Topic Introduction

The *Drosophila* Larval Neuromuscular Junction: Developmental Overview

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For decades, the *Drosophila* larval neuromuscular junction (NMJ) has been a go-to model for synaptic development. This simple, accessible system is composed of a repeating pattern of 33 distinct neurons that stereotypically innervate 30 muscles. Fundamental mechanisms that underlie diverse aspects of axon pathfinding, synaptic form, and function have been uncovered at the NMJ, and new pathways continue to be uncovered. These discoveries are fueled by the ease of dissections and an extensive array of techniques. Chief among these techniques are various microscopy approaches, including super-resolution and electron microscopy. Functionally, the *Drosophila* NMJ is glutamatergic, similar to the vertebrate central synapses, making it a great model to study normal development and neurological diseases. Here we provide a brief overview of the larval neuromuscular system, highlighting the connectivity patterns, development, and some of the mechanisms underlying these processes.

BACKGROUND

Synapses allow for the transmission of information in circuits and underlie all aspects of behavior. At chemical synapses, neurotransmitters released by the presynaptic terminal engage postsynaptic receptors to transduce an electrical signal, and this process is faithfully repeated at synapses across the nervous system. The repertoire of neurotransmitters and corresponding receptors is extensive and varies across different regions of the nervous system. For example, in vertebrates, the most common excitatory neurotransmitter in the central nervous system is glutamate, whereas acetylcholine is used at the neuromuscular system. Typically, presynaptic cells are neurons, and postsynaptic partners can be other neurons or nonneuronal cells, including muscles.

Investigations of neuromuscular junctions (NMJs)—the synapses formed between motor neurons and muscles—have provided significant insights into mechanisms that underlie axon guidance, synaptic partner recognition, synaptogenesis, synaptic maturation and maintenance, plasticity, and homeostasis (for reviews, see Wu et al. 2010; Shi et al. 2012; Slater 2017; Burden et al. 2018; Aponte-Santiago and Littleton 2020; Frank et al. 2020; Goel et al. 2020). The driving force of the vertebrate NMJ is the neurotransmitter acetylcholine and juxtaposed acetylcholine receptors (AchRs) on the muscle. Before formation of the vertebrate NMJ, AchRs are clustered at the central region of the muscle in a process termed prepatterning. Upon contact by a motor neuron terminal, anterograde and retrograde signaling instructs synapse stabilization and maturation. Motor neuron activity is impor-

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tant for maintenance of NMJs (Burgess 2006). To drive muscle contractions, patterned electrical activity in a motor neuron instructs the release of acetylcholine from its terminals, which binds to and activates nearby AchRs. The density of AchRs is enriched in a highly folded postsynaptic membrane to enhance the local depolarization and probability of muscle contraction (for excellent reviews on vertebrate NMJ development, see Sanes and Lichtman 1999; Wu et al. 2010; Shi et al. 2012; Burden et al. 2018). Although the vertebrate NMJ has revealed important molecules for formation, development, and maturation of cholinergic synapses, other synapse models have contributed to our understanding of vertebrate excitatory glutamatergic synapses.

In a serendipitous twist of fate, the main excitatory neurotransmitter in crustacean and insect NMJs is glutamate rather than acetylcholine, thus providing an easily accessible model for glutamatergic synapses. The postsynapse contains ionotropic glutamate receptors (GluRs) and the necessary scaffolding and signaling molecules (Kim et al. 2012; Ramos et al. 2015). Strikingly, these NMJ GluRs are homologous to the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type GluRs in the vertebrate brain. Early anatomists and physiologists turned to crustaceans and later to insects because of the ease of maintaining them in the laboratory and access to neuromuscular preparations. In the late 1800s, Thomas Huxley's book entitled *The Crayfish* (Huxley 1880) promoted the use of crustaceans for research by describing crayfish anatomy and physiology. Other scientists, including Charles Richet, were already examining crayfish NMJs to understand the physiology of the opener muscle, which is responsible for opening the claw (Richet 1879), and this work continued throughout the early 1900s (Bazemore et al. 1956; Atwood 1967; Sherman and Atwood 1971; Florey 1991; Cooper and Cooper 2009).

In the 1970s, Lily and Yuh Nung Jan (Jan and Jan 1976) introduced the *Drosophila* NMJ as a viable system with which to examine glutamate synapse morphology and physiology. In the following decades, the laboratories of Atwood, Bate, Bellen, Budnik, Davis, DiAntonio, Ganetzky, Goodman, Keshishian, Littleton, Stewart, Whitington, Wu, Zinn, and many others bolstered the use of the fly NMJ to examine all aspects of synapse biology and function. Importantly, this research has positioned the *Drosophila* NMJ as a powerful model for vertebrate excitatory synapses, as many of the vertebrate synaptic molecules have fly homologs.

NEUROMUSCULAR CIRCUIT DEVELOPMENT

The *Drosophila* larval neuromuscular circuit is established during embryonic development by corresponding motor neurons and muscles. Embryonic myogenesis generates a stereotyped set of muscles that perdure and expand significantly during larval development. Each muscle is formed by a special class of myoblasts called muscle founder cells (FCs) that individually recognize and fuse with specific subsets of neighboring fusion-competent myoblasts (FCMs) to form myotubes (Bate 1990; Dohrmann et al. 1990; Bourgouin et al. 1992; Bate et al. 1993; Bate and Rushton 1993). Recognition is governed by two pairs of immunoglobulin domain-containing transmembrane receptors: Dumbfounded/Roughest in FCs and Sticks and Stones/Hibris in FCMs (Nose et al. 1998; Bour et al. 2000; Artero et al. 2001; Duan et al. 2001; Strünkelnberg et al. 2001; Richardson et al. 2008; Ciglar et al. 2014). Receptor interactions signal bidirectionally to induce actin rearrangements and ultimately fusion. The specific FC determines the identity of the muscle cell and ultimately the recognition of appropriate presynaptic motor neuron partners (Rushton et al. 1995). At the end of embryonic development and upon entering the larval stage, muscles transition to a phase of drastic growth regulated by hormone signaling and the downstream dMyc effector (Demontis and Perrimon 2009; Kim and O'Connor 2021).

Drosophila larval motor neurons are generated in the embryonic ventral nerve cord (VNC) through division of progenitor cells called neuroblasts (Landgraf and Thor 2006; Meng and Heckscher 2021). Every neuroblast lineage has been mapped so that the identity and origin of most motor neurons are known (Landgraf et al. 1997; Schmid et al. 1999). Axons from embryonic motor

neurons exit the VNC in three nerve bundles: intersegmental (ISN), segmental (SN), or transverse (TN). Before innervating their respective muscles, the SN branches into SNa and SNc, and the ISN branches into ISN, ISNb, and ISNd at defined locations (Thomas et al. 1984; Canal and Ferrús 1986; Keshishian and Chiba 1993). The SN targets external muscles, and the ISN targets internal muscles. In the embryo, most laboratories visualize these nerves with anti-Fasciclin II (Fas2) staining (Fig. 1; Vactor et al. 1993). The pathways taken by motor axons are genetically programmed and have been extensively examined (Landgraf et al. 1997; Chisholm and Tessier-Lavigne 1999; Jeong 2021). After the ISN and SN bifurcate into smaller branches, motor axon filopodia explore the local region to faithfully recognize appropriate muscle targets.

