Homeostatic Control of Presynaptic Neurotransmitter Release

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Abstract

It is well established that the active properties of nerve and muscle cells are stabilized by homeostatic signaling systems. In organisms ranging from *Drosophila* to humans, neurons restore baseline function in the continued presence of destabilizing perturbations by rebalancing ion channel expression, modifying neurotransmitter receptor surface expression and trafficking, and modulating neurotransmitter release. This review focuses on the homeostatic modulation of presynaptic neurotransmitter release, termed presynaptic homeostasis. First, we highlight criteria that can be used to define a process as being under homeostatic control. Next, we review the remarkable conservation of presynaptic homeostasis at the *Drosophila*, mouse, and human neuromuscular junctions and emerging parallels at synaptic connections in the mammalian central nervous system. We then highlight recent progress identifying cellular and molecular mechanisms. We conclude by reviewing emerging parallels between the mechanisms of homeostatic signaling and genetic links to neurological disease.

INTRODUCTION

The emerging field of homeostatic plasticity can be subdivided into three areas that are defined by the way in which a cell responds to a perturbation, namely the homeostatic control of intrinsic excitability (1–7), neurotransmitter receptor expression (3, 7), and presynaptic neurotransmitter release (8–12). Together, these areas represent a suite of mechanisms that confer stability to nervous system function. A clear understanding of the underlying mechanisms at the cellular and molecular levels promises to provide fundamental new insights into neurological disease, as well as into phenomena ranging from memory formation to sleep. This review focuses exclusively on the homeostatic modulation of presynaptic neurotransmitter release, an evolutionarily conserved form of homeostatic control observed at synapses in systems that are as diverse as the glutamatergic neuromuscular junction (NMJ) of insects, the cholinergic NMJ of vertebrates, and central synapses throughout the mammalian brain (**Figure 1*a***). Recent reviews have examined the literature regarding other aspects of homeostatic plasticity (13–17).

The homeostatic adjustment of neurotransmitter release, termed presynaptic homeostasis, is a truly remarkable phenomenon. Homeostatic signaling systems can modulate presynaptic release by as little as 10% or by as much as 200% (**Figure 1*b***) (12). Furthermore, these adjustments can be achieved on timescales ranging from seconds to days (12, 18), can be sustained for weeks to years (19, 20), and appear to be synapse specific (see below)—an extraordinary fact given the diversity of cell types in the nervous system. The capacity of presynaptic homeostasis becomes even more remarkable if one considers the speed, the specificity, and the dynamic range of the synaptic vesicle fusion mechanism. Following the arrival of an action potential (AP) at a nerve terminal, the entire fusion reaction from calcium influx to full fusion occurs in less than 100 μs, and thus the entire protein complex involved in vesicle exocytosis must be readied prior to AP arrival. Synaptic vesicle fusion has a broad dynamic range, with some synapses able to release vesicles at rates spanning several orders of magnitude (21). Yet, in a stereotyped, synapse type–specific manner, different synapses respond to AP stimulation by releasing vesicles with different probabilities and with very different dynamics during repetitive stimulation. Understanding how homeostatic signaling interfaces with the macromolecular machine that executes AP-driven, calcium-coupled vesicle release remains a major challenge for cellular and molecular neuroscience.

DEFINITION OF HOMEOSTATIC SIGNALING

In general, a system can be defined as being under homeostatic control by satisfying three criteria. First, baseline function has to be measured and shown to be stable over time. Second, a quantifiable change in baseline function must be documented following an experimental or natural perturbation. Third, baseline function must be shown to be accurately restored in the continued presence of the perturbation. These criteria establish the action of an active signaling process that not only responds to the presence of a perturbation, but quantitatively offsets the magnitude of the perturbation with an opposing, compensatory response. The accuracy of a homeostatic response separates homeostasis from other forms of compensation and from systems that are simply robust to perturbation (22, 23).

A clear definition of homeostasis is important because it delineates the presence of a signaling system capable of accurate compensation. By analogy with engineered systems built for homeostatic control, biological systems under homeostatic control are thought to require several signaling modules. First, homeostatic systems require a set point that precisely defines the output of the system. This is also the state to which the system returns following a perturbation. Second, homeostatic control systems require sensors for the detection of a perturbation. Third,

