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Thinking Globally, Acting Locally: AMPA Receptor Turnover and Synaptic Strength

What determines the strength of a synapse, and how does activity modify that strength? Despite decades of research, these remain some of the most intensely debated problems in neuroscience. The difficulty in resolving these issues for local, synapse-specific forms of synaptic plasticity such as long-term potentiation (LTP) have arisen in part because the synapses that are modified are lost in a sea of unaltered synaptic connections. The recent identification of a strikingly different form of activity-dependent synaptic plasticity, where all of a neuron's synapses are scaled up or down together (Lissen et al., 1998; Turrigiano et al., 1998), has provided a highly tractable alternative for asking how activity modifies synaptic strengths. In a set of elegant experiments reported in this issue of *Neuron*, O'Brien et al. (1998) provide convincing evidence that global changes in excitatory synaptic strength occur through changes in the number of postsynaptic receptors clustered at the synapse, and that activity regulates the number of synaptic receptors by modifying the rate at which receptors are broken down. While these findings are unlikely to settle the debate over the site of change during LTP, they do provide an important insight into the factors that regulate postsynaptic strength and suggest a simple model for how activity could regulate the number of receptors at a synapse in both a local and a global manner.

Theoretical work suggests that two complementary mechanisms for regulating synaptic strengths during learning and development should coexist—mechanisms that locally change synaptic strengths in a synapse-specific manner, and mechanisms that globally regulate and stabilize the total synaptic strength of a neuron (Miller, 1996). While most work on synaptic plasticity has concentrated on synapse-specific mechanisms, a novel form of plasticity has recently been identified that globally scales all of a neuron's synaptic strengths up during periods of low activity, or down during periods of high activity (Lissen et al., 1998; Turrigiano et al., 1998). This synaptic scaling occurs through slow changes in the amplitude of AMPA-mediated miniature excitatory postsynaptic currents (mEPSCs). By stabilizing firing rates and regulating total synaptic strength, synaptic scaling can prevent the runaway synaptic potentiation produced by Hebbian learning rules and may allow neurons to remain optimally responsive to afferent input during development, when there are intense changes in synapse number and strength (Miller, 1996; Turrigiano, 1998).

But what is the site of expression of this form of synaptic plasticity? It has been suggested that synaptic scaling is due to changes in the number of AMPA receptors clustered at each synapse (Lissen et al., 1998; Turrigiano et al., 1998). O'Brien et al. (1998) now provide convincing evidence that this is so. Using quantitative immunohistochemistry to measure internal and surface expression of synaptic AMPA receptors, they find that activity-dependent changes in mEPSC amplitude are accompanied by changes in the accumulation of receptors at the synapse, and they demonstrate that the change in AMPA receptor number occurs through the activity-dependent regulation of receptor turnover. One of the most interesting observations of this paper is that when the intensity of the fluorescent signal arising from surface receptors at each synapse is quantified, there is a broad distribution of intensities that follows a rightward-skewed Gaussian distribution. This distribution resembles very closely the skewed Gaussian distribution of mEPSC amplitudes, suggesting that the number of AMPA receptors at a synapse is an important determinant of excitatory synaptic strength. A similar possibility was recently suggested for GABA receptors at inhibitory synapses (Nusser et al., 1997). These data raise the important but still elusive question of how different numbers of AMPA receptors are targeted to individual synapses and how this targeting is regulated (Craig, 1998).

A second key finding of O'Brien et al. (1998) is that long-term reductions in activity greatly increase the half-life of AMPA receptors. After 48 hr of reduced activity, the half-life is approximately doubled, and this is correlated with a shift in the entire distribution of synaptic AMPA receptor staining intensities toward larger values. While the total amount of AMPA receptor protein increases under these conditions, there is no change in the amount of mRNA encoding the receptor. Because there is ongoing turnover of surface AMPA receptors (Mammen et al., 1997), this change in the number of receptors at the synapse must arise either from an increase in the rate at which receptors are inserted into the membrane, or from a decrease in the rate at which they are removed (Figure 1). The correlation between increased receptor accumulation at all of a neuron's synapses and enhanced half-life of the receptor suggests two possible scenarios for how this change in accumulation occurs. The first possibility is that the increased stability of AMPA receptors generates a larger pool of receptors available for insertion into the membrane, thus globally increasing the insertion rate. The second possibility (illustrated in Figure 1) is that the removal rate of the receptors is globally decreased. This would increase receptor accumulation at the synapse and, by slowing removal, would tend to protect AMPA receptors from degradation and increase receptor half-life. While resolving this issue unequivocally will require separate measurements of insertion and removal rates, the latter interpretation is supported by the observation that the half-life of AMPA receptors increases as they begin to cluster at synapses (Mammen et al., 1997).

