Neuron*, Hashimoto and Kano

(2003) reveal that differences in glutamate release probabilities at presynaptic sites dictate the outcome of competition among climbing fibers (CF) for the sole innervation of Purkinje cells (PC) in the cerebellum. Due to its relative simplicity, the CF to PC synapse has provided an excellent model system to study activity-dependent synapse elimination in the developing central nervous system. In this system, each PC is initially innervated by multiple CFs in early postnatal life. Over the course of several weeks, however, every PC is eventually innervated by only one CF as the other innervating inputs are eliminated. Hashimoto and Kano obtained detailed measurements in physiological properties of CF to PC synapses during this transition from multiple to single innervation. They found that initially (postnatal day 3), PCs are innervated by multiple CFs, each having approximately similar synaptic strengths. As development proceeds, the synaptic strength of one CF becomes stronger than the others, with the weaker inputs presumably becoming eliminated. Interestingly, Hashimoto and Kano observed that the peak glutamate concentration in the synaptic cleft for weaker CFs was significantly lower than that for stronger CF. Furthermore, they found that the number of release sites but not the release probability is responsible for the difference in the amount of glutamate release between competing CFs. Such a difference in glutamate release appears to precede synapse elimination of weaker CFs while resulting in the stabilization and growth of the stronger CF. Together with similar studies of synaptic competition between different motor axons at developing neuromuscular junctions (Colman et al., 1997; Kopp et al., 2000), this study by Hashimoto and Kano underscores the importance of neurotransmitter release in the elimination and stabilization of synaptic connections.

Kater and colleagues speculated in 1995 (Kater and Lipton, 1995) that a series of fundamental questions regarding the role of neurotransmitters in the development of the nervous system would have been posed and addressed by now. Indeed, studies in the past few years including the two in this issue of *Neuron* clearly demonstrate that neurotransmitter activity is crucial in regulating synaptic connectivity. With the advent of transgenic mice expressing fluorescent proteins in the cytoplasm of axons and dendrites and the increased use of two-photon microscopy, one can envision that future studies will address directly in intact animals the role of neurotransmitters in synaptic plasticity and maintenance.

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Cholinergic Modulation of Skill Learning and Plasticity

The basal forebrain cholinergic system strongly influences both cortical plasticity and learning. Directly relating these two roles has proven difficult. New results indicate that nucleus basalis lesions prevent motor cortex map plasticity and impair skill learning. These results strengthen the hypothesis that nucleus basalis gates neural plasticity necessary for instrumental learning.

Our brains are learning machines that adapt constantly to meet our changing needs. With enough practice and dedication, even difficult skills like playing violin or reading Braille can be learned at any age. Modern imaging has made it possible to actually see brain changes that accompany skill learning (Ungerleider et al., 2002). Although there is no longer any question that neural networks can change, it is not at all clear how neurons determine what specific changes they should make to contribute to the development of a new skill. Presumably, individual neurons lack any real understanding of behavioral goals. So how do they determine which of their thousands of synaptic connections to strengthen and which to weaken?

Practicing Braille, violin, and many other skills causes more neurons to respond to inputs from the skin that are heavily engaged by these activities (Elbert et al., 1995). Since repetition is important for both skill learning and neural plasticity, it is possible that neurons learn new skills simply by strengthening active inputs (Rioult-Pedotti et al., 1998). The downside of such a strategy is that it would optimize our brains for frequent tasks without regard for their relative importance. Therefore, some mechanism must exist to prevent frequent experiences and actions from crowding out important but less common experiences and actions (Plautz et al., 2000). While many systems contribute to the regulation of cortical plasticity, several intriguing features of the basal forebrain cholinergic system suggest it plays a particularly important role in guiding both learning and plasticity.

Nucleus basalis magnocellularis (NBM) neurons located in the basal forebrain are activated by both rewards and punishments, proportional to the intensity of