Published in final edited form as:

Wiley Interdiscip Rev Dev Biol. 2017 September ; 6(5): . doi:10.1002/wdev.277.

Electrophysiological Analysis of Synaptic Transmission in *Drosophila*

Maria Bykhovskaia and Alexander Vasin

Abstract

Synaptic transmission is dynamic, plastic, and highly regulated. *Drosophila* is an advantageous model system for genetic and molecular studies of presynaptic and postsynaptic mechanisms and plasticity. Electrical recordings of synaptic responses represent a wide-spread approach to study neuronal signalling and synaptic transmission. We discuss experimental techniques that allow monitoring synaptic transmission in *Drosophila* neuromuscular and central systems. Recordings of synaptic potentials or currents at the larval neuromuscular junction (NMJ) are most common and provide numerous technical advantages due to robustness of the preparation, large and identifiable muscles, and synaptic boutons which can be readily visualized. In particular, focal macropatch recordings combined with the analysis of neurosecretory quanta enable rigorous quantification of the magnitude and kinetics of transmitter release. Patch-clamp recordings of synaptic transmission from the embryonic NMJ enable overcoming the problem of lethality in mutant lines. Recordings from the adult NMJ proved instrumental in the studies of temperature-sensitive paralytic mutants. Genetic studies of behavioural learning in *Drosophila* compel an investigation of synaptic transmission in the central nervous system (CNS), including primary cultured neurons and an intact brain. Cholinergic and GABAergic synaptic transmission has been recorded from the *Drosophila* CNS both *in vitro* and *in vivo*. *In vivo* patch-clamp recordings of synaptic transmission from the neurons in the olfactory pathway is a very powerful approach, which has a potential to elucidate how synaptic transmission is associated with behavioural learning.

Graphical abstract

Electrical recordings of synaptic responses from *Drosophila* neuromuscular and central nervous systems.

Introduction

Neurons communicate by releasing neuronal transmitters from nerve terminals. Transmitters are packed in synaptic vesicles and released into the synaptic cleft via the fusion of vesicles with the synaptic membrane. Transmitter molecules bind postsynaptic receptors and generate postsynaptic currents. Synaptic transmission is dynamic and plastic, and it is regulated at presynaptic and postsynaptic levels. Synaptic vesicles dock to the plasma membrane via a specialized protein complex termed SNARE, and a vesicle associated protein Synaptotagmin (Syt) triggers the fusion of vesicles with the plasma membrane and the release of neuronal transmitters¹. Transmitter molecules bind postsynaptic receptors, which generate synaptic currents. Electrical recordings of synaptic transmission is a widespread approach to study neuronal communication.

Peripheral nervous system in the fly is largely glutamatergic, and therefore *Drosophila* neuromuscular junction (NMJ) is an excellent model system to study the conserved features of glutamatergic transmission, including the protein machinery that governs transmitter release and the molecular biology of postsynaptic glutamate receptors. Since pioneering studies of Fatt and Katz², electrical recordings of synaptic transmission from the NMJ have been employed broadly to quantify the amount and kinetics of the transmitter release and the properties of postsynaptic receptors. Neuronal transmitters can be released in response to a stimulus, and fusion events also can occur spontaneously. Electrical recordings of postsynaptic responses at the NMJ allow detection of single fusion events, termed neurosecretory quanta. Changes in the frequency of spontaneous quanta usually reflect modifications in presynaptic processes, while alterations in amplitudes and shapes of quantal currents may reflect either postsynaptic or presynaptic mechanisms³. Statistical methods of quantal analysis of evoked multi-quantal responses enable evaluating the kinetics of evoked release⁴. NMJs of vertebrates and invertebrates, including frog, mouse, and crustacean have been broadly used for quantal analysis, since these preparations enable selective stimulation of an axon and recordings of currents or potentials from a limited number of identifiable synapses.

The *Drosophila* NMJ combines these advantages with the power of genetics. Genetic screens based on defects in photosensitivity⁵⁻⁹, altered neuronal structure or development¹⁰⁻¹², homeostasis^{13, 14}, or paralysis¹⁵⁻¹⁷ enabled identification of genes regulating the synaptic function. Respectively, numerous lines with genetically disrupted synaptic function have been generated. The larval NMJ has been used broadly to study transgenic lines with mutated synaptic proteins. Embryonic and adult NMJs have been also successfully employed to analyse special classes of mutants and to investigate the developmental changes in synaptic function.

Numerous *Drosophila* mutants with disruptions in behavioural learning have been generated, and these lines provided valuable insights into the genetics of behaviour¹⁸⁻²⁰. Thus, *Drosophila* represents an excellent model system to study synaptic pathways regulating behaviour, learning, and memory, and an investigation of synaptic transmission in the *Drosophila* central nervous system (CNS) is very compelling. Recordings of synaptic transmission at the *Drosophila* CNS are technically challenging because of a small size of fly CNS neurons and difficulties in obtaining functional synapses in primary cultures. In spite of these difficulties, synaptic transmission in the *Drosophila* CNS has been recorded both *in vitro* and *in vivo*. Recent advances in these approaches promise to promote our understanding of synaptic mechanisms that underlie behavioural learning.

Altogether, the *Drosophila* nervous system provides several fruitful experimental preparations for the investigation of synaptic transmission with electrophysiology methods. The power of *Drosophila* genetics combined with electrophysiology enable ground-breaking discoveries in molecular mechanisms of synaptic transmission and behaviour.

Neuromuscular System

The glutamatergic *Drosophila* NMJ is an advantageous experimental preparation widely used for characterizing the synaptic function. The NMJ is easily accessible for functional studies throughout the development. Electrical recordings were successfully employed to monitor synaptic transmission at different developmental stages, from embryos to adult flies, however the larval NMJ has been used most broadly.