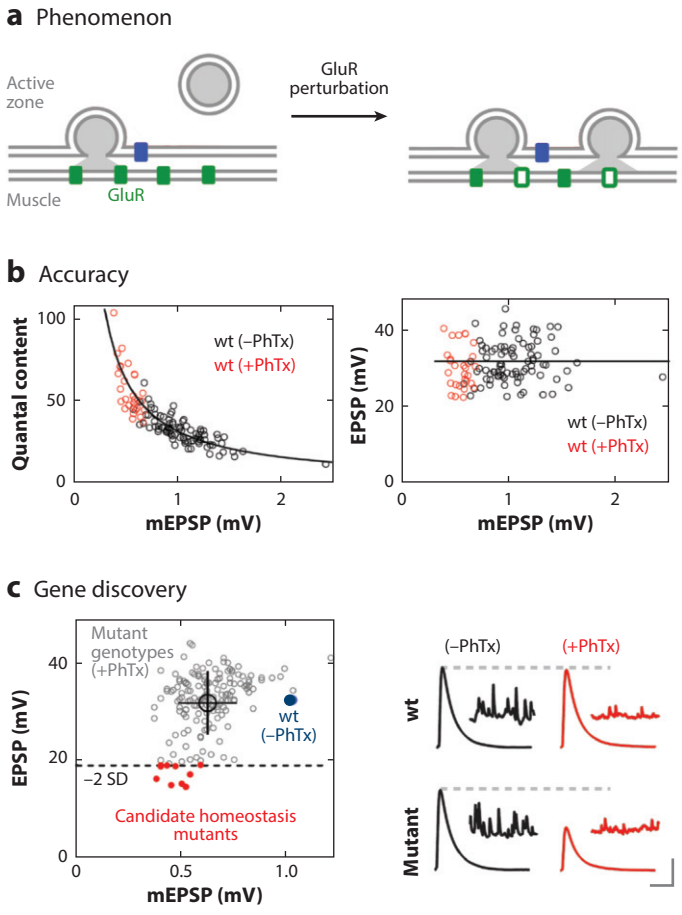

Figure 1

Presynaptic homeostasis. (*a*) Diagram depicting the homeostatic modulation of presynaptic vesicle release at a presynaptic active zone (voltage-gated calcium channels are indicated in *blue*) at baseline (*left*) and following the loss or inhibition of postsynaptic glutamate receptors (GluRs, *green squares*; *open green squares* indicate loss of or pharmacological inhibition of GluRs) (*right*). (*b*) (*Left*) The accuracy of presynaptic homeostasis is shown in a graph plotting quantal content versus miniature excitatory postsynaptic potential (mEPSP) amplitude. Each data point is average data from a recording at a single neuromuscular junction in normal saline [wt (−PhTx)] or in the presence of philanthotoxin [wt (+PhTx)]. The data fall along a curve representing perfect homeostatic compensation (12). (*Right*) Data from the same recordings reveal that action potential–evoked muscle depolarization [excitatory postsynaptic potential (EPSP)] is held constant with respect to mEPSP amplitude. (*c*) (*Left*) Results of a forward genetic screen for homeostatic plasticity mutants (data modified from Reference 28). Average data for wt are shown (*blue dot*). Each data point (*gray*) is an average from an independent genotype. The black open circle denotes the average across all genotypes [± 1 standard deviation (SD)]. Two SDs smaller than the EPSP amplitude population mean are indicated by a dotted horizontal line (−2 SD). Genotypes that reside at or below −2 SD are candidate homeostatic mutations (*red*; data modified from Reference 28). (*Right*) EPSP and mEPSP sample traces for wt (*top*) and a mutant (*bottom*) in which the homeostatic restoration of EPSP amplitude is blocked. Scale bars: 10 mV/2 mV and 100 ms/1s. wt denotes wild type. Data in panel *c* from Reference 56.

the difference between the set point and steady-state activity is encoded in an error signal that is used to promote a change in homeostatic effectors that drive compensatory change. Finally, most homeostatic systems involve feedback control to precisely retarget the set point (1).

HOMEOSTATIC CONTROL OF PRESYNAPTIC NEUROTRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION: PHENOMENOLOGY AND CONSERVATION

Phenomenology consistent with what we now understand to be the homeostatic control of presynaptic neurotransmitter release appeared in studies of the human neuromuscular disease myasthenia gravis (MG) (20), in mouse models of this condition (18), and in studies at the genetically tractable *Drosophila* NMJ (24–26). In each case, loss or impairment of postsynaptic neurotransmitter receptors decreased postsynaptic excitation in response to neurotransmitter release, documented as a decrease in the amplitude of spontaneous miniature excitatory postsynaptic potentials (mEPSPs). In each case, a compensatory increase in presynaptic neurotransmitter release precisely restored the amplitude of AP-evoked excitatory postsynaptic potentials (EPSPs). More specifically, at the NMJ of MG patients, substantially more acetylcholine (Ach) is released than at the normal NMJ, thereby offsetting a disease-related decrease in postsynaptic Ach receptor (AchR) levels (20). Similar observations were seen in mouse models of MG (18). For example, administration of the AchR antagonist α-bungarotoxin for a 2–6-week period creates a condition, termed toxin-induced MG, that includes a compensatory increase in presynaptic neurotransmitter release that offsets impaired muscle sensitivity to Ach (18). Similarly, at the *Drosophila* NMJ, genetic manipulations that impair the abundance or conductance of muscle-specific glutamate receptors cause a compensatory increase in AP-evoked neurotransmitter release. The increase in evoked release precisely compensates for the decrease in glutamate receptor sensitivity and, thereby, maintains synaptic excitation of the muscle cell at normal levels (**Figure 1**) (12, 24, 25).

The manner by which neurotransmitter release is modulated is remarkably similar at the *Drosophila* NMJ, mouse NMJ, and human NMJ, suggesting an evolutionarily conserved homeostatic process. Presynaptic release is enhanced without a change in the size of the presynaptic terminal and without a change in the number of presynaptic active zones (12, 27, 28). The increase in release, which can be as much as 200% over baseline, is observed in response to single-AP stimulation, implying a fundamental and precise change at the level of the molecular machinery that mediates synaptic vesicle fusion (18). Early review articles formalized a hypothesis that a highly conserved homeostatic signaling system is initiated in muscle following disruption of postsynaptic receptor function. The signal is then transmitted to the presynaptic nerve terminal, precisely adjusting neurotransmitter release to achieve normal muscle excitation (26, 29).

Rapid Induction and Sustained Expression: The Kinetics of Homeostatic Compensation

In all the aforementioned studies, the compensatory change in presynaptic release was an end point. The compensatory process had not been monitored in time to show that perturbing postsynaptic neurotransmitter receptor function induced a disruption that was subsequently corrected by homeostatic signaling. The process of homeostatic compensation was eventually followed in *Drosophila* by taking advantage of philanthotoxin (PhTx), a use-dependent open channel blocker of insect glutamate receptors that has a selective effect on muscle-specific glutamate receptors (12). Acute application of PhTx to the NMJ caused a rapid (~30-s) decrease in both mEPSP and EPSP amplitudes. Over the next 10 min, EPSP amplitudes climbed back to baseline levels

in the continued presence of PhTx. A statistically significant increase in release was observed as rapidly as 2 min following application of PhTx to the NMJ, indicating that the system detects a perturbation and initiates compensatory signaling in a time frame of seconds to minutes. This rapidly induced compensatory change in presynaptic release is highly accurate, as it can offset different magnitudes of postsynaptic receptor perturbation (**Figure 1*b***). Notably, similar kinetics of PhTx-induced homeostatic potentiation are also observed at adult *Drosophila* NMJs synapsing onto a proboscis muscle (19). Together, these data fulfilled the criteria for a homeostatic signaling system that is initiated in muscle and is mediated by a change in presynaptic neurotransmitter release (**Figure 1*a*,*b***).

Several observations followed in rapid succession, enabled by the acute PhTx-dependent assay. The rapid, PhTx-dependent induction of presynaptic homeostasis is independent of new transcription and translation (30). Current evidence suggests that the sustained expression of presynaptic homeostasis, caused by persistent genetic disruption of postsynaptic glutamate receptors, requires new transcription and translation based upon analysis of genetic mutations that disrupt these processes (see below) (30–33). Finally, the rapid, homeostatic restoration of EPSP amplitude is observed to asymptotically approach baseline values but is never observed to overshoot baseline (12). By analogy with engineered systems, this finding implies the existence of integral or proportional feedback signaling (34).

