Review

Development and structure of synaptic contacts in Drosophila

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Abstract

Structural synapses are key regulators of information flow in neuronal networks. To understand the function and formation of neuronal circuits, the development and function of synapses have therefore been intensely studied in both vertebrate and invertebrate species. Precise descriptions of synapses and their amenability to genetic analysis in the model organism Drosophila provide an efficient platform from which to explore mechanisms and principles of synapse formation, which find many counterparts in other animals. Here we summarise our knowledge of the structure of Drosophila synapses. Focusing on neuromuscular junctions and photoreceptor synapses, we provide an overview of mechanisms underlying the development of synaptic structure in Drosophila.

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Keywords: Drosophila; Synapse; Neuromuscular junction; Optic lobe; Photoreceptor

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1. Introduction

The term synapse (from the Greek, meaning to clasp) was introduced as a physiological concept by Charles Sherrington in 1897, but research leading to the discovery of synapses as structural entities, sites of contact between neurons, occurred only much later [21]. Synapses are the structures at which neurons form and maintain contact with other neurons in order to...
transmit information. During that transmission, the synapses filter, integrate or modify this information, and in doing so they act as key regulators of information flow in neuronal circuits. Each chemical synapse is a specialised cell junction with machinery for the release of neurotransmitter on the presynaptic side, precisely opposite to and matched by the machinery for neurotransmitter reception and integration on the postsynaptic side. The longstanding scientific attention given to the structure and function of synapses [21] has in recent decades been complemented by studies on synaptic development in both vertebrate and invertebrate species [45,110]. The strengths of Drosophila as a model organism for such studies are twofold. Firstly, Drosophila is amenable to genetic analyses [66] and to unbiased searches for developmental mechanisms [103] that are often conserved and translatable into other animals. Secondly, Drosophila has individually identifiable brain cells, which provide impressively detailed and refined cellular assays of gene function [86]. Here we will provide an overview of synaptic structure in Drosophila. Focussing on the fly's neuromuscular junctions (NMJ) and photoreceptor synapses, we will summarise insights into the mechanisms of synaptic development.

2. The locations of synapses in the Drosophila nervous system

As in vertebrates, Drosophila has a central nervous system (CNS) and a stomatogastric nervous system, which is loosely comparable to the autonomic nervous system of vertebrates [41]. Sites of neuronal release of neurotransmitter are located in the CNS (Figs. 1–3) and, in the periphery, on somatic muscles (Fig. 1), the adult heart, and various organs (gut, glands, reproductive organs) [16,27,36,59]. However, not all of these sites harbour synapses, as defined by characteristic structural features (see Section 3.2), but instead represent sites of non-synaptic, mostly pedipodicger release. So far, descriptions of synapses in Drosophila have been reported for NMJs and a number of regions in the CNS.

NMJs are manifestly amenable to experimentation and easy to visualise [15,86]. They are established in the periphery in predictable combinations between individual motor neurons and muscles [57]. In contrast, central synapses are located within the neuropile, the soma-free central compartment of the CNS. The neuropile contains the neurites of sensory, inter-, and motor neurons, and synapses are often restricted to dendritic and presynaptic compartments within these neurites [64,95]. The neuropile is bounded by an ensheathing layer of glial cells (Fig. 1) [44], which is believed to create a physiologically favourable ionic environment distinct from the haemolymph [3,55]. But the glial layer also constitutes a confounding barrier to experimental penetration, and thus to the analysis of CNS synapses. As typical of higher invertebrates, Drosophila neurons in the CNS are usually uni- or monopolar [18,95]; their cell bodies are excluded from the synaptic neuropile but lie in the cortex, the outer rind of the CNS (Fig. 1). As a consequence of this segregation between soma and neurites, central synapses form between neurites (Fig. 1) but usually not upon somata, as in vertebrates. Although these differences between vertebrate and invertebrate neurons appear severe, they show clear homologous traits [95].

