Mini-Symposium

New Approaches for Studying Synaptic Development, Function, and Plasticity Using *Drosophila* as a Model System

C. Andrew Frank,[1] Xinnan Wang,[2] Catherine A. Collins,[3] Avital A. Rodal,[4] Quan Yuan,[5] Patrik Verstreken,[6] and Dion K. Dickman[7]

[1]Department of Anatomy and Cell Biology, University of Iowa Carver College of Medicine, Iowa City, Iowa 52242, [2]Stanford Institute for Neuro-innovation and Translational Neurosciences and Department of Neurosurgery, Stanford University School of Medicine, Palo Alto, California 94305, [3]Department of Molecular Cellular and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109, [4]Department of Biology, Brandeis University, Waltham, Massachusetts 02453, [5]National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda, Maryland 20892, [6]VIB, Center for the Biology of Disease and KU Leuven, Department for Human Genetics, 3000 Leuven, Belgium, and [7]Department of Biology, University of Southern California, Los Angeles, California 90089

The fruit fly *Drosophila melanogaster* has been established as a premier experimental model system for neuroscience research. These organisms are genetically tractable, yet their nervous systems are sufficiently complex to study diverse processes that are conserved across metazoans, including neural cell fate determination and migration, axon guidance, synaptogenesis and function, behavioral neurogenetics, and responses to neuronal injury. For several decades, *Drosophila* neuroscientists have taken advantage of a vast toolkit of genetic and molecular techniques to reveal fundamental principles of neuroscience illuminating to all systems, including the first behavioral mutants from Seymour Benzer's pioneering work in the 1960s and 1970s, the cloning of the first potassium channel in the 1980s, and the identification of the core genes that orchestrate axon guidance and circadian rhythms in the 1990s. Over the past decade, new tools and innovations in genetic, imaging, and electrophysiological technologies have enabled the visualization, *in vivo*, of dynamic processes in synapses with unprecedented resolution. We will review some of the fresh insights into synaptic development, function, and plasticity that have recently emerged in *Drosophila* with an emphasis on the unique advantages of this model system.

Introduction

Drosophila melanogaster has been a prominent model organism in biological research for more than a century. During this time, fruit fly researchers have made groundbreaking discoveries in numerous fields, including neuroscience (Bier, 2005; Bellen et al., 2010). Beginning in the 1960s, Seymour Benzer pioneered the field of *Drosophila* neurogenetics, hypothesizing that complex behavioral traits could be mapped to unique genetic loci (Benzer, 1967; Greenspan, 2008). In an era before recombinant DNA technology, work initiated in the Benzer laboratory led to fundamental discoveries that reverberate today, including the identification and characterization of ion channel gene mutations and their regulators (Jan et al., 1977; Wu and Ganetzky, 1980; Wu et al., 1983), the elucidation of the circadian clock (Konopka and Benzer, 1971), and the identification of genes that influence the complex process of learning and memory (Dudai et al., 1976).

The 1980s and1990s brought forth an impressive expansion of the *Drosophila* genetic toolkit, including transgenic animals (Rubin and Spradling, 1982), transposon-mediated mutagenesis (Bellen et al., 1989; Bier et al., 1989; Wilson et al., 1989), mosaic analysis (Golic and Lindquist, 1989; Golic, 1991), and tissue-specific control of gene expression (Fischer et al., 1988; Brand and Perrimon, 1993; Greig and Akam, 1993; Kaiser, 1993). In the past decade, there have been advances that enable the labeling of genetically distinct neurons or lineages (Lee and Luo, 2001) and the targeting of any gene in the fly genome for mutation (Rong and Golic, 2000) or knockdown (Dietzl et al., 2007; Ni et al., 2008, 2009). In the realm of neuroscience, these advances have been combined with classical forward genetic screening approaches to usher in a wealth of new information in diverse fields, including synaptic transmission (Schwarz, 2006), axon guidance (Dickson and Zou, 2010; Evans and Bashaw, 2010), eye development (Thomas and Wassarman, 1999), and olfaction (Davis, 1996, 2005; Fiala, 2007).