Like vertebrate central and peripheral circuits (Katz and Shatz 1996; Hua and Smith 2004; Yamamoto and López-Bendito 2012; Doll and Broadie 2014; Koropouli and Kolodkin 2014; Arroyo and Feller 2016; Pan and Monje 2020), fly axons initially form exuberant contacts across many potential synaptic partners, and as development progresses, inappropriate contacts are pruned to reveal the mature wiring map (Halpern et al. 1991; Sink and Whitington 1991; Vonhoff and Keshishian 2017a). In the embryonic neuromuscular circuit, electrical activity is required for this refinement, as suppression of activity in motor neurons impedes elimination of incorrect synapses (Jarecki and Keshishian 1995; Carrillo et al. 2010). As with most activity-dependent processes, Ca^{2+} and its downstream effectors are key components. NMJ refinement requires the voltage-gated Ca^{2+} channel Cacophony (Cac) and several downstream signaling pathways, including the Ca^{2+}/calmodulin-dependent serine/threonine kinase II (CaMKII) (Carrillo et al. 2010) and the Ca^{2+}-dependent adenylyl cyclase Rutabaga (Vonhoff and Keshishian 2017b). Withdrawal of inappropriate contacts requires presynaptic Semaphorin 2a (Sema2a) signaling, and activity/Ca^{2+} may modulate the Sema2a/Plexin B chemorepulsion pathway (Carrillo et al. 2010).

After synaptic refinement, the remaining axon terminal contacts will develop into presynaptic terminal swellings called boutons that contain active zones—the sites where neurotransmitter vesicles accumulate and are released. After contacts are established, Ca^{2+} channels (Kittel et al. 2006; Liu et al. 2011; Graf et al. 2012), neurotransmitter vesicles (Winther et al. 2015), and the machinery for vesicle release and recycling (Broadie et al. 1995; Aravamudan et al. 1999; Vilinsky et al. 2002) are presynaptically clustered. Simultaneously, scaffolding proteins such as Discs large (Dlg; an ortholog of the vertebrate PSD-95), GluRs, and signaling messengers accumulate at the postsynaptic site (Mathew et al. 2005; Yoshihara et al. 2005; Liebl et al. 2008). Although prepatterning of postsynaptic molecules has not been extensively examined at the *Drosophila* NMJ, the cell adhesion molecule Fasciclin 3 (Fas3) was observed at NMJ sites independent of innervation (Broadie and Bate 1993), similar to the localization of vertebrate muscle AchRs (Wu et al. 2010; Shi et al. 2012; Slater 2017; Burden et al. 2018). The persistence of Fas3 at synaptic sites, however, is lost if innervation does not occur,

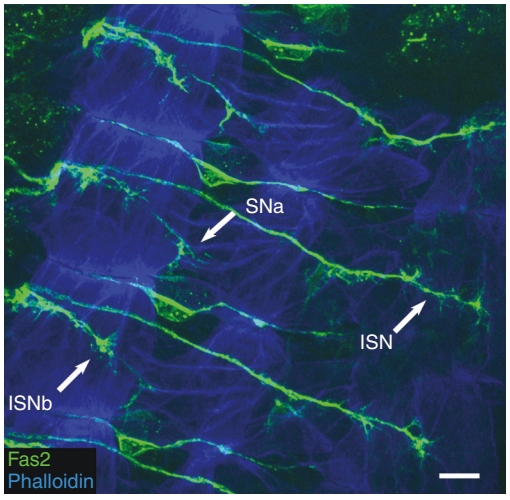

FIGURE 1. Embryonic axons exploring muscle area. Stage 16 embryo depicting muscles labeled with phalloidin (blue) and peripheral nerves (green). Anti-Fas2 staining clearly highlights the motor nerves; depicted here are intersegmental nerve b (ISNb) and segmental nerve a (SNa). Scale bar, 10 µm.

suggesting that motor neuron synapses are required to maintain Fas3. Fas3 is only expressed in two ventral longitudinal muscles (Broadie and Bate 1993; Kose et al. 1997), so whether other muscles have similar prepatterning molecules has yet to be determined. Interestingly, the NMJ at each muscle appears in a spatially restricted area, suggesting that genetic mechanisms instruct these boundaries.

NEUROMUSCULAR CIRCUIT MAP

A significant advantage of studying synaptic development in the *Drosophila* larval neuromuscular circuit is the simplicity of the circuit architecture. Unlike the dense neuropil in the central nervous system, where synaptic connections are difficult to identify, the neuromuscular innervation pattern forms a stereotyped, genetically encoded circuit in which individual synapses can be readily identified and imaged. Even in this "simple" circuit, we lack a complete understanding of the molecular mechanisms that govern synaptic wiring. In each hemisegment of this circuit, developmental programs must instruct 33 motor neurons in the embryonic VNC to extend their axonal projections to the periphery, where they must decide which of the 30 muscle fibers to innervate. This program is repeated across 16 abdominal hemisegments (eight bilaterally symmetrical segments) and similarly at six thoracic hemisegments (Fig. 2). It is important to note that, even within the abdominal segments, there are some differences in the muscle patterns in the more anterior and posterior segments. For example, in the first abdominal segment, A1, there are 29 muscles due to the absence of muscles 6 and 7, and the addition of muscle 31.

Larval motor neurons are grouped into three types—I, II, and III—and type I neurons are further divided into types Ib (big) and Is (small) (Atwood et al. 1993; Jia et al. 1993; Lnenicka and Keshishian 2000; Choi et al. 2004; He et al. 2009). The motor neuron types differ in many key characteristics that support their excitatory or neuromodulatory roles. Type I motor neuron synapses provide the major glutamatergic excitatory drive and are surrounded by complex, membranous folds known as the subsynaptic reticulum (SSR). Dlg labels the SSR and provides a convenient marker to differentiate Ib and Is NMJs because the SSR is significantly thicker around Ib boutons (Guan et al. 1996; see Fig. 2 in Protocol: **Immunohistochemistry and Morphometric Analysis of *Drosophila* Larval Body Wall Neuromuscular Junction Preparations** [Ashley and Carrillo 2024b]). Type Ib motor neurons innervate single muscles with a few exceptions: (1) the Ib neuron that innervates both muscles 6 and 7 (MN6/7-Ib; also called RP3 in the embryo), (2) the Ib motor neurons that target several lateral transverse muscles, and (3) two Ib motor neurons that innervate muscles 15, 16, and 17 (Hoang and Chiba 2001). Is motor neurons innervate groups of muscles: The ventral common exciter (vCE; also called RP5 in the embryo and MNISNb/d-Is in the larva) targets the ventral muscles, and the dorsal common exciter (dCE; also called RP2 in the embryo and MNISN-Is in the larva) targets the dorsal muscle group (Takizawa et al. 2007). A third Is motor neuron has been reported to innervate the lateral transverse muscles (Hoang and Chiba 2001), but we did not observe Is innervation of these muscles in our studies (Wang et al. 2021, 2022).

Unlike those of crustaceans, *Drosophila* muscles do not receive inhibitory input in addition to excitatory glutamatergic input (Atwood 1967; Wolf and Lang 1994; Cooper and Cooper 2009; Pflüger and Duch 2011). Instead, muscles are coinnervated by neuromodulatory neurons: type II and type III (Gorczyca et al. 1993; Stocker et al. 2018). Unlike type Ib motor neurons that contain glutamate-filled clear vesicles, type II motor neurons are aminergic and contain dense core vesicles filled with octopamine (Monastirioti et al. 1995, 1996; Monastirioti 1999; Stocker et al. 2018), analogous to adrenaline in vertebrates. Type II boutons are also smaller than type I boutons (1–2 µm vs. 2–5 µm, respectively). Type II motor neurons—dorsal unpaired median neurons and ventral unpaired median neurons—in the VNC send bilaterally symmetrical axons into the body wall, where they innervate groups of muscles. Type II boutons are only sparsely labeled with the active zone scaffolding protein BRP (Koon and Budnik 2012), in contrast to the accumulation of BRP at type I boutons. Autocrine signaling, mediated by the Octβ2R receptor, promotes the elaboration of new type II

