Homeostatic plasticity at the Drosophila neuromuscular junction

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Abstract

In biology, homeostasis refers to how cells maintain appropriate levels of activity. This concept underlies a balancing act in the nervous system. Synapses require flexibility (i.e. plasticity) to adjust to environmental challenges. Yet there must also exist regulatory mechanisms that constrain activity within appropriate physiological ranges. An abundance of evidence suggests that homeostatic regulation is critical in this regard.

In recent years, important progress has been made towards identifying molecules and signaling processes required for homeostatic forms of neuroplasticity. The *Drosophila melanogaster* third instar larval neuromuscular junction (NMJ) has been an important experimental system in this effort. Drosophila neuroscientists combine genetics, pharmacology, electrophysiology, imaging, and a variety of molecular techniques to understand how homeostatic signaling mechanisms take shape at the synapse. At the NMJ, homeostatic signaling mechanisms couple retrograde (muscle-to-nerve) signaling with changes in presynaptic calcium influx, changes in the dynamics of the readily releasable vesicle pool, and ultimately, changes in presynaptic neurotransmitter release.

Roles in these processes have been demonstrated for several molecules and signaling systems discussed here. This review focuses primarily on electrophysiological studies or data. In particular, attention is devoted to understanding what happens when NMJ function is challenged (usually through glutamate receptor inhibition) and the resulting homeostatic responses. A significant area of study not covered in this review, for the sake of simplicity, is the homeostatic control of synapse growth, which naturally, could also impinge upon synapse function in myriad ways.

1. The Drosophila NMJ exhibits robust homeostatic control of synaptic function

Biological systems require physiologically tolerable ranges of activity. By definition, homeostatic mechanisms must respond to challenges that could push activity outside of acceptable ranges. The nervous system is no exception. At the level of synapses and circuits, examples of robust homeostatic plasticity have been characterized by many labs employing a panoply of invertebrate and vertebrate experimental systems (Davis, 2006; Marder, 2012; Marder and Goaillard, 2006; Perez-Otano and Ehlers, 2005; Pozo and Goda, 2010; Turrigiano, 2012; Turrigiano, 2008). A major task is to delineate the signaling processes that drive synaptic homeostasis – and to identify which ones are universally conserved. Based on

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current progress, studies at the *Drosophila melanogaster* neuromuscular junction (NMJ) promise to be valuable.

The Drosophila NMJ is a glutamatergic synapse that is readily accessible for electrophysiological analysis (Jan and Jan, 1976a, b) (Figure 1A). The NMJ exhibits a strong homeostatic response to changes in excitability (Figure 1B). The main type of perturbation or "homeostatic challenge" employed at this synapse is impairment of postsynaptic glutamate receptor function. Pioneering studies in the 1990s demonstrated that deletion of a Drosophila glutamate receptor subunit gene (*GluRIIA*) (DiAntonio et al., 1999; Petersen et al., 1997) and muscle-specific expression of constitutively active Protein Kinase A (PKA) (Davis et al., 1998) greatly diminish muscle response to single vesicles of glutamate (quantal size). The NMJ responds to this decrease in quantal size with a robust, homeostatic increase in presynaptic neurotransmitter release (quantal content). This increase in quantal content restores evoked muscle responses to normal levels (Davis et al., 1998; DiAntonio et al., 1999; Petersen et al., 1997) (Figure 1B). PKA-mediated diminishment of quantal size requires GluRIIA-containing receptors, suggesting that both genetic perturbations act through the same mechanism (Davis et al., 1998).

The *GluRIIA* and *GluRIIB* glutamate receptor subunit genes are redundant for fruit fly viability, but the corresponding gene products behave differently electrophysiologically (DiAntonio et al., 1999). Decrease in quantal size – and therefore homeostatic pressure to increase presynaptic glutamate release – appears to occur specifically when GluRIIA-containing receptors are eliminated or reduced (DiAntonio et al., 1999; Petersen et al., 1997). Of note, when levels of the essential GluRIII (also known as GluRIIC) glutamate receptor subunit are lowered, one also observes greatly diminished quantal size, accompanied by enhanced quantal content (Marrus et al., 2004).

Similar results have corroborated these original findings. Postsynaptic factors that ensure proper levels of glutamate receptor clustering reveal the homeostatic properties of the NMJ. For example, loss-of-function mutations in Drosophila *p21 activated kinase* (*Pak*) (Rho-type GTPase effector gene) (Albin and Davis, 2004), mutations in *dorsal* and *cactus* (NFκB and IκB genes) (Heckscher et al., 2007), or loss of the translational repressor gene *nanos* (Menon et al., 2009) all diminish glutamate receptor clusters at the NMJ. In each case, the NMJs of mutants show reduced quantal size, coupled with homeostatically increased quantal content (Albin and Davis, 2004; Heckscher et al., 2007; Menon et al., 2009).

However, there are exceptions, and these exceptions may lead to new information about how homeostatic signals are propagated. For instance, the Drosophila Neto protein (Neuropilin and Tolloid-like) is required to cluster glutamate receptors at the NMJ (Kim et al., 2012). Hypomorphic *neto* mutant larvae can survive, but by electrophysiology, their NMJs display dramatic decreases in quantal size (Kim et al., 2012). However, there is no associated homeostatic increase in quantal content (Kim et al., 2012). One possibility that could explain these results is that Neto-mediated signaling processes are required for *both* proper glutamate receptor clustering and for propagation of retrograde homeostatic signals. Follow-up experiments are needed to test this idea.

2. Rapid synaptic homeostasis by pharmacology

Genetic impairments of glutamate receptor function last throughout development. For a third instar Drosophila larva raised at 25° C, this developmental process takes less than a week. But how quickly does the NMJ respond to a challenge to its glutamate receptor function? Is it a process that must take place over a period of days? Or is it more rapid? To measure the

speed of homeostatic responses, it has been helpful to exploit pharmacological reagents that challenge synapse function on a short timescale.

It is known that invertebrate glutamate receptor function can be blocked with drugs such as Argiotoxin-636, which is derived from spider toxin (Broadie and Bate, 1993; DiAntonio et al., 1999; Jackson and Usherwood, 1988; Jarecki and Keshishian, 1995; Zhong and Pena, 1995). Another effective glutamate receptor blocker is Philanthotoxin-433 (PhTx), a non-competitive, open channel blocker derived from wasp venom (Eldefrawi et al., 1988; Karst and Piek, 1991). Injections of sub-blocking concentrations of PhTx into third instar Drosophila larvae paralyzes them for about 30 minutes, but then they recover the ability to move (Frank et al., 2006). Electrophysiological analyses of the NMJs of injected animals reveal decreased quantal size with homeostatically increased quantal content (Frank et al., 2006), reminiscent of *GluRIIA* mutant NMJs (DiAntonio et al., 1999; Petersen et al., 1997).

The fact that PhTx-injected larvae regain movement and show robust evoked electrophysiological responses on a time scale of minutes (as opposed to hours or days) points to a rapid system of compensation at the NMJ. Acute toxin exposure bears this out: Direct applications of PhTx-433, PhTx-343, and the spider neurotoxin NSTX-3 to dissected NMJ preparations reveal that significant homeostatic compensation can occur within ten minutes; and pharmacogenetic data suggest that PhTx works by specifically impairing GluRIIA-containing receptors (Frank et al., 2006). Surprisingly, this rapid form of homeostatic compensation can occur in the absence of either protein translation or evoked neurotransmission (Frank et al., 2006).