Drosophila neuroscientists are uniquely positioned to address fundamental questions in synapse biology. At the primary amino acid level, synaptic proteins in *Drosophila* are, on average, >70% similar to their mammalian counterparts; and with few exceptions, every mammalian protein has a fruit fly ortholog (Littleton

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Correspondence should be addressed to Dr. Dion K. Dickman, Department of Biology, University of Southern California, HNB 309, 3641 Watt Way, Los Angeles, CA 90089. E-mail: dickman@usc.edu.

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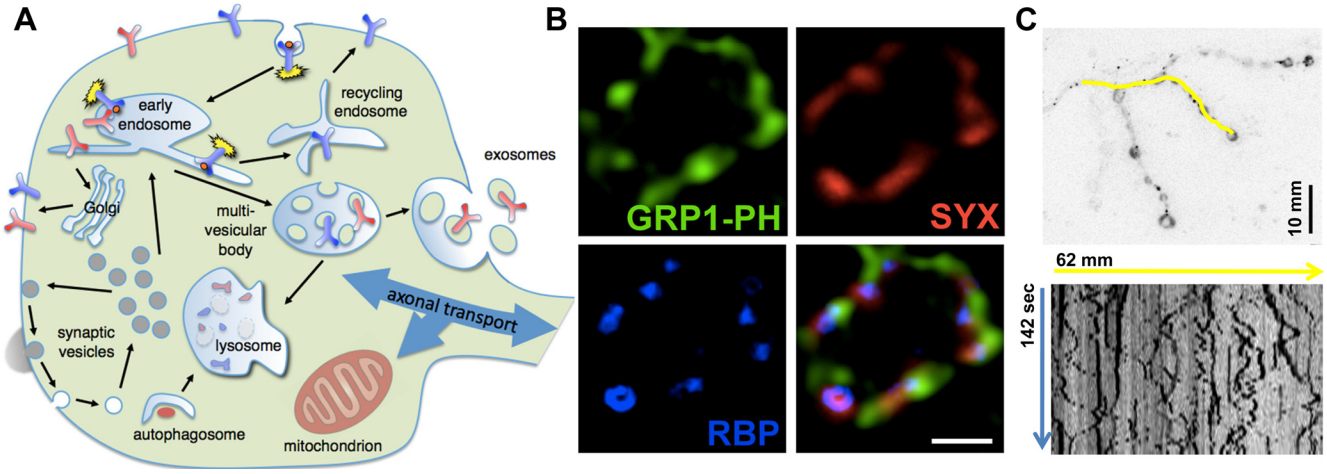

Figure 1. Visualization of membrane trafficking dynamics in *Drosophila*. ***A***, Schematic of intersecting trafficking pathways in synaptic boutons at the *Drosophila* NMJ. ***B***, Super resolution imaging (photobleaching microscopy with nonlinear processing) (Munck et al., 2012) of a larval synaptic bouton expressing N- and C-Split Venus-GRP1-PH (green), a marker for $PI(3,4,5)P_3$, and immunolabeled with anti-Syntaxin1A (red) and anti-Rim Binding Protein (RBP, blue) (Khuong et al., 2013). The images indicate that $PI(3,4,5)P_3$ and Syntaxin1A cluster preferentially at active zones that are marked by RBP. Scale bar, 500 nm. Image courtesy of Thang Manh Khuong (KU Leuven, Leuven, Belgium). ***C***, Live imaging reveals trafficking of the BMP receptor Tkv-mCherry in synaptic boutons (top), and dynamic movement along axon terminals (indicated in yellow as a kymograph; bottom).

and Ganetzky, 2000; Littleton, 2000; Lloyd et al., 2000). Given an extensive array of powerful approaches, *Drosophila* neuroscientists are in pole-position to elucidate the functions of synaptic proteins and the many processes in which they participate.