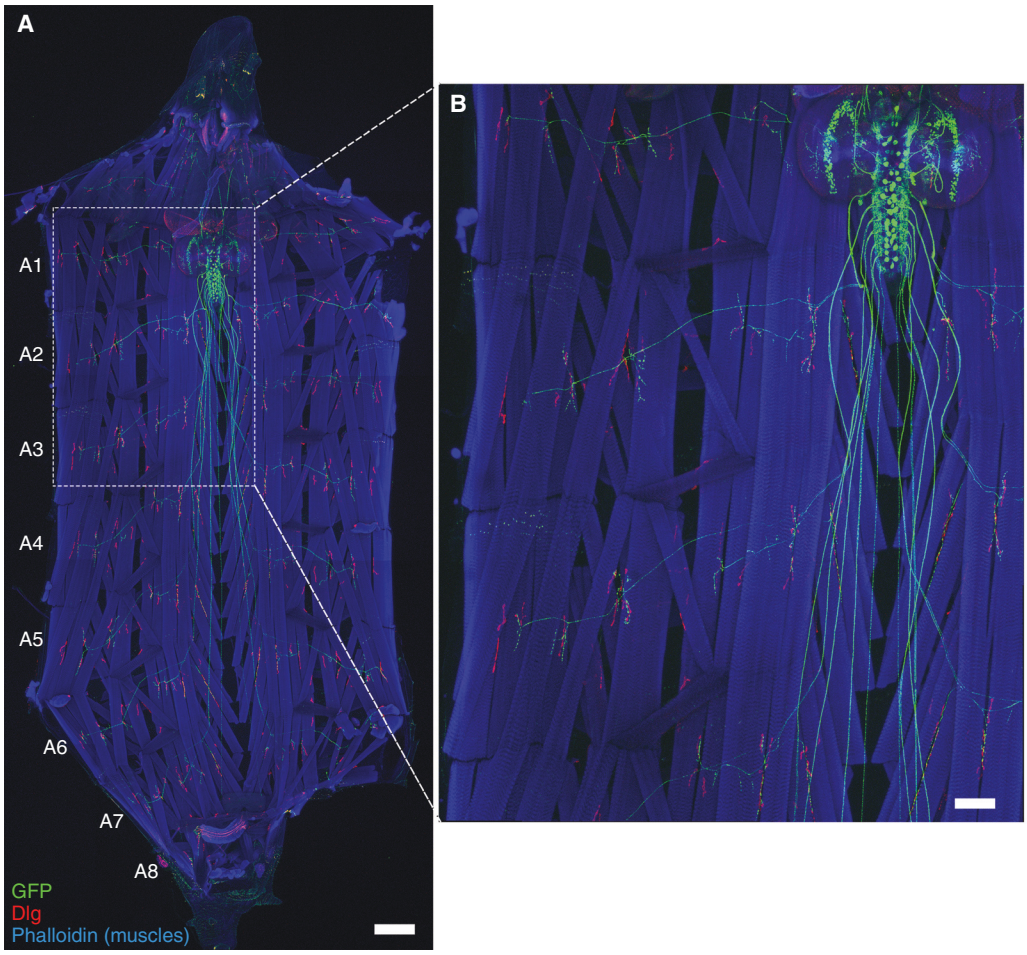

FIGURE 2. Third-instar larval body wall fillet. (*A*,*B*) Larval body wall fillet highlighting muscles (phalloidin; blue), ventral common exciter (vCE) and dorsal common exciter (dCE) motor neurons (anti-GFP staining; green), and neuromuscular junctions (NMJs; anti-Dlg staining; red). (*A*) Low-magnification tiled image. (*B*) High-magnification tiled image. Scale bars: *A*, 250 µm; *B*, 50 µm.

endings (Koon et al. 2011) that is required for an increase in locomotion upon starvation. Another receptor, Octβ1R, counteracts the positive effects and inhibits synaptic growth of both type II and type I motor terminals (Koon and Budnik 2012). The remaining type of neuromodulatory motor neuron is the peptidergic type III neuron, which innervates muscle 12. Type III boutons are 2–5 µm in diameter and contain dense core vesicles and *Drosophila* insulin-like peptide (Dilp) (Gorczyca et al. 1993; Wong et al. 2012). These neurons, however, are not widely studied, and thus much less is known about their function compared to the other motor neuron types.

MOTOR NEURON–MUSCLE RECOGNITION

The matching of motor neurons and their muscle targets must be highly specific, as patterned recruitment of muscles drives all motor behaviors. A number of mutant screens for alterations in NMJ morphology or patterning in the larval neuromuscular system have been performed (Nose et al. 1992; Vactor et al. 1993). Loss-of-function (LOF) mutants in a small subset of genes cause motor axons to fail to arborize onto any muscle fibers, resulting in large-scale alterations in innervation patterns. However, many of these phenotypes are due to perturbations in axon pathfinding (e.g.,

Beaten Path) (Fambrough and Goodman 1996; Pipes et al. 2001) rather than synaptic partner matching; thus, it is important to carefully examine when during circuit assembly the phenotype arises.

Several studies have identified local molecules that contribute to the invariant NMJ map. These studies, like most fly NMJ research, have mainly focused on two pairs of neighboring muscles: m6/7 and m13/12. Synaptic partner-matching molecules include both secreted and membrane-bound proteins. Netrin-B, for example, is a local attractive cue secreted by muscles 6/7 and attracts the RP3 neuron (MN6/7-Ib) through the Frazzled receptor (Mitchell et al. 1996; Winberg et al. 1998). Most cell surface proteins required for recognition fall into the leucine-rich repeat family or the immunoglobulin superfamily (IgSF). Fas3 is an IgSF protein normally expressed in muscles 6/7 and RP3, and ectopic expression of Fas3 in nontarget muscles results in RP3 innervating these muscles, suggesting that Fas3 is a homophilic recognition molecule (Chiba et al. 1995). In the early 1990s, the Goodman laboratory uncovered Connectin (Con) as a putative homophilic recognition molecule, and gain-of-function (GOF) studies, similar to Fas3 studies, revealed that Con can rewire neuromuscular connections (Nose et al. 1992, 1994). Capricious (Shishido et al. 1998; Taniguchi et al. 2000; Kohsaka and Nose 2009), Tartan (Kurusu et al. 2008), Toll (Rose et al. 1997; Ritzenthaler and Chiba 2003), Teneurin (Mosca et al. 2012; DePew et al. 2019), Hattifattener (Kurusu et al. 2008), Convoluted (Kurusu et al. 2008), and others (Nose 2012) have also been implicated in synaptic partner matching in the neuromuscular circuit. However, LOF mutations in any of these genes produce only low-penetrance targeting defects, suggesting partially redundant targeting cues.

To uncover novel interactions between cell surface proteins, the Garcia laboratory developed the extracellular interaction assay and tested 202 proteins bearing leucine-rich repeat, IgSF, and Fibronectin type III domains. In collaboration with the Zinn laboratory, they identified 83 novel interactions, including those between two subfamilies of the IgSF: defective proboscis response (Dpr) proteins and Dpr-interacting proteins (DIPs) (Özkan et al. 2013; Carrillo et al. 2015; also see Protocol: **Labeling of Cell Surface Proteins at the *Drosophila* Larval Neuromuscular Junction Using Binding Partner Peptides** [Ashley and Carrillo 2024c]). The 21 Dprs and the 11 DIPs interact heterophilically, and a small subset can also interact homophilically (Özkan et al. 2013; Carrillo et al. 2015; Cosmanescu et al. 2018; Cheng et al. 2019). Dprs and DIPs were found to be expressed throughout the fly visual circuit, and intriguingly, many Dpr–DIP interactors were found in respective synaptic partners (Özkan et al. 2013; Carrillo et al. 2015; Tan et al. 2015; Cosmanescu et al. 2018; Xu et al. 2018), suggesting that these proteins may be part of the synaptic recognition code. Indeed, several laboratories used LOF and GOF studies to demonstrate a role for Dprs and DIPs in wiring subsets of photoreceptors and medulla neurons (Carrillo et al. 2015; Cosmanescu et al. 2018; Xu et al. 2018; Courgeon and Desplan 2019; Xu et al. 2019). Recently, we began to examine the function of these IgSF proteins in the embryonic/larval neuromuscular circuit. We observed expression of several *dpr* and *DIP* genes in motor neurons and muscles (Carrillo et al. 2015; Ashley et al. 2019) and recently found that each motor neuron expresses a unique subset of *dpr* and *DIP* genes (Wang et al. 2022). For example, *DIP-α* is expressed in two type Is motor neurons (vCE and dCE), and the gene for one of its binding partners, *dpr10*, is expressed in muscles. Surprisingly, loss of *DIP-α* or *dpr10* results in the highly penetrant loss of innervation of specific muscles including muscle 4 and, to a lesser extent, muscle 3 (Ashley et al. 2019). Whether other Dprs and DIPs are required for neuromuscular assembly has yet to be determined. Overall, these studies suggest that homophilic and heterophilic interactions between cell surface proteins instruct motor neuron–muscle synaptic partner matching, and likely, multiple attractive and repulsive cues act in combination to map neuromuscular circuits (Winberg et al. 1998; Nose 2012).