Activity Independence: Accuracy Without Feedback Control?

If AP-evoked postsynaptic depolarization is homeostatically stabilized, then this parameter should provide the information necessary for the feedback signaling that adjusts presynaptic release. One hypothesis is that EPSP amplitude is somehow monitored and incorporated into feedback signaling such that, as the EPSP approaches baseline values, the homeostatic potentiation of release is gradually attenuated (11). However, accurate homeostatic restoration of EPSP amplitude, in both flies and mice, occurs in the absence of AP-evoked EPSPs (12, 35). For example, during prolonged recordings from the *Drosophila* NMJ, PhTx was applied, and, despite a complete absence of AP-evoked neurotransmitter release during a 10-min incubation period, presynaptic homeostasis was fully expressed (12). Likewise, α-bungarotoxin–induced homeostatic potentiation at the mouse NMJ occurs normally in the presence of AP blockade by a tetrodotoxin cuff applied to the motor nerve (35). Thus, an EPSP amplitude–based feedback system can seemingly be discarded because homeostatic compensation is accurately achieved in the absence of EPSPs. The information contained in the spontaneous release of synaptic vesicles appears to be sufficient to account for the fully accurate expression of presynaptic homeostasis (12). The information contained in spontaneous miniature events may be fed into an open loop or feed-forward system without feedback, and the individual components of the homeostatic signaling system may function because they are homeostatically controlled. Alternatively, we have not yet discovered the signal being monitored and used as feedback within the neuromuscular systems of flies, mice, and humans.

HOMEOSTATIC CONTROL OF NEUROTRANSMITTER RELEASE IN THE CENTRAL NERVOUS SYSTEM

Phenomena consistent with presynaptic homeostasis are observed at mammalian central synapses in response to perturbed target innervation (36), in response to altered postsynaptic excitability (37–39; but see Reference 40), following sensory deprivation (41), and following chronic inhibition of neural activity (42–45). In some instances, the observed presynaptic changes are sufficient to

restore postsynaptic neuron firing to baseline, set-point levels, consistent with a true homeostatic effect (37).

Most studies examining homeostatic plasticity in the central nervous system have documented postsynaptic modulation of neurotransmitter receptor abundance, an effect referred to as quantal scaling, which is observed at both excitatory and inhibitory synapses (3, 7). There is, as yet, no clear indication why an activity perturbation leads to quantal scaling in some instances while causing a change in synapse number or a change in presynaptic release in others (see **Figure 1**; see Reference 46 for review). The developmental stage at which a perturbation is applied can influence the locus of expression of homeostatic plasticity (47–50). The locus of expression may also depend on cell type (see below).

The quantification of presynaptic release is among the many challenges associated with analysis of synaptic transmission in the central nervous system. Altered presynaptic release is often inferred from changes to the rate of spontaneous mEPSP release and/or from changes to paired-pulse stimulation. Although a change during a paired-pulse paradigm is indicative of altered release probability, the lack of an effect during a paired-pulse paradigm need not be diagnostic if altered release is achieved through changes in the size of the pool of releasable vesicles, a situation documented at the fly NMJ (51, 52). The studies that have reported homeostatic alterations in presynaptic release in the central nervous system have done so by quantifying (*a*) changes in AP-driven presynaptic calcium influx, (*b*) vesicle recycling with vital dyes or with GFP-based reporters of membrane recycling (42, 45, 47), and (*c*) correlated changes in synaptic ultrastructure (47).

Given the diversity of cell types in the brain, the mode of homeostatic signaling could be expressed in a cell type–specific manner. Indeed, cell type–specific differences in the locus of expression of homeostatic plasticity have been documented (43, 53). In one study, the response to chronic activity blockade was remarkably synapse specific when three sets of synapses in hippocampal cultures were examined (53; see also Reference 44). In another study, excitatory synapses onto parvalbumin-positive (PV) or somatostatin-positive (SA) interneurons were examined by using paired electrophysiological recordings in organotypic neocortical cultures (43). After 5 days of AP blockade, the synapses onto both types of interneurons became stronger, an effect that may be indicative of homeostatic plasticity. No major changes in synaptic short-term dynamics or in spontaneous miniature excitatory postsynaptic current (mEPSC) amplitude were observed at synapses onto PV interneurons, indicating that a change in synapse number and/or in release-ready vesicle number is responsible for the increase in synaptic strength. By contrast, excitatory synapses onto SA neurons displayed a significant decrease in synaptic facilitation, consistent with modulation of presynaptic neurotransmitter release probability. Remarkably, these differences are consistent across cells that are connected to each other, implying a cell type–specific homeostatic expression mechanism that is shared among neurons within a neural circuit. Taken together, these findings indicate that presynaptic homeostasis is observed at mammalian central synapses, although the parameters associated with induction and cell type–specific expression remain to be fully defined.

SEARCHING FOR MECHANISMS: AN ELECTROPHYSIOLOGY-BASED FORWARD GENETIC SCREEN

Although the phenomenon of presynaptic homeostasis is robust and highly conserved, the underlying molecular mechanisms are only just emerging. The search for molecular mechanisms has been driven, in part, by a candidate-based approach in mammalian cell culture and the *Drosophila* NMJ, with some notable successes, including the implication of CDK5 (cyclin-dependent kinase 5) (42, 44), mTOR (54, 55), and microRNA-based signaling (31, 33). However, if the ultimate goal

is to define the molecular architecture of a homeostatic regulatory system that includes poorly defined parameters such as a set point, error signal, and proportional feedback, then there are no candidates to try. This is where the power of an unbiased, forward genetic approach in a model organism such as *Drosophila* has become essential.

The discovery that acute application of PhTx to the *Drosophila* NMJ can induce a rapid, homeostatic adjustment of presynaptic neurotransmitter release opened the door to forward genetic approaches for candidate homeostatic plasticity genes. A forward genetic screen based upon intracellular recordings from the NMJ was used to assay homeostatic compensation following PhTx application (27, 28, 56). For each mutation in this screen, three to ten intracellular recordings were performed, and numerous parameters—including mEPSP amplitude, EPSP amplitude, quantal content, muscle resting membrane potential, and input resistance—were quantified (**Figure 1*c***). To date, the screen has tested more than 1,000 independent mutations or RNAi lines. It was essential that presynaptic homeostasis was quantified directly, because many of the mutations that were found to block presynaptic homeostasis had no effect on either the anatomical development of the NMJ or baseline synaptic electrophysiology in the absence of PhTx (27). These mutations selectively blocked homeostatic plasticity. As such, this forward genetic screen demonstrated that homeostatic plasticity is a process that can be genetically separated from the mechanisms of normal neuromuscular development (27). The remainder of this review focuses on emerging presynaptic mechanisms. **Figure 2*a*** summarizes the genes recently implicated in presynaptic homeostasis at the *Drosophila* NMJ.