If evoked neurotransmission is not required, then what is triggering the homeostatic signaling process to begin? One possibility is that the approximately 2000 spontaneous miniature release events that occur in ten minutes of PhTx exposure communicate sufficient information (Frank et al., 2006). The idea that spontaneous miniature events could shape the time course and robustness of homeostatic signaling mirrors data from rodent hippocampal neurons and slices in which NMDA receptor blockade induces a rapid form of synaptic scaling (Sutton et al., 2006). However, one notable difference is that rapid homeostatic scaling in the rodent system proceeds via a protein-synthesis-dependent mechanism (Sutton et al., 2006).

Do the acute (PhTx) and chronic (*GluRIIA* mutant) challenges to neuromuscular function induce homeostatic increases in quantal content through identical signaling mechanisms? This question is still being investigated, but it seems unlikely. Several factors appear to control the sustained expression of synaptic homeostasis throughout life, but not the rapid induction of it. The existence of such molecules likely means that some signaling events need to occur rapidly in response to an acute homeostatic challenge – and then over time, additional factors are required for the NMJ to consolidate that response.

3. Alternative modes of triggering homeostatic signaling mechanisms

Is there only one way to induce a homeostatic response at the Drosophila NMJ – postsynaptic inhibition of glutamate receptors that results in increased presynaptic release? No. There are several perturbations that do not target the glutamate receptors directly but nevertheless induce homeostatic forms of compensation.

A manipulation independent of glutamate receptor function is general impairment of muscle excitability. Postsynaptic expression of the Kir2.1 potassium channel impairs muscle depolarization (Paradis et al., 2001). Kir2.1-expressing muscles have severely reduced input resistance and hyperpolarized resting potentials (Paradis et al., 2001). Increased quantal content compensates for reduced muscle excitability, reminiscent of *GluRIIA* mutant NMJs;

however, in contrast to *GluRIIA* mutant NMJs, voltage clamp recordings reveal that there does not appear to be any measurable deficit in glutamate receptor function in the Kir2.1 muscles (Paradis et al., 2001).

Another perturbation is to alter the amount of innervation that individual muscles receive. This has been achieved by misexpressing the cell adhesion molecule Fasciclin II (Davis et al., 1997; Grenningloh et al., 1991; Lin and Goodman, 1994). In an elegant set of experiments, electrophysiological recordings were performed on two neighboring muscles that were either hyper-innervated or hypo-innervated due to misexpression of Fasciclin II (Davis and Goodman, 1998). Single bouton recordings were also performed. Despite having dramatically increased numbers of synaptic boutons, hyper-innervated muscles exhibit normal electrophysiological properties because the probability of release at single boutons is homeostatically downregulated (Davis and Goodman, 1998). By contrast, recordings from hypo-innervated muscles reveal increases in quantal size, accompanied by reduced quantal content (Davis and Goodman, 1998). In sum, the Drosophila NMJ is capable of homeostatically responding to multiple perturbations of function – not simply the loss of glutamate receptor function – and possibly through a diverse array of mechanisms.

4. Is it possible to downregulate neurotransmitter release by homeostatic mechanisms

Decreased glutamate receptor function (*GluRIIA* mutation or PhTx) and decreased muscle excitability (Kir2.1 overexpression) are sufficient to induce homeostatic increases in presynaptic glutamate release. Is the opposite true? Do *increases* in quantal size directly correlate with *decreases* in quantal content at the Drosophila NMJ? This appears to be the case with hypo-innervated muscles (Davis and Goodman, 1998), but more generally, do insect neuromuscular synapses have built-in safeguards against excessive neurotransmission? The data are mixed.

One example of homeostatic downregulation of synaptic function involves overexpression of the Drosophila vesicular glutamate transporter (DVGLUT) in motor neurons (Daniels et al., 2004). This is a presynaptic perturbation that results in glutamatergic vesicles of increased size (by electron microscopy) (Daniels et al., 2004). Electrophysiologically, there is a significant increase in quantal size (~ 60%). This increase in quantal size is offset by a homeostatic decrease in quantal content, and evoked responses are normal (Daniels et al., 2004).

Similar phenotypes (increased vesicle size, increased quantal size, and decreased quantal content) also result from loss-of-function mutations in synaptic vesicle endocytosis genes such as *endophilin* (Dickman et al., 2005; Verstreken et al., 2002), *synaptojanin* (Dickman et al., 2005; Verstreken et al., 2003), *AP180* (Bao et al., 2005; Zhang et al., 1998), and *Dap160* (Koh et al., 2004; Marie et al., 2004). Disrupted clathrin-mediated endocytosis or impaired retrieval of vesicles from bulk membrane cisternae could be responsible for the large synaptic vesicles (and hence, large quantal size). But what mechanisms are responsible for the decreases in quantal content? The answer is not entirely clear. Indeed, there are confounding issues caused by endocytic mutations, such as abnormal synaptic bouton growth (Koh et al., 2004; Marie et al., 2004; Verstreken et al., 2002), mislocalized or decreased amounts of other synaptic proteins (Bao et al., 2005; Koh et al., 2004; Marie et al., 2004; Verstreken et al., 2003; Zhang et al., 1998), and – in the case of *AP180* – quantal content that is decreased so severely that evoked responses are far below normal (Bao et al., 2005). Nevertheless, several endocytic mutant NMJs have normal or only mildly diminished evoked responses to single stimuli (Koh et al., 2004; Marie et al., 2004; Verstreken et al.,

2002; Verstreken et al., 2003). Therefore, as with DVGLUT overexpression, it is possible that the underlying decreases in quantal content are homeostatic in nature.

Collectively, these data suggest that the Drosophila NMJ has built-in mechanisms to downregulate presynaptic release after increases in quantal size. Yet this is not always the case. As counterexamples, increases in GluRIIA expression (relative to GluRIIB expression) and postsynaptic inhibition of PKA function cause *increases* in quantal size with *no corresponding decreases* in quantal content (Davis et al., 1998; DiAntonio et al., 1999; Petersen et al., 1997).

Questions abound. What is the difference between the increases in quantal size driven by postsynaptic GluRIIA/GluRIIB ratios and the increases in quantal size induced by DVGLUT activity or endocytosis mutations? Could the NMJ respond differently depending upon whether the original perturbation is pre- or postsynaptic? One idea that has been postulated is that glutamate in the synaptic cleft serves as a trigger that drives down presynaptic release (Daniels et al., 2004). Such a mechanism would imply that excessive cleft glutamate (or spillover glutamate) signals through glutamate receptors that are distinct from the postsynaptic GluRIIA-containing receptors. Consistent with this idea, loss of presynaptic group II metabotropic glutamate receptors at the NMJ results in increased presynaptic excitability (Howlett et al., 2008). The concept of synaptic tissues detecting "extra" neurotransmitter as a signal that eventually drives homeostatic changes is not unprecedented. For example, in mammalian hippocampal neurons, homeostatic synaptic scaling is mediated in part by glial-derived TNFα signaling, and glial detection of spillover glutamate is a key trigger of this signaling paradigm (Stellwagen and Malenka, 2006).