NMJ ELABORATION DURING LARVAL DEVELOPMENT

The initiation of bouton/synapse formation marks a critical transition from the signaling that guides the stereotyped patterns of motor neuron–muscle connections in the embryo to the differentiative program that stabilizes and elaborates the synaptic connections on each muscle in the larva. Although the connectivity map of the neuromuscular circuit is preserved during larval development, a major

challenge encountered is that of maintaining synaptic efficacy as the animal grows: The muscle expands 100-fold in surface area, and the motor neuron terminal arbors must expand to maintain adequate signaling. Morphologically, to expand synaptic connections and complexity, NMJs elaborate by adding and retracting boutons. A foundational study by Karen Zito and colleagues used chimeric green fluorescent proteins tagged to the postsynaptic membrane for time-lapse imaging of postsynaptic structures that outline boutons (Zito et al. 1999). These studies revealed that new bouton formation occurs either between pre-existing boutons or at the end of an NMJ branch. These increases in bouton number can be observed by dissecting late embryos and late-stage larvae (see Protocol: ***Drosophila* Late Embryonic through Late Larval Stage Body Wall Dissection: Dissection Tools and Techniques** [Ashley and Carrillo 2024a] and Protocol: **Immunohistochemistry and Morphometric Analysis of *Drosophila* Larval Body Wall Neuromuscular Junction Preparations** [Ashley and Carrillo 2024b]). More recent studies showed that the NMJ can respond morphologically to acute changes in activity (Eaton et al. 2002; Yoshihara et al. 2005; Ataman et al. 2008), and NMJs continually grow if the life span of the larva is extended (Miller et al. 2012).

A plethora of genetic and biochemical studies have examined the signaling mechanisms that promote or inhibit NMJ growth. For example, muscles release the BMP ligand Glass bottom boat (Gbb), which activates the presynaptic receptor Wishful Thinking (Wit), resulting in phosphorylation of Mad (pMad) and translocation to the nucleus to change expression of a subset of genes (Aberle et al. 2002; Marqués et al. 2002; McCabe et al. 2003; Rawson et al. 2003; Ball et al. 2010; Berke et al. 2013). Another signaling pathway relies on the release of Wingless (Wg) from motor neurons and glia and binding to its receptor, DFrizzled2 (DFz2), thus triggering postsynaptic RNA localization (Packard et al. 2002; Mathew et al. 2005; Ataman et al. 2008; Mosca and Schwarz 2010; Speese et al. 2012; Kerr et al. 2014). Recently, the Mosca laboratory discovered that cleavage of DFz2 by presenilin is required for NMJ development (Restrepo et al. 2022). The interactions between DFz2 and presenilin was confirmed by proximity ligation assay (see Protocol: **Using the Proximity Ligation Assay to Visualize Colocalization of Proteins at the *Drosophila* Larval Neuromuscular Junction** [Ashley and Carrillo 2024d]). We direct readers to several excellent reviews that describe the signaling pathways underlying NMJ elaboration during larval development (Keshishian and Kim 2004; Menon et al. 2013; Chou et al. 2020).

Recently, our laboratory and others discovered that motor neuron elaboration can also be modified when neighboring motor neurons die; we ablated Is motor neurons and observed NMJ expansion at the adjacent Ib motor neurons (Aponte-Santiago and Littleton 2020; Wang et al. 2021, 2023; Han et al. 2022). These studies suggest that motor neuron morphology is highly dynamic not only during normal development but also when confronted with perturbations in the surrounding environment. The methods to ablate motor neurons and other cells are introduced in Protocol: **Cell Ablation Techniques for the Larval *Drosophila* Neuromuscular Junction** (Ashley and Carrillo 2024e).

The *Drosophila* NMJ is used by many laboratories across the world and continues to shed light on fundamental programs underlying synaptic development and function. The fortuitous utilization of glutamate at these synapses provides a powerful model system for excitatory vertebrate synapses, and certainly many exciting new discoveries await to be uncovered.

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REFERENCES

Aberle H, Haghighi AP, Fetter RD, McCabe BD, Magalhães TR, Goodman CS. 2002. Wishful thinking encodes a BMP type II receptor that regulates synaptic growth in *Drosophila*. *Neuron* **33:** 545–558. doi:10.1016/S0896-6273(02)00589-5

Aponte-Santiago NA, Littleton JT. 2020. Synaptic properties and plasticity mechanisms of invertebrate tonic and phasic neurons. *Front Physiol* **11:** 611982. doi:10.3389/fphys.2020.611982

Aravamudan B, Fergestad T, Davis WS, Rodesch CK, Broadie K. 1999. *Drosophila* UNC-13 is essential for synaptic transmission. *Nat Neurosci* **2:** 965–971. doi:10.1038/14764

Arroyo DA, Feller MB. 2016. Spatiotemporal features of retinal waves instruct the wiring of the visual circuitry. *Front Neural Circuits* **10:** 54. doi:10.3389/fncir.2016.00054

Artero RD, Castanon I, Baylies MK. 2001. The immunoglobulin-like protein Hibris functions as a dose-dependent regulator of myoblast fusion and is differentially controlled by Ras and Notch signaling. *Development* **128:** 4251–4264. doi:10.1242/dev.128.21.4251

Ashley J, Carrillo RA. 2024a. *Drosophila* late embryonic through late larval stage body wall dissection: dissection tools and techniques. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108499

Ashley J, Carrillo RA. 2024b. Immunohistochemistry and morphometric analysis of *Drosophila* larval body wall neuromuscular junction preparations. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108500

Ashley J, Carrillo RA. 2024c. Labeling of cell surface proteins at the *Drosophila* larval neuromuscular junction using binding partner peptides. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108501

Ashley J, Carrillo R. 2024d. Using the proximity ligation assay to visualize colocalization of proteins at the *Drosophila* larval neuromuscular junction. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108502

Ashley J, Carrillo RA. 2024e. Cell ablation techniques for the larval *Drosophila* neuromuscular junction. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot108503

Ashley J, Sorrentino V, Lobb-Rabe M, Nagarkar-Jaiswal S, Tan L, Xu S, Xiao Q, Zinn K, Carrillo RA. 2019. Transsynaptic interactions between IgSF proteins DIP-α and Dpr10 are required for motor neuron targeting specificity. *eLife* **8:** e42690. doi:10.7554/eLife.42690

Ataman B, Ashley J, Gorczyca M, Ramachandran P, Fouquet W, Sigrist SJ, Budnik V. 2008. Rapid activity-dependent modifications in synaptic structure and function require bidirectional Wnt signaling. *Neuron* **57:** 705–718. doi:10.1016/j.neuron.2008.01.026

Atwood HL. 1967. Variation in physiological properties of crustacean motor synapses. *Nature* **215:** 57–58. doi:10.1038/215057a0

Atwood HL, Govind CK, Wu CF. 1993. Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. *J Neurobiol* **24:** 1008–1024. doi:10.1002/neu.480240803

Ball RW, Warren-Paquin M, Tsurudome K, Liao EH, Elazzouzi F, Cavanagh C, An BS, Wang TT, White JH, Haghighi AP. 2010. Retrograde BMP signaling controls synaptic growth at the NMJ by regulating trio expression in motor neurons. *Neuron* **66:** 536–549. doi:10.1016/j.neuron.2010.04.011

Bate M. 1990. The embryonic development of larval muscles in *Drosophila*. *Development* **110:** 791–804. doi:10.1242/dev.110.3.791

Bate M, Rushton E. 1993. Myogenesis and muscle patterning in *Drosophila*. *C R Acad Sci III* **316:** 1047–1061.

