such structural adaptations are involved in mediating behavioral long-term adaptation processes in the motor-system of flies. Given the great possibilities for cell type–specific genetic intervention, coupled with high microscopic and physiological access, the *Drosophila* neuromuscular junction is a prime system for studying such scenarios.

Koon *et al.*[1] also show that genetic suppression of activity in the octopamine-secreting neurons leads to severe undergrowth or even complete elimination of type-II arbors. Such a severe effect of activity deprivation on neuronal structure is rather unusual, as it was thought that neurons devoid of all synaptic transmission can still form basic terminals[9,10]. A closer examination of the activity dependence of the development, stability and plasticity of aminergic terminals in other systems is also an attractive avenue for future research.

Finally, Koon and colleagues[1] demonstrate a fundamental similarity between the pathways mediating hunger-induced octopamine-derived synaptic plasticity and learning and memory processes. Following octopamine release upon starvation, cyclic AMP levels are increased, which leads to the activation of the transcription factor CREB and, subsequently, to transcription and translation of CREB-regulated genes (**Fig. 1**). Accordingly, altering cyclic AMP levels acutely or chronically in each case leads to changes in the numbers of synaptopods. Notably, both octopamine signaling and the cAMP/CREB pathway are engaged during the formation of associative memories[11]. Thus, similar molecular plasticity mechanisms seem to be at work in two superficially very different behavioral scenarios: 'noble' learning and 'ordinary' crawling. But maybe these behaviors are not as different as they might appear—octopamine secretion could mediate food-oriented, appetitive locomotion behavior in general. In olfactory conditioning of *Drosophila*, activation of octopaminergic neurons is sufficient as a replacement for reward in classical conditioning[12]. Such classical conditioning involves the pairing of an odor with a reward. In honeybees, aminergic neurons are activated by the reward and not by the odor before conditioning. But after conditioning, learned odors directly activate octopaminergic neurons in the central brain[13]. As odors learned in this way induce learned appetitive search[14], a common denominator of octopamine function might be the organization of appetitive search behaviors. From the perspective of the motor system, it would not matter whether food search was originally induced by starvation, by an unexpected reward or by reward-predicting odors. Clearly, this hypothesis needs to be taken with a grain of salt, as the exact contributions of different octopaminergic neurons are incompletely understood. For example, aggression, another arousal state associated with intense locomotion, is triggered by a small group of octopamine-positive neurons in the fly brain[15].

In most experiments, Koon *et al.*[1] modified the complete set of octopaminergic neurons—the ones in the central brain in addition to peripheral motor neurons. Therefore, adaptations of crawling speed could, to some degree, also be explained by altered octopaminergic signaling in the CNS. The specific contributions of different neuronal populations to the observed phenotypes should be addressed in future studies—for example, by using genetic mosaic techniques.

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Ubiquitin regulation of neuronal excitability

Sriharsha Kantamneni, Kevin A Wilkinson & Jeremy M Henley

Demonstrating the common mechanism of proteasome-dependent degradation of ion channels, two studies in this issue of *Nature Neuroscience* show that ubiquitin-dependent protein degradation can modulate neuronal excitability.

Protein modification by ubiquitin and subsequent proteasomal degradation has emerged as a key regulator of neuronal activity[1]. However, many of the specific proteins targeted and the underlying molecular mechanisms involved in this process have not yet been determined. Two papers in this issue of *Nature Neuroscience* report direct ubiquitination-mediated degradation of two types of ion channels critical for neuronal function, namely the L-type voltage-gated calcium channel subunit Cav1.2 and the AMPA receptor (AMPAR) subunit GluR1 (**Fig. 1**). These papers provide important new insights into how neuronal excitability is modulated in different cellular compartments by different ubiquitin ligases and highlight the crucial roles of ubiquitination and proteasomal degradation at different stages of the biosynthetic pathway.

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For L-type calcium channels, Altier *et al.*[2] demonstrate that the accessory subunit Cavβ is necessary for 'quality control' during Cav1.2 channel biosynthesis. In the absence of Cavβ, Cav1.2 channels are ubiquitinated, targeted to the endoplasmic reticulum (ER)-associated protein degradation (ERAD) complex and degraded at the proteasome. For AMPARs, Fu *et al.*[3] describe how chronic elevation of synaptic activity increases ephrin signaling, which reduces synaptic strength by invoking direct ubiquitination and proteasomal degradation of the GluR1 subunit of functional AMPARs. These findings increase understanding of the specific mechanisms that determine the trafficking and stability of two proteins essential for regulating neuronal excitability. In the wider context, they provide important new examples of how ubiquitination and proteasomal degradation can regulate proteins in different cell compartments at different stages of their assembly and maturation to control neuronal excitability and synaptic transmission.