5. Presynaptic molecules and targets of homeostatic signaling at the NMJ

In response to postsynaptic decrease in quantal size, there is a homeostatic increase in presynaptic release at the Drosophila NMJ. How does this process work? What is the sensor that detects an "error" in the system? What is (are) the retrograde signal(s)? What are the signaling targets? In recent years, genetic approaches have been tremendously beneficial in helping to start to answer these questions. So far, many of molecules identified that are required for homeostatic signaling at the Drosophila NMJ have been placed on the presynaptic side of the synapse. They are reviewed in this section. Postsynaptic factors and retrograde signaling processes are examined in subsequent sections. A summary of many molecules discussed is in Table 1, and a collective cartoon model is depicted in Figure 2.

Ca$_V$2-type calcium channels, enhanced calcium influx, and presynaptic active zones

Ubiquitous in metazoan nervous systems, presynaptic voltage-gated N-, P/Q-, and R-type calcium channels are critical for many types of rapid neurotransmission, and their regulation has been an intense area of study (Catterall, 2000; Currie, 2010; Dolphin, 2009; Tedford and Zamponi, 2006). Human mutations in the Ca$_V$2.1 α1 channel subunit gene *CACNA1A* are associated with forms of migraine and ataxia (Ophoff et al., 1996; Pietrobon, 2010; Zhuchenko et al., 1997). Since synaptic homeostasis is the process by which stable synaptic activity is maintained, an attractive idea is that this can be achieved, in part, by tight regulation of presynaptic calcium channel function. Conversely, misregulation of calcium channels could lead to neuronal instability and neurological disorders such as migraine and ataxia.

The Drosophila α1 subunit of Ca$_V$2-type calcium channels is encoded by the gene *cacophony* (*cac*) (Smith et al., 1996). Null *cac* mutants do not survive past the late embryonic stage of development (Kulkarni and Hall, 1987; Kurshan et al., 2009), but there are several hypomorphic, missense mutants that are viable and fertile (Brooks et al., 2003;

Kawasaki et al., 2000; Smith et al., 1998); furthermore, it is possible to remove one copy of *cac* and have viable animals. Partial loss of *cac* function blocks the homeostatic increase in presynaptic release at the NMJ after glutamate receptor impairment (Frank et al., 2006; Frank et al., 2009). This result is independent of *cac* baseline neurotransmission defects, and it is consistent with the idea that functional, intact Ca_V2 channels are needed to properly upregulate neurotransmission after a homeostatic challenge (Frank et al., 2006; Frank et al., 2009).

How does this process work? This is an intense area of study. Recent work illustrates that researchers can visualize homeostatic changes at the synapse. Imaging of presynaptic Ca^{2+} transients during evoked stimulation shows that *GluRIIA* deletion (or PhTx application) induces homeostatic, presynaptic increases in calcium influx (Müller and Davis, 2012). By contrast, double mutant cac^S; *GluRIIA* NMJs (and cac^S NMJs exposed to PhTx) show no more presynaptic calcium influx than cac^S mutants alone (Müller and Davis, 2012). In addition, a combination of electrophysiology and imaging has shown that PhTx application results in a rapid increase in the quantity of presynaptic active zone protein, Bruchpilot (Brp) (an ELKS/CAST homolog), an increase in the size of the presynaptic cytomatrix structure, and an increase in the size of the readily releasable pool (RRP) of presynaptic vesicles (Weyhersmüller et al., 2011). Therefore, an increase in presynaptic calcium influx, an increase in the size of the RRP, or both could facilitate increased glutamate release at the Drosophila NMJ (Figure 1B).

The miR-310 micro RNA cluster and the kinesin family member Khc-73

The above results spur another question: What are the signaling processes that control Ca_V2 function, enhanced presynaptic calcium influx, and enhanced RRP size? The answers are not clear, but molecules and regulatory systems that could control these aspects of synaptic homeostasis are subjects of active discovery. One such regulatory system is the miR-310 cluster of four microRNAs (miRNAs).

Genomic deletion of the miR-310 cluster results in markedly enhanced baseline NMJ neurotransmission, as well as a significant increase in the quantity of presynaptic Brp expression (Tsurudome et al., 2010). These phenotypes suggest that presynaptic calcium levels could be increased. Direct measurement of calcium transients confirms this hypothesis (Tsurudome et al., 2010). miRNAs regulate target gene expression, and in the Drosophila genome, there could be many miR-310 cluster targets. In the context of NMJ neurotransmission, the most important regulatory target appears to be the *Khc-73* gene, which encodes a kinesin super family member. Partial loss of *Khc-73* function can suppress the effects of miR-310 deletion, while neuronal overexpression of a *Khc-73* transgene phenocopies miR-310 loss (Tsurudome et al., 2010). Importantly, miR-310 cluster overexpression and *Khc-73* gene knockdown both block homeostatic compensation in response to a *GluRIIA* deletion (Tsurudome et al., 2010).

How is presynaptic Khc-73 exerting control over calcium influx, neurotransmission, and ultimately, synaptic homeostasis? The answer is not known, but a compelling hypothesis is that as a kinesin, Khc-73 helps to traffic synaptic active zone proteins (or other crucial synaptic proteins), resulting in the potentiation of Ca_V2 function (Tsurudome et al., 2010). This hypothetical function of Khc-73 could be similar to functions described for a Drosophila kinesin 3 family member, Unc-104/Imac (Barkus et al., 2008; Pack-Chung et al., 2007). There is no direct evidence that Khc-73 participates in axon transport or interacts directly with active zone components (Tsurudome et al., 2010), however, it has been shown to associate with microtubules (Kner et al., 2009).

Eph Receptor-Ephexin signaling

What other signaling processes could be important for regulating Ca_V2 function? Genetic and electrophysiological data suggest that a signaling system consisting of the Eph receptor tyrosine kinase, the Rho-type guanine exchange factor Ephexin, and Rho-type GTPases (Rho1, Rac1, and Cdc42) functions upstream of Ca_V2/Cacophony in regulating synaptic homeostasis (Frank et al., 2009). Loss of function of some of these factors alone (e.g. *Ephexin*) or in double heterozygous genetic combination with each other (e.g. *Cdc42*/+; *Ephexin*/+ or *cac*/+; *Ephexin*/+) results in a complete block of *GluRIIA* deletion-induced synaptic homeostasis (Frank et al., 2009). In the case of *Ephexin* mutants, this block can be partially ameliorated by overexpressing a transgenic copy of *cac* (Frank et al., 2009; Kawasaki et al., 2004).

As with miR-310 and Khc-73, it is unknown precisely how Ephexin and related signaling molecules might impinge upon Ca_V2 function. They may facilitate the trafficking of additional channels to the synapse, or they may mediate the modulation of channels already present at the synapse (Frank et al., 2009). Furthermore, Ephexin could work in conjunction with, or in parallel to, Khc-73-mediated processes. What we do know is that in other biological systems, pre- and postsynaptic Ephexin family members canonically signal through Rho-type GTPases to mediate neurite, growth cone, or dendritic spine collapse and neuromuscular development (Fu et al., 2007; Knoll and Drescher, 2004; Sahin et al., 2005; Shamah et al., 2001; Shi et al., 2010a; Shi et al., 2010b). It is intriguing to speculate that components of this conserved developmental signaling process might be co-opted by the NMJ to modulate presynaptic neurotransmitter release. Since Drosophila Eph receptor and Ephexin can bind one another, important muscle-to-nerve information may be transduced through Eph receptor tyrosine kinase (Frank et al., 2009). Notably, Ephexin is dispensable for rapid homeostatic compensation (PhTx application) but is required for long-term homeostatic compensation (*GluRIIA* deletion) (Frank et al., 2009). This result suggests that there are important mechanistic differences between the induction process of synaptic homeostasis and its long-term maintenance and consolidation.