3. The structure of Drosophila synapses

3.1. Physiological considerations

The broad repertoire of neurotransmitters, neuropeptides, ionotropic and metabotropic transmitter receptors now reported for Drosophila [62,78,86] reflects the existence of many physiologically distinct types of synapses. For example, somatic and adult cardiac muscles are innervated by glutamatergic terminals [27,46], whereas nerve terminals on larval gut muscles contain serotonin, glutamate and FMRFamide [16]. Terminals of interneurons are predominantly cholinergic [115]. Further transmitters or neuromodulators reported for interneurons are GABA (about 20% of neurons in the embryonic/larval ventral nerve cord), glutamate, taurine, aspartate, dopamine, histamine, octopamine (also in some efferent neurons) or serotonin [54,58,74,77,106,114]. Terminals of adult photoreceptors are histaminergic [40] as probably are head mechanosensory neurons [14], whereas most other sensory receptor neurons are believed to be cholinergic [115]. In addition, neuropeptides are found throughout the CNS and in the periphery [59,78]. Such diversity among transmitters and neuromodulators is accompanied by differences at the neurophysiological level. For example, glutamatergic NMJs or cholinergic central synapses execute low-output neurotransmitter release evoked by presynaptic spikes [12,92]. In contrast, release at high-output histaminergic photoreceptor synapses is tonic and evoked by tiny, light-evoked, graded depolarisations [47]. The spectrum of physiological properties detected at Drosophila synapses is steadily increasing, and includes synaptic plasticity at both glutamatergic and cholinergic synaptic contacts [55,92,101,112].

