Neurons are able to restore their activity when challenged by external or internal perturbations. This type of homeostatic plasticity is important in the maintenance of neuronal or network stability during development and normal brain functioning. Homeostatic regulation is a negative feedback response that returns the system from a shifted position to an intrinsic physiological "set point," usually indicated as a stable firing rate of action potentials. Homeostatic plasticity can be achieved through adjustments in the strength of synaptic inputs, neuronal excitability, neuronal connectivity, or the balance between excitation and inhibition. Among these possibilities, regulation of synaptic strength is believed to be the primary cellular mechanism, which is also known as homeostatic synaptic plasticity.1-7

In the brain, most of the excitatory synaptic transmission is mediated by glutamate and its receptors including AMPA receptors (AMPAR) and NMDA receptors (NMDAR). Given the dynamic behavior of glutamate receptors, mainly AMPARs, receptor numbers at synaptic sites can be altered by means of receptor trafficking in response to neuronal activity or intracellular signaling, resulting in synaptic plasticity such as long-term potentiation (LTP) and depression (LTD).8-13 Similarly, homeostatic synaptic plasticity is expressed via the regulation of AMPAR synaptic accumulation. During homeostatic regulation, AMPAR numbers at synapses are up- or downwardly regulated in response to activity deprivation or overexcitation, respectively. When cultured cortical neurons are incubated with tetrodotoxin (TTX) to chronically silence network activity, the synapse responds in a compensatory manner, resulting in an increase in synaptic AMPAR accumulation and the strength of synaptic transmission.14-20 Conversely, an increase in neural network activity results in reduced accumulation of synaptic AMPARs.

Most experimental evidence for homeostatic plasticity comes from studies in which activity of the entire neuronal network is chronically altered. Global homeostatic plasticity leads to an overall up- or downregulation of the entire synapse population, presumably to a level proportional to the original strength of each synapse, i.e., synaptic scaling.2 In addition to global regulation, studies have revealed that homeostatic plasticity can also occur at the level of a single neuron or at subcellular domains, such as dendrite branches or a group of local synapses.21-23 An important question is whether homeostatic regulation also applies to individual single synapses.

To this end, we have adopted paradigms in cultured neurons to selectively alter activities of single synapses to study homosynaptic homeostatic response. We find that when activity at a single synapse is continuously suppressed by abolishing presynaptic firing, more AMPARs are accumulated at the postsynaptic site, indicating an attempt by the inhibited synapse to restore its activity.24 This homosynaptic regulation is highly selective, because receptor amounts at the neighboring synapses remain unchanged. Also, we find that the presynaptic suppression-induced homosynaptic recruitment of AMPARs is dependent on the activity of GluA2-lacking AMPARs, presumably via calcium cascades, and PI3-kinase (PI3K) signaling.24

To selectively enhance the activity of a single synapse, we transfected an engineered light-activatable glutamate receptor GluR6

Keywords: Homeostatic synaptic plasticity, scaling, glutamate receptor, AMPA receptor, trafficking, synaptic transmission, single synapse

Neurons are able to restore their activity to a set-point level when challenged by external or internal perturbations. This type of homeostatic plasticity is important in the maintenance of neuronal or network stability during development and normal brain functioning. One of the major cellular events underlying the expression of homeostatic regulation is the alteration of glutamatergic AMPA receptor (AMPAR) accumulation and thus, synaptic strength. Traditional global homeostatic plasticity is believed to adjust the input strength of all synapses. Since each individual synapse receives different input with varied levels of activity and distinct history of synaptic plasticity, an input-specific homeostatic regulation is necessary to restrain synaptic activity within a physiological range. Our studies suggest that at the single synapse level, homeostatic plasticity is expressed via input-specific alterations of AMPAR amounts. This homosynaptic homeostatic regulation is expected to play an important role in preventing the deleterious situations imposed by Hebbian plasticity to secure long-term synaptic stability.

Input-specific homeostatic regulation of AMPA receptor accumulation at central synapses

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Keywords: Homeostatic synaptic plasticity, scaling, glutamate receptor, AMPA receptor, trafficking, synaptic transmission, single synapse
(LiGluR) into cultured hippocampal neurons. The LiGluR system is based on the photoisomerization of a tethered agonist, maleimide-azobenzene-glutamate (MAG), between its trans and cis configuration. Upon UV (UV, 380 nm) light exposure, a switch from trans to cis mode brings MAG to the agonist binding site on LiGluR and causes receptor channel opening, leading to membrane depolarization and neuronal firing of action potentials.

The LiGluR neuron and the synaptic maker protein synapsin-YFP, activity was induced by UV exposure and AMPAR amount at the activated synapse was reduced.

