As one is probing deeper into the mechanistic details of voltage gating, it was important to confirm that this structural feature captured in the crystal environment is in accord with observations based on functional channels in membrane bilayers. The modular nature of the voltage sensor and pore domains is also consistent with the functional chimeras engineered by substituting the pore domain of the KcsA channel into the voltage-gated Shaker channel (Lu et al., 2001) and naturally compatible with the allosteric model of channel gating developed by Aldrich and coworkers (Ledwell and Aldrich, 1999). The discovery of voltage sensors with high sequence similarity to the S1–S4 helices in two unrelated membrane-associated proteins lacking any channel-like central pore domain leaves no doubts about its recurrent and modular nature (Murata et al., 2005; Ramsey et al., 2006; Sasaki et al., 2006).

Once the concept of relatively independent voltage-sensing and pore domains is established, it begs the question of how and where they are coupled. In an extreme view, one might hypothesize that each S1–S4 helical bundle constitutes a complete functional voltage-sensing unit, able to work on its own in the membrane. In other words, the voltage-sensing domains float like "buoys" in the membrane and only need to be loosely attached to the central pore to confer voltage-gating properties. The careful study by Soler-Llavina et al. reveals that the coupling between the voltage sensor and the pore domain is, in fact, more complex than suggested by this naive view. They identified two regions where clusters of mutations display very different functional phenotypes. Mutations near the extracellular end of S5 affect mainly the $R \rightarrow A$ transition by making the activated state more unfavorable. Mutations toward the intracellular end of S5 seem to disrupt the coupling between the voltage sensors and the gate to the pore. On the one hand, these mutations make the transition $R \rightarrow A$ easier, but on the other hand, they make the concerted transition $C \rightarrow O$ much more difficult. By inspection of the $K_v 1.2$ structure, those mutations are in physical proximity to the so-called ILT mutations in the S4 helix that are known to have a pronounced effect on the concerted transition $C \rightarrow O$ initially discovered by Aldrich and coworkers (Ledwell and Aldrich, 1999; Smith-Maxwell et al., 1998a, 1998b).

According to the $K_v 1.2$ structure, the S4–S5 linker makes strong van der Waals contacts with the S6 helix forming the pore gate (Long et al., 2005b), a feature that was previously found to be essential for functional channels from the engineered Shaker-KcsA chimeras (Lu et al., 2001). Further examination also shows that 10 out of the 20 residues forming the transmembrane part of S4 are positioned within 4 Å of the S5 helix from the adjacent subunit. Thus, the idea that the voltage sensor lacks extensive interactions with the pore domain, while generally correct, needs to be carefully qualified in trying to dissect the coupling mechanism. The results of Soler-Llavina et al. indicate that the interactions between one face of the S4 helix with the S5 helix of the adjacent subunit most likely underlie the concerted transition leading to the channel opening.

These findings advance our fundamental understanding of the gating mechanism in K_v channels and also raise numerous questions about the voltage-sensing modules in the phosphatase (Murata et al., 2005) and the proton pore (Ramsey et al., 2006; Sasaki et al., 2006) with those of K_v channels. For example, does the modular unit formed by a single S1–S4 anticlockwise helical bundle have the ability to function as a voltage-sensing electromechanical "device" on its own? What molecular interactions are responsible for the transduction of the voltage-sensing signal to another protein? What aspect of those interactions might be conserved across different systems?

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Synaptic Homeostasis on the Fast Track

Synaptic homeostasis is a phenomenon that prevents the nervous system from descending into chaos. In this issue of *Neuron*, Frank et al. overturn the notion that synaptic homeostasis at *Drosophila* NMJs is a slow developmental process. They report that postsynaptic changes are offset within minutes by a homeostatic increase in neurotransmitter release that requires the presynaptic Ca^{2+} channel *Cacophony*.

Synaptic homeostasis maintains postsynaptic excitability within a narrow range by regulating synaptic strength. This stabilizes neuronal circuits and ensures the fidelity of communication within complex networks that require continuous remodeling to accommodate activity—and/or growth-dependent changes. Although synaptic homeostasis is acknowledged as an essential phenomenon of the nervous system, its mechanisms remain enigmatic.

Synaptic homeostasis can be distinguished from cellular homeostasis since it requires two-way communication between the pre- and postsynaptic cell to adjust synaptic performance. Like other forms of homeostasis, synaptic homeostasis requires the support of a closed feedback loop (Figure 1A). Since information from both sides of the synapse needs to be integrated, the loop requires signaling pathways that encompass both sides (Figure 1B). A homeostatic change involves a change on one side of the synapse that is offset by a change on the opposite side. If a retrograde signal is not required, the change may be a cell-autonomous response and may represent cellular homeostasis rather than synaptic homeostasis (Figure 1C). However, synaptic homeostasis is not seen as being independent of cellular homeostasis. Instead, *trans*-synaptic signaling pathways may recruit and/or interact with cellular homeostatic mechanisms to maintain excitability by regulating synaptic performance.