3.2. General features of Drosophila synapses

The various synapse types reported for Drosophila display diameters of less than 1 μm and can usually be identified by features such as specialisations within the synaptic cleft, by presynaptic dense bodies (see Section 3.3), postsynaptic densities, and/or by the darker appearance of pre- and postsynaptic membranes (Fig. 1F and G). NMJs and giant fibre terminals form monadic synapses, in which one presynaptic site displays a continuous junction with just a single postsynaptic site (Figs. 1D, E and 2A, C). In contrast, most central synapses have two, or sometimes three, or even four or more postsynaptic elements opposite a single presynaptic release site, in constellations commonly referred to as dyads, triads, tetrads, etc. (Figs. 1C, 2H and 3E–G). This form of synaptic organisation contrasts with the situation in the vertebrate brain, in which synapses are monadic in composition except at specialised sites such as the retina, in which synaptic microcircuits incorporate many dyad and triad configurations [26]. Synaptic configurations with more than two partner cells have been intensively studied in the visual system of the fly (details in Fig. 3). Different explanations have been advanced for the existence of multiple
Fig. 1. Principles of Drosophila nervous system organisation in the embryonic or larval trunk. (A) Horizontal view of a Drosophila larva (anterior to the top; ml, midline) showing the head skeleton (he), muscles (mu), brain (br), ventral nerve cord (vn), and two neurons (red and blue lines). (B) Transverse section through trunk (plane of interrupted line in A). Some of interneurons (green, i), motor neurons (red/orange, m) lie in the cortex (cx) surrounded by glial cells (yellow). Ipsilateral (coloured), contralateral (black), ascending (circle with ×) and descending (circle with dot) neurites establish synaptic networks (triangles, presynaptic; black bars, postsynaptic) in the neuropile (np) enmeshed by glia. Motor neuron dendrites are restricted to the dorsal (red shaded), sensory neurites to the ventral (blue shaded) neuropile [59]. Sensory (s) and motor neurons (m) project through segmental nerves (sn) enmeshed by glia, insulating from circulating haemolymph (hl), except for a 10–20 μm gap at the distal ends (curved arrow) [3]. Motor neurons form NMJs on muscles (arrow heads). (C) Synapse in the neuropile at late embryonic/early larval stage with one presynaptic bouton (*) connecting to two postsynaptic densities (p) (compare [89]). (D–I) Details of NMJs (*, nerve terminal; mu/beige, muscle) in the late embryo (st.17; D, F, H) or at the late larval stage (E, G, I) [86]. Many features of NMJs change during larval life: the terminal increases in size, detaches from the basement membrane (bm) into the interior of the muscle, the muscle membrane forms infoldings (SSR, subsynaptic reticulum), the density of vesicles increases and ribosomes enrich at postsynaptic sites [100] (indicated in E). In contrast, characteristics of synapses remain constant (H, I; black arrow-heads in F, G, synapses in D–E); they are identifiable (even in the absence of T-bars; white arrow head in G) by evenly-spaced electron-dense membranes, dashed material in the postsynaptic half of the synaptic cleft, and usually have a presynaptic T-bar ribbon surrounded by vesicles (see endocytotic events: I and white arrow head in G); the extrasynaptic cell junction (open arrows) is less electron-dense and tends to undulate. Scale bar 200 nm in C, 600 nm in F, G and 250 nm in H, I.
Fig. 2. Central synapses of the giant fibre axon and mushroom body calyx. (A) Terminals of the giant fibre axon (GF) display chemical synapses (black arrow-heads; see C), beside gap junctions (white arrow heads; see D) [5]. (B) Somata of giant fibre neurons (curved arrow) located in the adult brain (br), have axons that descend through the head connective (hc) into the ventral nerve cord (vn), where they synapse via chemical and electrical synapses onto both peripherally synapsing interneurons (PSI) and tritocerebral motor neurons (TTM), which in turn project into segmental nerves (sn) to innervate motor neurons of the dorsal longitudinal flight muscle (not shown) and the jump muscle of the middle leg (mu), respectively [5]. (C) Chemical synapses display T-bar ribbons with vesicles and an intercellular space of 13–20 nm filled with electron-dense filamentous material. (D) Gap junctions have a close membrane apposition (2–4 nm) and a single layer of electron-lucent vesicles 30–55 nm in diameter along the GF membrane, forming a regular, hexagonal array when viewed en face (not shown) [5]. (E) Large, irregularly shaped terminals of projection neurons (p) in the mushroom body calyx are surrounded by smaller neuritic profiles (mostly Kenyon cell dendrites, but also neurites of other extrinsic neurons) upon which they form synapses (black arrows, compare F-H) [114]. (F) The wider context of these neurons [106,114]: projection neurons (p) receive synaptic input from antennal olfactory neurons (o) and further interneurons (not shown) in the antennal lobe (al), project through the middle (not shown) or inner (ia) antennocerebral tracts into the lateral horn (lh) or calyx (ca); neurites of mushroom body-intrinsic Kenyon cells (k) establish the neuropiles of mushroom bodies, the calyx (ca), pedunculus (pe), α-, β- and γ-lobes; further extrinsic interneurons such as GABA-positive cells (g) contribute to these neuropiles. (G) Candidate GABAergic terminals (g; here identified by anti-GABA labelling) display synapses onto projection neurons (yellow arrow) and other profiles (most likely Kenyon cell dendrites, white arrows, compare F) [114]. (H) Output synapses of projection neurons (p) have presynaptic T-bar ribbons (black arrows) surrounded by synaptic vesicles and opposite multiple postsynaptic elements with pronounced postsynaptic densities (orange arrows). Images A, C, D were taken with permission from [5] and E, G, H from [114]. Scale bar 570 nm in A, 180 nm in C, D, H, 1100 nm in E, 500 nm in G.

postsynaptic elements at these synapses [73]: (a) that energy requirements for presynaptic release [61] are cost-shared at each synapse among several postsynaptic cells; (b) that target neurons receive evenly-balanced synaptic inputs at shared synapses; and (c) that synaptic divergence and circuit complexity are achieved in Drosophila by increasing the compositional complexity of each neuron’s synapses (neuron-rich vertebrates, by contrast, in which most synapses are monadic, attain network complexity by increasing the number of neurons).