Advances in synaptic trafficking

Motor neurons in third-instar *Drosophila* larvae are exceptional models to study organelle trafficking in an organismal context (Fig. 1*A*), given the unique and powerful combination of modern molecular genetic, electrophysiological, and imaging technologies available (Venken et al., 2011). Motor neuron axons and terminals are found near the surface of the muscle, where they are directly accessible for imaging and electrophysiology in filleted preparations. In addition, neurons and subsynaptic structures can be easily and repeatedly imaged through the cuticle over many days of development in intact larvae (Füger et al., 2007; Schmid et al., 2008; Ghannad-Rezaie et al., 2012). These assays in fly axons and synapses have enabled some of the first *in vivo* measurements of synaptic trafficking rates ever obtained in a model organism and have advantages over cultured systems because they reveal neuronal processes within an intact developmental context. In contrast, *in vivo* studies of comparable resolution and sophistication are difficult in mammalian models because of challenges in accessing structures deep in tissue, the long generation times, and the often limited reagents necessary for complex genetic experiments. Furthermore, the impact of membrane trafficking processes in fly neurons can be effectively determined within the context of higher-order processes, such as synapse formation, transmission, motor function, and neurodegeneration, making *Drosophila* a powerful system to understand molecular, cellular, synaptic, circuit, and behavioral functions. Here, we will discuss recent developments, using the larval neuromuscular junction (NMJ), that have led to novel insights into the trafficking of synaptic vesicles, endosomes, and mitochondria.

Synaptic boutons at the *Drosophila* larval NMJ are quite large (2–5 μm diameter) and are thus easily amenable to light microscopy. Recent advances in super resolution imaging have enabled the visualization of synaptic structures beyond the diffraction limit, yielding molecular insight into the organization, structure, and function of synaptic vesicle release sites (Owald et al., 2010, 2012; Liu et al., 2011; Miśkiewicz et al., 2011). We have used super resolution imaging and a novel lipid binding reagent we developed to reveal new insights into the active zone at the presynaptic membrane. Using a split-Venus fluorophore fused to the general receptor for phosphoinositides 1 pleckstrin homology domain (GRP1-PH), we demonstrated that a very low-abundance lipid, $PI(3,4,5)P_3$, localizes to synaptic vesicle release sites and is necessary and sufficient to cluster Syntaxin1A, a protein required for vesicle fusion (Khuong et al., 2013) (Fig. 1*B*). These and numerous other studies in *Drosophila* have established a foundation to start building a molecular model for how proteins and lipids interact to regulate neurotransmitter release in healthy and diseased neurons (Lauwers and Verstreken, 2013).

The ability to image synaptic processes at the larval NMJ has also enabled us to delineate the function of endosomes in the synaptic vesicle cycle. Although compartments that harbor endosomal markers are present at synapses (Wucherpfennig et al., 2003), their role in the synaptic vesicle cycle has remained controversial (Zenisek et al., 2000; Opazo et al., 2010). Our synaptic transmission screens isolated a novel and evolutionary conserved GTPase-activating protein that we named Skywalker (Sky). Using live imaging of synaptic vesicles and time course electron microscopy and tomography, we found that most synaptic vesicles are forced to traffic via an endosomal compartment in *sky* mutants (Uytterhoeven et al., 2011). To our surprise, synaptic transmission in *sky* mutants is significantly increased, and the size of the readily releasable synaptic vesicle pool is markedly larger. We reasoned that dysfunctional proteins may be sorted locally in synaptic terminals for degradation through endosomal compartments, and this process may be facilitated in *sky* mutants. These synapses, in turn, may have a higher ratio of synaptic vesicles with "more functional" proteins, leading to increased neurotransmitter release. To test this hypothesis, we constructed an artificial synaptic vesicle protein as a substrate for degradation. This protein was indeed very efficiently degraded in *sky* mutant animals compared with wild-type (Henne et al., 2011). Hence, genetic and dynamic imaging approaches revealed that endosomes may serve as sorting stations for the local degradation of dysfunctional synaptic vesicle proteins. Given that the human homolog of *sky*,

TBC1D24, has been found mutated in epileptic patients and has recently also been linked to neuronal survival (Poduri and Lowenstein, 2011), endosomal sorting of synaptic vesicles may also be medically relevant.