HOMEOSTATIC SIGNALING TARGETS BOTH CALCIUM INFLUX AND THE RELEASE-READY VESICLE POOL

To be released upon AP stimulation, a vesicle has to fulfill two requirements: First, due to the relatively low calcium affinity of the sensor for synchronous vesicle fusion, the vesicle needs to "see" a sufficiently high elevation of the intracellular concentration of free calcium ions. Second, the vesicle must be release competent, meaning that all molecular interactions required for vesicle fusion in response to the calcium trigger have taken place. Vesicles that satisfy both criteria are referred to as release-ready vesicles. Evidence to date supports the conclusion that the homeostatic modulation of presynaptic release occurs at the intersection of two processes within the presynaptic terminal: an increase in presynaptic calcium influx through $Ca_V2.1$ channels and an increase in the readily releasable pool (RRP) of synaptic vesicles (**Figure 2*a*,*b***). The strength of these conclusions is based on the identification of mutations that selectively impair each process and block presynaptic homeostasis.

Homeostatic Modulation of Presynaptic Calcium Influx via Synaptic ENaC Channel Insertion

A change in the amplitude of single-AP-induced, spatially averaged calcium transients that is potentially sufficient to explain the homeostatic change in presynaptic release has been observed at the *Drosophila* NMJ (**Figure 2*b***) (57). This change occurs after homeostatic challenge imposed by genetic ablation of a glutamate receptor subunit (encoded by *GluRIIA*) and after acute application of PhTx to the NMJ (57). In these experiments, there were no apparent changes in the rate of decay of the calcium signal, indicating that the observed change in calcium transient amplitude is not a secondary consequence of altered calcium buffering or extrusion. In both instances, a 20–30% increase in the spatially averaged calcium signal correlated with a near doubling of presynaptic vesicle release. Given the highly cooperative relationship between intraterminal

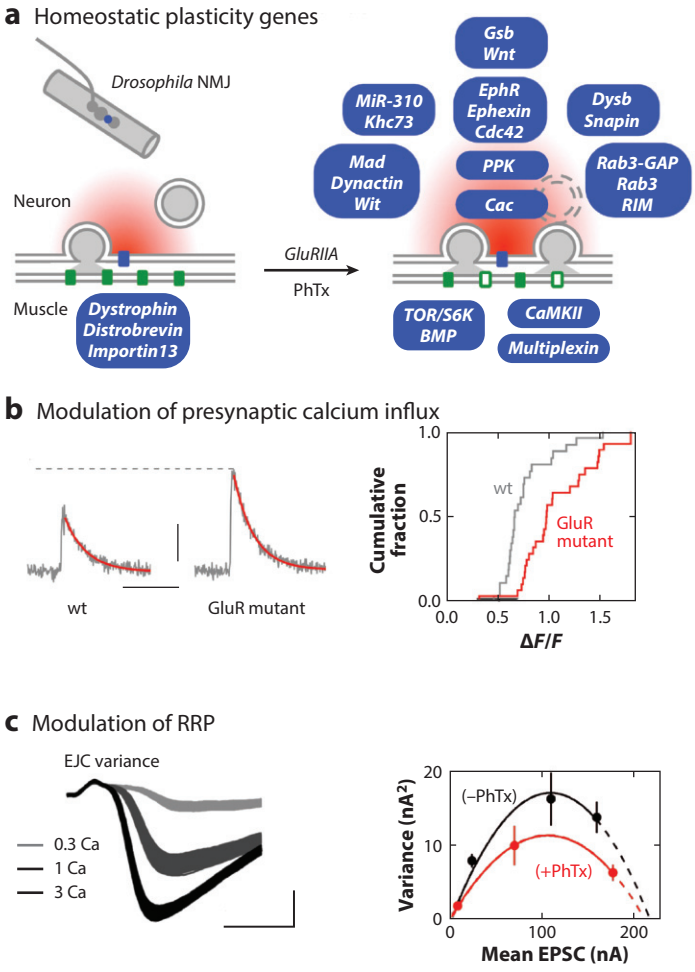

Figure 2

Mechanisms of presynaptic homeostasis at the *Drosophila* neuromuscular junction (NMJ). (*a*) (*Left*) Cartoon of a synapse with a local calcium domain (*red*). Shown are postsynaptic genes implicated in the control of baseline presynaptic release, including those encoding Dystrophin, Distrobrevin (86, 110), and Importin13 (85). (*Right*) Following philanthotoxin (PhTx) or glutamate receptor (GluR) perturbation, there is an increase in presynaptic calcium influx (*red*; 57) and an increase in the readily releasable pool (RRP) of vesicles (51, 52). Each indicated gene disrupts presynaptic homeostasis when mutated. Genes are grouped when molecular, genetic, or biochemical information is consistent with function in a similar pathway. Genes include those encoding Multiplexin (90); bone morphogenetic protein (BMP) (30, 81); TOR/S6 kinase (S6K) (55); CaMKII (81); Mothers Against Decapentaplegic (Mad) (30); Dynactin (30); Wishful Thinking (Wit) (30); miR-310 and kinesin heavy chain 73 (Khc73) (31); EphR, Ephexin, and Cdc42 (87); Gooseberry (Gsb) and Wnt (32); the calcium channel Cacophony (Cac); an ENaC channel (PPK); and the Rab3-GAP (Rab3-GTPase-activating protein)/Rab3/RIM (Rab3-interacting molecule) signaling system (28, 52). (*b*) (*Left*) Example traces for single-action-potential-induced, presynaptic, spatially averaged calcium transients at baseline and following induction of presynaptic homeostasis (*GluRIIA*). Scale bar: 0.5 $\Delta F/F$, 0.5 s. (*Right*) Calcium transient peak amplitude data are summarized as a cumulative frequency distribution. Panel *b* modified from Reference 57. (*c*) Sample data showing the modulation of the RRP based on variance-mean analysis. (*Left*) Superimposed excitatory junctional current (EJC) wave forms at different calcium concentrations (in millimolar). (*Right*) Parabolic fits to EJC amplitude variance-mean data, from which estimates of the binomial parameter N can be derived, indicate an increase in RRP size. Panel *c* modified from Reference 52.

calcium concentration and vesicle release, a power relationship with an exponent of 3–5 at most synapses (58, 59), the modest increase in calcium influx may be sufficient to fully account for the homeostatic change in presynaptic release.