Recordings from the larval NMJ—Since Jan and Jan seminal study²¹, the third instar larvae has been broadly used to study evoked and spontaneous synaptic transmission by monitoring excitatory junction potentials (EJPs) or currents (EJCs), as well as miniature EJPs or EJCs (mEJPs or mEJCs). The preparation has eight abdominal segments with easily identifiable muscles (Fig. 1 A-C). The dissection procedure is described in²². The "standard" solution composition described in Jan and Jan²¹ is optimal for the dissection, while electrical recordings are usually performed in HL3²³ or modified HL3²⁴ solutions, which improve recording stability and enable the nerve stimulation in the absence of muscle contractions. The recordings are most commonly performed from segments 2-4 and muscles 6 and 7 (Fig. 1 B, C), which are large and positioned on the surface of the dissected preparation. Recordings from other muscles, including muscles 4 and 5, also have some advantages, for example a limited number of synaptic boutons^{25, 26}. The muscles 6 and 7 are innervated by

two axons, which form two types of synaptic boutons, 1b and 1s (Fig. 1D). The 1b (Fig. 1E) and 1s boutons differ by their morphology²⁷ and synaptic properties^{25, 26}.

Intracellular recordings of synaptic potentials and currents: Typically, synaptic responses are recorded intracellularly from the larval NMJ. Recordings are performed by a sharp glass micro-electrode (Fig. 2A). This technique has been used broadly in different preparations, including frog^{2, 28, 29}, mouse^{30, 31}, crayfish^{32, 33}, as well as *Drosophila*^{21, 23, 34}. The recorded EJPs reflect the activity of the entire NMJ, including all the boutons making synapses at the given muscle fibre. (Fig. 2B). Importantly, muscle fibers in *Drosophila* are essentially iso-potential and do not show significant attenuation²¹. Indeed, mEJPs recorded by two electrodes positioned at a distance of 200 μm are similar in shape and size (Fig. 2C). The nerves are typically stimulated via a suction electrode (Fig. 2A). The two axons innervating the muscle fibres 6 and 7 often have different excitation thresholds (Fig. 2D), which enables selective stimulation of low-threshold axons^{21, 35}.

EJP and mEJP amplitudes and decays can be used to evaluate the amount of transmitter release, the release time-course, and the activity of postsynaptic receptors. In particular, run down of EJP amplitudes during high frequency stimulation can serve as a measure of endocytic defects^{36, 37}. The amplitudes of mEJPs can serve to assess the activity of postsynaptic receptors and homeostatic plasticity^{14, 38, 39}.

The number of neurosecretory quanta released in response to a stimulus, the quantal content, can be derived from the sizes of EJP and mEJP (Sidebar 1). However, the standard intracellular recording technique has several limitations. First, the EJP shape and decay may be affected by a repolarization in the muscle membrane. Second, when the membrane depolarization of the muscle approaches the reversal potential, the estimates of the quantal content become inaccurate (Sidebar 1). Indeed, the quantal content can be derived from the EJP amplitude only when the condition of linear summation is met. This condition is met when the synaptic response produces a relatively small change in the membrane potential, so the reversal potential is not approached. For example, this would be the case at low Ca^{2+} concentrations, when few quanta are released, and the membrane depolarization is on the order of several millivolts. However, at physiological Ca^{2+} concentrations, hundreds of quanta are released in response to an action potential at the innervations of muscles 6 and 7⁴⁰⁻⁴². At these conditions, the membrane potential approaches the reversal potential. When membrane depolarization is close to the reversal potential, postsynaptic currents are not generated, and therefore at these conditions EJPs cannot serve as a measure of transmitter release.

To overcome these limitations, two-electrode voltage clamp recordings^{23, 40-42} have been employed (Fig. 2E). The command voltage enables clamping the muscle at a constant membrane potential (typically -80 mV), and under this configuration the recorded currents, EJC and mEJC can be used to quantify synaptic transmission, including its magnitude and time-course. In *Drosophila* larval NMJ, this approach has been pioneered by Zhong and Wu⁴⁰ who investigated Ca^{2+} dependence of synaptic transmission in learning mutants with an impaired cAMP cascade, *dunce* and *rutabaga* (Fig. 2F). This study used EJC and mEJC amplitudes to derive the quantal content. It was shown subsequently that the charge transfer

obtained by integration of EJC and mEJC traces represents the most accurate measure of the EJC and mEJC sizes, since it accounts for a possible asynchrony in the release events comprising EJCs^{41, 42}.

Focal extracellular recordings—It was originally demonstrated by Fat and Katz⁴⁶ that synaptic transmission at the frog NMJ can be recorded extracellularly by positioning a micropipette tip near the nerve ending. The recorded currents represented synaptic responses produced by a single endplate. This technique was further developed by Dudel, who optimized the macropatch recoding electrodes^{47, 48}. This approach enabled reliable recordings of EJCs and mEJCs using fire polished electrodes of a tip diameter ranging from 1–3 to 10 μm ^{47, 48}, and it closely matches the loose-patch-clamp method⁴⁹. Focal macropatch recordings have been successfully employed at several NMJ preparations, including frog^{50–52}, mouse^{53–55}, crustacean^{56–63}, and *Drosophila*^{23, 64–69}. Simultaneous recordings by two or three macropatch electrodes from the same NMJ^{50, 52}, as well as moving the electrode along the NMJ^{69, 70}, demonstrated that a macropatch electrode predominantly records the activity of synaptic boutons under the electrode tip, although the boutons positioned under the electrode rim and those in the immediate proximity to the electrode tip can make a minor contribution to the recorded EJCs.

The *Drosophila* larval NMJ has clearly defined synaptic boutons, and numerous transgenic lines with genetically encoded neuronal fluorescent tags are available. These advantages enable recordings of EJCs and mEJCs from a selected bouton (Fig. 3 A, B). Respectively, this technique is advantageous for quantitative studies of release kinetics in the mutants with enhanced spontaneous activity and altered release time-course⁶⁸ (Fig. 3 C), such the *complexin* null mutant^{42, 71}. The rapid mEJC time-course and limited number of release sites enable accurate detection of neurosecretory quanta, which can be performed automatically (Fig. 3D)⁴⁵. This technique has been also employed to analyse alterations in the shapes of neurosecretory quanta produced by mutations in the presynaptic proteins⁶⁵.

The ability to monitor activity of a single bouton can be potentially combined with recent imaging technologies. For example, it is a promising approach to couple acute local photoinactivation of synaptic proteins (FIAsH-FALI)^{72, 73} with selective recordings of synaptic activity from the boutons which underwent the photoinactivation. Another promising approach is to couple focal recordings of EJCs and mEJCs with optical detection of activity at individual active zones, enabled by recent groundbreaking advances in imaging GCaMP tethered to postsynaptic specializations⁷⁴. These powerful combinations of microscopy and electrophysiology have a promise to reveal new insights into synaptic mechanisms.