Most of what we know about synaptic homeostasis stems from studies employing cultured mammalian CNS neurons or the *Drosophila* neuromuscular junction (NMJ). Two early in vitro studies were influential in establishing that both pre- and postsynaptic neurons can alter their physiology to offset changes imposed by pharmacological agents (O'Brien et al., 1998; Turrigiano et al., 1998). For example, blocking glutamate receptors for days increased the sensitivity of the postsynaptic membrane to glutamate. Reducing the firing rate of presynaptic neurons by chronic application of TTX also increased the sensitivity of the postsynaptic membrane to glutamate. Conversely, increasing the presynaptic firing rate using antagonists for GABA or glycine receptors decreased postsynaptic glutamate sensitivity. Interestingly, after 2 days, presynaptic neurons adapted to the GABA antagonist, and their firing frequency returned to pretreated levels, indicative of a strong cellular homeostatic mechanism. Subsequent studies of cultured neurons then reported compensatory changes across synapses that likely require a retrograde signal, indicating true synaptic homeostasis (Bacci et al., 2001; Burrone et al., 2002; Murthy et al., 2001).

At the same time, genetic studies at the *Drosophila* NMJ provided evidence for synaptic homeostasis. Two genetic manipulations reduced the sensitivity of the postsynaptic membrane to glutamate while a third decreased the excitability of the postsynaptic muscle: mutations in the glutamate receptor subunit GluRIIA (Petersen et al., 1997), postsynaptic expression of a constitutively active catalytic subunit of PKA (Davis et al., 1998), and postsynaptic expression of the Kir2.1 K^+ channel (Paradis et al., 2001). All postsynaptic manipulations triggered an increase in presynaptic transmitter release to reestablish almost wild-type levels of muscle excitability, suggesting a retrograde signaling mechanism that maintains postsynaptic excitability throughout development.

All of these elegant experiments suggested a slow time course of synaptic homeostasis. Pharmacological agents provoked significant homeostatic changes in cultured CNS neurons only over several days, while homeostatic changes in response to genetic manipulations at *Drosophila* NMJs were only examined after several days of development. The study of Frank et al. (2006 [this issue of *Neuron*]) revises our notion of homeostatic mechanisms by providing two important insights. First, they reveal a surprisingly rapid time course for the induction of synaptic homeostasis, providing some insight into potential retrograde signaling candidates. Second, they reveal the identity of a potential presynaptic effector molecule—the high-voltage-activated presynaptic Ca^{2+} channel *Cacophony*.

The authors show that within minutes of applying synthetic philanthotoxin (PhTox) to partially antagonize glutamate receptors, nerve stimulation evokes the release of more neurotransmitter quanta from the presynaptic terminal. Remarkably, neither axonal firing in motor patterns nor low-frequency nerve stimulation is required to induce this homeostatic response. However, the homeostatic effect was absent when the pore-forming subunit of the presynaptic Ca^{2+} channel *Cacophony* is mutated, suggesting that this Ca^{2+} channel is an essential effector element of the mechanism conferring synaptic homeostasis. There is a remote possibility that PhTox acts directly on presynaptic glutamate receptors. Reassuringly, PhTox is unable to elicit a change in neurotransmitter in larvae mutant for GluRIIA, suggesting that PhTox does not significantly alter targets other than GluRIIA.

With the help of *cacophony* mutations, Frank et al. go on to forge a link between the fast form of synaptic homeostasis and the previously documented slow form. As the Goodman group showed earlier (Petersen et al., 1997), GluRIIA mutant NMJs exhibit an increase in quantal content that almost perfectly compensates for the loss of excitation induced by the GluRIIA mutation. However, Frank et al. demonstrate that NMJs mutant for both GluRIIA and *cacophony* lack a significant homeostatic increase in quantal content, suggesting that the slow and the fast form of synaptic homeostasis are mechanistically linked.