The correlation between presynaptic calcium influx and homeostatic plasticity was then confirmed genetically. The pore-forming $\alpha 1$ subunit of the $Ca_V2.1$ channel—the sole calcium channel responsible for synaptic transmission at the *Drosophila* NMJ—is encoded by the *cacophony* gene. A point mutation (F1029I in the sixth transmembrane domain of the third repeat of the $\alpha 1$ subunit) in the *cacophony* gene (cac^S) (60, 61) causes a modest impairment of both presynaptic calcium influx and neurotransmitter release under baseline conditions but completely blocks the homeostatic modulation of both calcium influx and neurotransmitter release (12). The F1029I point mutation resides in a transmembrane domain outside the voltage-sensing region of this channel. This mutation does not prevent increased calcium flux through the channel in response to AP broadening or elevated extracellular calcium (57). Thus, the F1029I point mutation appears to render the $Ca_V2.1$ channel unresponsive to the homeostatic signaling system that adjusts presynaptic release.

In principle, the observed change in presynaptic calcium influx may be mediated by a change in calcium channel number or function. Forward genetics has uncovered a mechanism that argues for the latter possibility (56): The involvement of a presynaptic DEG/ENaC sodium leak channel was uncovered in the aforementioned genetic screen. Channels in this superfamily are voltage insensitive and are assembled as either homomeric or heteromeric trimers with a large extracellular loop implicated in responding to diverse extracellular stimuli (62–64). DEG/ENaC channels have been implicated as part of the mechanotransduction machinery (65) and in taste perception in both invertebrate and vertebrate systems (66, 67). Beyond this, little is known regarding the function of pH-insensitive DEG/ENaC channels in the nervous system. Two genes encoding DEG/ENaC channel subunits were identified as candidate homeostatic plasticity genes. Investigators showed that mutations in either one of the two DEG/ENaC channel subunits, *ppk11* or *ppk16*, block presynaptic homeostasis and that both genes function in presynaptic motoneurons (56). It was also shown that both genes are transcriptionally upregulated (by 400%) in a glutamate receptor mutant background, consistent with increased demand for these gene products during the long-term maintenance of presynaptic homeostasis. These and other data support the conclusion that these DEG/ENaC channel subunits are homeostatic plasticity genes (56).

A subsequent set of experiments demonstrated that ENaC channel function is coupled to the homeostatic control of presynaptic calcium influx. These experiments showed that benzamil, an ENaC channel antagonist, erases the expression of presynaptic homeostasis at the NMJ. Remarkably, whereas benzamil has no effect on baseline calcium influx, application of benzamil to a glutamate receptor mutant caused a dramatic decrease in presynaptic calcium influx that correlated with the erasure of presynaptic homeostasis (56). An emerging model is based upon the well-established regulation of ENaC channel trafficking in the collecting duct of the kidney during the homeostatic control of blood pressure (68). This model proposes that presynaptic DEG/ENaC channels reside on an intracellular vesicle pool prior to the induction of presynaptic homeostasis. When presynaptic homeostasis is induced, DEG/ENaC channels are inserted at or near the nerve terminal, where they may cause low-voltage modulation of the presynaptic resting potential due to sodium leak and subsequent potentiation of presynaptic calcium influx (see **Figure 4** below) (56). This model is attractive because it provides an analog mechanism that could quantitatively tune presynaptic calcium influx according to the demands of the homeostatic signaling system. Moreover, the model is consistent with previous work demonstrating low-voltage modulation of neurotransmitter release in systems ranging from the crayfish NMJ to the rodent hippocampus (69–71). However, links to homeostatic plasticity had not been previously established before this work (56).

The action of presynaptic DEG/ENaC channels may also explain two previous results. First, the block of homeostasis by the F1029I point mutation in the $Ca_V2.1$ channel can be explained if this mutation restrains the movement of the S6 domain and, thereby, renders the channel less susceptible to low-voltage modulation. Thus, although the point mutation does not alter the ability of the $Ca_V2.1$ channel to pass calcium, it may render it insensitive to low-voltage membrane modulation during presynaptic homeostasis. Second, it was previously shown that presynaptic overexpression of a potassium channel, either Shaker or Kir2.1, blocks presynaptic homeostasis (4, 72, 73). If increased potassium leak prevents ENaC channel–dependent depolarization of the presynaptic membrane, then this could explain why potassium channel overexpression blocks the expression of presynaptic homeostasis.

Homeostatic Control of the Readily Releasable Vesicle Pool of Synaptic Vesicles

Several experiments at the *Drosophila* NMJ demonstrate that presynaptic homeostasis requires an increase in the number of release-ready vesicles, assayed either by quantification of pool size during a stimulus train or by EPSC amplitude fluctuation analysis during single-AP stimulation (**Figure 2*c***) (51, 52). Depending on the condition, the magnitude of the observed increase ranged between ~160% and ~200% of control. This effect could, in principle, be secondary to the observed homeostatic increase in presynaptic calcium influx. There is a positive correlation, although sublinear, between RRP size and presynaptic calcium influx (74, 75). However, analysis of NMJs that lack RIM (Rab3-interacting molecule) argues against this possibility. Experiments showed that presynaptic homeostasis is blocked in *rim* mutants but that the homeostatic potentiation of presynaptic calcium influx is unaltered (52). Subsequent experiments showed that loss of *rim* prevents the homeostatic modulation of RRP size (52). Thus, presynaptic homeostasis seems to require two genetically separable processes: an increase in presynaptic calcium influx (57) and a parallel increase in the RRP (51, 52). At present, how RIM could be modulated during homeostatic plasticity at the *Drosophila* NMJ is unclear. RIM biochemically interacts with a number of presynaptic proteins, including ELKS/CAST, the presynaptic voltage-gated calcium channel, and munc13 (76). Additional support for the action of RIM-interacting molecules during presynaptic homeostasis is the required function of Rab3-GTPase-activating protein functioning in concert with its cognate small GTPase, Rab3 (28).