The larval NMJ is also an outstanding system to study how neurons integrate intracellular membrane trafficking pathways to transduce growth signals and how these events may be altered in disease. During larval development, synaptic growth is driven through diverse mechanisms involving neuronal activity, glial influences, and target-derived signals (Packard et al., 2002; Marqués and Zhang, 2006; Fuentes-Medel et al., 2012) and depends on trafficking of growth factors and presynaptic signaling receptor complexes (Dickman et al., 2006; O'Connor-Giles et al., 2008; Rodal et al., 2008, 2011). Indeed, pioneering forward genetic screens in *Drosophila* based on visual observation of intact larval synapses have discovered novel and conserved regulators of synaptic growth, including highwire/rpm1/Pam/Phr1 and bone morphogenic proteins (BMPs) (Wan et al., 2000; Aberle et al., 2002; Marqués et al., 2002). Work in *Drosophila* has revealed that endosomal cargoes take remarkably varied yet intersecting routes within presynaptic terminals (Fig. 1*A*), trafficking between diverse synaptic compartments (Wang et al., 2007; Rodal et al., 2008, 2011; Korkut et al., 2009; Kim et al., 2010; Koles et al., 2012), yet synaptic endosomal function remains poorly understood.

We recently developed live-imaging assays to directly visualize receptor signaling and endosomal dynamics simultaneously (Fig. 1*C*). These compartmental movements occur at very high speeds (up to several micrometers per second) and were impossible to measure until recent advances in spinning disk confocal technology and genetically encoded fluorophores. These studies have revealed that transient interactions between the membrane-remodeling proteins Nervous Wreck (Nwk) and Sorting Nexin 16 on presynaptic early and recycling endosomes correlate with down-regulation of receptor signaling activity (Rodal et al., 2011). This led us to investigate the membrane-deforming activity of Nwk, a member of the F-BAR protein family (Becalska et al., 2013). We found that Nwk exhibits an unconventional higher-order zigzag assembly of the F-BAR domain, leading to novel deforming activities: membrane ridging, scalloping, and negative curvature. This suggests that cargo transfer between these synaptic endosomes occurs by a unique mechanism distinct from conventional tubulation-mediated sorting (Campelo and Malhotra, 2012). In addition, we have recently monitored the trajectory of two synaptic growth-promoting transmembrane cargoes, the BMP receptor Thickveins (Tkv) and the amyloid precursor protein (A. Becalska, Z. Feiger, M. Zunitch, and A. Rodal, unpublished data). Within the same nerve terminal, these two cargoes traffic via distinct endosomal pathways and are profoundly affected in neurodegenerative disease models. Tkv trafficking and signaling are perturbed in a fly model of amyotrophic lateral sclerosis, whereas amyloid precursor protein traffic is altered upon manipulation of the endosomal retromer complex, which is linked to Alzheimer's disease (Muhammad et al., 2008; Small, 2008; Wen et al., 2011). These observations suggest that the compromised function of different endocytic pathways can lead to distinct disease manifestations. One particularly interesting area of future research will be to investigate how these endosomal pathways integrate with those regulating the synaptic vesicle cycle.

Mitochondrial transport and distribution in neurons are critical for synaptic integrity and function, given their vital roles in ATP synthesis, Ca^{2+} buffering, and apoptosis. Forward genetic screens in *Drosophila* have identified two mitochondrial adaptors, Milton and Miro, which are necessary for anterograde movement of mitochondria in axons (Stowers et al., 2002; Guo et al., 2005; Glater et al., 2006). Imbalances in mitochondrial trafficking have been linked to human neurological disease and modeled in flies (Shidara and Hollenbeck, 2010; Wang et al., 2011; Iijima-Ando et al., 2012). For example, two hereditary forms of Parkinson's disease (PD) arise from mutations in the Ser/Thr kinase PINK1 and the E3 ubiquitin ligase Parkin (Kitada et al., 1998; Valente et al., 2004). Genetic and live-imaging studies in *Drosophila* were the first to establish that PINK1 functions in part upstream of Parkin to control mitochondrial motility and morphology (Clark et al., 2006; Park et al., 2006; Yang et al., 2006; Exner et al., 2007; Poole et al., 2008; Wang et al., 2011; Liu et al., 2012). Mammalian work has also established this pathway in damaged mitochondrial clearance via mitophagy (Narendra et al., 2008, 2012; Geisler et al., 2010; Haddad et al., 2013). Recent work using large-scale genomic or proteomic approaches in *Drosophila* has further demonstrated the importance of PINK1 in maintaining the mitochondrial electron transport chain and protein turnover *in vivo* (Morais et al., 2009; Vilain et al., 2012; Vos et al., 2012; Esposito et al., 2013; Vincow et al., 2013). Together, these approaches in *Drosophila* have revealed mechanistic insights into the regulatory dynamics of mitochondria and their significance in the etiology of PD.