Bate M, Rushton E, Frasch M. 1993. A dual requirement for neurogenic genes in *Drosophila* myogenesis. *Dev Suppl* **1993:** 149–161.

Bazemore A, Elliott KA, Florey E. 1956. Factor I and γ-aminobutyric acid. *Nature* **178:** 1052–1053. doi:10.1038/1781052a0

Berke B, Wittnam J, McNeill E, Van Vactor DL, Keshishian H. 2013. Retrograde BMP signaling at the synapse: a permissive signal for synapse maturation and activity-dependent plasticity. *J Neurosci* **33:** 17937–17950. doi:10.1523/JNEUROSCI.6075-11.2013

Bour BA, Chakravarti M, West JM, Abmayr SM. 2000. *Drosophila* SNS, a member of the immunoglobulin superfamily that is essential for myoblast fusion. *Genes Dev* **14:** 1498–1511. doi:10.1101/gad.14.12.1498

Bourgouin C, Lundgren SE, Thomas JB. 1992. Apterous is a *Drosophila* LIM domain gene required for the development of a subset of embryonic muscles. *Neuron* **9:** 549–561. doi:10.1016/0896-6273(92)90192-G

Broadie K, Bate M. 1993. Innervation directs receptor synthesis and localization in *Drosophila* embryo synaptogenesis. *Nature* **361:** 350–353. doi:10.1038/361350a0

Broadie K, Prokop A, Bellen HJ, O'Kane CJ, Schulze KL, Sweeney ST. 1995. Syntaxin and synaptobrevin function downstream of vesicle docking in *Drosophila*. *Neuron* **15:** 663–673. doi:10.1016/0896-6273(95)90154-X

Burden SJ, Huijbers MG, Remedio L. 2018. Fundamental molecules and mechanisms for forming and maintaining neuromuscular synapses. *Int J Mol Sci* **19:** 490. doi:10.3390/ijms19020490

Burgess RW. 2006. The formation of the vertebrate neuromuscular junction: roles for the extracellular matrix in synaptogenesis. In *Molecular mechanisms of synaptogenesis* (ed. Dityatev A, El-Husseini A), Springer, Boston, MA.

Canal I, Ferrús A. 1986. The pattern of early neuronal differentiation in *Drosophila melanogaster*. *J Neurogenet* **3:** 293–319. doi:10.3109/01677068609106856

Carrillo RA, Olsen DP, Yoon KS, Keshishian H. 2010. Presynaptic activity and CaMKII modulate retrograde semaphorin signaling and synaptic refinement. *Neuron* **68:** 32–44. doi:10.1016/j.neuron.2010.09.005

Carrillo RA, Özkan E, Menon KP, Nagarkar-Jaiswal S, Lee PT, Jeon M, Birnbaum ME, Bellen HJ, Garcia KC, Zinn K. 2015. Control of synaptic connectivity by a network of *Drosophila* IgSF cell surface proteins. *Cell* **163:** 1770–1782. doi:10.1016/j.cell.2015.11.022

Cheng S, Ashley J, Kurleto JD, Lobb-Rabe M, Park YJ, Carrillo RA, Özkan E. 2019. Molecular basis of synaptic specificity by immunoglobulin superfamily receptors in *Drosophila*. *eLife* **8:** e41028. doi:10.7554/eLife.41028

Chiba A, Snow P, Keshishian H, Hotta Y. 1995. Fasciclin III as a synaptic target recognition molecule in *Drosophila*. *Nature* **374:** 166–168. doi:10.1038/374166a0

Chisholm A, Tessier-Lavigne M. 1999. Conservation and divergence of axon guidance mechanisms. *Curr Opin Neurobiol* **9:** 603–615. doi:10.1016/S0959-4388(99)00021-5

Choi JC, Park D, Griffith LC. 2004. Electrophysiological and morphological characterization of identified motor neurons in the *Drosophila* third instar larva central nervous system. *J Neurophysiol* **91:** 2353–2365. doi:10.1152/jn.01115.2003

Chou VT, Johnson SA, Van Vactor D. 2020. Synapse development and maturation at the *Drosophila* neuromuscular junction. *Neural Dev* **15:** 11. doi:10.1186/s13064-020-00147-5

Ciglar L, Girardot C, Wilczyński B, Braun M, Furlong EE. 2014. Coordinated repression and activation of two transcriptional programs stabilizes cell fate during myogenesis. *Development* **141:** 2633–2643. doi:10.1242/dev.101956

Cooper AS, Cooper RL. 2009. Historical view and physiology demonstration at the NMJ of the crayfish opener muscle. *J Vis Exp* **33:** 1595. doi:10.3791/1595-v

Cosmanescu F, Katsamba PS, Sergeeva AP, Ahlsen G, Patel SD, Brewer JJ, Tan L, Xu S, Xiao Q, Nagarkar-Jaiswal S, et al. 2018. Neuron-subtype-specific expression, interaction affinities, and specificity determinants of DIP/Dpr cell recognition proteins. *Neuron* **100:** 1385–1400.e6. doi:10.1016/j.neuron.2018.10.046

Courgeon M, Desplan C. 2019. Coordination between stochastic and deterministic specification in the *Drosophila* visual system. *Science* **366:** eaay6727. doi:10.1126/science.aay6727

Demontis F, Perrimon N. 2009. Integration of Insulin receptor/Foxo signaling and dMyc activity during muscle growth regulates body size in *Drosophila*. *Development* **136:** 983–993. doi:10.1242/dev.027466

DePew AT, Aimino MA, Mosca TJ. 2019. The tenets of teneurin: conserved mechanisms regulate diverse developmental processes in the *Drosophila* nervous system. *Front Neurosci* **13:** 27. doi:10.3389/fnins.2019.00027

Dohrmann C, Azpiazu N, Frasch M. 1990. A new *Drosophila* homeobox gene is expressed in mesodermal precursor cells of distinct muscles during embryogenesis. *Genes Dev* **4:** 2098–2111. doi:10.1101/gad.4.12a.2098

Doll CA, Broadie K. 2014. Impaired activity-dependent neural circuit assembly and refinement in autism spectrum disorder genetic models. *Front Cell Neurosci* **8:** 30. doi:10.3389/fncel.2014.00030

Duan H, Skeath JB, Nguyen HT. 2001. *Drosophila* Lame duck, a novel member of the Gli superfamily, acts as a key regulator of myogenesis by controlling fusion-competent myoblast development. *Development* **128:** 4489–4500. doi:10.1242/dev.128.22.4489

Eaton BA, Fetter RD, Davis GW. 2002. Dynactin is necessary for synapse stabilization. *Neuron* **34:** 729–741. doi:10.1016/S0896-6273(02)00721-3

Fambrough D, Goodman CS. 1996. The *Drosophila* beaten path gene encodes a novel secreted protein that regulates defasciculation at motor axon choice points. *Cell* **87:** 1049–1058. doi:10.1016/S0092-8674(00)81799-7

Florey E. 1991. GABA: history and perspectives. *Can J Physiol Pharmacol* **69:** 1049–1056. doi:10.1139/y91-156

Frank CA, James TD, Müller M. 2020. Homeostatic control of *Drosophila* neuromuscular junction function. *Synapse* **74:** e22133. doi:10.1002/syn.22133

Goel P, Nishimura S, Chetlapalli K, Li X, Chen C, Dickman D. 2020. Distinct target-specific mechanisms homeostatically stabilize transmission at pre- and post-synaptic compartments. *Front Cell Neurosci* **14:** 196. doi:10.3389/fncel.2020.00196