L-type calcium channels comprise a pore-forming α-subunit (for example, Cav1.2),

a membrane-anchored α2δ-subunit and a cytosolic β-subunit. Although it is well established that the α2δ and β subunits act synergistically to promote surface expression, the underlying mechanisms have not been defined. Now Altier *et al.*[2] show that expression of Cav1.2 with Cavβ leads to an increase in both total and surface expression of Cav1.2. In the absence of Cavβ there is less Cav1.2, and what remains is almost exclusively confined to the ER. Thus, Cavβ seems to facilitate ER export and either enhances translation or protects Cav1.2 from degradation.

ERAD is a quality control mechanism in which misfolded membrane proteins are translocated from the ER membrane into the cytosol, ubiquitinated by an ER-resident ubiquitin ligase and degraded by the proteasome[4]. Here Altier *et al.*[2] show that Cav1.2 is ubiquitinated by the ER-associated ubiquitin ligase RFP2 but in the presence of Cavβ ubiquitination is reduced. In addition, the ERAD proteins Derlin-1 and p97 coimmunoprecipitate with Cav1.2, and this association is also decreased by Cavβ. Thus, it seems that in the absence of Cavβ the Cav1.2 channel is recognized as a misfolded protein, undergoes RFP2-mediated ubiquitination and is targeted for proteasomal degradation by ERAD. Therefore, the Cavβ subunit is a critical determinant of channel stability and function because it allows Cav1.2 to bypass ERAD and promotes exit from the ER. These findings demonstrate that the interplay between Cavβ and the ubiquitin ligase RFP2 defines a trafficking checkpoint to ensure that only correctly assembled Cav1.2 channels reach the surface. Furthermore, several calcium channel subtypes contain Cavβ subunits, potentially implicating this control mechanism as a general determinant of calcium channel trafficking and stability.

Fu *et al.* show that AMPAR degradation is controlled by direct ubiquitination of the GluR1 subunit[3]. Synaptic scaling is the process by which neurons adjust their excitability to compensate for changes in network activity by regulating synaptic AMPARs[5]. One mechanism for reducing synaptic responsiveness upon prolonged elevation of activity involves direct ubiquitination and proteasome-dependent degradation of the AMPAR subunit GluR1 by means of a pathway dependent on the ephrin receptor EphA4 (ref. 3). Eph receptors are receptor tyrosine kinases activated by membrane-bound ephrin ligand proteins expressed on the surface of adjacent cells, allowing the initiation of bidirectional signaling cascades[6]. Although ubiquitination of AMPAR-associated proteins has been reported previously[7,8], the study by Fu and colleagues shows for the first time that direct ubiquitination of GluR1 leads to its proteasomal degradation[3].