There is strong evidence that the degree of postsynaptic excitability is the variable detected by the sensor (Paradis et al., 2001). However, Frank et al. demonstrate that evoked postsynaptic potentials are not required to induce synaptic homeostasis. Does this indicate that locally restricted synaptic depolarization by spontaneous release activity is integrated to trigger a homeostatic response? Although untested, it is a fascinating proposition that a change in "mini" amplitudes may trigger swift homeostatic changes in the presynaptic terminal. Considering that postsynaptic Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity is important for at least the slow form of synaptic homeostasis at the *Drosophila* NMJ (Haghighi et al., 2003), Ca^{2+} remains a reasonable candidate for the variable that trips the sensor. Since glutamate receptors at fly NMJs are permeable to Ca^{2+}, it is possible that postsynaptic Ca^{2+} entry rather than the voltage change across the membrane is the integrated
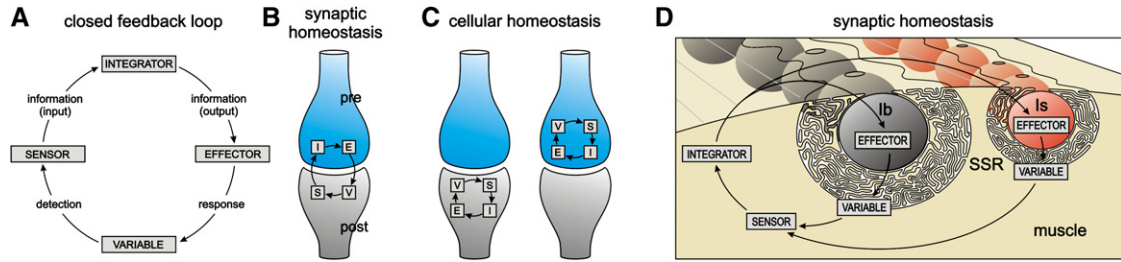
Figure 1. Principle of Homeostasis at Synapses

(A) A closed feedback loop may support homeostasis, essentially as described in control theory. VARIABLE, the characteristic that is controlled; SENSOR, detects the value of the variable; INTEGRATOR, compares the sensed value of the variable to a predetermined set-point value; EFFECTOR, mechanism that can change the variable.

(B) A neuron-neuron synapse, typical of cultured CNS neurons, with an embedded feedback loop that might support synaptic homeostasis.

(C) Cell-autonomous feedback loops embedded in either side of the synapse, incapable of supporting synaptic homeostasis.

(D) The neuromuscular junction of *Drosophila* larvae with its dual innervation by two different motor axons forming synaptic boutons (type-1b and type-1s) that are surrounded by the subsynaptic reticulum (SSR) of the muscle. A feedback loop is overlaid to show the putative locations of synaptic homeostasis components.

variable of activity. It is also possible that Ca^{2+} entry through postsynaptic L-type Ca^{2+} channels is the relevant variable (Thiagarajan et al., 2005). If this is also the case in *Drosophila*, one expects that mutations in the *Drosophila* L-type Ca^{2+} channel homolog *DmCa1D/* Ca_v1 will effectively block synaptic homeostasis.

The most exciting aspects of the Frank et al. study are the very rapid retrograde signaling and effector mechanisms that are in operation. Although the speed of the response does not rule out conventional morphogens such as the TGF-β pathway (Marqués, 2005), the present study challenges current models. In particular, pathways that require protein translation can be eliminated, since Frank et al. show that the homeostatic response persists when protein biosynthesis is acutely inhibited.

There are numerous retrograde signaling molecules that work on a fast time scale, such as cannibinoids, glutamate, or gases like carbon monoxide (CO) and nitric oxide (NO). While effects of glutamate are unlikely for reasons discussed earlier, only NO has so far been implicated in synaptic function at *Drosophila* NMJs (Wildemann and Bicker, 1999). Consideration of possible retrograde signals should include not just readily diffusible molecules but also mechanisms that span the synaptic cleft. Molecules that form multimeric *trans*-synaptic connections, like integrins, could constitute part of the signaling machinery and transduce the signal across the synaptic cleft. The peculiar observation that synaptic homeostasis is disrupted by stretching the NMJ leaves a signal-transducing physical link across the synaptic cleft as an intriguing possibility.

The study by Frank and colleagues offers a new perspective that views synaptic homeostasis as a fast form of synaptic plasticity that may also give meaning to spontaneous "mini" release amplitudes. It rules out a canonical signaling cascade and forces us to rethink retrograde signaling mechanisms. Considering that synaptic homeostasis is accountable for stable and continuous transmission while accommodating rapid change, perhaps we shouldn't be surprised that synaptic homeostasis held the power of rapid response all along. Only the future can tell us whether the revelation of fast-tracked synaptic homeostasis makes it easier or more difficult to reconcile how homeostatic mechanisms are interweaved with activity-dependent changes in synaptic strength.

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