Patch-clamp recordings from the embryonic NMJ—Although the larval NMJ represents an excellent model system to study synaptic transmission, this preparation does not allow an investigation of embryo lethal mutations. This is an important limitation, since loss-of-function mutations in synaptic machinery, such as SNARE proteins or Syt, often produce lethal phenotypes. Patch-clamp recordings of synaptic transmission from the embryonic NMJ (Fig. 4 A, B) overcome this limitation, although this technique is technically challenging. Synaptic currents can be observed at embryonic NMJs within 13–24

hours after egg laying (AEL)^{76, 77}. In most cases the muscle 6 is used for recordings, and EJC's are recorded in whole-cell configuration (Fig. 4 B-D), with the muscle being voltage clamped at a holding potential of -60 mV. This approach promoted understanding of how the SNARE proteins regulate release kinetics⁷⁸⁻⁸² and enabled characterization of *syt* loss-of-function mutants⁸³.

Recordings from adult NMJs of temperature sensitive paralytic mutants—A special class of *Drosophila* mutants, including *shr*⁸⁴⁻⁸⁶ and *comt*^{15, 87} demonstrate temperature dependent paralysis in adult flies. These lines have mutations in the genes encoding endocytic synaptic proteins dynamin and NSF, respectively, and thus provide an opportunity to correlate synaptic transmission and motor activity in adult flies. To monitor synaptic activity in adult *Drosophila*, recordings of neuromuscular transmission from dorsal longitudinal muscles (DLM) have been employed⁸⁸. Each DLM muscle receives multiple inputs from a motor neuron in the thoracic ganglion (Fig. 4A). The motor neurons can be stimulated via the cervical connective nerve bundle by placing two metal electrodes in the neck region (Fig. 4 B)⁸⁸. Alternatively, the stimulation of the brain can be performed via a glass electrode, or the mesothoracic nerve can be isolated and stimulated via a suction electrode⁸⁹. Intracellular recordings of excitatory endplate potentials (EPP) from DLM muscles of *comt* mutant at permissive and restrictive temperatures (Fig. 4 C) enabled defining the role of NSF in neurotransmitter release^{89, 90}. Endplate currents (EPC) were also recorded from DLM muscles of *shi* mutants employing two electrode voltage clamp technique⁹¹ to investigate the functional role of dynamin in the recovery of synaptic transmission.

Central Nervous System

Although *Drosophila* NMJ is an attractive preparation, understanding learning, memory, and behaviour compels an investigation of synaptic transmission at the CNS. The capabilities of *Drosophila* genetics offer exciting capabilities of generating and investigating behavioural mutants with deficits in learning and memory. In particular, *Drosophila* mutants with altered olfactory learning⁹²⁻⁹⁴ represent an attractive model to study the link between synaptic plasticity and behaviour. The insect olfactory neuronal pathway includes olfactory receptor neurons connecting to cholinergic projection neurons (PN), and the information is processed in higher olfactory centres of the brain, including Kenyon cells (KC) in the mushroom body⁹⁴⁻⁹⁷ (Fig. 6). Synaptic connections within this pathway are not easily accessible, and therefore functional studies of *Drosophila* CNS synapses are challenging. However, methodologies for the investigation of synaptic transmission within the *Drosophila* olfactory pathway have been developed *in vitro*⁹⁸ and *in vivo*⁹⁹, promising to bridge molecular genetics, synaptic function, and behaviour.

Recordings of synaptic activity *in vitro*—Cultured differentiated *Drosophila* neurons derived from embryonic¹⁰⁰ and larval¹⁰¹ preparations have been used extensively for genetic studies of neuronal excitability and the function of ion channels. However, obtaining functional synaptic connections in cultured CNS neurons proved to be challenging. Initially, functional synapses were obtained for embryonic giant *Drosophila* neurons co-cultured with *Xenopus* myocytes, which served as postsynaptic detectors¹⁰². Subsequently, a culture

system was developed from pupae brain neurons, and both cholinergic excitatory postsynaptic currents (EPSCs) and GABAergic inhibitory postsynaptic currents (IPSCs) were recorded (Fig. 7)⁹⁸. This system enabled the first characterization of EPSCs and IPSCs from genetically labelled KSs^{98, 103, 104}. The methodology subsequently developed by Gu and O'Dowd^{105, 106} enabled whole-cell recordings of spontaneous EPSCs from KSs and PNs¹⁰⁷ in the dissected whole-brain preparation.

Synaptic activity *in vivo*—The first *in vivo* recordings of cholinergic synaptic transmission from the *Drosophila* CNS were obtained by Rohrbough and Broadie on the larval preparation¹⁰⁸. This study identified dorsal neurons of the ventral nerve cord, which were readily visualized, accessible for whole-cell recordings, and exhibited endogenous synaptic activity. Subsequently, a ground-breaking study of Wilson et al.⁹⁹ demonstrated recordings of IPSCs from PNs connected to LNs in an intact antennal lobe (Fig. 8). This powerful technique¹⁰⁹ enabled a characterization of synaptic transmission in the *Drosophila* antennal lobe *in vivo*. This approach was subsequently adapted to Kenyon cells¹¹⁰, providing an insight to the mechanisms by which synaptic connectivity contributes to olfactory coding.

Conclusion

Drosophila represents an advantageous model organism to study synaptic transmission. Numerous *Drosophila* lines with mutated synaptic proteins enabled critical advances in understanding the molecular mechanisms of neurotransmitter release and postsynaptic activation. The invertebrate neuromuscular system has glutamatergic synapses, and therefore the *Drosophila* NMJ has been used broadly for the studies of the mechanisms of glutamatergic transmission conserved between invertebrates and vertebrates. The larval NMJ is especially suitable for such studies, since this preparation has easily identifiable muscles and readily visualized synaptic boutons, and this enables an examination of synaptic transmission with a remarkable degree of rigor. The kinetics of transmitter release can be quantified in this preparation employing quantal analysis, which includes direct counts of synaptic events and the analysis of their amplitudes and the time-course. Several recording configurations have been used at the larval NMJ, including intracellular recordings of synaptic potentials, recordings of synaptic currents with two electrode voltage clamp, and focal macropatch recordings of synaptic currents. The latter technique allows precise quantification of synaptic transmission at visualized boutons. Since some of the mutations in synaptic proteins can produce embryo lethal phenotype, embryonic NMJ has been also successfully used to study synaptic transmission, and this fruitful preparation enabled critical advances in understanding the mechanisms of the synaptic vesicle fusion. Finally, synaptic transmission in temperature sensitive paralytic mutants has been extensively studied at the adult flight NMJ, since these mutants demonstrate pronounced behavioural phenotype at the adult stage.