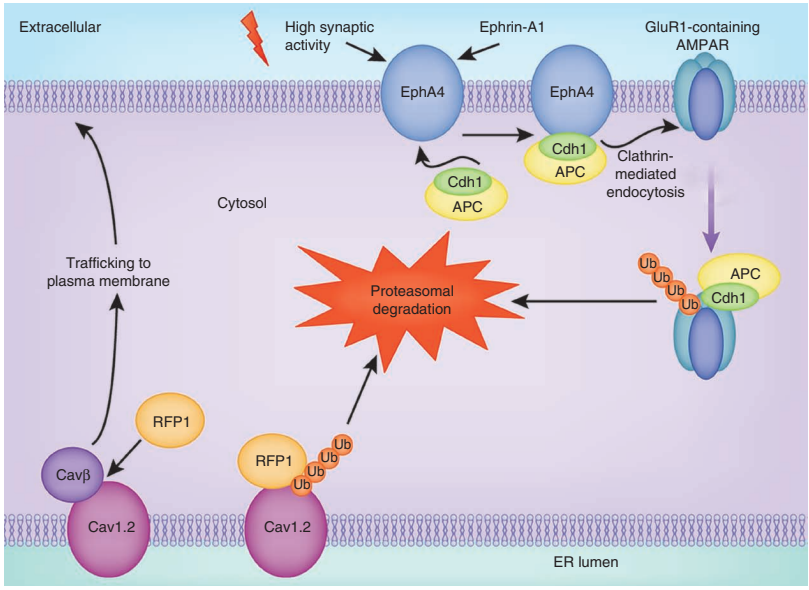

Figure 1 Ubiquitin controls neuronal excitability and synaptic transmission by regulating ion channel stability and trafficking. For AMPARs (top), chronically elevated synaptic activity or direct stimulation with ephrin-A1 activates EphA4 receptors. This leads to recruitment of the Cdh1 component of the multiprotein ubiquitin ligase anaphase-promoting complex (APC) that, in turn, binds to and ubiquitinates the GluR1 subunit of AMPARs. These ubiquitinated AMPARs are targeted for degradation in the proteasome, providing a scaling mechanism to downregulate synaptic responsiveness during prolonged periods of synaptic activity. For L-type calcium channels (bottom), the Cav1.2 pore-forming subunit must assemble with the cytosolic β-subunit for correct surface expression and protein stability. In the absence of Cavβ, Cav1.2 binds to and is ubiquitinated by the ER-associated ubiquitin ligase RFP2 and is targeted for proteasomal degradation by ERAD.

Fu *et al.*[3] show that ephrin-A1 activation of one of its cognate receptors, EphA4, causes a reduction in AMPAR miniature excitatory postsynaptic current frequency and amplitude, indicating fewer functional synapses and fewer AMPAR at individual synapses, respectively. Consistent with this, a reduction in total and surface GluR1 and a reduction in spine number in response to ephrin-A1 were observed in corresponding biochemical experiments. To define the physiological relevance of this ephrin signaling, Fu *et al.*[3] induced synaptic scaling by prolonged incubation of cortical neurons with the $GABA_A$ antagonist bicuculline, which enhances network activity. As expected, this elevated synaptic activity activated the EphA4 receptor and reduced total expression of GluR1 in wild-type neurons. In *Epha4*$^{-/-}$ neurons, bicuculline had no effect on GluR1 levels. These findings suggest that chronic elevation of synaptic activity results in the activation of ephrin-EphA4 signaling, which, in turn, downregulates AMPAR expression, resulting in homeostatic scaling.

The EphA4-dependent downregulation of GluR1 requires both clathrin-mediated endocytosis and proteasomal activity. To investigate the molecular mechanisms underlying this effect, Fu *et al.*[3] performed yeast two-hybrid screens for EphA4 interactors and isolated Cdh1, a component of the multiprotein ubiquitin ligase anaphase-promoting complex (APC). Of key importance, ephrin-A1 increases the interaction between APC and EphA4 in neurons, indicating that APC is recruited to EphA4 in a ligand-dependent manner. Furthermore, in heterologous cells, APC can bind to and ubiquitinate GluR1, decreasing both its surface and total expression. Consistent with this, knockdown of Cdh1 in neurons prevents both the ephrin-A1– and bicuculline-induced decrease in GluR1. Overall these results demonstrate that APC-mediated ubiquitination of GluR1 represents a physiologically important regulator of synaptic strength.

Although the actual lysine(s) that are ubiquitinated GluR1 remain to be defined, a surprising observation was that the site of APC binding to GluR1 is located in the extracellular N-terminal region of GluR1. Although the authors do not provide direct evidence, they speculate that the extracellular N-terminal region of GluR1 interacts with components of the APC ubiquitin ligase complex after retrotranslocation into cytoplasm. Indeed, this retrotranslocation event seems mechanistically analogous to the Cav1.2 translocation during ERAD reported by Altier *et al.*[2], but how this occurs for GluR1 is unclear.

Both papers present findings that significantly advance understanding of the roles of

ubiquitin in controlling membrane protein stability and trafficking events that, in turn, regulate neuronal excitability and synaptic transmission. As always, however, these new findings raise further questions. Most immediate among these is identification of the specific lysine residues that are ubiquitinated in both Cav1.2 and GluR1 so that nonubiquitinatable mutants can be made and tested. Further work is also required to define exactly how Cavβ prevents the ERAD machinery recognizing Cav1.2. Possible mechanisms include steric hindrance and/or a role in folding of Cav1.2. For GluR1, what are the mechanisms for retrotranslocation, as this apparently occurs before ubiquitination, and how does EphA4-mediated recruitment of APC lead it to ubiquitinate GluR1?

