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SPOTLIGHT

Neurons regulate synaptic strength through homeostatic scaling of active zones

Karen L. Cunningham and J. Troy Littleton

How neurons stabilize their overall synaptic strength following conditions that alter synaptic morphology or function is a key question in neuronal homeostasis. In this issue, Goel et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201807165) find that neurons stabilize synaptic output despite disruptions in synapse size, active zone number, or postsynaptic function by controlling the delivery of active zone material and active zone size.

Neurons show a remarkable ability to modulate their functional output in response to a variety of stimuli and signaling pathways that allow brain circuits to learn and store relevant environmental information. Hebbian forms of plasticity change neuronal output such that learned stimuli evoke larger or smaller responses between connected partners depending on the circuit and stimulus. In contrast, a discrete form of circuit modification, known as homeostatic plasticity, functions to counteract these shifts in activity to stabilize overall output within a physiological range defined by specific set-points. Mechanisms that regulate these forms of plasticity have been described for many systems, and their effect can be mediated through changes in the presynaptic or postsynaptic partner that alter synaptic strength. A popular model for characterizing homeostatic plasticity is the *Drosophila melanogaster* larval neuromuscular junction (NMJ; 1, 2). At this connection, the glutamatergic motor neuron forms multiple en passant presynaptic varicosities on the muscle surface. Neurotransmitter release occurs over a population of several hundred individual release sites, termed active zones (AZs), that are clustered within individual varicosities. The AZs function to organize synaptic vesicles and presynaptic calcium channels at release sites while aligning the synaptic vesicle release machinery to postsynaptic glutamate receptor fields. Individual AZs at the *Drosophila* NMJ have variable release properties that largely rely on their abundance of AZ material (3, 4). Remarkably, the NMJ is capable of maintaining relatively normal synaptic transmission despite major perturbations in synaptic growth and structure, as well as altered function of postsynaptic glutamate receptors (5–7). How neurons manage to stabilize output under these conditions represents a key aspect of homeostatic plasticity. In the current issue, Goel et al. used mutants that alter synaptic growth, AZ density, and postsynaptic sensitivity to push the dynamic range of synaptic architecture and explore how overall synaptic output can be maintained despite these changes (8). The authors demonstrate that neurons can control release across the NMJ by regulating the trafficking and availability of key AZ proteins, thereby scaling AZ size across the entire population.

Using two well-established mutants that increase or decrease AZ number and synapse area, the authors find that motor neurons homeostatically correct for dramatic changes in synapse architecture to stabilize functional output at control levels. *endo* mutants disrupt a key BAR domain–containing protein (Endophilin) required for endocytosis, leading to dramatic synaptic overgrowth and excess AZs (due to defective termination of synaptic growth signals) and bigger individual synaptic vesicles (secondary to alterations in synaptic vesicle recycling; 6). Despite this large increase in synaptic vesicle size and AZ number, *endo* mutants maintain functional synaptic output (the overall evoked response measured physiologically). In contrast, mutations in the synaptic vesicle Rab3 protein disrupt AZ formation and have fewer fully formed AZs due to a defect in the distribution of AZ material (9). In *rab3* mutants, a large fraction of AZs is devoid of key presynaptic components, including the voltage-gated calcium channel and several AZ scaffolding proteins, while the remaining AZs have an overabundance of these components. Strikingly, despite these major differences in synaptic architecture, the evoked response is homeostatically regulated to match control levels in both genotypes (Fig. 1).

Though AZ number varies between *endo*, *rab3*, and control NMJs, Goel et al. find that the total amount of several key AZ components at the NMJ is conserved (8), pointing toward a synapse-wide mechanism for regulating total release independent of AZ number. When summed across all release sites at the NMJ, *rab3* and *endo* mutants maintain wild-type levels of the core AZ scaffold Bruchpilot (BRP), voltage-gated calcium channels, and the AZ-resident Unc13A proteins. Recent findings indicate that *Drosophila* AZs are composed of "nanomodules" that can be added or removed to regulate the probability of vesicle fusion (10). Here, the authors used a similar analysis to demonstrate that AZ area and module number change during homeostatic potentiation, although the ratio of BRP, Unc13A, and presynaptic calcium channels