Drosophila represents an attractive model to study the genetics of behaviour, and numerous mutant lines with impairments in olfactory and visual learning have been generated. The need to understand how these genetically encoded impairments affect synaptic transmission in the brain compelled an electrophysiological investigation of *Drosophila* CNS neurons.

Cultured primary CNS neurons derived from embryos and pupae were demonstrated to form functional synaptic connections, and spontaneous synaptic transmission has been recorded in cultures. The neurons forming an olfactory pathway were also investigated in an intact brain both *in vitro* and *in vivo*. Although recordings of synaptic transmission in an intact brain of *Drosophila* are very challenging due to a small size of the neurons and complex circuitry, these studies created a promise of unravelling the link between synaptic transmission and behavioural learning.

References

1. Sudhof TC. Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. *Neuron*. 2013; 80:675–690. [PubMed: 24183019]
2. Fatt P, Katz B. An analysis of the end-plate potential recorded with an intracellular electrode. *J Physiol*. 1951; 115:320–370. [PubMed: 14898516]
3. Van der Kloot W. The regulation of quantal size. *Prog Neurobiol*. 1991; 36:93–130. [PubMed: 1847748]
4. Redman S. Quantal analysis of synaptic potentials in neurons of the central nervous system. *Physiol Rev*. 1990; 70:165–198. [PubMed: 2404288]
5. Dickman DK, Kurshan PT, Schwarz TL. Mutations in a *Drosophila* alpha2delta voltage-gated calcium channel subunit reveal a crucial synaptic function. *J Neurosci*. 2008; 28:31–38. [PubMed: 18171920]
6. Dickman DK, Horne JA, Meinertzhagen IA, Schwarz TL. A slowed classical pathway rather than kiss-and-run mediates endocytosis at synapses lacking synaptojanin and endophilin. *Cell*. 2005; 123:521–533. [PubMed: 16269341]
7. Verstreken P, Ly CV, Venken KJ, Koh TW, Zhou Y, Bellen HJ. Synaptic mitochondria are critical for mobilization of reserve pool vesicles at *Drosophila* neuromuscular junctions. *Neuron*. 2005; 47:365–378. [PubMed: 16055061]
8. Mehta SQ, Hiesinger PR, Beronja S, Zhai RG, Schulze KL, Verstreken P, Cao Y, Zhou Y, Tepass U, Crair MC, et al. Mutations in *Drosophila* sec15 reveal a function in neuronal targeting for a subset of exocyst components. *Neuron*. 2005; 46:219–232. [PubMed: 15848801]
9. Stowers RS, Schwarz TL. A genetic method for generating *Drosophila* eyes composed exclusively of mitotic clones of a single genotype. *Genetics*. 1999; 152:1631–1639. [PubMed: 10430588]
10. Dickman DK, Lu Z, Meinertzhagen IA, Schwarz TL. Altered synaptic development and active zone spacing in endocytosis mutants. *Curr Biol*. 2006; 16:591–598. [PubMed: 16546084]
11. Pielage J, Cheng L, Fetter RD, Carlton PM, Sedat JW, Davis GW. A presynaptic giant ankyrin stabilizes the NMJ through regulation of presynaptic microtubules and transsynaptic cell adhesion. *Neuron*. 2008; 58:195–209. [PubMed: 18439405]
12. Salzberg A, D'Evelyn D, Schulze KL, Lee JK, Strumpf D, Tsai L, Bellen HJ. Mutations affecting the pattern of the PNS in *Drosophila* reveal novel aspects of neuronal development. *Neuron*. 1994; 13:269–287. [PubMed: 8060613]
13. Frank CA, Pielage J, Davis GW. A presynaptic homeostatic signaling system composed of the Eph receptor, ephexin, Cdc42, and CaV2.1 calcium channels. *Neuron*. 2009; 61:556–569. [PubMed: 19249276]
14. Muller M, Pym EC, Tong A, Davis GW. Rab3-GAP controls the progression of synaptic homeostasis at a late stage of vesicle release. *Neuron*. 2011; 69:749–762. [PubMed: 21338884]
15. Littleton JT, Chapman ER, Kreber R, Garment MB, Carlson SD, Ganetzky B. Temperature-sensitive paralytic mutations demonstrate that synaptic exocytosis requires SNARE complex assembly and disassembly. *Neuron*. 1998; 21:401–413. [PubMed: 9728921]
16. Palladino MJ, Bower JE, Kreber R, Ganetzky B. Neural dysfunction and neurodegeneration in *Drosophila* Na⁺/K⁺ ATPase alpha subunit mutants. *J Neurosci*. 2003; 23:1276–1286. [PubMed: 12598616]