Our studies and those of others have also revealed that mutations in several of the PD-related genes, including *pink1*, *parkin*, and *LRRK2*, lead to defects in synaptic vesicle trafficking in both flies and rodents (Gautier et al., 2008; Morais et al., 2009; Lee et al., 2010; Piccoli et al., 2011; Poduri and Lowenstein, 2011; Matta et al., 2012; Haddad et al., 2013). Although loss of *pink1* and *parkin* may affect synaptic transmission because of mitochondrial defects (Verstreken et al., 2005), we discovered that fly LRRK and mammalian LRRK2 directly phosphorylate EndophilinA (Matta et al., 2012), a critical gene for synaptic vesicle endocytosis (Verstreken et al., 2002; Dickman et al., 2005; Milosevic et al., 2011). Misregulation in LRRK-dependent EndophilinA phosphorylation causes defects in membrane association and synaptic vesicle endocytosis; and interestingly, Parkin has been shown to ubiquitinate EndophilinA (Trempe et al., 2009). These studies not only reveal a novel LRRK2 substrate but implicate dysfunction in synaptic vesicle trafficking in the etiology of PD. Thus, powerful genetic and live imaging approaches developed in *Drosophila* have provided a unique tool to uncover fundamental mechanisms of synaptic organelle traffic in health and disease.

Advances in homeostatic synaptic plasticity

Recent studies have demonstrated that the nervous system is endowed with potent and adaptive mechanisms that respond to perturbations that disrupt electrical activity and restore normal functionality, termed homeostatic plasticity. Homeostatic regulation has been identified in nervous systems of both vertebrates and invertebrates, indicating that this is an evolutionarily conserved form of neural regulation (Davis, 2006; Nelson and Turrigiano, 2008; Turrigiano, 2008; Vitureira et al., 2012). Synapses, as fundamental units of nervous system function, are key substrates to achieve and maintain the homeostatic control of neural function. However, the genes and molecular mechanisms that orchestrate these complex and fundamental signaling systems are virtually unknown. Although vertebrate studies have demonstrated robust homeostatic signaling and found a few intriguing molecules involved, including retinoic acid, BDNF, TNF-α, and