BLOC-1 complex and SNARE complex members

An electrophysiology-based screen was conducted by applying PhTx to Drosophila NMJs carrying mutations in neuronally expressed genes. This screen revealed that the schizophrenia susceptibility gene *dysbindin* is critical for homeostatic plasticity at the Drosophila NMJ (Dickman and Davis, 2009). Dysbindin functions presynaptically, where it associates with synaptic vesicles during homeostatic compensation (Dickman and Davis, 2009). Dysbindin expression levels alter the calcium dependence of synaptic vesicle release. Yet, unlike Ephexin, genetic and electrophysiological analyses do not suggest that Dysbindin acts in conjunction with Ca_V2-type calcium channels in the presynaptic neuron. Rather, Dysbindin appears to act downstream or independently of calcium influx (Dickman and Davis, 2009). This information is consistent with a role for Dysbindin in modulating synaptic vesicle release probability in response to a homeostatic challenge.

Mammalian Dysbindin is a member of the BLOC-1 complex (biogenesis of lysosome-related organelles) (Starcevic and Dell'Angelica, 2004). Work from several systems suggests Dysbindin can influence many cellular processes, including synaptic vesicle biogenesis and exocytosis (Ghiani and Dell'Angelica, 2011; Ghiani et al., 2010). These facts fit well with a role for Dysbindin in the homeostatic upregulation of vesicle release. Importantly, follow-up work at the Drosophila NMJ demonstrates roles in synaptic homeostasis for Snapin (also a BLOC-1 complex member) and for SNAP25 (a component of the SNARE complex and a binding partner of Snapin) (Dickman et al., 2012). An intriguing possibility is that Dysbindin and Snapin coordinately exert control over

homeostatic increases in synaptic vesicle release by modulating SNARE-mediated fusion events (Dickman et al., 2012).

Rab3-GAP, RIM, and the readily releasable vesicle pool

Additional electrophysiology-based screening uncovered an important presynaptic role for Rab3-GAP (Rab3 GTPase Activating Protein) in synaptic homeostasis (Müller et al., 2011). In mammalian systems, both Rab3 GTPase and Rab3-GAP have been implicated in regulating neurotransmission at a late stage of vesicle release (Sakane et al., 2006; Südhof, 2004). At the Drosophila NMJ, electrophysiological data show that presynaptic Rab3-GAP is required for synaptic homeostasis, and that Rab3-GAP relieves an opposing influence on homeostasis that is catalyzed by Rab3 (Müller et al., 2011). But how exactly does this process work? Could Rab3-GAP be acting at the same time point or process as Dysbindin – possibly by regulating SNARE-mediated fusion events?

New, illuminating data have emerged by examining Drosophila RIM (Rab3 interacting molecule). RIMs constitute a conserved family of proteins. Similar to roles described for its mammalian counterparts, Drosophila RIM localizes to the active zone, interacts with Rab3 GTPase molecules, and interacts with calcium channels to help them localize them to the presynaptic active zone (Graf et al., 2012; Müller et al., 2012). Beyond these similarities with mammalian RIM, Drosophila RIM is required for homeostatic compensation (Müller et al., 2012).

Given that RIM helps to localize calcium channels, a simple hypothesis would be that RIM plays some role upstream of Ca_V2 channels in regulating calcium influx during homeostatic compensation. Perhaps RIM constitutes a missing link between Eph/Ephexin signaling (or Khc-73 kinesin activity) and Ca_V2 – helping to traffic more calcium channel components to the active zone to enhance calcium influx. However, *rim* loss-of-function mutants have no difficulty in upregulating calcium influx in response to glutamate receptor inhibition (Müller et al., 2012). This result suggests that the role RIM plays in synaptic homeostasis is independent of its role in Ca_V2 regulation. It was recently demonstrated that the homeostatic increase in vesicle release is correlated with an increase in the size of the readily releasable vesicle pool (RRP) (Müller et al., 2012; Weyhersmüller et al., 2011). Follow-up electrophysiological experiments utilizing high frequency stimulation and EPSC amplitude fluctuation analysis point to a role for RIM in regulating the size of the RRP during homeostatic compensation (Müller et al., 2012). Finally, genetic data suggest that RIM could be performing this function in conjunction with Rab3-GAP (Müller et al., 2012).

Gooseberry transcription factor

Gooseberry (Gsb) – a pair-rule transcription factor – was identified as an essential component of *GluRIIA* mutant-induced synaptic homeostatic compensation (Marie et al., 2010). Gsb has long been known to be important for embryonic neuroblast determination (Skeath et al., 1995), but it is also expressed in mature motor neurons (Marie et al., 2010). Like Ephexin, Gsb is dispensable for the rapid induction of synaptic homeostasis (Marie et al., 2010), demonstrating once again that it is possible to uncouple the short-term induction of homeostatic increases in presynaptic release from the long-term maintenance of those increases.

Interestingly, Gsb assumes an opposing role with Wnt/Wingless (Wg) signaling during embryonic cell fate specification (Bhat et al., 2000; Duman-Scheel et al., 1997). Genetic and electrophysiological data suggest that this antagonistic relationship is maintained during synaptic homeostasis (Marie et al., 2010). In the context of the NMJ, this is an especially intriguing result because Wnt/Wg signaling also plays important roles in NMJ development

(Korkut et al., 2009; Mathew et al., 2005; Miech et al., 2008). This underscores possible links between NMJ developmental processes and long-term maintenance of synapse function.

Potassium channels and a hierarchy of homeostatic signals

An additional protein that emerged from searches for homeostatic factors is Drosophila Shal (Shaker cognate I). Shal is a K_V4-type potassium channel protein, which along with Drosophila Shaker (K_V1) mediates potassium current in the neuronal cell body. *shal* loss-of-function mutants have completely impaired homeostatic responses to PhTx or *GluRIIA* mutant challenges (Bergquist et al., 2010).

At first glance, this result is puzzling. One would expect the loss of a potassium channel to *enhance* the excitability of a cell – and possibly to drive a robust increase in transmitter release. However, in this particular case, quantitative RT-PCR experiments show that *shal* and *Shaker* gene function and expression are inversely coupled (possibly due to a separate homeostatic maintenance of potassium current). Decreased *shal* gene function leads to increased *Shaker* gene expression and *vice versa* (Bergquist et al., 2010). As a result, the increased Shaker protein expressed in *shal* mutants completely impairs the cell's ability to respond to homeostatic challenges. Simple overexpression of Shaker has the same effect: silencing of homeostatic compensation (Bergquist et al., 2010).

Why does overexpression of Shaker block synaptic homeostasis? Channel localization may be the answer. Shal channels are restricted to the cell body (Bergquist et al., 2010; Tsunoda and Salkoff, 1995), whereas Shaker can localize to the synaptic terminal (Ganetzky and Wu, 1982; Jan et al., 1977; Wu et al., 1983). NMJ blockade of Shaker potassium channels using 4-aminopyradine successfully restores homeostatic responses in *shal* mutants (Bergquist et al., 2010). Taken all together, these results highlight the importance of a neuron's overall excitability in conferring competence to respond to a homeostatic challenge.