Protein ubiquitination is now recognized as a key regulator of neuronal responsiveness. Clearly, these new studies raise questions concerning roles for ubiquitination in the regulated trafficking and stability of other proteins. Are equivalent processes required for other members of the voltage-gated ion channel and ligand-gated ion channel superfamilies, and more generally for other neuronal membrane proteins? For example, given the role of ERAD in the quality control of Cav1.2, it certainly seems plausible that an analogous mechanism could regulate AMPAR surface expression, possibly through one or more of the AMPAR accessory subunits or binding proteins such as TARPs[9]. Similarly, as shown for GluR1, ubiquitin may regulate the degradation of surface-expressed Cav1.2 channels. Much remains to be discovered, but it is already evident that ubiquitin and ubiquitin-like post-translational modification of neuronal proteins represents a crucial and, as yet, poorly understood regulatory system. The findings presented in this issue by Altier *et al.*[2] and Fu *et al.*[3] provide further impetus to this exciting and expanding area of research.

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Integration and autonomy in axons

Barry W Connors & Omar J Ahmed

A new study shows that even under normal conditions repetitive spiking in some cortical interneurons can trigger spontaneous spiking that originates from distal axons and lasts for tens of seconds.

Axons are exquisitely adapted for communicating neural signals rapidly and efficiently over long distances. Most neurons in the vertebrate brain send action potentials from the axon initial segment toward distal axon terminals, propagating orthodromically (literally "racing in the right direction"). It is the nature of axons, however, that spikes can propagate about equally well in both directions. This is easily demonstrated in the lab by stimulating distal axons and triggering antidromic propagation. But perhaps the bidirectionality of axonal conduction is actually a bug, not a feature. Antidromic spikes arise spontaneously from distal ectopic sites during seizures, nerve injury and inflammation[1]. Ectopic spiking may distribute and enhance paroxysms and pain. A provocative possibility is that distal axons also initiate spikes under normal circumstances, but support for this has been sparse. An article by Sheffield *et al.*[2] in this issue provides new evidence that such ectopic spiking occurs in the normal cerebral cortex.

The experiments of Sheffield *et al.*[2] were relatively simple, but the results are remarkable. They studied brain slices from transgenic mice *in vitro* and recorded from a subtype of hippocampal interneuron identified by its expression of green fluorescent protein (GFP) under the *Htr5b* promoter. The behavior of these cells was unexceptional until the authors had triggered hundreds of action potentials (on average, 800) with current injections. At that point, about 80% of the cells commenced firing spontaneously and persistently for tens of seconds, hitting maximum spike rates of about 50 Hz on average (**Fig. 1**). Neither induction nor maintenance of persistent firing required fast glutamate or GABA receptors, extracellular Ca^{2+} or particular patterns or frequencies of spikes. Various other interneuron types from the hippocampus and neocortex of mice and rats could also generate persistent firing, but firing was most common and robust in the Htr5b-GFP hippocampal interneurons.

The phenomenon of induced persistent firing prompts two initial questions: what is the nature of the process that senses and integrates spikes during the induction phase, and where and how are spontaneous spikes generated? Sheffield *et al.*[2] showed that the interneuron must integrate a few hundred spikes over a minute or two to trigger spontaneous spiking. They also demonstrated that some part of the distal axon itself is doing this integration—direct stimulation of distal axons induced persistent firing, even when full spikes were prevented in the soma. Distal regions of the axons also seemed to be the source of autonomous spikes (**Fig. 1**). As viewed from the soma, the voltage trajectory of spontaneous spikes rose sharply from resting potential, and thresholds were far below those of somatically triggered spikes. Small spikelets were sometimes observed, suggesting that axonal spikes had not invaded the soma completely. Computational models of interneurons could reproduce variably sized somatic spikes and spikelets when axonal spikes failed to propagate past branch points or the proximal axon[3]. The authors' experiments did not reveal the precise origin of spontaneous spikes, but it was almost certainly far distal from the axon initial segment.

Perhaps the most extraordinary result of this study is that evoked action potentials in one interneuron could sometimes induce persistent firing in a neighboring interneuron (**Fig. 1**). This occurred in only 3 of 19 tested pairs, but taken at face value it raises a third important question: how can spiking in one interneuron induce autonomous spiking in a neighboring interneuron? The authors assumed the interneuron pairs interacted through their distal axons because somatic recordings did not reveal chemical or electrical synaptic connections; but what is the signal?

Is autonomous axonal spiking common? Spontaneous ectopic spiking has been observed

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