Gorczyca M, Augart C, Budnik V. 1993. Insulin-like receptor and insulin-like peptide are localized at neuromuscular junctions in *Drosophila*. *J Neurosci* **13:** 3692–3704. doi:10.1523/JNEUROSCI.13-09-03692.1993

Graf ER, Valakh V, Wright CM, Wu C, Liu Z, Zhang YQ, DiAntonio A. 2012. RIM promotes calcium channel accumulation at active zones of the *Drosophila* neuromuscular junction. *J Neurosci* **32:** 16586–16596. doi:10.1523/JNEUROSCI.0965-12.2012

Guan B, Hartmann B, Kho YH, Gorczyca M, Budnik V. 1996. The *Drosophila* tumor suppressor gene, *dlg*, is involved in structural plasticity at a glutamatergic synapse. *Curr Biol* **6:** 695–706. doi:10.1016/S0960-9822(09)00451-5

Halpern ME, Chiba A, Johansen J, Keshishian H. 1991. Growth cone behavior underlying the development of stereotypic synaptic connections in *Drosophila* embryos. *J Neurosci* **11:** 3227–3238. doi:10.1523/JNEUROSCI.11-10-03227.1991

Han Y, Chien C, Goel P, He K, Pinales C, Buser C, Dickman D. 2022. Botulinum neurotoxin accurately separates tonic vs. phasic transmission and reveals heterosynaptic plasticity rules in *Drosophila*. *eLife* **11:** e77924. doi:10.7554/eLife.77924

He T, Singh V, Rumpal N, Lnenicka GA. 2009. Differences in Ca^{2+} regulation for high-output Is and low-output Ib motor terminals in *Drosophila* larvae. *Neuroscience* **159:** 1283–1291. doi:10.1016/j.neuroscience.2009.01.074

Hoang B, Chiba A. 2001. Single-cell analysis of *Drosophila* larval neuromuscular synapses. *Dev Biol* **229:** 55–70. doi:10.1006/dbio.2000.9983

Hua JY, Smith SJ. 2004. Neural activity and the dynamics of central nervous system development. *Nat Neurosci* **7:** 327–332. doi:10.1038/nn1218

Huxley TH. 1880. *The crayfish: an introduction to the study of zoology*. D Appleton and Company, New York.

Jan LY, Jan YN. 1976. Properties of the larval neuromuscular junction in *Drosophila melanogaster*. *J Physiol (Lond)* **262:** 189–214. doi:10.1113/jphysiol.1976.sp011592

Jarecki J, Keshishian H. 1995. Role of neural activity during synaptogenesis in *Drosophila*. *J Neurosci* **15:** 8177–8190. doi:10.1523/JNEUROSCI.15-12-08177.1995

Jeong S. 2021. Molecular mechanisms underlying motor axon guidance in *Drosophila*. *Mol Cells* **44:** 549–556. doi:10.14348/molcells.2021.0129

Jia XX, Gorczyca M, Budnik V. 1993. Ultrastructure of neuromuscular junctions in *Drosophila*: comparison of wild type and mutants with increased excitability. *J Neurobiol* **24:** 1025–1044. doi:10.1002/neu.480240804

Katz LC, Shatz CJ. 1996. Synaptic activity and the construction of cortical circuits. *Science* **274:** 1133–1138. doi:10.1126/science.274.5290.1133

Kerr KS, Fuentes-Medel Y, Brewer C, Barria R, Ashley J, Abruzzi KC, Sheehan A, Tasdemir-Yilmaz OE, Freeman MR, Budnik V. 2014. Glial wingless/Wnt regulates glutamate receptor clustering and synaptic physiology at the *Drosophila* neuromuscular junction. *J Neurosci* **34:** 2910–2920. doi:10.1523/JNEUROSCI.3714-13.2014

Keshishian H, Chiba A. 1993. Neuromuscular development in *Drosophila*: insights from single neurons and single genes. *Trends Neurosci* **16:** 278–283. doi:10.1016/0166-2236(93)90182-L

Keshishian H, Kim YS. 2004. Orchestrating development and function: retrograde BMP signaling in the *Drosophila* nervous system. *Trends Neurosci* **27:** 143–147. doi:10.1016/j.tins.2004.01.004

Kim MJ, O'Connor MB. 2021. *Drosophila* Activin signaling promotes muscle growth through InR/TORC1-dependent and -independent processes. *Development* **148:** dev190868.

Kim YJ, Bao H, Bonanno L, Zhang B, Serpe M. 2012. *Drosophila* Neto is essential for clustering glutamate receptors at the neuromuscular junction. *Genes Dev* **26:** 974–987. doi:10.1101/gad.185165.111

Kittel RJ, Wichmann C, Rasse TM, Fouquet W, Schmidt M, Schmid A, Wagh DA, Pawlu C, Kellner RR, Willig KI, et al. 2006. Bruchpilot promotes active zone assembly, Ca^{2+} channel clustering, and vesicle release. *Science* **312:** 1051–1054. doi:10.1126/science.1126308

Kohsaka H, Nose A. 2009. Target recognition at the tips of postsynaptic filopodia: accumulation and function of Capricious. *Development* **136:** 1127–1135. doi:10.1242/dev.027920

Koon AC, Budnik V. 2012. Inhibitory control of synaptic and behavioral plasticity by octopaminergic signaling. *J Neurosci* **32:** 6312–6322. doi:10.1523/JNEUROSCI.6517-11.2012

Koon AC, Ashley J, Barria R, DasGupta S, Brain R, Waddell S, Alkema MJ, Budnik V. 2011. Autoregulatory and paracrine control of synaptic and behavioral plasticity by octopaminergic signaling. *Nat Neurosci* **14:** 190–199. doi:10.1038/nn.2716

Koropouli E, Kolodkin AL. 2014. Semaphorins and the dynamic regulation of synapse assembly, refinement, and function. *Curr Opin Neurobiol* **27:** 1–7. doi:10.1016/j.conb.2014.02.005

Kose H, Rose D, Zhu X, Chiba A. 1997. Homophilic synaptic target recognition mediated by immunoglobulin-like cell adhesion molecule Fasciclin III. *Development* **124:** 4143–4152. doi:10.1242/dev.124.20.4143

Kurusu M, Cording A, Taniguchi M, Menon K, Suzuki E, Zinn K. 2008. A screen of cell-surface molecules identifies leucine-rich repeat proteins as key mediators of synaptic target selection. *Neuron* **59:** 972–985. doi:10.1016/j.neuron.2008.07.037

Landgraf M, Thor S. 2006. Development and structure of motoneurons. *Int Rev Neurobiol* **75:** 33–53. doi:10.1016/S0074-7742(06)75002-4

Landgraf M, Bossing T, Technau GM, Bate M. 1997. The origin, location, and projections of the embryonic abdominal motorneurons of *Drosophila*. *J Neurosci* **17:** 9642–9655. doi:10.1523/JNEUROSCI.17-24-09642.1997

Liebl FL, Wu Y, Featherstone DE, Noordermeer JN, Fradkin L, Hing H. 2008. Derailed regulates development of the *Drosophila* neuromuscular junction. *Dev Neurobiol* **68:** 152–165. doi:10.1002/dneu.20562

Liu KS, Siebert M, Mertel S, Knoche E, Wegener S, Wichmann C, Matkovic T, Muhammad K, Depner H, Mettke C, et al. 2011. RIM-binding protein, a central part of the active zone, is essential for neurotransmitter release. *Science* **334:** 1565–1569. doi:10.1126/science.1212991

Lnenicka GA, Keshishian H. 2000. Identified motor terminals in *Drosophila* larvae show distinct differences in morphology and physiology. *J Neurobiol* **43:** 186–197. doi:10.1002/(SICI)1097-4695(200005)43:2<186::AID-NEU8>3.0.CO;2-N

Marqués G, Bao H, Haerry TE, Shimell MJ, Duchek P, Zhang B, O'Connor MB. 2002. The *Drosophila* BMP type II receptor Wishful Thinking regulates neuromuscular synapse morphology and function. *Neuron* **33:** 529–543. doi:10.1016/S0896-6273(02)00595-0