Survival Motor Neuron 1

An intriguing link has been made between synaptic homeostasis and the Drosophila homolog of Survival Motor Neuron 1 (Smn) protein. Smn family members were previously shown to regulate small nuclear ribonucleoprotein (snRNP) biogenesis (Fischer et al., 1997), and loss of human *SMN1* or *SMN2* function causes spinal muscular atrophy (Coovert et al., 1997; Lefebvre et al., 1997). Several studies of Smn in invertebrate model systems have been conducted to help elucidate the mechanisms by which it acts and to generate invertebrate genetic models of spinal muscular atrophy (Briese et al., 2009; Chan et al., 2003; Chang et al., 2008; Rajendra et al., 2007; Sleigh et al., 2011).

By taking advantage of RNA interference (RNAi)-based tools and tissue specific gene knockdown, it was recently demonstrated that presynaptic Drosophila Smn is required for long-term homeostatic compensation (Timmerman and Sanyal, 2012). Yet, as with Ephexin and Gsb, Smn is dispensable for rapid homeostatic compensation (Timmerman and Sanyal, 2012). These new results raise the intriguing possibility that regulation of small nuclear RNAs or mRNA metabolism could be involved in consolidating long-term homeostatic responses. They also raise possible connections between homeostatic plasticity and disorders like spinal muscular atrophy.

6. Postsynaptic molecules regulating retrograde increases in presynaptic release

The muscle-to-nerve signals regulated by the proteins reviewed in this section increase quantal content, even in the absence of a homeostatic challenge, like *GluRIIA* deletion. These proteins may represent core components (or in some cases, negative regulators) of postsynaptic signaling processes downstream of glutamate receptor inhibition.

Target of Rapamycin

One factor in the muscle that propagates a retrograde signal in order to increase presynaptic release is Drosophila Target of Rapamycin (TOR) (Penney et al., 2012). Postsynaptic overexpression of TOR – or postsynaptic expression of a constitutively active TOR target, S6 Kinase (S6K) – induces increases in presynaptic quantal content (Penney et al., 2012). These results suggest that TOR is capable of propagating an *instructive* signal through S6K to increase presynaptic neurotransmitter release. Is this TOR-induced signal activated when postsynaptic GluRIIA-containing receptors are absent? It appears that it is. Homeostatic compensation is completely blocked in *GluRIIA; Tor* double loss-of-function mutants. Furthermore, when TOR is overexpressed postsynaptically in a *GluRIIA* mutant background, there is no additive effect on release – i.e., there is no further increase in quantal content compared to *GluRIIA* mutants alone (Penney et al., 2012). Collectively, these results suggest that *GluRIIA* mutant-induced forms of synaptic homeostasis proceed through TOR signaling.

Canonically, TOR promotes protein translation – either by phosphorylating S6K, which promotes cap-dependent translation (Holz et al., 2005; Ma and Blenis, 2009), or by inhibiting the activity of the eIF4E binding protein, 4E-BP (Gingras et al., 2001; Sonenberg and Hinnebusch, 2009). At the Drosophila NMJ, could a similar promotion of protein translation result in the production of factors needed for synaptic homeostasis? It appears so. Not only does S6K activation *promote* enhanced presynaptic release, but also, transgenic expression of a TOR-independent form of 4E-BP in the muscle *impairs* homeostatic compensation (Penney et al., 2012). Superficially, these results appear to be in conflict with prior data that rapid homeostatic compensation at the NMJ does not require protein translation (Frank et al., 2006). However, in the present case, the authors were not examining PhTx-induced compensation; instead they were examining *GluIIA* loss-induced compensation. As stated before, it is likely that distinct (or partially overlapping) mechanisms are required for different forms of homeostatic compensation: in this case, rapid, protein translation-independent homeostatic plasticity (PhTx), and long-term, protein translation-dependent homeostatic plasticity (*GluRIIA*).

The fact that Drosophila TOR is required for NMJ homeostasis matches nicely with the fact that TOR has been implicated in forms of synaptic plasticity in mammalian systems (Hoeffer and Klann, 2010; Jaworski and Sheng, 2006). Of particular interest, mammalian Target of Rapamycin (mTOR) has recently been shown to participate in a retrograde form of homeostatic plasticity that results in increased presynaptic release (Henry et al., 2012). This signaling process is gated (at least in part) by presynaptic calcium channel function (Jakawich et al., 2010). These findings mirror salient features of retrograde homeostatic signaling at the Drosophila NMJ (Frank et al., 2006; Penney et al., 2012) and raise the possibility for ancient roles in homeostatic plasticity for factors like TOR and presynaptic calcium channels. However, there are important distinctions. Perhaps most notably, the retrograde signaling system in mammalian neurons proceeds through release of Brain-Derived Neurotrophic Factor (BDNF) (Henry et al., 2012). BDNF and its canonical TrkB receptors are absent from Drosophila.

Dystrophin and Crossveinless-c

Drosophila TOR promotes increased presynaptic release at the NMJ. Loss of TOR blocks *GluRIIA* deletion-induced homeostatic compensation. Are there postsynaptic factors that behave in the opposite way – factors whose loss leads to *increased* presynaptic release and whose activity could be *opposing* TOR? The answer is yes; three Drosophila proteins that fit this description are Dystrophin, the RhoGAP Crossveinless-c (Cv-c), and Importin 13 (Imp13).

Human *dystrophin* mutations cause muscular dystrophy (Hoffman et al., 1987), Dystrophin functions to couple muscle cytoskeleton to the extracellular matrix, which helps to maintain muscle strength and integrity. In addition, Dystrophin interacts with intracellular signaling molecules (Rando, 2001). Genetic model studies of Dystrophin have sought to elucidate how it might regulate signaling processes relevant to disease.

At the Drosophila NMJ, large isoforms of muscle Dystrophin localize at synaptic and extrasynaptic sites (van der Plas et al., 2006). In *dystrophin* mutants that lack these isoforms, NMJ development appears completely normal (van der Plas et al., 2006). By electrophysiology, *dystrophin* mutant NMJ quantal size is normal, but quantal content is significantly increased, resulting in enhanced evoked amplitudes (van der Plas et al., 2006). Postsynaptic Dystrophin rescues this electrophysiological phenotype. Therefore, postsynaptic Dystrophin downregulates presynaptic neurotransmitter release, the opposite result of what has been documented for TOR.

A genetic screen found that *cv-c* loss-of-function mutations interact genetically with *dystrophin* mutations in Drosophila wing cross vein formation (Pilgram et al., 2011). Examination of the *dystrophin/cv-c* genetic relationship at the NMJ showed that postsynaptic loss of *cv-c* phenocopies *dystrophin* loss (normal quantal size, and significantly increased quantal content) (Pilgram et al., 2011). Dystrophin appears to act downstream of Cv-c in the muscle. Postsynaptic overexpression of *dystrophin* partially suppresses the enhanced quantal content phenotype of *cv-c* mutants. By contrast, postsynaptic expression of transgenic *cv-c* does *not* suppress enhanced quantal content of *dystrophin* mutants (Pilgram et al., 2011).

As a RhoGAP, Cv-c negatively regulates Rho-type GTPase function. Interestingly, Rho-type GTPase *Cdc42* loss-of-function mutations partially suppress *cv-c* mutations (Pilgram et al., 2011). Together with studies of the RhoGEF Ephexin, this result potentially places Cdc42 function at *both* pre- and postsynaptic sites in the execution of retrograde homeostatic signaling (Frank et al., 2009; Pilgram et al., 2011).