The reported cleft widths of synapse types in Drosophila all lie within 10–20 nm (Figs. 1–3), in contrast to vertebrates, in which the synaptic cleft at NMJs is significantly wider than at central synapses [85]. Within the synaptic cleft, many central synapses of Drosophila display diffuse, evenly distributed grey material (Figs. 1C, 2C, H, and 3G). In contrast, synapses at NMJs show an asymmetric distribution of cleft material, which associates with the postsynaptic membrane and displays a dashed or hexagonal pattern, depending on the plane of section [86].
Fig. 3. Synaptic connections in the lamina. (A) The retina (re) is part of the peripheral nervous system and composed of about 750 ommatidia (om) each containing eight photoreceptor neurons (R1–R8) which project into the two outermost optic neuropiles of the brain, the lamina (la) and medulla (me); central to these lie the lobula (lo) and lobula plate (lp). R7 and R8 terminate in the medulla, R1–R6 of each ommatidium sort into adjacent cartridges in the lamina (see B). (B) R1–R6 terminals are joined by projections of interneurons, the cell bodies of which lie in the surrounding cortex (ellipses; anatomical details in [71]); here shown are L1 (pink) and L2 (orange) cells, a T1 (grey) and an amacrine cell (am, brown), but additional cells exist, some indicated in C. Between these projections synaptic contacts are established which are reproducible in composition and number. For example, R1–R6 terminals each form about 50 tetradic synapses in Drosophila [73]. This redundancy is thought to improve the signal-to-noise ratio of transmission [24]. Opposite each tetrad site are precisely four postsynaptic elements, always containing lamina cells L1 and L2, and two other contributions from either a local amacrine cell, an L3 cell and/or epithelial glial cell [32,79] (arrow-head in B; L3 and glia not shown). The second example (curved arrow) shows amacrine cells synapsing on T1 and back on the photoreceptor terminal [71]. (C) These regular synaptic arrangements have enabled a wiring diagram of synaptic microcircuits to be worked out, based on the tetrads (green box) and including the most numerously represented synaptic pathways illustrated here (for further details see [73]). (D) A cross-sectional cartridge (section plane of D shown as interrupted line in B), reveals its conserved spatial organisation, like a footprint; each column comprises a ring of 6 photoreceptor terminals (each from a different ommatidium) surrounding L1 and L2 at the centre, separated by the paired neurites of amacrine (am) and T1 cells, and other interneurons (open circles: L3-L5, C2, C3), and is associated with the R7/8 axons from the overlying ommatidium. As illustrated here, L1 and L2 emit regular rows of paired spines (arrow; as in B) which combine with those from amacrine and L3 cells to form a common postsynaptic cluster, like a multiplex connector (tetrad). (E) Idealised scheme of the synaptic tetrad in C showing a presynaptic T-bar ribbon of the photoreceptor terminal, postsynaptic cisternae (cs) anchored via whiskers (w) in spines of lamina cells L1 and L2, and capitate projections (cp) from surrounding epithelial glia (gl) that penetrate the photoreceptor terminal, sites of vesicle endocytosis (ec) and possibly also of histamine recovery [31,71]. (F) Micrograph of two R1–R6 photoreceptor terminals synapsing on the same L1 and L2 spines, thus forming two opposing tetrads (arrowheads; another pair of postsynaptic elements not visible in this plane of section; es, endosome; mc, mitochondria; other abbreviations as in E). Capitate projections are about 0.2 μm deep with a head diameter of about 175 nm displaying specialized densities of unknown function [71]. (G) Each synapse is a single release site with a presynaptic ribbon surrounded by clear 30-nm synaptic vesicles [71] about 0.25 μm × 0.5 μm in diameter and with four postsynaptic elements (only L1 and L2 postsynaptic elements seen in this section plane) [32,79]. Scale bar 320 nm in F, 150 nm in G.

(Fig. 3H, I). We would predict that any material in the synaptic cleft should represent extracellular matrix, except basement membrane, or extracellular domains of adhesion molecules and transmitter receptors. None of these components has so far been clearly identified, however.

Postsynaptic membranes are usually lined by postsynaptic densities (PSDs) on their cytoplasmic faces, which are expected to contain signalling molecules, scaffolding proteins, and the cytoplasmic portions of transmitter receptors, other ion channels and adhesion molecules [98]. PSDs are pronounced at many central synapses of Drosophila (Figs. 1C and 2H), but are less visible at neuromuscular and photoreceptor synapses (Figs. 1F-1 and 3G). Differences in the PSDs most likely correlate with physiological differences. For example, GABAergic synapses in vertebrates are usually type 2 or symmetric synapses with a sparse PSD [85], and the same might be true for GABAergic synapses in Drosophila [95] (F.-W. Schürmann, personal communication).