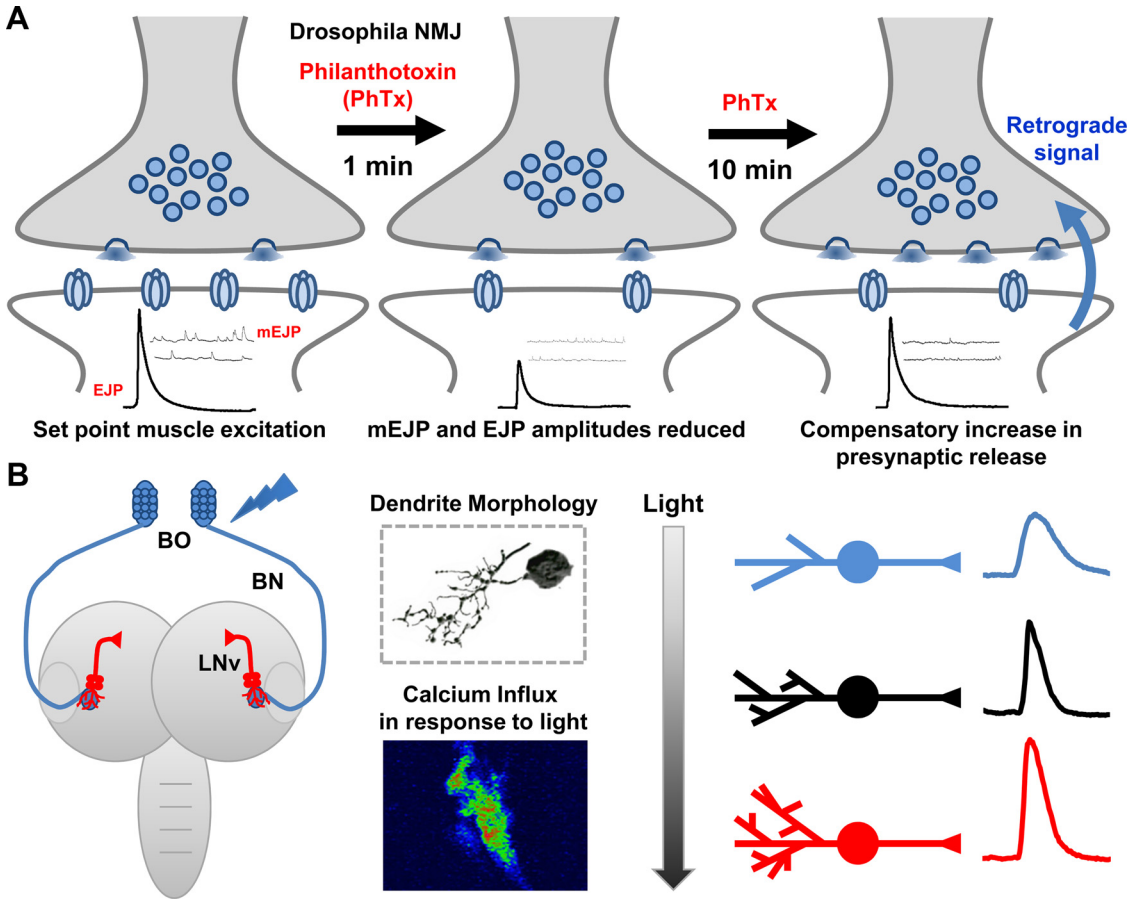

Figure 2. *Drosophila* models of homeostatic synaptic plasticity. ***A***, Schematic of homeostatic compensation after application of the postsynaptic glutamate receptor antagonist philanthotoxin (PhTx) to the larval NMJ. Application of PhTx initially causes an ~50% decrease in miniature excitatory junction potential (mEJP) amplitude and a parallel ~50% decrease in evoked excitatory junction potential (EJP) amplitude. After 10 min incubation in PhTx, mEJP amplitudes remain depressed whereas EJP amplitudes increase to baseline values resulting from a homeostatic enhancement of presynaptic neurotransmitter release (quantal content). ***B***, Schematic of the larval visual circuit; the light sensing BO sends an axon projection (BN) into the larval brain where it synapses with the LNv (left). LNvs exhibit activity-dependent structural and functional plasticity, demonstrated by 3D tracing and calcium imaging (middle). Light exposure bidirectionally alters dendrite length and synaptic activity (right).

Arc (Pozo and Goda, 2010), progress in gene discovery in these systems is limited. Recently, the larval NMJ and visual system in *Drosophila* have emerged as powerful models for studying homeostatic synaptic plasticity, enabling the elucidation of these processes in a system amenable to forward genetic approaches.