17. Rieckhof GE, Yoshihara M, Guan Z, Littleton JT. Presynaptic N-type calcium channels regulate synaptic growth. *J Biol Chem*. 2003; 278:41099–41108. [PubMed: 12896973]
18. Gerstner JR, Lyons LC, Wright KP Jr, Loh DH, Rawashdeh O, Eckel-Mahan KL, Roman GW. Cycling behavior and memory formation. *J Neurosci*. 2009; 29:12824–12830. [PubMed: 19828795]
19. Davis RL. Physiology and biochemistry of *Drosophila* learning mutants. *Physiol Rev*. 1996; 76:299–317. [PubMed: 8618959]
20. Engel JE, Wu CF. Genetic dissection of functional contributions of specific potassium channel subunits in habituation of an escape circuit in *Drosophila*. *J Neurosci*. 1998; 18:2254–2267. [PubMed: 9482810]
21. Jan LY, Jan YN. Properties of the larval neuromuscular junction in *Drosophila melanogaster*. *J Physiol*. 1976; 262:189–214. [PubMed: 11339]
22. Smith R, Taylor JP. Dissection and imaging of active zones in the *Drosophila* neuromuscular junction. *J Vis Exp*. 2011
23. Stewart BA, Atwood HL, Renger JJ, Wang J, Wu CF. Improved stability of *Drosophila* larval neuromuscular preparations in haemolymph-like physiological solutions. *J Comp Physiol A*. 1994; 175:179–191. [PubMed: 8071894]
24. Feng Y, Ueda A, Wu CF. A modified minimal hemolymph-like solution, HL3.1, for physiological recordings at the neuromuscular junctions of normal and mutant *Drosophila* larvae. *J Neurogenet*. 2004; 18:377–402. [PubMed: 15763995]
25. He T, Singh V, Rumpal N, Lnenicka GA. Differences in Ca²⁺ regulation for high-output Is and low-output Ib motor terminals in *Drosophila* larvae. *Neuroscience*. 2009; 159:1283–1291. [PubMed: 19409207]
26. Lnenicka GA, Keshishian H. Identified motor terminals in *Drosophila* larvae show distinct differences in morphology and physiology. *J Neurobiol*. 2000; 43:186–197. [PubMed: 10770847]
27. Atwood HL, Govind CK, Wu CF. Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. *J Neurobiol*. 1993; 24:1008–1024. [PubMed: 8409966]
28. Del Castillo J, Katz B. Quantal components of the end-plate potential. *J Physiol*. 1954; 124:560–573. [PubMed: 13175199]
29. Kita H, van der Kloot W. Time course and magnitude of effects of changes in tonicity on acetylcholine release at frog neuromuscular junction. *J Neurophysiol*. 1977; 40:212–224. [PubMed: 300428]
30. Bain AI, Quastel DM. Quantal transmitter release mediated by strontium at the mouse motor nerve terminal. *J Physiol*. 1992; 450:63–87. [PubMed: 1359125]
31. Guan YY, Quastel DM, Saint DA. Single Ca²⁺ entry and transmitter release systems at the neuromuscular synapse. *Synapse*. 1988; 2:558–564. [PubMed: 2847344]
32. Wojtowicz JM, Atwood HL. Maintained depolarization of synaptic terminals facilitates nerve-evoked transmitter release at a crayfish neuromuscular junction. *J Neurobiol*. 1983; 14:385–390. [PubMed: 6137513]
33. Wojtowicz JM, Atwood HL. Presynaptic long-term facilitation at the crayfish neuromuscular junction: voltage-dependent and ion-dependent phases. *J Neurosci*. 1988; 8:4667–4674. [PubMed: 2904490]
34. Zhang B, Stewart B. Electrophysiological recording from *Drosophila* larval body-wall muscles. *Cold Spring Harb Protoc*. 2010; 2010:prot5487.
35. Ruffner ME, Cromarty SI, Cooper RL. Depression of synaptic efficacy in high- and low-output *Drosophila* neuromuscular junctions by the molting hormone (20-HE). *J Neurophysiol*. 1999; 81:788–794. [PubMed: 10036278]
36. Verstreken P, Koh TW, Schulze KL, Zhai RG, Hiesinger PR, Zhou Y, Mehta SQ, Cao Y, Roos J, Bellen HJ. Synaptojanin is recruited by endophilin to promote synaptic vesicle uncoating. *Neuron*. 2003; 40:733–748. [PubMed: 14622578]
37. Koh TW, Verstreken P, Bellen HJ. Dap160/intersectin acts as a stabilizing scaffold required for synaptic development and vesicle endocytosis. *Neuron*. 2004; 43:193–205. [PubMed: 15260956]

38. Muller M, Genc O, Davis GW. RIM-binding protein links synaptic homeostasis to the stabilization and replenishment of high release probability vesicles. *Neuron*. 2015; 85:1056–1069. [PubMed: 25704950]
39. Davis GW, Muller M. Homeostatic control of presynaptic neurotransmitter release. *Annu Rev Physiol*. 2015; 77:251–270. [PubMed: 25386989]
40. Zhong Y, Wu CF. Altered synaptic plasticity in *Drosophila* memory mutants with a defective cyclic AMP cascade. *Science*. 1991; 251:198–201. [PubMed: 1670967]
41. Delgado R, Maureira C, Oliva C, Kidokoro Y, Labarca P. Size of vesicle pools, rates of mobilization, and recycling at neuromuscular synapses of a *Drosophila* mutant, shibire. *Neuron*. 2000; 28:941–953. [PubMed: 11163278]
42. Jorquera RA, Huntwork-Rodriguez S, Akbergenova Y, Cho RW, Littleton JT. Complexin controls spontaneous and evoked neurotransmitter release by regulating the timing and properties of synaptotagmin activity. *J Neurosci*. 2012; 32:18234–18245. [PubMed: 23238737]
43. Jan LY, Jan YN. L-glutamate as an excitatory transmitter at the *Drosophila* larval neuromuscular junction. *J Physiol*. 1976; 262:215–236. [PubMed: 186587]
44. Zhang, B., Stewart, B. Synaptic Electrophysiology of the *Drosophila* NMJ. In: Zhang, B., editor. *Drosophila Neurobiology: A Laboratory Manual*. Cold Spring Harbo: CSHL Press; 2012.
45. Bykhovskaia M. Making quantal analysis more convenient, fast, and accurate: user-friendly software QUANTAN. *J Neurosci Methods*. 2008; 168:500–513. [PubMed: 18045692]
46. Fatt P, Katz B. Spontaneous subthreshold activity at motor nerve endings. *J Physiol*. 1952; 117:109–128. [PubMed: 14946732]
47. Dudel J. The effect of reduced calcium on quantal unit current and release at the crayfish neuromuscular junction. *Pflugers Arch*. 1981; 391:35–40. [PubMed: 6269044]
48. Dudel J. Contribution of Ca²⁺ inflow to quantal, phasic transmitter release from nerve terminals of frog muscle. *Pflugers Arch*. 1992; 422:129–142. [PubMed: 1362607]
49. Marrero, HG., Lemos, JR. Loose-Patch-Clamp method. 2 ed. Humana Press; 2007.
50. Macleod GT, Gan J, Bennett MR. Vesicle-associated proteins and quantal release at single active zones of amphibian (*Bufo marinus*) motor-nerve terminals. *J Neurophysiol*. 1999; 82:1133–1146. [PubMed: 10482733]
51. Macleod GT, Farnell L, Gibson WG, Bennett MR. Quantal secretion and nerve-terminal cable properties at neuromuscular junctions in an amphibian (*Bufo marinus*). *J Neurophysiol*. 1999; 81:1135–1146. [PubMed: 10085340]
52. Zefirov A, Benish T, Fatkullin N, Cheranov S, Khazipov R. Localization of active zones. *Nature*. 1995; 376:393–394. [PubMed: 7630413]
53. Macleod GT, Lavidis NA, Bennett MR. Calcium dependence of quantal secretion from visualized sympathetic nerve varicosities on the mouse vas deferens. *J Physiol*. 1994; 480(Pt 1):61–70. [PubMed: 7853226]
54. Samigullin D, Bill CA, Coleman WL, Bykhovskaia M. Regulation of transmitter release by synapsin II in mouse motor terminals. *J Physiol*. 2004; 561:149–158. [PubMed: 15388780]
55. Coleman WL, Bykhovskaia M. Rab3a-mediated vesicle recruitment regulates short-term plasticity at the mouse diaphragm synapse. *Mol Cell Neurosci*. 2009; 41:286–296. [PubMed: 19348946]
56. Atwood HL, Parnas H, Parnas I, Wojtowicz JM. Quantal currents evoked by graded intracellular depolarization of crayfish motor axon terminals. *J Physiol*. 1987; 383:587–599. [PubMed: 2888878]
57. Parnas H, Dudel J, Parnas I. Neurotransmitter release and its facilitation in crayfish. I Saturation kinetics of release, and of entry and removal of calcium. *Pflugers Arch*. 1982; 393:1–14. [PubMed: 6123979]
58. Wojtowicz JM, Marin L, Atwood HL. Activity-induced changes in synaptic release sites at the crayfish neuromuscular junction. *J Neurosci*. 1994; 14:3688–3703. [PubMed: 8207482]
59. Zucker RS. Crayfish neuromuscular facilitation activated by constant presynaptic action potentials and depolarizing pulses. *J Physiol*. 1974; 241:69–89. [PubMed: 4153766]
60. Zucker RS. Changes in the statistics of transmitter release during facilitation. *J Physiol*. 1973; 229:787–810. [PubMed: 4348351]