A current challenge is to understand why homeostatic plasticity is blocked by mutations that selectively affect either modulation of calcium influx or modulation of the RRP. One model proposes that a homeostatic increase in calcium influx is effective only when it is coupled to an increase in the number of release-ready vesicles that take advantage of the increase in presynaptic calcium influx to potentiate release. An alternative explanation is that all the presynaptic processes—ENaC channel insertion, altered calcium influx, and an enhanced RRP—are interdependent processes under separate homeostatic control. The presynaptic system fails if any of these processes is interrupted. How this might occur at a molecular level remains unknown.

PARALLELS WITH THE MECHANISMS OF PRESYNAPTIC HOMEOSTASIS AT MAMMALIAN CENTRAL SYNAPSES

Parallels exist at mammalian central synapses, consistent with the homeostatic modulation of both vesicle pools and presynaptic calcium influx. **Figure 3*a*** summarizes genes recently implicated in presynaptic homeostasis in the mammalian central nervous system. Chronic activity blockade causes a correlated increase in both presynaptic release and calcium influx. Calcium influx and presynaptic release were imaged simultaneously through coexpression of transgenic reporters for vesicle fusion and presynaptic calcium (**Figure 3*b***) (45). Mechanistically, presynaptic CDK5 has

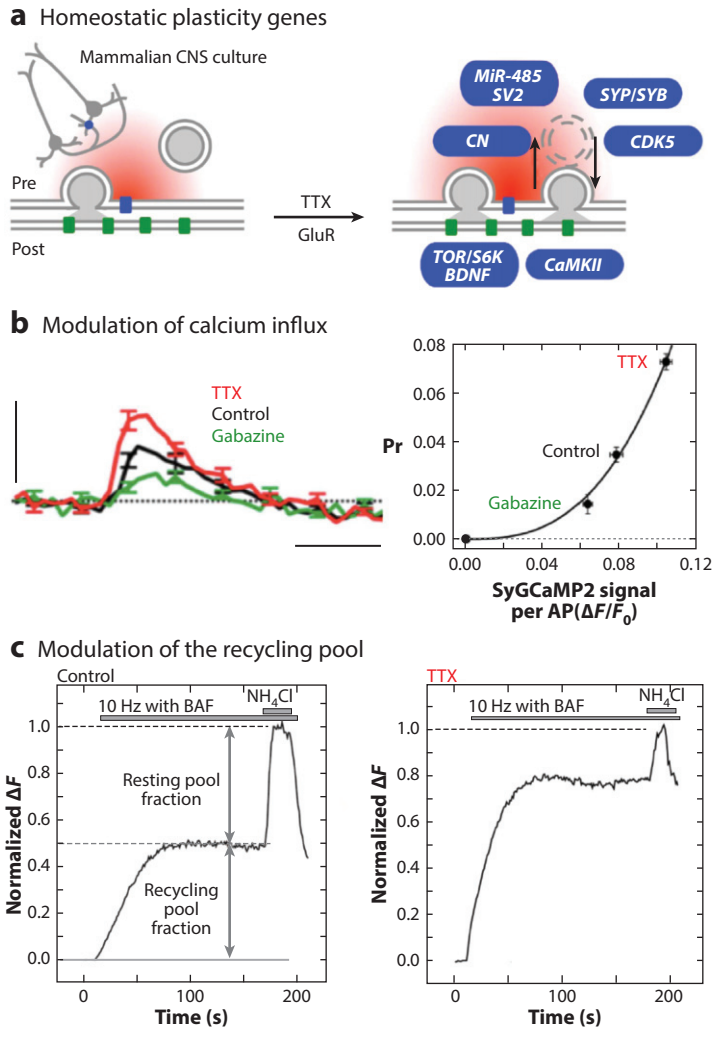

Figure 3

Homeostatic modulation of neurotransmitter release in the mammalian central nervous system (CNS). (*a*) Cartoon showing a CNS synapse before (*left*) and after (*right*) prolonged tetrodotoxin (TTX) treatment or prolonged glutamate receptor (GluR) inhibition. TTX application increases both presynaptic calcium influx (45) and recycling vesicle pool size (42). Calcineurin A (CN) and CDK5 are implicated in the modulation of both presynaptic calcium influx (77) and recycling vesicle pool size (42). Additional mechanisms include the involvement of *miR-485* and SV2 (33), as well as synaptophysin/synaptobrevin complexes (SYP/SYB) (80) following bicuculline/4-AP and APV/CNQX treatment, respectively. Postsynaptic TOR signaling was recently implicated in transsynaptic modulation of presynaptic release (54), possibly through increased release of BDNF (111). Postsynaptic CaMKII has also been implicated (112). (*b*) (*Left*) Single-action-potential-induced presynaptic calcium transients from cultured hippocampal neurons measured by SyGCaMP2 under control conditions and after prolonged TTX or gabazine treatment (average ± SEM; scale bars: 0.1 $\Delta F/F_0$, 0.5 s). (*Right*) Neurotransmitter release probability (Pr, based on the synaptic vesicle fusion reporter SypHy) as a function of calcium transient peak amplitude for the indicated conditions (45). Panel *b* modified from Reference 45. (*c*) SynaptopHluorin fluorescence change induced by continuous 10-Hz stimulation in the presence of bafilomycin (BAF) normalized to the maximal fluorescence change induced by NH_4Cl treatment under control conditions (*left*) and after prolonged TTX treatment (*right*) (42).

been implicated in presynaptic homeostasis. Loss or inhibition of CDK5 potentiates presynaptic release by promoting calcium influx and enhancing access to a recycling pool of synaptic vesicles (42, 77). Chronic activity suppression phenocopies these effects and decreases synaptic CDK5, implying a causal link (42). The activity of CDK5 is balanced by calcineurin A, and together these molecules act via the $Ca_V2.2$ channel (77). CDK5 has also been implicated in presynaptic homeostasis at the hippocampal mossy fiber synapse following activity blockade, indicating a generalized mechanism (44). CA3 pyramidal neuron paired recordings revealed that activity blockade causes a reallocation of synaptic weights, strengthening some connections and weakening others in a CDK5-dependent manner (44). Enhanced synaptic strength occurred through increased presynaptic release probability without a change in quantal size. These effects are considered homeostatic at the level of network stability (44). Remarkably, the CDK5/calcineurin-dependent modulation of presynaptic release has sufficient signaling capacity to cause the silencing and unsilencing of individual active zones in hippocampal cultures (77). There are several other examples in which activity perturbation leads to apparent changes in functional synapse number (43, 47), and CDK5 may be involved.