Importin 13

Importin receptors facilitate transport through nuclear pore complexes. In Drosophila, Imp13 localizes to nuclei in both neurons and muscles (Giagtzoglou et al., 2009). Electrophysiologically, *imp13* loss-of-function mutations phenocopy *dystrophin* and *cv-c* losses of function: normal quantal size, increased quantal content (Giagtzoglou et al., 2009). Expressing transgenic *imp13* in the neurons fails to rescue the enhanced release phenotype, but expressing it in the muscle does rescue (Giagtzoglou et al., 2009). The electrophysiology is corroborated by presynaptic calcium imaging experiments showing increased levels of presynaptic intracellular calcium in *imp13* mutants; this phenotype is also rescued by postsynaptic expression of transgenic *imp13* (Giagtzoglou et al., 2009).

Since loss of postsynaptic *imp13* leads to presynaptic increases in quantal content, a simple model could be that Imp13 somehow controls the expression of the glutamate receptors, which results in retrograde control of presynaptic release. However, quantal size is not

affected in *imp13* mutants, and neither are expression levels of GluRIIA or GluRIIB subunits (Giagtzoglou et al., 2009). Currently, it is unknown how Imp13 may interact with Dystrophin, Cv-c, or, TOR function in the muscle, or even if *imp13* loss of function combined with TOR gain of function has additive or non-additive effects on presynaptic release. Future experiments along these lines could help illustrate how retrograde homeostatic signaling processes take shape in the muscle.

7. What are the retrograde homeostatic signals at the NMJ

It has been clear for a long while that retrograde signaling mechanisms are important for synaptic homeostasis at the NMJ. To date, however, the precise, instructive retrograde signals required for synaptic homeostasis are elusive.

Evidence against canonical retrograde signaling

Retrograde signals abound in mammalian neuronal systems such as endocanabinoids, Brain-Derived Neurotrophic Factor (BDNF), and nitric oxide (NO). Yet these signals do not appear to constitute retrograde homeostatic signals at the Drosophila NMJ. Neither orthologs of cannabinoid receptors, nor BDNF, nor the BDNF TrkB receptor are found in Drosophila. NO is a prominent signaling molecule in insects, but at the Drosophila NMJ, evidence suggests that NO originates in the presynaptic nerve, rather than the postsynaptic muscle. Application of NO donors to the NMJ results in cyclic GMP immunoreactivity presynaptically, but not postsynaptically (Wildemann and Bicker, 1999a, b).

Another prominent retrograde signaling molecule at the Drosophila NMJ is Synaptotagmin 4 (Syt4). Syt4 is reported to act as a postsynaptic calcium sensor that induces a retrograde signaling process controlling spontaneous miniature activity, evoked neurotransmission, and local synapse development (Barber et al., 2009; Yoshihara et al., 2005). The effects on neurotransmission make Syt4 an excellent candidate for controlling synaptic homeostasis. Drastic changes in the frequency of spontaneous release could reflect relevant changes to the presynaptic RRP or release probability. However, a direct test of *Syt4* mutant NMJs for a role in rapid homeostatic compensation did not reveal one (Dickman and Davis, 2009), and to date, it is unclear what (if any) role Syt4 might play in the homeostatic regulation of synapse function.

Bone Morphogenetic Protein signaling

One retrograde system that is required for NMJ homeostasis is Bone Morphogenetic Protein (BMP) signaling. Together, a pair of studies has convincingly demonstrated that the muscle-derived BMP ligand Glass Bottom Boat (Gbb) and its presynaptic receptor Wishful Thinking (Wit) are needed for both rapid (PhTx) and long-term (*GluRIIA* mutant) forms of synaptic homeostasis (Goold and Davis, 2007; Haghighi et al., 2003). Presynaptic Wit appears to act through retrograde axonal transport to activate the Drosophila Mad (Smad) transcription factor (McCabe et al., 2003), and this event is necessary for PhTx-induced synaptic homeostasis (Goold and Davis, 2007). In addition, BMP signaling is required for increases in presynaptic release that are driven either by postsynaptic calcium/calmodulin-dependent protein kinase II (CaMKII) inhibition (Haghighi et al., 2003) or by *dystrophin* mutation (van der Plas et al., 2006). Taken together, these results ideally position BMP signaling to propagate retrograde information to induce increased neurotransmitter release.

Yet current data do not support an instructive role for BMP/Mad signaling during rapid homeostatic compensation. Rather, BMP/Mad function appears permissive. First, severing the segmental motor axons – hence eliminating retrograde transport to the nucleus – does not impair PhTx-induced increases in quantal content (Frank et al., 2006). Second, inhibition

of BMP/Mad signaling at various points in developmental time demonstrates a requirement for signaling well before PhTx application (Goold and Davis, 2007). This latter result was achieved by exploiting the *P[Switch]* expression system, which allows Drosophila researchers exquisite control over both the spatial and temporal expression of target *UAS* transgenes (Osterwalder et al., 2001; Roman et al., 2001). Using this system, expression of a transgenic Mad inhibitor, *UAS-dad,* blocks synaptic homeostasis. The longer during development that *UAS-dad* is expressed, the more severe the block (Goold and Davis, 2007). One day of *UAS-dad* expression leaves rapid, PhTx-induced homeostatic compensation largely in tact, while longer periods of *UAS-dad* expression render a severe block (Goold and Davis, 2007).

At this juncture, it may be premature to conclude that BMP/Mad signaling plays no instructive role during homeostatic compensation at the NMJ. The above results point to a permissive role for BMP/Mad during PhTx-induced homeostatic compensation (Frank et al., 2006; Goold and Davis, 2007). Yet it is quite possible BMP/Mad signaling instructively consolidates long-term homeostatic changes that are needed after *GluRIIA* loss. Furthermore, acute removal of Gbb or Wit (which could test for instructive signaling roles for the BMP ligand and receptor) has not been attempted. Therefore, there exists a formal possibility of instructive, non-Mad-mediated BMP signaling during rapid synaptic homeostasis.

8. Concluding Remarks

The Drosophila NMJ compared with vertebrate models of homeostatic synaptic plasticity

As detailed above, the Drosophila NMJ shares several aspects of homeostatic plasticity with vertebrate central synapses. Retrograde signaling driven by postsynaptic TOR function is prominent for both the Drosophila NMJ and hippocampal synapses (Henry et al., 2012; Penney et al., 2012). The requirement of presynaptic calcium channel function is also an important similarity (Frank et al., 2006; Frank et al., 2009; Jakawich et al., 2010), as is the demonstration that challenges to synaptic function result in increases in presynaptic calcium influx (Müller and Davis, 2012; Zhao et al., 2011). There is also evidence that spontaneous miniature synaptic events shape the time course and robustness of homeostatic responses in Drosophila and mammalian systems (Frank et al., 2006; Sutton et al., 2006).

But how do the ultimate homeostatic expression systems compare between insect and vertebrate synapses? Homeostatic signaling at the Drosophila NMJ redounds to changes in presynaptic quantal content. This fact appears to represent an important difference between the Drosophila NMJ and hippocampal synapses that employ postsynaptic scaling mechanisms to globally strengthen or weaken synaptic efficacy via AMPA-type glutamate receptor addition or subtraction (O'Brien et al., 1998; Turrigiano et al., 1998). However, depending upon the preparation used or the manipulation employed, hippocampal synapses also display homeostatic changes in presynaptic release properties and RRP size, not unlike the Drosophila NMJ (Burrone et al., 2002; Murthy et al., 2001; Thiagarajan et al., 2005).