Cell-surface AMPARs are known to be dynamic, constantly exchanging with intracellular pools via receptor endocytosis and membrane insertion. We find that UV exposure causes enhanced AMPAR endocytosis at activated individual synapses, suggesting that the removal of surface receptors results from receptor internalization. However, if the internalized receptors remain intact in the spine, the total synaptic receptors should remain unaltered. The observed UV stimulation-induced reduction in total receptor amount could be a consequence of either receptor diffusion out of the spine, or removal in situ via protein degradation. To evaluate outward diffusion of AMPARs after internalization, we examined changes in receptor immunointensity at the activated synaptic spine. AMPAR subunit GluA1 was immunostained following UV stimulation, and immunointensity was plotted along the spine at LiGluR sites (Fig. 2A-C). The plot shows a typical dendritic base, spine neck and head pattern, with the highest signals at the spine head region. If AMPARs diffuse out of the spine, GluA1 intensity at the synapse neck should be higher at activated LiGluR synapses. However, against the possibility of receptor outward diffusion, GluA1 relative intensity at the spine neck of UV-treated LiGluR sites shows no difference from control LiGluR synapses treated only by blue light (Fig. 2D). Further, to confirm the sensitivity of receptor trafficking through the spine neck, we transfected neurons with DsRed as a structural marker and GFP-GluA1, and performed live imaging prior to and immediately after the induction of chemical LTP by glycine application. Indeed, we observed a significant increase in GFP-GluA1 intensity at the spine head as well as the spine neck, indicating a rapid translocation of AMPARs into the spine (data not shown). Next, we examined GluA1 intensity in the dendritic shaft flanking the LiGluR synaptic spine. If receptors diffuse out of the spine, a gradient in GluA1 distribution should exist. We measured the base of the spine, as well as two sites 0.5 μm and 1.5 μm away on each side of the spine. Consistent with the line plot measurement, no significant difference was detected among the locations adjacent to the activated spine (Fig. 2E and F). These results indicate that receptor diffusion is likely not responsible for AMPAR removal from the activated synapse. Also, we find that local protein synthesis is not responsible for AMPAR reduction because pretreatment with the protein synthesis inhibitor anisomycin does block UV-induced homeostatic downregulation.

In contrast, UV-induced total GluA1 reduction is prevented by the proteasome inhibitor MG-132, but not the lysosome inhibitor chloroquine, indicating proteasome-mediated degradation. The input-specific AMPAR reduction by UV activation can also be observed at dendritic branches that are physically isolated from the soma, strongly indicating that receptor degradation occurs locally in the spine. For proteosomal digestion, target proteins need to be modified by ubiquitination. This is a likely scenario given that AMPARs and other synaptic proteins are subject to ubiquitination. In agreement with this, we find that at UV-activated spines, levels of ubiquitinated proteins and the AMPAR E3 ligase Nedd4 are increased compared with surrounding normal spines. Our findings suggest a process in which synaptic activation causes AMPAR ubiquitination, followed by internalization and local degradation by the proteasomal pathway.

The synaptic activation-induced homeostatic AMPAR reduction reveals similar features with NMDAR-dependent LTD, which is also input-specific and dependent on AMPAR internalization. However, we find that the underlying signaling cascades

![Figure 1. Illustration of UV-induced activation of a single synapse. (A) In a neuron expressing LiGluR, a brief UV exposure (1 sec, arrow) triggered rapid depolarization and high-frequency firing of action potentials, which was terminated by a brief blue light (0.3 sec) stimulation. (B) In a neuron expressing both LiGluR and a synaptic marker protein synapsin-YFP, activity was induced by UV exposure and AMPAR amount at the activated synapse was reduced.](Image)
make adequate adjustments so as to restrain their activity within a normal physiological range. Thus, input-specific homeostatic responses at individual synapses, acting independently or coupled to global homeostatic regulation, function to restore synapse function, allowing efficient flow and storage of information in the nervous system.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 2. Examination of AMPAR diffusion into the dendritic shaft. Following GluA1 immunostaining in UV-treated neurons, LIGluR synapses were selected as indicated by synapsin-YFP (arrows) for line plotting (A and B). GluA1 intensity across the spine and base dendritic region was plotted (C). GluA1 intensity ratio of spine neck vs. base dendrite showed no difference between control and UV treated samples (D). (E and F) GluA1 intensity was measured at different dendritic sites flanking the activated spine. For each set of measurements, values were normalized to the mean of the two end sites (sites A and E). Control was from neurons transfected with the same constructs (LIGluR and syn-YFP), but treated with blue light only.
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