Analysis of presynaptic homeostasis in vitro also centers on the presynaptic matrix of synaptic proteins involved in the control of vesicle secretion. Prolonged glutamate receptor blockade in hippocampal cultures increased RIM levels at a subset of synapses, whereas overall RIM expression decreased (78). Conversely, prolonged exposure to elevated extracellular potassium concentration decreased RRP size and synaptic RIM levels (79). Thus, homeostatic signaling may affect synaptic RIM levels, as well as the levels of other presynaptic matrix–associated proteins (15). A current model suggests the activity-dependent modulation of the presynaptic ubiquitin proteasome system (15).

Another lead is the identification of *miR-485*, which is expressed in both the hippocampus and the cortex (33). The data indicate that *miR-485* is regulated by activity and is required for an adaptive change in synapse number following chronic activity blockade (33). Although there are many downstream targets, control of SV2 has been highlighted as an important link to this homeostatic response (33). Finally, one of the earliest mechanistic insights into presynaptic homeostasis was the observation that chronic inhibition of glutamate receptor function in hippocampal cultures decreases synaptophysin/synaptobrevin complexes assayed by immunoprecipitation (80). This observation was followed by the demonstration that a peptide that inhibits the binding of synaptophysin to synaptobrevin dramatically increases spontaneous vesicle release in control cultures but that this effect is blocked in cultures incubated in glutamate receptor antagonists (80). A remaining challenge will be to determine whether these emerging signaling systems confer quantitatively accurate homeostatic modulation, as has been observed at the NMJ. This will require the use of manipulations that perturb, rather than completely block, neuronal function and experimental paradigms that can follow a change in neurotransmitter release over time. Notably, such an experiment has been achieved using sparse expression of the Kir2.1 channel in cultured mammalian neurons, where a correlation exists between the emergence of enhanced presynaptic release and the restoration of neuronal firing properties (37).

POSTSYNAPTIC MECHANISMS: SCAFFOLDS, SENSORS, AND SIGNALING

The evidence is clear at the NMJs of flies and mice that postsynaptic disruption of neurotransmitter receptor function is coupled to a homeostatic change in presynaptic release and that parallels exist at mammalian central synapses (1). As such, there must be postsynaptic signaling systems in place

that can detect the perturbation and relay this information to the presynaptic terminal. At the *Drosophila* NMJ, several postsynaptic proteins have been implicated in the homeostatic control of presynaptic neurotransmitter release (**Figure 3**). Perturbing CaMKII function in muscle cells disrupted the homeostatic modulation of release in a *GluRIIA* mutant background (81). There is also evidence for an involvement of TOR/S6K signaling. Loss-of-function mutations in TOR and S6K prevented the homeostatic modulation of presynaptic release in a *GluRIIA* mutant background (55). In addition, postsynaptic overexpression of either TOR or a constitutively active S6K was sufficient to increase presynaptic release, but these manipulations did not further enhance release when TOR or S6K was postsynaptically expressed in the *GluRIIA* mutant (55). The authors went on to provide evidence that the essential function of TOR signaling is through enhanced protein synthesis, as demonstrated by showing that expression of a TOR-independent form of 4E-BP impaired homeostatic plasticity (55). Important questions remain. Because muscle-specific expression of a constitutively active S6K does not enhance release beyond that observed in *GluRIIA* alone, mechanisms must be in place to limit the effect of this retrograde signaling system. In addition, loss of S6K does not appear to impair the rapid, PhTx-mediated induction of homeostatic plasticity (82), a protein synthesis–independent process. Therefore, some other system must detect an acute perturbation and instruct the initial, rapid homeostatic potentiation of release.

An intriguing possibility is that the TOR/S6K signaling system represents a metabolic sensor that detects prolonged changes in synaptic transmission. This idea is consistent with experiments implicating a function for TOR-dependent signaling downstream of AMPA receptor blockade in mammalian neurons (54). The potential importance of this signaling system for homeostasis in vivo is emphasized in experiments demonstrating that TOR signaling is essential for balanced network excitation and inhibition in mammals (83). In many systems, TOR signaling is used to detect quantitative changes in the cellular environment and, thereby, regulates cellular homeostasis and growth (84). As such, it is a candidate for detecting quantitative changes in neural function underlying the homeostatic modulation of presynaptic release.

Finally, at the *Drosophila* NMJ, several additional loss-of-function mutations (**Figure 3*a***), acting in postsynaptic muscle, influence baseline presynaptic release probability. Examples include mutations in the nuclear import gene *importin13* (85), in the genes encoding components of the Dystrophin/Dystroglycan protein complex (86), and in genes encoding Rho-GAP/Cdc42-mediated signaling (87); see Reference 13 for a more extensive review of these signaling systems. However, until these signaling systems are tested in the background of a homeostatic challenge, it is not possible to ascribe an activity that is directly related to homeostatic plasticity.

TRANSSYNAPTIC HOMEOSTATIC SIGNALING

One of the most remarkable features of presynaptic homeostasis is that a signal from muscle to nerve is required for accurate modulation of presynaptic release, precisely offsetting the magnitude of the postsynaptic perturbation. Either quantitative information is conveyed in the retrograde signal, or the signaling system is under feedback control such that the magnitude or the effect of the retrograde signal is quantitatively controlled. Bone morphogenetic protein (BMP)-dependent signaling couples the growth of the postsynaptic muscle cell to the growth of the presynaptic motoneuron (88) in a manner that is consistent with the trophic theory of neural development (89). Current evidence supports the conclusion that BMP signaling is a permissive requirement for homeostatic plasticity (30). If BMP signaling is impaired at the level of the motoneuron nucleus but persists locally at the NMJ, where ligand-receptor binding occurs, then the rapid induction of synaptic homeostasis is prevented. Thus, the action of BMP signaling at the NMJ is