61. Worden MK, Bykhovskaia M, Hackett JT. Facilitation at the lobster neuromuscular junction: a stimulus-dependent mobilization model. *J Neurophysiol.* 1997; 78:417–428. [PubMed: 9242290]
62. Bykhovskaia M, Hackett JT, Worden MK. Asynchrony of quantal events in evoked multiquantal responses indicates presynaptic quantal interaction. *J Neurophysiol.* 1999; 81:2234–2242. [PubMed: 10322062]
63. Bykhovskaia M, Polagaeva E, Hackett JT. Mechanisms underlying different facilitation forms at the lobster neuromuscular synapse. *Brain Res.* 2004; 1019:10–21. [PubMed: 15306233]
64. Cooper RL, Stewart BA, Wojtowicz JM, Wang S, Atwood HL. Quantal measurement and analysis methods compared for crayfish and *Drosophila* neuromuscular junctions, and rat hippocampus. *J Neurosci Methods.* 1995; 61:67–78. [PubMed: 8618427]
65. Pawlu C, DiAntonio A, Heckmann M. Postfusional control of quantal current shape. *Neuron.* 2004; 42:607–618. [PubMed: 15157422]
66. Akbergenova Y, Bykhovskaia M. Synapsin maintains the reserve vesicle pool and spatial segregation of the recycling pool in *Drosophila* presynaptic boutons. *Brain Res.* 2007; 1178:52–64. [PubMed: 17904536]
67. Akbergenova Y, Bykhovskaia M. Enhancement of the endosomal endocytic pathway increases quantal size. *Mol Cell Neurosci.* 2009; 40:199–206. [PubMed: 19026748]
68. Vasin A, Volfson D, Littleton JT, Bykhovskaia M. Interaction of the Complexin Accessory Helix with Synaptobrevin Regulates Spontaneous Fusion. *Biophys J.* 2016; 111:1954–1964. [PubMed: 27806277]
69. Wong K, Karunanithi S, Atwood HL. Quantal unit populations at the *Drosophila* larval neuromuscular junction. *J Neurophysiol.* 1999; 82:1497–1511. [PubMed: 10482765]
70. Kapitsky S, Zueva L, Akbergenova Y, Bykhovskaia M. Recruitment of synapses in the neurosecretory process during long-term facilitation at the lobster neuromuscular junction. *Neuroscience.* 2005; 134:1261–1272. [PubMed: 16084655]
71. Huntwork S, Littleton JT. A complexin fusion clamp regulates spontaneous neurotransmitter release and synaptic growth. *Nat Neurosci.* 2007; 10:1235–1237. [PubMed: 17873870]
72. Heerssen H, Fetter RD, Davis GW. Clathrin dependence of synaptic-vesicle formation at the *Drosophila* neuromuscular junction. *Curr Biol.* 2008; 18:401–409. [PubMed: 18356056]
73. Marek KW, Davis GW. Transgenically encoded protein photoinactivation (FlAsH-FALI): acute inactivation of synaptotagmin I. *Neuron.* 2002; 36:805–813. [PubMed: 12467585]
74. Melom JE, Akbergenova Y, Gavornik JP, Littleton JT. Spontaneous and evoked release are independently regulated at individual active zones. *J Neurosci.* 2013; 33:17253–17263. [PubMed: 24174659]
75. Nishikawa K, Kidokoro Y. Junctional and extrajunctional glutamate receptor channels in *Drosophila* embryos and larvae. *J Neurosci.* 1995; 15:7905–7915. [PubMed: 8613729]
76. Broadie K, Bate M. Activity-dependent development of the neuromuscular synapse during *Drosophila* embryogenesis. *Neuron.* 1993; 11:607–619. [PubMed: 7691105]
77. Broadie KS. Synaptogenesis in *Drosophila*: coupling genetics and electrophysiology. *J Physiol Paris.* 1994; 88:123–139. [PubMed: 7833856]
78. Schulze KL, Broadie K, Perin MS, Bellen HJ. Genetic and electrophysiological studies of *Drosophila* syntaxin-1A demonstrate its role in nonneuronal secretion and neurotransmission. *Cell.* 1995; 80:311–320. [PubMed: 7834751]
79. Aravamudan B, Fergestad T, Davis WS, Rodesch CK, Broadie K. *Drosophila* UNC-13 is essential for synaptic transmission. *Nat Neurosci.* 1999; 2:965–971. [PubMed: 10526334]
80. Fergestad T, Wu MN, Schulze KL, Lloyd TE, Bellen HJ, Broadie K. Targeted mutations in the syntaxin H3 domain specifically disrupt SNARE complex function in synaptic transmission. *J Neurosci.* 2001; 21:9142–9150. [PubMed: 11717347]
81. Yoshihara M, Ueda A, Zhang D, Deitcher DL, Schwarz TL, Kidokoro Y. Selective effects of neuronal-synaptobrevin mutations on transmitter release evoked by sustained versus transient Ca^{2+} increases and by cAMP. *J Neurosci.* 1999; 19:2432–2441. [PubMed: 10087058]
82. Deitcher DL, Ueda A, Stewart BA, Burgess RW, Kidokoro Y, Schwarz TL. Distinct requirements for evoked and spontaneous release of neurotransmitter are revealed by mutations in the *Drosophila* gene neuronal-synaptobrevin. *J Neurosci.* 1998; 18:2028–2039. [PubMed: 9482790]