An important aspect of synaptic scaling in cortical neurons is that changes in synaptic strength occur multiplicatively (Turrigiano, 1998; Turrigiano et al., 1998). Decreased or increased activity scales synaptic strengths up or down by multiplying or dividing each synaptic strength by the same factor. This has the important

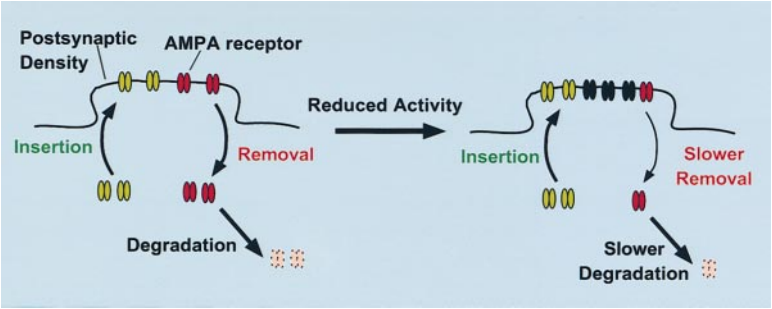

Figure 1. Activity Regulates AMPA Receptor Accumulation at the Synapse

consequence of preserving the relative differences between synapses, such as those produced by LTP or long-term depression (LTD), while allowing the neuron to adjust its total synaptic strength. The data of O'Brien et al. (1998) also support this model, because the change in the distribution of receptor intensities occurs without a change in the coefficient of variation (CV), which is consistent with a multiplicative, but not an additive, change in the distribution. While O'Brien et al. do not directly address the issue of multiplicative scaling, their data suggest a simple model for how such scaling could occur (Figure 2). In this model, the number of receptors at a given synapse is the result of a dynamic equilibrium between the insertion and removal rates of receptors. Imagine that the insertion rate at a synapse is 1 receptor per hour. Now imagine that once a receptor reaches the membrane, the probability that it will be removed is 10% in any given hour. Then this synapse (synapse 1) will grow in size until the removal rate equals the insertion rate, which will occur when the synapse has 10 receptors (that is, when 10% of the total number of receptors equals the number inserted per hour). If synapse 2 has an insertion rate of 2 receptors per hour, it will reach a steady-state size of 20 receptors, and if synapse 3 has an insertion rate of 0.5 receptors per hour, it will reach a steady-state size of 5 receptors. By locally varying the insertion rate across synapses, this model can reproduce the variability in the number of AMPA receptors at individual synapses observed by O'Brien et al.

Now consider what happens if we model the effects of reduced activity on AMPA receptor accumulation as a change in the probability of receptor removal, from 10% per hour to 5% per hour. Each synapse will double in size—in other words, the strength of each synapse will be multiplied by a factor of two. Conversely, if increased activity doubles the removal rate, the original strength of each synapse will be divided by a factor of two (Figure 2). It is worth noting that this scheme also works with a global insertion rate and a removal rate that varies across synapses. This model provides a simple and elegant means of scaling synaptic strengths without requiring that each synapse "keep track" of how many receptors it has. In addition, this model suggests that activity could regulate postsynaptic strength either globally or locally, by selectively targeting either a global variable (modeled as removal rate) or a synapse-specific variable (modeled as insertion rate).

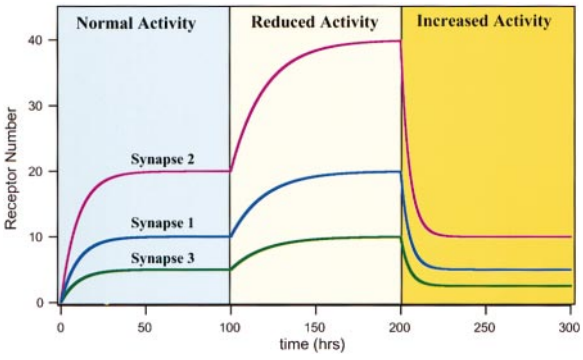

Figure 2. Multiplicative Scaling of the Number of Synaptic AMPA Receptors

Investigation into the function and mechanism of synaptic scaling is still in its infancy, and many of the details required to evaluate these ideas are unresolved. For example, is synaptic scaling really a global process? The data so far are most consistent with this interpretation, but because studies to date have varied the activity of a neuron and all its inputs together, this issue is still open. What are the relative roles of AMPA receptor activation and changes in firing rates in scaling synaptic strengths, and are NMDA receptors involved (Lissen et al., 1998) or unimportant (O'Brien et al., 1998; Turrigiano et al., 1998) in synaptic scaling? What is the signal linking changes in activity to the global scaling of synaptic strengths? In cortical neurons, synaptic scaling is mediated through the activity-dependent release of brain-derived neurotrophic factor (BDNF) (Rutherford et al., 1998), raising the question of how this neurotrophin influences AMPA receptor turnover. Finally, the present study raises the intriguing possibility that some forms of synapse-specific plasticity are expressed through local changes in the rates of receptor insertion or removal. By thinking globally, we may be poised to uncover the local changes that differentiate one synapse from another.