Concerning peripheral synapses, fascinating similarities exist between the glutamatergic Drosophila NMJ and the cholinergic mammalian NMJ. For example, in the human neuromuscular disorder myasthenia gravis, acetylcholine receptor function is impaired. Electrophysiological recordings show that myasthenic muscle endplates have dramatically reduced quantal size, coupled with increased quantal content (Cull-Candy et al., 1980; Plomp et al., 1995). The same is also true for a rat model of myasthenia gravis induced by α-bungarotoxin (Plomp et al., 1995; Plomp et al., 1992, 1994). At a basic level of homeostatic compensation, these results are strikingly reminiscent of the Drosophila NMJ model.

Challenges for the future

Moving forward, there are several important challenges to address at the Drosophila NMJ. An obvious goal is to identify and characterize the retrograde muscle-to-nerve signals that result in homeostatic increases (or decreases) of neurotransmitter release. It is also necessary to conduct further characterization of the molecules already identified that participate in synaptic homeostasis (Table 1). Which ones act upstream of Ca_V2 to control levels of presynaptic calcium influx? How do they do it? Which ones act upstream or in conjunction with RIM to control RRP size? How do the known molecules connect with each other to form coherent signaling pathways?

A major question for any molecule implicated in synaptic homeostasis is whether it *instructively drives* homeostatic adjustments in quantal content or whether it *permissively guides* the synapse to be competent to respond to homeostatic challenges. To help address this problem, there are many tools that Drosophilists can use to acutely add or subtract homeostatic factors from the synapse such as RNAi-based transgenic constructs (Dietzl et al., 2007), the aforementioned *P[Switch]* expression system (Osterwalder et al., 2001; Roman et al., 2001), protein photoinactivation techniques like FlAsH-FALI (Heerssen et al., 2008; Marek and Davis, 2002; Poskanzer et al., 2003), and traditional pharmacology. It is tempting to assume that molecules required for the rapid, PhTx-induced form of synaptic homeostasis are more likely to represent instructive signals, while molecules required for the sustained, *GluRIIA* mutant-induced form are more likely to represent permissive ones. Yet neither assumption is necessarily true. It is possible that some molecules – like BMP-induced Mad – are required to provide a developmental framework to allow the rapid induction of synaptic homeostasis. Conversely, it is possible that other molecules are required for continuous, active signaling during the long-term maintenance of synaptic homeostasis.

Clearly, the Drosophila NMJ is a genetically tractable system. But ultimately, will this insect model help us to identify molecules and signaling processes that are important for neuronal stability in mammalian central and peripheral nervous systems? Is the NMJ home to important universal signaling processes that direct synaptic stability? Will these signaling processes prove relevant to human health and physiology? It is extremely likely. The list of Drosophila proteins implicated in synaptic homeostasis at the NMJ includes several that have human homologs with roles in neurological or muscular disorders such as: ataxia and migraine ($Ca_V2.1$/Cacophony) (Adams and Snutch, 2007; Pietrobon, 2010); schizophrenia (Dysbindin) (Ross et al., 2006); pain, epilepsy, and heart arrhythmia (K_V4/Shal) (Birnbaum et al., 2004; Castro et al., 2001); spinal muscular atrophy (Smn) (Coovert et al., 1997; Lefebvre et al., 1997); and muscular dystrophy (Dystrophin) (Hoffman et al., 1987). It is entirely possible that some of these conditions are caused or exacerbated by underlying defects in synaptic homeostasis. Thus, basic molecular information uncovered about the homeostatic regulation of Drosophila NMJ function may be relevant to human neurological disorders.

Acknowledgments

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Highlights

- A summary of homeostatic plasticity at the Drosophila neuromuscular junction (NMJ)

- Electrophysiological results demonstrating homeostatic control of NMJ function

- Current understanding of molecular mechanisms that drive synaptic homeostasis

- Presynaptic and postsynaptic molecules controlling NMJ homeostasis

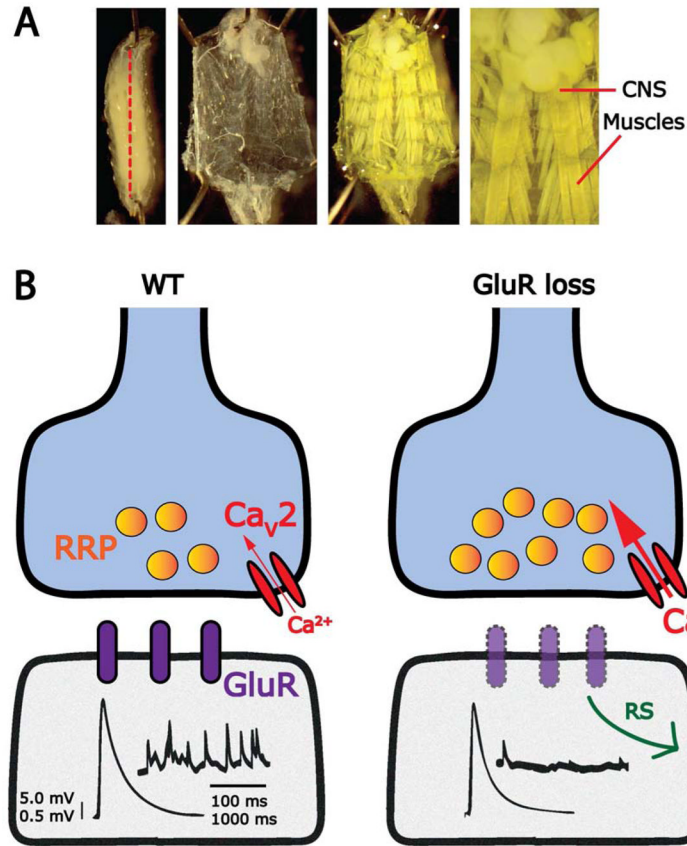- Notable connections with other experimental systems