The landmark publication demonstrating homeostatic control of synaptic function was made by Gina Turrigiano and colleagues ~15 years ago (Turrigiano et al., 1998). Around this time, genetic mutants and manipulations to postsynaptic glutamate receptors at the *Drosophila* NMJ revealed the surprising existence of a retrograde, homeostatic synaptic signaling mechanism (Petersen et al., 1997; Davis et al., 1998). Here, an increase in presynaptic release was observed that precisely compensated for reduced postsynaptic receptor function, adaptively restoring normal postsynaptic activity. Diverse perturbations have been shown to induce this phenomenon, including altered synaptic inputs (Davis and Goodman, 1998), reduced excitability of the postsynaptic muscle (Paradis et al., 2001), and alterations in postsynaptic glutamate receptor classes, expression levels, or sensitivity (for review, see Frank, 2013). Further, acute pharmacological blockade of postsynaptic receptors demonstrated that this form of homeostatic compensation was surprisingly rapid, occurring in 10 min (Frank et al., 2006). Finally, the homeostatic control of presynaptic function is bidirectional. Some genetic perturbations induce increased presynaptic vesicle size. This can result in increased quantal size and a concomitant homeostatic reduction in presynaptic release (quantal content), restoring normal postsynaptic responsiveness (Daniels et al., 2004).

Using an acute pharmacological assay (detailed in Fig. 2*A*), we undertook an electrophysiology-based forward genetic screen of >350 mutants for potential roles in homeostatic synaptic plasticity, isolating seven novel mutations. This screen identified the *Drosophila* homolog of the vertebrate gene *dysbindin* to be required for adaptive synaptic plasticity (Dickman and Davis, 2009). Interestingly, the human homolog of this gene has been found to be a susceptibility gene for schizophrenia (Ross et al., 2006), raising the intriguing possibility that defects in homeostatic plasticity may contribute to the etiology of complex neuropsychiatric diseases. Dysbindin is part of large protein complex called the Biogenesis of Lysosome-related Organelles Complex (Ghiani and Dell'Angelica, 2011), and we recently showed that another member of this complex, the synaptic vesicle protein Snapin, is also required for synaptic homeostasis (Dickman et al., 2012). Thus, this forward screening approach identified an unanticipated protein complex in presynaptic homeostatic plasticity and revealed intriguing links with neuropsychiatric disease.

This screen also led to the discovery of unanticipated roles for potassium channels in homeostatic regulation (Bergquist et al., 2010), as well as a novel calcium-binding protein and neurotransmitter receptor (D. K. Dickman et al., unpublished data). In addition, subsequent screens and other studies have found diverse genes and processes to be involved in synaptic homeostasis, including Rab3, Ephexin, calcium signaling, synaptic vesicle pool sizes, axonal transport, and postsynaptic translation (Frank et al., 2009; Tsurudome et al., 2010; Müller et al., 2011, 2012; Penney et al., 2012; Frank, 2013). A challenge for the future will be to delineate how the homeostat integrates these complex and diverse signaling pathways to precisely modulate synaptic strength. Together with dynamic imaging methods that are being developed (Müller and Davis, 2012), the *Drosophila* NMJ will continue to be a powerful system to identify the genes and elucidate the mechanisms that orchestrate the homeostatic control of synaptic function.

In addition to the *Drosophila* NMJ, we recently identified robust homeostatic modulation of both synaptic structure and function in the fly larval visual system (Yuan et al., 2011). Visual experience during larval development in the CNS drastically modifies the dendritic arbor of ventral lateral neurons (LNvs), a group of second-order cells postsynaptic to the primary larval photoreceptor, Bolwig's organ (BO) (Schmucker et al., 1992; Malpel et al., 2002). Light stimulation induces structural changes in LNv dendritic complexity and size as well as physiological responses inverse to the amount of light stimulation (Fig. 2*B*). Indeed, increased light exposure reduces dendritic arbors and synaptic responses in LNvs, whereas reduced light exposure leads to increased dendritic size and responsiveness. These compensatory changes are similar in principle to the homeostatic adjustment of synaptic strength observed at the NMJ. Because LNvs are critical components in the neural circuits controlling circadian rhythms and larval light avoidance behaviors (Helfrich-Forster, 1998; Mazzoni et al., 2005), this modulation of dendritic morphology and physiology may serve important functions in the behavioral adaptation to changing environmental conditions.