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Dissecting Semaphorin Signaling

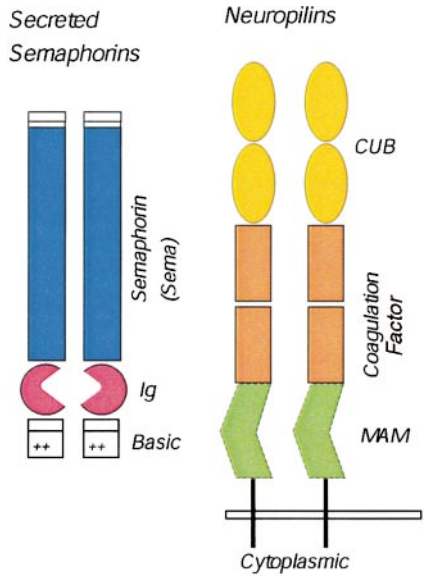
The precise wiring of neural architecture requires numerous signals for governing axon targeting. While chemotropic effects have been known for some time, only in the last decade has the importance of repulsive chemical cues become apparent. Several families of molecules have now been identified that are involved in inhibiting or repelling axon growth. Members of the Class III semaphorin family are secreted molecules that have been shown to act as repulsive factors for specific axonal populations. The first identified, SemaIII (also known as Collapsin-1 or SemaD), causes growth cone collapse and axonal retraction and repulsion in sensory and sympathetic axons in culture. The receptor for SemaD was identified last year as neuropilin-1, a transmembrane protein expressed in specific cell populations (see Kolodkin and Ginty, 1997). The importance of the Sema3/neuropilin-1 interaction for proper nervous system development in vivo was demonstrated by studies showing that both SemaD and neuropilin-1 mutations resulted in identical axonal projection defects (Kitsukawa et al., 1997; Taniguchi et al., 1997). Other semaphorins with repulsive activity in specific neuronal populations have also been identified. For instance, the related molecules Sema A, SemaE, and SemaIV repel sympathetic axons but have no effect on sensory axons. Three recent studies begin to clarify the mechanisms for the biological specificity of the semaphorins.

Takahashi et al. (1998) and Giger et al. (1998 [this issue of *Neuron*]) report that neuropilin-2, identified last year in a homology screen, is the functional receptor for SemaA and SemaE (Takahashi et al., 1998) and SemaIV (Giger et al., 1998). Giger et al. show that neuropilin-2 is present in postganglionic sympathetic neurons, neuronal populations that respond to SemaIV, and they present evidence that SemaIV and neuropilin-2 are present during development in specific complementary patterns. They also demonstrate that expression of neuropilin-2 is necessary and sufficient to produce a collapse response to SemaIV.

These studies begin to paint a picture in which repulsion of axons of specific neuronal subtypes is mediated by the interaction of specific semaphorin and neuropilin family members. However, we know that semaphorin/neuropilin binding alone is not sufficient for the repulsive activity. Neuropilin-1 binds SemaD, SemaA, SemaE, and SemaIV with high affinity, yet only SemaD leads to growth cone collapse in neuropilin-1-expressing neurons (Chen et al., 1997; Koppel et al., 1997; Giger et al., 1998). Two studies in this issue of *Neuron* begin to dissect the molecular basis of the specificity of semaphorin/neuropilin interactions.

Secreted class III semaphorins are known to have a conserved semaphorin domain, an Ig domain, and a basic tail (Chen et al., 1998; Kolodkin and Ginty, 1997). The semaphorin domain has been shown to be responsible for the binding specificities of the semaphorins in situ (Feiner et al., 1997), while the Ig-basic domain also exhibits binding properties. The neuropilins have two N-terminal domains similar to complement binding domains, called CUB domains; two coagulation factor domains; a C-terminal MAM domain; a transmembrane domain; and a short cytoplasmic tail (see figure). Both Giger et al. (1998) and Nakamura et al. (1998) made constructs lacking specific portions of the semaphorin and neuropilin proteins. Ligands had an alkaline phosphatase moiety fused to portions of chick SemaD, or human or rat SemaIII. Neuropilin-1 receptor constructs were made lacking a, b, or c domains, singly and in combination.

Two types of assays were used to determine the specificity of the ligand/receptor interactions. Physical interactions were determined through binding studies performed when these molecules were expressed in COS cells, while the functional specificity of responses was assayed by monitoring growth cone collapse in neurons expressing the receptor constructs. Binding studies clearly demonstrated that the CUB domain is necessary for physical binding of the sema domain (Giger et al., 1998; Nakamura et al., 1998). Strittmatter and colleagues (Nakamura et al., 1998) also tested whether the CUB domain alone was sufficient to confer binding specificity



Semaphorin and Neuropilin Domains