Figure 1. The Drosophila NMJ exhibits robust homeostatic control of synaptic function
A) A series of images displaying the Drosophila larval NMJ preparation. A larva is pinned at its anterior and posterior, a shallow dorsal incision is made (dotted line), and a hexagonal filet exposes muscles and nerves for electrophysiological analysis or staining. Bouin's fixative is added to this preparation to better visualize the muscles. The final image of the fixed preparation shows a close-up view of the central nervous system (CNS) and adjacent muscles. **B)** A basic cartoon model of how homeostatic upregulation of neurotransmitter release proceeds at the Drosophila NMJ. The muscle is gray and the neuron is blue. Only a few key components are shown. Pharmacological or genetic inhibition of muscle glutamate receptors greatly diminishes quantal size. This is evident from decreases in spontaneous miniature excitatory postsynaptic potentials (small traces). However, relatively normal evoked potentials (large traces) are achieved because of homeostatic, muscle-to-nerve retrograde signaling processes (RS - green arrows) that increase quantal content. At least two mechanisms facilitate this increase in quantal content: 1) increased presynaptic calcium influx (presumably through Ca_V2-type calcium channels); and 2) increased size of the readily releasable pool of synaptic vesicles.
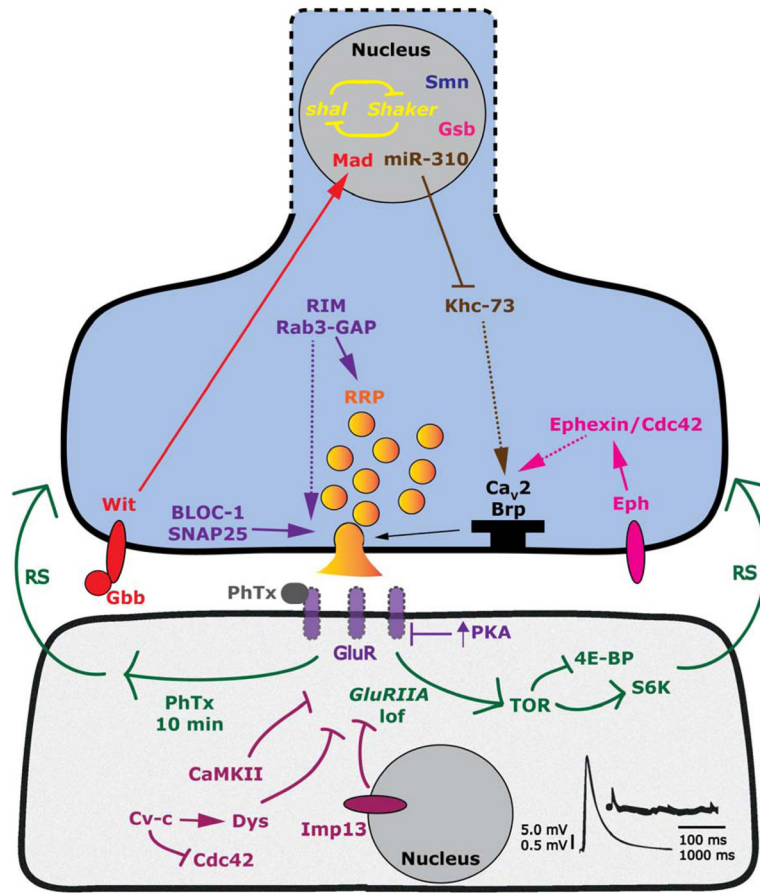
Figure 2. Model for selected molecules and processes regulating the homeostatic upregulation of presynaptic release

Refer to text and Table 1 for additional details. Refer to Figure 1 for a simplified model. Dashed arrows represent speculative or likely signaling connections, based primarily on indirect supporting evidence.

Genetic (*GluRIIA* deletion) or pharmacological (PhTx) inhibition of glutamate receptor function initiates retrograde signaling processes (RS - green) that result in increased presynaptic neurotransmitter release. Postsynaptic CaMKII, Crossveinless-c (Cv-c), Dystrophin (Dys), and Importin 13 (Imp13) all oppose retrograde signaling-induced increases in presynaptic quantal content. Downstream of GluRIIA subunit receptor loss, postsynaptic Target of Rapamycin (TOR) appears to initiate a translation-dependent retrograde signaling process through S6 kinase activation (S6K). Based on genetic and electrophysiological data, some retrograde signaling may operate through Bone Morphogenetic Protein (BMP) pathway members Glass Bottom Boat (Gbb) and Wishful Thinking (Wit), or the Eph receptor tyrosine kinase (or other unknown processes). Presynaptic proteins such as Eph/Ephexin and kinesin Khc-73 may act to potentiate presynaptic active zone (black T-bar) activity by regulating the localization or function of active zone molecules, such as Ca_V2-type calcium channels and the ELKS/CAST homolog, Bruchpilot (Brp). In parallel, Rab3 Interacting Molecule (RIM) and Rab3-GAP may coordinately enhance the size of the readily releasable pool of presynaptic vesicles (RRP) and also work in conjunction with members of the BLOC-1 (Dysbindin, Snapin) and SNARE (SNAP25) complexes to enhance vesicle release through SNARE-mediated fusion events.

In the presynaptic nucleus, overall neuronal excitability is shaped in part by reciprocal regulation of expression of the *shal* and *Shaker* potassium channel-encoding genes. Transcription factors Mad and Gooseberry (Gsb), as well as the Survival of Motor Neuron 1 (Smn) protein, confer competence upon the neuron to undergo homeostatic increases in synaptic release. Additionally, the miR-310 microRNA cluster negatively regulates expression of the *Khc-73* gene.

Table 1

Selected molecules controlling homeostatic regulation of Drosophila NMJ function

Most molecules listed promote homeostatic increases in presynaptic release following a glutamate receptor inhibition. Gray shading indicates an opposing function: decrease in presynaptic release.

Molecule	Tissue	Proposed Homeostatic Role	References
Cacophony(Ca$_V$2)	neuron	increase of presynaptic Ca^{2+} influx	(Frank et al., 2006; Frank et al., 2009; Müller and Davis, 2012)
Bruchpilot	neuron	increase active zone components	(Weyhersmüller et al., 2011)
miR-310 cluster	neuron	negative regulation of *Khc-73* expression	(Tsurudome et al., 2010)
Khc-73	neuron	kinesin function upstream of increased Ca$_V$2/Ca^{2+} influx	(Tsurudome et al., 2010)
Eph	neuron	signaling upstream of Ca$_V$2	(Frank et al., 2009)
Ephexin	neuron	signaling upstream of Ca$_V$2	(Frank et al., 2009)
Cdc42	both	signaling upstream of Ca$_V$2 (neuron) or opposing RhoGAP Cv-c (muscle)	(Frank et al., 2009; Pilgram et al., 2011)
Dysbindin	neuron	presynaptic vesicle release	(Dickman and Davis, 2009)
Snapin	neuron	presynaptic vesicle release	(Dickman et al., 2012)
SNAP25	neuron	presynaptic vesicle release	(Dickman et al., 2012)
Rab3-GAP	neuron	presynaptic vesicle release/RRP size	(Müller et al., 2011)
RIM	neuron	presynaptic RRP size regulation	(Müller et al., 2012)
Gooseberry	neuron	prolonged maintenance of homeostasis	(Marie et al., 2010)
Shal (K$_V$4)	neuron	K$^+$ current and Shaker inhibition	(Bergquist et al., 2010)
Shaker (K$_V$1)	neuron	K$^+$ current and Shal inhibition	(Bergquist et al., 2010)
Smn	neuron	prolonged maintenance of homeostasis	(Timmerman and Sanyal, 2012)
TOR	muscle	promotion of retrograde signaling	(Penney et al., 2012)
S6 kinase	muscle	promotion of retrograde signaling	(Penney et al., 2012)
Dystrophin	muscle	inhibition of retrograde signaling	(Pilgram et al., 2011; van der Plas et al., 2006)
Cv-c	muscle	inhibition of retrograde signaling	(Pilgram et al., 2011)
Importin 13	muscle	inhibition of retrograde signaling	(Giagtzoglou et al., 2009)
CaMKII	muscle	inhibition of retrograde signaling	(Haghighi et al., 2003)
Gbb	muscle	retrograde BMP signaling (ligand)	(Goold and Davis, 2007; McCabe et al., 2003)
Wit	neuron	retrograde BMP signaling (receptor)	(Goold and Davis, 2007; Haghighi et al., 2003)
Mad	neuron	retrograde BMP signaling (effector)	(Goold and Davis, 2007; McCabe et al., 2003)