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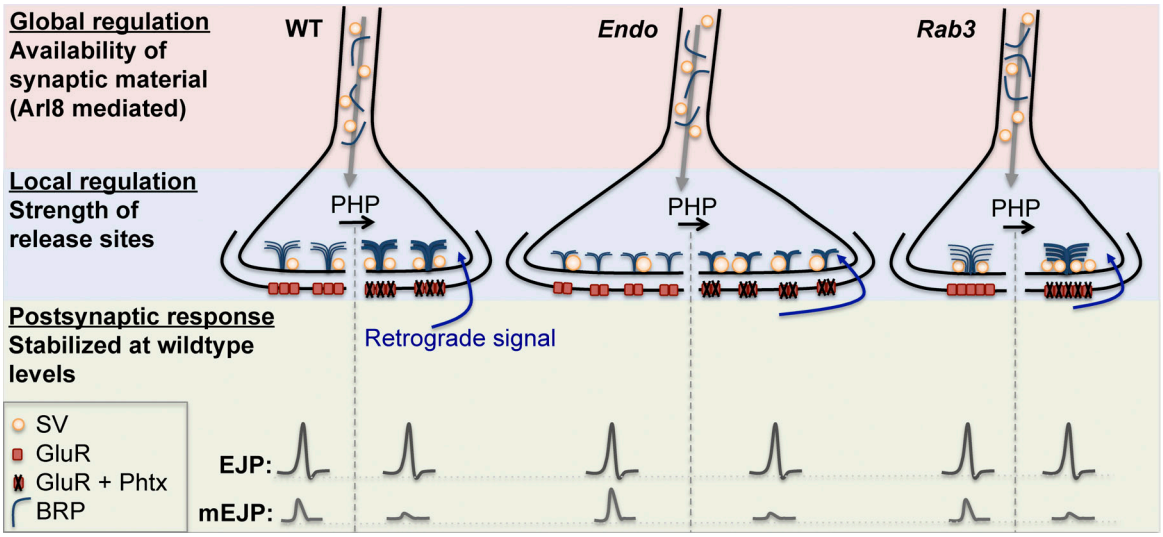

Figure 1. **Model illustrating the levels of regulation that tune synaptic output at *Drosophila* motor neuron terminals.** Although AZ number, synapse area, and synaptic vesicle (SV) size vary significantly at wild-type (left), *endo* (middle), and *rab3* (right) NMJs, their baseline postsynaptic response to evoked stimuli (evoked junctional potential [EJP]) is homeostatically stabilized at wild-type levels by changing AZ size and corresponding release strength. Arl8-mediated anterograde axonal transport of key synaptic components regulates synaptic strength upstream of synaptic architecture. All three genotypes can undergo PHP following philanthotoxin application that blocks postsynaptic glutamate receptors and reduces quantal size (miniature excitatory junctional potential [mEJP]). This rapid restoration of synaptic strength secondary to retrograde signaling can restore evoked amplitudes to baseline levels, suggesting multiple forms of homeostatic regulation can be expressed simultaneously to maintain synaptic output at the NMJ.

in individual modules remains constant. Given that the amount of AZ material correlates well with release probability at the single AZ level (3, 4), scaling the abundance of AZ material and release strength across the population to maintain total synaptic output is a salient model for global regulation of synaptic strength.

Since the amount of synaptic material is conserved across genotypes independent of AZ number, the authors probed whether anterograde trafficking regulates the abundance of AZ material at the NMJ. Recent work has demonstrated that the kinesin adapter Arl8 is critical for delivering synaptic vesicle and AZ material to the synapse (11). At wild-type terminals, increasing Arl8 abundance results in increased AZ material and increased neurotransmitter release. Conversely, loss of Arl8 reduces synaptic material and synaptic release (11). Goel et al. demonstrate that increasing or decreasing Arl8-mediated trafficking in *endo* and *rab3* mutants has a similar effect on AZ material and synaptic function (8). These findings suggest that Arl8-mediated transport is capable of bidirectionally scaling overall AZ material delivery despite changes in local synaptic structure.

Two major types of presynaptic homeostatic plasticity have been reported in *Drosophila*. Overexpression of the vesicular glutamate transporter leads to bigger synaptic vesicles, with a homeostatic presynaptic depression pathway acting to decrease release probability to offset this increase in quantal size (7). In contrast, presynaptic homeostatic potentiation (PHP) increases presynaptic release probability to compensate for decreased postsynaptic function due to changes in postsynaptic glutamate receptor density or function. PHP can occur developmentally secondary to mutations in the postsynaptic glutamate receptor subunits, or acutely following application of a drug that blocks postsynaptic receptors (1). A recent study demonstrated that both acute and chronic PHP involve an increase in the abundance of several AZ components (10). Goel et al. sought to determine if homeostatic AZ scaling as seen in *rab3* and *endo* mutants can be coexpressed along with PHP. They applied the glutamate receptor blocker philanthotoxin to *endo* and *rab3* mutants and observed that acute presynaptic potentiation successfully occurred and produced visible increases in the intensity of synaptic proteins (8), as previously demonstrated in controls (10). They also found that presynaptic homeostatic depression is functional on top of homeostatic AZ scaling. These results indicate that the AZ homeostat can coexist with other homeostatic plasticity pathways to precisely tune synaptic output. In conclusion, the current study provides evidence that neurons may make a set amount of AZ proteins that are delivered across the synaptic field, independent of the number of synapses formed, providing a robust system to ensure overall homeostatic set-points in presynaptic release independent of synapse number.

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