Mathew D, Ataman B, Chen J, Zhang Y, Cumberledge S, Budnik V. 2005. Wingless signaling at synapses is through cleavage and nuclear import of receptor DFrizzled2. *Science* **310:** 1344–1347. doi:10.1126/science.1117051

McCabe BD, Marqués G, Haghighi AP, Fetter RD, Crotty ML, Haerry TE, Goodman CS, O'Connor MB. 2003. The BMP homolog Gbb provides a retrograde signal that regulates synaptic growth at the *Drosophila* neuromuscular junction. *Neuron* **39:** 241–254. doi:10.1016/S0896-6273(03)00426-4

Meng JL, Heckscher ES. 2021. Development of motor circuits: from neuronal stem cells and neuronal diversity to motor circuit assembly. *Curr Top Dev Biol* **142:** 409–442. doi:10.1016/bs.ctdb.2020.11.010

Menon KP, Carrillo RA, Zinn K. 2013. Development and plasticity of the *Drosophila* larval neuromuscular junction. *Wiley Interdiscip Rev Dev Biol* **2:** 647–670. doi:10.1002/wdev.108

Miller DL, Ballard SL, Ganetzky B. 2012. Analysis of synaptic growth and function in *Drosophila* with an extended larval stage. *J Neurosci* **32:** 13776–13786. doi:10.1523/JNEUROSCI.0508-12.2012

Mitchell KJ, Doyle JL, Serafini T, Kennedy TE, Tessier-Lavigne M, Goodman CS, Dickson BJ. 1996. Genetic analysis of *Netrin* genes in *Drosophila*:

Netrins guide CNS commissural axons and peripheral motor axons. *Neuron* **17:** 203–215. doi:10.1016/S0896-6273(00)80153-1

Monastirioti M. 1999. Biogenic amine systems in the fruit fly *Drosophila melanogaster*. *Microsc Res Tech* **45:** 106–121. doi:10.1002/(SICI)1097-0029(19990415)45:2<106::AID-JEMT5>3.0.CO;2-3

Monastirioti M, Gorczyca M, Rapus J, Eckert M, White K, Budnik V. 1995. Octopamine immunoreactivity in the fruit fly *Drosophila melanogaster*. *J Comp Neurol* **356:** 275–287. doi:10.1002/cne.903560210

Monastirioti M, Linn CE Jr., White K. 1996. Characterization of *Drosophila tyramine β-hydroxylase* gene and isolation of mutant flies lacking octopamine. *J Neurosci* **16:** 3900–3911. doi:10.1523/JNEUROSCI.16-12-03900.1996

Mosca TJ, Schwarz TL. 2010. The nuclear import of Frizzled2-C by Importins-β11 and α2 promotes postsynaptic development. *Nat Neurosci* **13:** 935–943. doi:10.1038/nn.2593

Mosca TJ, Hong W, Dani VS, Favaloro V, Luo L. 2012. Trans-synaptic Teneurin signalling in neuromuscular synapse organization and target choice. *Nature* **484:** 237–241. doi:10.1038/nature10923

Nose A. 2012. Generation of neuromuscular specificity in *Drosophila*: novel mechanisms revealed by new technologies. *Front Mol Neurosci* **5:** 62. doi:10.3389/fnmol.2012.00062

Nose A, Mahajan VB, Goodman CS. 1992. Connectin: a homophilic cell adhesion molecule expressed on a subset of muscles and the motoneurons that innervate them in *Drosophila*. *Cell* **70:** 553–567. doi:10.1016/0092-8674(92)90426-D

Nose A, Takeichi M, Goodman CS. 1994. Ectopic expression of connectin reveals a repulsive function during growth cone guidance and synapse formation. *Neuron* **13:** 525–539. doi:10.1016/0896-6273(94)90023-X

Nose A, Isshiki T, Takeichi M. 1998. Regional specification of muscle progenitors in *Drosophila*: the role of the *msh* homeobox gene. *Development* **125:** 215–223. doi:10.1242/dev.125.2.215

Özkan E, Carrillo RA, Eastman CL, Weiszmann R, Waghray D, Johnson KG, Zinn K, Celniker SE, Garcia KC. 2013. An extracellular interactome of immunoglobulin and LRR proteins reveals receptor-ligand networks. *Cell* **154:** 228–239. doi:10.1016/j.cell.2013.06.006

Packard M, Koo ES, Gorczyca M, Sharpe J, Cumberledge S, Budnik V. 2002. The *Drosophila* Wnt, wingless, provides an essential signal for pre- and postsynaptic differentiation. *Cell* **111:** 319–330. doi:10.1016/S0092-8674(02)01047-4

Pan Y, Monje M. 2020. Activity shapes neural circuit form and function: a historical perspective. *J Neurosci* **40:** 944–954. doi:10.1523/JNEUROSCI.0740-19.2019

Pflüger HJ, Duch C. 2011. Dynamic neural control of insect muscle metabolism related to motor behavior. *Physiology (Bethesda)* **26:** 293–303. doi:10.1152/physiol.00002.2011

Pipes GC, Lin Q, Riley SE, Goodman CS. 2001. The Beat generation: a multigene family encoding IgSF proteins related to the Beat axon guidance molecule in *Drosophila*. *Development* **128:** 4545–4552. doi:10.1242/dev.128.22.4545

Ramos CI, Igiesuorobo O, Wang Q, Serpe M. 2015. Neto-mediated intracellular interactions shape postsynaptic composition at the *Drosophila* neuromuscular junction. *PLoS Genet* **11:** e1005191. doi:10.1371/journal.pgen.1005191

Rawson JM, Lee M, Kennedy EL, Selleck SB. 2003. *Drosophila* neuromuscular synapse assembly and function require the TGF-β type I receptor saxophone and the transcription factor Mad. *J Neurobiol* **55:** 134–150. doi:10.1002/neu.10189

Restrepo LJ, DePew AT, Moese ER, Tymanskyj SR, Parisi MJ, Aimino MA, Duhart JC, Fei H, Mosca TJ. 2022. γ-secretase promotes *Drosophila* postsynaptic development through the cleavage of a Wnt receptor. *Dev Cell* **57:** 1643–1660.e7. doi:10.1016/j.devcel.2022.05.006

Richardson BE, Nowak SJ, Baylies MK. 2008. Myoblast fusion in fly and vertebrates: new genes, new processes and new perspectives. *Traffic* **9:** 1050–1059. doi:10.1111/j.1600-0854.2008.00756.x

Richet C. 1879. Contribution a la physiologic des centres nerveux et des muscles de l'ecrevisse. *Arch de Physio* **6:** 522–576.

Ritzenthaler S, Chiba A. 2003. Myopodia (postsynaptic filopodia) participate in synaptic target recognition. *J Neurobiol* **55:** 31–40. doi:10.1002/neu.10180

Rose D, Zhu X, Kose H, Hoang B, Cho J, Chiba A. 1997. Toll, a muscle cell surface molecule, locally inhibits synaptic initiation of the RP3 motoneuron growth cone in *Drosophila*. *Development* **124:** 1561–1571. doi:10.1242/dev.124.8.1561

Rushton E, Drysdale R, Abmayr SM, Michelson AM, Bate M. 1995. Mutations in a novel gene, myoblast city, provide evidence in support of the founder cell hypothesis for *Drosophila* muscle development. *Development* **121:** 1979–1988. doi:10.1242/dev.121.7.1979

Sanes JR, Lichtman JW. 1999. Development of the vertebrate neuromuscular junction. *Annu Rev Neurosci* **22:** 389–442. doi:10.1146/annurev.neuro.22.1.389

Schmid A, Chiba A, Doe CQ. 1999. Clonal analysis of *Drosophila* embryonic neuroblasts: neural cell types, axon projections and muscle targets. *Development* **126:** 4653–4689. doi:10.1242/dev.126.21.4653