The bidirectional homeostatic plasticity observed in the *Drosophila* CNS provides an opportunity to use genetic screens to identify genes involved in these processes. We have recently performed genome-wide RNAi and loss-of-function mutant screens, identifying a number of interesting candidates, including novel cell adhesion molecules, to be required for homeostatic structural plasticity in dendrites (Yuan et al., 2011). This system has also provided insights into local inhibitory inputs in regulating experience-dependent plasticity, which mammalian work has found to be the driving force for critical period plasticity in the primary visual cortex (Hensch, 2004; Espinosa and Stryker, 2012). In the fly larval visual system, anatomical evidence has suggested that both excitatory and inhibitory inputs interact with the BO-LNv synapses (Hamasaka et al., 2005, 2007). By manipulating activity of inhibitory neurons and receptor expression on LNvs, we observed strong influences on LNv dendritic plasticity (Q. Yuan et al., unpublished data). We are currently searching for developmental and environmental cues that modulate wiring plasticity in the fly visual circuit. New genetic screens and calcium imaging technologies will continue to provide mechanistic insights into the homeostatic control of structural and functional synaptic plasticity.

Advances in synaptic degeneration and repair

A dramatic form of synaptic plasticity is the loss and clearance of defective or damaged synapses and the structural remodeling necessary to generate new connections. A number of assays have recently been developed in *Drosophila* to study the degeneration and regeneration of axons, dendrites, and synapses (Leyssen et al., 2005; Hoopfer et al., 2006; MacDonald et al., 2006; Massaro et al., 2009; Stone et al., 2010; Xiong et al., 2010, 2012; Tao and Rolls, 2011; Fang and Bonini, 2012; Song et al., 2012; Xiong and Collins, 2012). Hence, the power of *Drosophila* genetics can now be brought to bear upon these topics, which are of great therapeutic interest.

Not surprisingly, discoveries in flies have been shown to hold true for vertebrates as well. A striking example of this is the essential role for a conserved axonal Dileucine Zipper kinase named Wallenda (Wnd) in *Drosophila* in the regeneration of injured axons in multiple model organisms (Hammarlund et al., 2009; Yan et al., 2009; Xiong et al., 2010; Shin et al., 2012). Wnd becomes acutely activated by axonal injury and mediates a transcriptional response to promote new axonal growth (Xiong et al., 2010). Wnd was originally identified as a target of the Highwire (Hiw) E3 ubiquitin ligase during synaptic development in a forward genetic suppressor screen. Excessive levels (and activity) of Wnd protein lead to synaptic overgrowth in *hiw* mutants (Wan et al., 2000; Collins et al., 2006). We have recently identified a new role for Wnd in organizing microtubules during synaptic development at the NMJ (Klinedinst et al., 2013), demonstrating that Wnd has dual functions in both synaptic development and axonal regeneration. We hypothesize that the turnover of Wnd by Hiw may serve as a sensor for the state of the synapse, promoting new synaptic growth during development and after injury.

The mechanisms driving axonal degeneration remain poorly understood. A prevailing hypothesis has been that axons degenerate through an active "self destruction" pathway, analogous to, but molecularly distinct from, apoptosis (Wang et al., 2012). This hypothesis was the basis of a recent brute-force genetic screen in *Drosophila*, which discovered a novel gene, Sarm, to be essential for axonal degeneration (Osterloh et al., 2012). We have found that Hiw also plays a similarly critical role in axonal degeneration by regulating the levels of the NAD biosynthetic enzyme NMNAT (Xiong et al., 2012). Subsequent work has shown that homologs of both Hiw and Sarm function in axonal degeneration in mice (Osterloh et al., 2012; Babetto et al., 2013). Future studies using genetically encoded reporters for cellular events, including mitochondrial movement, calcium imaging, and cytoskeletal dynamics, will be used to study both regeneration and degeneration within the context of native circuits in a living animal. Therefore, *Drosophila* is certain to be a leading system to enable discoveries of additional players and their mechanistic relationships in the mysterious process of neuronal degeneration.

In conclusion, we have highlighted several examples of the current approaches being used in *Drosophila*, which have merged genetic, imaging, and electrophysiological technologies to reveal new insights into synaptic development, function, and plasticity. With these powerful tools and a new generation of researchers, synaptic studies in *Drosophila* will continue to pioneer new discoveries in diverse areas of neuroscience research.

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