83. Yoshihara M, Littleton JT. Synaptotagmin I functions as a calcium sensor to synchronize neurotransmitter release. *Neuron*. 2002; 36:897–908. [PubMed: 12467593]
84. Chen MS, Obar RA, Schroeder CC, Austin TW, Poodry CA, Wadsworth SC, Vallee RB. Multiple forms of dynamin are encoded by shibire, a *Drosophila* gene involved in endocytosis. *Nature*. 1991; 351:583–586. [PubMed: 1828536]
85. van der Bliek AM, Meyerowitz EM. Dynamin-like protein encoded by the *Drosophila* shibire gene associated with vesicular traffic. *Nature*. 1991; 351:411–414. [PubMed: 1674590]
86. Kelly LE, Suzuki DT. The effects of increased temperature on electroretinograms of temperature-sensitive paralysis mutants of *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*. 1974; 71:4906–4909. [PubMed: 4216025]
87. Pallanck L, Ordway RW, Ganetzky B. A *Drosophila* NSF mutant. *Nature*. 1995; 376:25. [PubMed: 7596428]
88. Siddiqi O, Benzer S. Neurophysiological defects in temperature-sensitive paralytic mutants of *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*. 1976; 73:3253–3257. [PubMed: 184469]
89. Kawasaki F, Mattiuz AM, Ordway RW. Synaptic physiology and ultrastructure in comatose mutants define an in vivo role for NSF in neurotransmitter release. *J Neurosci*. 1998; 18:10241–10249. [PubMed: 9852561]
90. Kawasaki F, Ordway RW. The *Drosophila* NSF protein, dNSF1, plays a similar role at neuromuscular and some central synapses. *J Neurophysiol*. 1999; 82:123–130. [PubMed: 10400941]
91. Kawasaki F, Hazen M, Ordway RW. Fast synaptic fatigue in shibire mutants reveals a rapid requirement for dynamin in synaptic vesicle membrane trafficking. *Nat Neurosci*. 2000; 3:859–860. [PubMed: 10966613]
92. Gasque G, Labarca P, Delgado R, Darszon A. Bridging behavior and physiology: ion-channel perspective on mushroom body-dependent olfactory learning and memory in *Drosophila*. *J Cell Physiol*. 2006; 209:1046–1053. [PubMed: 16924658]
93. Perisse E, Burke C, Huetteroth W, Waddell S. Shocking revelations and saccharin sweetness in the study of *Drosophila* olfactory memory. *Curr Biol*. 2013; 23:R752–763. [PubMed: 24028959]
94. Hong W, Luo L. Genetic control of wiring specificity in the fly olfactory system. *Genetics*. 2014; 196:17–29. genetic. [PubMed: 24395823]
95. Jefferis GS, Hummel T. Wiring specificity in the olfactory system. *Semin Cell Dev Biol*. 2006; 17:50–65. [PubMed: 16439169]
96. Oswald D, Waddell S. Olfactory learning skews mushroom body output pathways to steer behavioral choice in *Drosophila*. *Curr Opin Neurobiol*. 2015; 35:178–184. [PubMed: 26496148]
97. Masse NY, Turner GC, Jefferis GS. Olfactory information processing in *Drosophila*. *Curr Biol*. 2009; 19:R700–713. [PubMed: 19706282]
98. Su H, O'Dowd DK. Fast synaptic currents in *Drosophila* mushroom body Kenyon cells are mediated by alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors and picrotoxin-sensitive GABA receptors. *J Neurosci*. 2003; 23:9246–9253. [PubMed: 14534259]
99. Wilson RI, Turner GC, Laurent G. Transformation of olfactory representations in the *Drosophila* antennal lobe. *Science*. 2004; 303:366–370. [PubMed: 14684826]
100. Seecof RL, Alleaume N, Teplitz RL, Gerson I. Differentiation of neurons and myocytes in cell cultures made from *Drosophila* gastrulae. *Exp Cell Res*. 1971; 69:161–173. [PubMed: 4330919]
101. Wu CF, Suzuki N, Poo MM. Dissociated neurons from normal and mutant *Drosophila* larval central nervous system in cell culture. *J Neurosci*. 1983; 3:1888–1899. [PubMed: 6310066]
102. Yao WD, Rusch J, Poo M, Wu CF. Spontaneous acetylcholine secretion from developing growth cones of *Drosophila* central neurons in culture: effects of cAMP-pathway mutations. *J Neurosci*. 2000; 20:2626–2637. [PubMed: 10729343]
103. Rohrbough J, O'Dowd DK, Baines RA, Broadie K. Cellular bases of behavioral plasticity: establishing and modifying synaptic circuits in the *Drosophila* genetic system. *J Neurobiol*. 2003; 54:254–271. [PubMed: 12486708]
104. Jiang SA, Campusano JM, Su H, O'Dowd DK. *Drosophila* mushroom body Kenyon cells generate spontaneous calcium transients mediated by PLTX-sensitive calcium channels. *J Neurophysiol*. 2005; 94:491–500. [PubMed: 15772240]