Sherman RG, Atwood HL. 1971. Synaptic facilitation: long-term neuromuscular facilitation in crustaceans. *Science* **171:** 1248–1250. doi:10.1126/science.171.3977.1248

Shi L, Fu AK, Ip NY. 2012. Molecular mechanisms underlying maturation and maintenance of the vertebrate neuromuscular junction. *Trends Neurosci* **35:** 441–453. doi:10.1016/j.tins.2012.04.005

Shishido E, Takeichi M, Nose A. 1998. *Drosophila* synapse formation: regulation by transmembrane protein with Leu-rich repeats, CAPRICIOUS. *Science* **280:** 2118–2121. doi:10.1126/science.280.5372.2118

Sink H, Whitington PM. 1991. Pathfinding in the central nervous system and periphery by identified embryonic *Drosophila* motor axons. *Development* **112:** 307–316. doi:10.1242/dev.112.1.307

Slater CR. 2017. The structure of human neuromuscular junctions: some unanswered molecular questions. *Int J Mol Sci* **18:** 2183. doi:10.3390/ijms18102183

Speese SD, Ashley J, Jokhi V, Nunnari J, Barria R, Li Y, Ataman B, Koon A, Chang YT, Li Q, et al. 2012. Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling. *Cell* **149:** 832–846. doi:10.1016/j.cell.2012.03.032

Stocker B, Bochow C, Damrau C, Mathejczyk T, Wolfenberg H, Colomb J, Weber C, Ramesh N, Duch C, Biserova NM, et al. 2018. Structural and molecular properties of insect type II motor axon terminals. *Front Syst Neurosci* **12:** 5. doi:10.3389/fnsys.2018.00005

Strünkelnberg M, Bonengel B, Moda LM, Hertenstein A, de Couet HG, Ramos RG, Fischbach KF. 2001. *rst* and its paralogue *kirre* act redundantly during embryonic muscle development in *Drosophila*. *Development* **128:** 4229–4239. doi:10.1242/dev.128.21.4229

Takizawa E, Komatsu A, Tsujimura H. 2007. Identification of common excitatory motoneurons in *Drosophila melanogaster* larvae. *Zool Sci* **24:** 504–513. doi:10.2108/zsj.24.504

Tan L, Zhang KX, Pecot MY, Nagarkar-Jaiswal S, Lee PT, Takemura SY, McEwen JM, Nern A, Xu S, Tadros W, et al. 2015. Ig superfamily ligand and receptor pairs expressed in synaptic partners in *Drosophila*. *Cell* **163:** 1756–1769. doi:10.1016/j.cell.2015.11.021

Taniguchi H, Shishido E, Takeichi M, Nose A. 2000. Functional dissection of *Drosophila* capricious: its novel roles in neuronal pathfinding and selective synapse formation. *J Neurobiol* **42:** 104–116. doi:10.1002/(SICI)1097-4695(200001)42:1<104::AID-NEU10>3.0.CO;2-V

Thomas JB, Bastiani MJ, Bate M, Goodman CS. 1984. From grasshopper to *Drosophila*: a common plan for neuronal development. *Nature* **310:** 203–207. doi:10.1038/310203a0

Vactor DV, Sink H, Fambrough D, Tsoo R, Goodman CS. 1993. Genes that control neuromuscular specificity in *Drosophila*. *Cell* **73:** 1137–1153. doi:10.1016/0092-8674(93)90643-5

Vilinsky I, Stewart BA, Drummond J, Robinson I, Deitcher DL. 2002. A *Drosophila* SNAP-25 null mutant reveals context-dependent redundancy with SNAP-24 in neurotransmission. *Genetics* **162:** 259–271. doi:10.1093/genetics/162.1.259

Vonhoff F, Keshishian H. 2017a. Activity-dependent synaptic refinement: new insights from *Drosophila*. *Front Syst Neurosci* **11:** 23. doi:10.3389/fnsys.2017.00023

Vonhoff F, Keshishian H. 2017b. Cyclic nucleotide signaling is required during synaptic refinement at the *Drosophila* neuromuscular junction. *Dev Neurobiol* **77:** 39–60. doi:10.1002/dneu.22407

Wang Y, Lobb-Rabe M, Ashley J, Anand V, Carrillo RA. 2021. Structural and functional synaptic plasticity induced by convergent synapse loss in the *Drosophila* neuromuscular circuit. *J Neurosci* **41:** 1401–1417. doi:10.1523/JNEUROSCI.1492-20.2020

Wang Y, Lobb-Rabe M, Ashley J, Chatterjee P, Anand V, Bellen HJ, Kanca O, Carrillo RA. 2022. Systematic expression profiling of Dpr and DIP genes

reveals cell surface codes in *Drosophila* larval motor and sensory neurons. *Development* **149:** dev200355. doi:10.1242/dev.200355

Wang Y, Zhang R, Huang S, Valverde PTT, Lobb-Rabe M, Ashley J, Venkatasubramanian L, Carrillo RA. 2023. Glial Draper signaling triggers cross-neuron plasticity in bystander neurons after neuronal cell death in *Drosophila*. *Nat Commun* **14:** 4452. doi:10.1038/s41467-023-40142-y

Winberg ML, Mitchell KJ, Goodman CS. 1998. Genetic analysis of the mechanisms controlling target selection: complementary and combinatorial functions of netrins, semaphorins, and IgCAMs. *Cell* **93:** 581–591. doi:10.1016/S0092-8674(00)81187-3

Winther AM, Vorontsova O, Rees KA, Näreoja T, Sopova E, Jiao W, Shupliakov O. 2015. An endocytic scaffolding protein together with synapsin regulates synaptic vesicle clustering in the *Drosophila* neuromuscular junction. *J Neurosci* **35:** 14756–14770. doi:10.1523/JNEUROSCI.1675-15.2015

Wolf H, Lang DM. 1994. Origin and clonal relationship of common inhibitory motoneurons CI1 and CI3 in the locust CNS. *J Neurobiol* **25:** 846–864. doi:10.1002/neu.480250709

Wong MY, Zhou C, Shakiryanova D, Lloyd TE, Deitcher DL, Levitan ES. 2012. Neuropeptide delivery to synapses by long-range vesicle circulation and sporadic capture. *Cell* **148:** 1029–1038. doi:10.1016/j.cell.2011.12.036

Wu H, Xiong WC, Mei L. 2010. To build a synapse: signaling pathways in neuromuscular junction assembly. *Development* **137:** 1017–1033. doi:10.1242/dev.038711

Xu S, Xiao Q, Cosmanescu F, Sergeeva AP, Yoo J, Lin Y, Katsamba PS, Ahlsen G, Kaufman J, Linaval NT, et al. 2018. Interactions between the Ig-superfamily proteins DIP-α and Dpr6/10 regulate assembly of neural circuits. *Neuron* **100:** 1369–1384.e6. doi:10.1016/j.neuron.2018.11.001

Xu C, Theisen E, Maloney R, Peng J, Santiago I, Yapp C, Werkhoven Z, Rumbaut E, Shum B, Tarnogorska D, et al. 2019. Control of synaptic specificity by establishing a relative preference for synaptic partners. *Neuron* **103:** 865–877.e7. doi:10.1016/j.neuron.2019.06.006

Yamamoto N, López-Bendito G. 2012. Shaping brain connections through spontaneous neural activity. *Eur J Neurosci* **35:** 1595–1604. doi:10.1111/j.1460-9568.2012.08101.x

Yoshihara M, Adolfsen B, Galle KT, Littleton JT. 2005. Retrograde signaling by Syt 4 induces presynaptic release and synapse-specific growth. *Science* **310:** 858–863. doi:10.1126/science.1117541

Zito K, Parnas D, Fetter RD, Isacoff EY, Goodman CS. 1999. Watching a synapse grow: noninvasive confocal imaging of synaptic growth in *Drosophila*. *Neuron* **22:** 719–729. doi:10.1016/S0896-6273(00)80731-X

The *Drosophila* Larval Neuromuscular Junction: Developmental Overview

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