3.3. A common presynaptic component of fly synapses: the T-bar ribbon

In spite of the differences described in Section 3.2, most presynaptic terminals in Drosophila share a common type of presynaptic organelle, a pronounced dense body comprising a base or pedestal surrounded by a platform. These so-called T-bar ribbons are found at NMJs (Fig. 1), in photoreceptor terminals (Fig. 3), many other central synapses (Figs. 1C and 2), and potentially even in neuroendocrine terminals [4]. In contrast, only rather specialised vertebrate neurons, such as sensory...
receptor neurons or their second-order targets, display characteristic synaptic dense bodies [83,116]. Obvious cases are seen in the vertebrate retina, and are thought to be specialised for high rates of delivery of synaptic vesicles to the presynaptic membrane, either acting as a canal per se or to tether vesicles for release as multi-unit packages [83,102]. Similarly, at the T-bar ribbons of flies, vesicles often appear physically attached [50,80]. Furthermore, vesicle exocytosis occurs at the presynaptic plasma membrane beneath the T-bar (Fig. 1E) [5,86,94,109]. Such synaptic release would be expected to depend on local induction of high (Ca^{2+}) microdomains [63]. Consistent with this idea, T-bars are closely associated with clusters of membrane particles likely to be calcium channels [32,48,86] similar to the clustering of calcium channels at vertebrate ribbons [107,116]. Vertebrate ribbons contain structural molecules such as Ribeye, Bassoon, Piccolo and RIM (Rab3-interacting molecule), but also the molecular motor protein KIF3A [75,107]. Some of these lack fly homologues, however, and the first components of T-bar ribbons, such as the nect2 antigen [113], are only starting to emerge.

T-bar ribbons are usually surrounded by clear, round vesicles (Figs. 1–3), which may contain subpopulations of pleiomorphic or dense-cored vesicles. For example, during larval development neuromuscular boutons become increasingly filled with vesicles (Fig. 1E, G) that constitute readily releasable and reserve pools [56]. Similarly, photoreceptor R1–R6 terminals are each filled with a uniform population of about 43,000 vesicles [7,71]. Whereas a fraction of these vesicles would be expected to be derived from the Golgi apparatus and delivered to the synapse via axonal transport [39,76], another route is through local vesicle recovery from the plasma membrane [29,50]. At NMJs, clathrin-mediated recovery occurs extrasynaptically (Fig. 1D, E) with some vesicles travelling through the endosomal compartment [113]. In R1–R6 terminals, clathrin-mediated recovery occurs extrasynaptically at so-called capitate projections (Fig. 3E; details in Section 3.4) [31]. In addition to clathrin-mediated recovery, immediate vesicle exocytosis occurs at kiss-and-run release has been suggested to take place below T-bar ribbons in motor neuronal and photoreceptor terminals [50,109], but is now thought not to occur in the former [28].

3.4. Other synaptic specialisations

Other prominent specialisations at synapses have been reported and are briefly summarised here. Capitate projections are found in the terminals of R1–R6 in the first optic neuropile, or lamina [31] and of R7/R8 in the second neuropile, or medulla (I.A.M., unpublished) (Fig. 3A), suggesting that these organelles are specific to photoreceptors or to histaminergic transmission. Each is a regularly-shaped glial invagination that penetrates the photoreceptor terminals (Fig. 3E, F) as a dynamic organelle which invaginates from, and retracts to, the plasma membrane, like an organ stop. The relative numbers of these different stages can change within periods of the order of 10 min, but the duty cycle of a single penetrating capitate projection is not known. Capitate projections are specialised regions for membrane retrieval by clathrin-mediated endocytosis [31], possibly required to accommodate the high rates of recycling required at fly photoreceptor terminals. Thus, an R1–R6 terminal in Drosophila harbours 50 tetrads, each thought to release up to 100 vesicles per second [7,60]. Capitate projections are also proposed sites for neurotransmitter reuptake [31] after released histamine is metabolised by the catalytic activity of Ebony [6,91]. Capitate projections made their appearance early in the evolution of diptera [97]. Possible counterparts of glial invaginations into the presynaptic nerve terminal with endocytic intermediates have been found at frog neuromuscular synapses [42].