105. Gu H, O'Dowd DK. Cholinergic synaptic transmission in adult *Drosophila* Kenyon cells in situ. *J Neurosci*. 2006; 26:265–272. [PubMed: 16399696]
106. Gu H, O'Dowd DK. Whole cell recordings from brain of adult *Drosophila*. *J Vis Exp*. 2007; 248
107. Gu H, Jiang SA, Campusano JM, Iniguez J, Su H, Hoang AA, Lavian M, Sun X, O'Dowd DK. Cav2-type calcium channels encoded by *cac* regulate AP-independent neurotransmitter release at cholinergic synapses in adult *Drosophila* brain. *J Neurophysiol*. 2009; 101:42–53. [PubMed: 19004991]
108. Rohrbough J, Broadie K. Electrophysiological analysis of synaptic transmission in central neurons of *Drosophila* larvae. *J Neurophysiol*. 2002; 88:847–860. [PubMed: 12163536]
109. Murthy M, Turner G. Whole-cell in vivo patch-clamp recordings in the *Drosophila* brain. *Cold Spring Harb Protoc*. 2013; 2013:140–148. [PubMed: 23378646]
110. Hige T, Aso Y, Rubin GM, Turner GC. Plasticity-driven individualization of olfactory coding in mushroom body output neurons. *Nature*. 2015; 526:258–262. [PubMed: 26416731]

Further Reading

111. Kandel E, Jessell T, Sigelbaum S, Schwarz JH, Hudspeth AJ. Principles of neural science. McGraw Hill. 2012

Fig 1.

Drosophila larval NMJ preparation. **A** Bright-field image of a dissected larvae. The rectangle marks segment 4. **B** Enlarged segment showing the muscle fibers. **C**. The diagram denoting the muscle fibers on the surface of the preparation. **D**. Innervation of the muscles 6 and 7 labelled by horseradish peroxidase. The enlarged boxed area is shown in the bottom, 1b and 1s innervation types are labeled. Scale bar: 10 μ m. **E** Electron micrograph showing a 1b type bouton. SSR- subsynaptic reticulum, AZ-active zone, SV-synaptic vesicle.

Fig 2.

Intracellular recordings. **A.** The recording configuration. The axon is pulled in the stimulating suction electrode, and EJPs are recorded from the muscle with a sharp microelectrode. **B.** Diagram representing a position of the recording electrode in relation to the NMJ. Scale bar: 10 μm. **C.** mEJPs recorded by two sharp electrodes positioned at a distance of 200 μm are similar, showing that the muscle is iso-potential (reproduced with permission from²¹). **D.** Recordings of EJPs at different stimulation intensities show two peaks produced by response of two axons. Lower intensities produce a single peak, corresponding to a response of a single axon with a lower stimulation threshold. (reproduced with permission from²¹). **E.** Two-electrode voltage clamp configuration. Two sharp microelectrodes maintain command voltage V_{cmd} and record synaptic currents. **F.** Characterization of *dunce* (*dnc*) and *rutabaga* (*rut*) mutants employing two electrode voltage clamp (reproduced with permission from⁴⁰). Traces show EJCs and mEJCs, and Ca^{2+} dependence of the quantal content is shown on the right.

Fig 3.

Focal macropatch recordings in the *Drosophila* larval NMJ. **A.** A macropatch electrode is positioned on the top of a bouton. Neuronal membranes tagged with CD8-GFP show clearly defined boutons (A.1, arrows), which are also visible in bright-field with DIC optics (A.2, arrows). An electrode can be positioned on the top of the bouton (A.3), which is confirmed by an overlay of the fluorescent and DIC images (A.4). Scale bar : 10 μ m. **B.** A current trace showing EJC and mEJC. **C.** Recordings of EJCs and mEJCs from *complexin* null mutant show a quantifiable increase in mEJC frequency and a prolonged EJC decay. **D.** Automated analysis of mEJCs employing Quantan software⁴⁵. Quantal peaks (red arrows) and current deviations from the baseline (green) are detected.

Fig 4. Patch clamp recordings from the embryonic NMJ. **A.** Dissected *Drosophila* embryo (reproduced with permission from⁵⁶). **B.** A recording configuration. **C.** Synaptic currents recorded at different holding potentials (reproduced with permission from⁵⁶). **D.** The relationship between the EJC amplitude and the holding membrane potential (reproduced with permission from⁷⁵).

Fig 5.

Intracellular recordings from DLM in adult *Drosophila*. **A.** A diagram showing the DLM innervation. **B.** Recording configuration. **C.** Recordings from DLM in wild type and *comt* flies at different temperature in response to a repeated stimulation (reproduced with permission from⁶⁹).

Fig 6.

A diagram showing an olfactory neuronal pathway in *Drosophila*.

Fig 7. Recording of synaptic activity from cultured KCs. **A.** Neurons in culture. Top panels show live cultures imaged with Nomarski optics. Bottom panels show labelled cholinergic and GABAergic neurons. **B.** Whole-cell recordings of cholinergic and GABAergic synaptic currents (reproduced with permission from⁷⁸).

Fig 8.

Paired patch-clamp recordings from *Drosophila* CNS *in vivo*. **A.** A diagram showing the recording configuration. **B.** Visualized LN (green) and PN (red) neurons. **C.** Individual recordings from LN aligned with the presynaptic PN depolarization (reproduced with permission from⁷⁹).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Sidebar 1
Membrane depolarization and quantal content

Quantal content	$m = \text{EJP}/Q$, where Q is the quantal size
Linear summation	$\text{EJP} = m \cdot Q$
Resting potential	$V_{\text{rest}} \sim -60 \div -50 \text{ mV}$ ^{21, 23}
Reversal potential	$V_{\text{rev}} \sim -10 \div -1 \text{ mV}$ ^{43, 44}
Quantal size	$Q \sim 0.5 \text{ mV}$ (average mEJP amplitude) ²¹
Quantal content	$m \sim 10 \div 20$ at $[\text{Ca}^{2+}]_o = 0.1 \div 0.2$ ²¹
	100 at $[\text{Ca}^{2+}]_o = 0.4$ ⁴⁰
	300 at $[\text{Ca}^{2+}]_o = 2.5$ ⁴¹