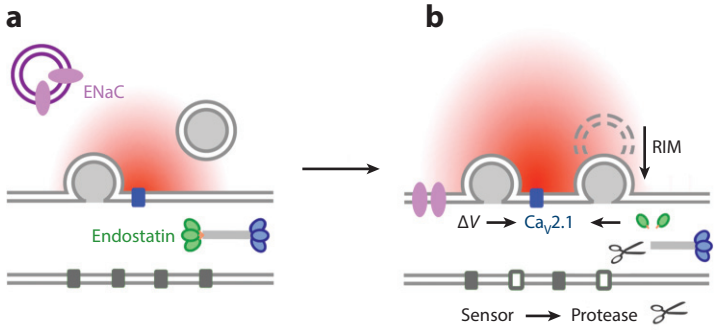

Figure 4

Emerging model of presynaptic homeostasis at the *Drosophila* neuromuscular junction. The model is based on mutations that regulate either presynaptic calcium influx or readily releasable pool (RRP) size. (*a*) At baseline, ENaC channels are sequestered intracellularly (*pink ovals* and *purple vesicle*). (*b*) The induction of presynaptic homeostasis includes the insertion of ENaC channels (*pink ovals*) into the membrane, causing a modest depolarization of the resting membrane potential (ΔV) that, subsequently, potentiates presynaptic calcium influx (*red*) (56). Signaling from muscle to nerve includes the action of an unknown protease that cleaves the matrix protein Multiplexin, releasing Endostatin, which is a transsynaptic signal necessary for the potentiation of presynaptic calcium influx (see Reference 90). Rab3-interacting molecule (RIM) is necessary for the potentiation of the RRP of synaptic vesicles, coupling increased calcium influx through $Ca_V2.1$ channels (57) to increased neurotransmitter release (52).

insufficient (30). The possibility that BMPs have an essential, permissive, target-derived function to gate homeostatic plasticity is interesting, as such a function may underlie developmental, cell type–specific, or age-dependent changes in the expression of presynaptic homeostasis.

Another candidate transsynaptic signal was recently identified in the aforementioned forward genetic screen for PhTx-dependent homeostatic signaling genes. Mutations in the *multiplexin* gene were identified that block both the rapid induction and the sustained expression of presynaptic homeostasis (90). *Multiplexin* is the *Drosophila* homolog of human Collagens XV and XVIII, matrix molecules that are expressed in vascular and epithelial basement membranes throughout the body (91). The C terminus of Collagen XVIII, encoding an Endostatin domain, can be cleaved proteolytically (92, 93), and Collagen XVIII functions as an antiangiogenesis factor to inhibit tumor progression (94–96). At the *Drosophila* NMJ, *multiplexin* appears to control the abundance of presynaptic calcium channels, suggesting a matrix–calcium channel interaction similar to that shown for Laminins at the mammalian NMJ (97). Remarkably, the Endostatin domain of Multiplexin appears to be specifically required for the homeostatic modulation of presynaptic calcium influx, independently of effects on baseline calcium channel number (90). A current model suggests that the proteolytic cleavage of Multiplexin releases Endostatin at the synaptic cleft, where it is a transsynaptic signal for presynaptic homeostasis (**Figure 4**). Many questions remain, including whether Endostatin can encode the quantitative information necessary for accurate compensation. In addition, the model predicts that presynaptic homeostasis is initiated by the activity of a synaptic protease to drive the cleavage of Multiplexin and the release of Endostatin. This idea is consistent with recent evidence that transsynaptic linkages mediated by Neurexin-Neuroligin receptor-ligand signaling are controlled by the activity-dependent action of a matrix metalloprotease (98). Numerous transsynaptic protein-protein interactions, including those mediated by Elfn1 (99) and NGL2 (100), influence baseline presynaptic release. As of the writing of this review, however, there is little evidence for a link between these transsynaptic interactions and presynaptic homeostasis (but see Reference 101).

GENES IMPLICATED IN BOTH HOMEOSTATIC PLASTICITY AND NEUROLOGICAL DISEASE

There are emerging molecular parallels between homeostatic plasticity and neurological disease that revolve around genes that, when mutated, block homeostatic plasticity in a model system and that have been linked to disease in human populations (16). For example, mutations in the schizophrenia-associated gene *dysbindin* block presynaptic homeostasis in *Drosophila* (27). A *Drosophila* model of spinal muscular atrophy includes failure of persistent presynaptic homeostasis (102). In mammals, signaling of Homer and mGluR, both of which are required for the homeostatic control of neurotransmitter receptor abundance, is implicated in mouse models of fragile X syndrome (103), as is retinoic acid (104). Others have speculated about an involvement of disrupted homeostatic signaling in posttraumatic epilepsy (29, 105), Rett syndrome (106), and autism spectrum disorders (107). An important consideration is whether the loss of homeostatic plasticity causes disease. An alternative is that the loss of homeostatic signaling sensitizes the nervous system to the effects of disease, speeding disease onset or progression. The latter hypothesis is supported by data showing that homeostatic plasticity at the mouse NMJ is blocked by mutations in muscle-specific kinase (MuSK) (108). Interestingly, one form of MG is caused by MuSK mutations, and patients harboring these mutations progress to paralysis more rapidly compared with patients with other forms of MG in which homeostatic plasticity remains intact (108). Homeostatic signaling systems are ubiquitous in animal physiology, and disruption of these systems causes disease (68). As such, causal links between homeostatic neuronal plasticity and disease seem likely, but such discoveries will await clear molecular definition of the homeostatic signaling pathways in the nervous system.

CONCLUSIONS

There has been remarkable progress characterizing the cellular processes and molecular mechanisms underlying the homeostatic modulation of presynaptic release. It is now clear that there are fundamental similarities in the mechanisms of presynaptic homeostasis at synapses in organisms as diverse as *Drosophila*, mice, and humans. It stands to reason that emerging molecular mechanisms will be broadly conserved. Despite this progress, many questions remain unanswered. How are perturbations detected, and how is this signaling information converted, via some of the recently identified homeostatic plasticity genes, into an accurate modification of the presynaptic release apparatus? There is evidence for homeostatic depression of presynaptic neurotransmitter release (109), but there is no mechanistic insight into this form of presynaptic homeostasis or how bidirectional control might be achieved. Given the diversity of cell types in the nervous system, many of which express unique synaptic properties, how are homeostatic signaling systems tailored to sustain the function of different cell types? How do the mechanisms of presynaptic homeostasis interface with widespread, activity-dependent plasticity? Answers will be facilitated by new cellular and molecular insights driven by unbiased discovery. Yet, with an increasing number of homeostatic plasticity genes identified, it seems equally important to define the biochemical processes that describe how individual proteins are organized into a signaling system capable of homeostatic control of presynaptic neurotransmitter release.

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