Post synaptic elements at photoreceptor tetrads display specialisations called cisterneae. These membranous organelles underlie the postsynaptic membrane in spines of the lamina cells L1 and L2 and are connected to the plasma membrane via protein bridges called whiskers (Fig. 3E–G) [71]. They are part of the endoplasmic reticulum, but their contents and function have not so far been reported.

Motor neuron terminals at the larval NMJ are surrounded by the subsynaptic reticulum (Fig. 1E, G), reticular invaginations of the muscle membrane (SSR). The SSR starts developing only after embryogenesis is complete [37] (Fig. 1D–G), and its function is still not clear. The SSR should not be equated with postsynaptic junctional folds at the vertebrate NMJ, whereas openings of the SSR are always extrasynaptic (Fig. 1E, G), the cleft-shaped junctional folds at the vertebrate NMJ harbour acetylcholine receptors at their neck and are aligned opposite the often elongated release sites of the motor neuron end-plate [85]. At giant-fibre synapses in Drosophila, chemical synapses with typical T-bar ribbons co-exist with gap junctions (Fig. 2A–D) [5], a constellation also observed in vertebrates [84]. Giant fibre gap junctions are 0.5–2 μm in diameter, and show extremely close membrane apposition (2–4 nm). A singlelayer of electron-lucent vesicles (30–55 nm) of yet unknown function is arrayed in a hexagonal pattern on the cytoplasmic face of the giant fibre membrane. Similar junctions have been found in various sites in the fly’s brain [105].

4. The development of synapses at NMJs and photoreceptor terminals

The formation of synapses is a stepwise process. First, neurites of appropriate synaptic partner cells have to establish contact, and this decision is reached during two consecutive phases of neuronal growth, pathfinding and target recognition. Second, synapses have to assemble at these contacts in the right number, with the correct molecular composition and spatial distribution. The spatial distribution and cell-specificity of synapses determine the complexity and operational roles of neuronal circuits. The number and specific molecular composition of synapses at synaptic contacts will determine their synaptic strength. In the following sections we will focus on insights into the formation of neuromuscular and photoreceptor contacts.
4.1. Connectivity in the neuromuscular system

In the neuromuscular system, one muscle can be innervated by a single motor neuron exclusively or by two or more motor neurons [43]. Multiply innervated muscles (see muscle 2 in Fig. 1B) need to assign the right quantities and types of GluRs to each contact (black and white arrow-heads in Fig. 1B) [65]. Conversely, single motor neurons can either innervate a single muscle or distribute their terminals on several different muscles [43,68], in which case motor neurons (dark red in Fig. 1B) distribute their release sites in balanced and genetically regulated ways on their different targets (black arrow-heads in Fig. 1B) [23,49]. Taken together, both muscle and neuron have to assign the right numbers and types of pre- and postsynaptic structures to their various contacts, thus determining the extent to which each individual neuromuscular contact contributes to the activation of any particular muscle [104].

4.2. Formation of neuromuscular contacts

Pathfinding and target recognition in the neuromuscular system are reviewed elsewhere [19]. In brief, during the period of pathfinding each motor axon grows to its adequate CNS exit point, and either pioneers or joins the appropriate nerve or nerve branch to reach its target area (Fig. 1B). Target recognition succeeds pathfinding without interruption and is initiated when motor axons defasciculate from their nerves at neuron-specific positions to contact their appropriate target muscle(s). The current view is that highly reproducible regulatory regimes during birth and development give rise to individually identifiable neurons and muscles, both of which follow individual genetic programmes to achieve, eventually, a perfect match in which a particular muscle is always innervated by the same identifiable motor neuron(s) [57,86].

At these newly formed neuromuscular contacts, synapse assembly starts at about 13h (±60%) of embryonic development and the contacts reach structural and physiological maturity at the time of hatching [86]. However, during larval life, NMJs increase dramatically in size and yet another phase of de novo or re-formation of NMJs occurs during metamorphosis at the pupal stage. These postembryonic phases of NMJ development also involve synapse assembly. At each stage of development, NMJs adopt a stereotypic morphology, defined by nerve entry point, and either pioneers or joins the appropriate nerve or nerve branch to reach its target area (Fig. 1B). Target recognition succeeds pathfinding without interruption and is initiated when motor axons defasciculate from their nerves at neuron-specific positions to contact their appropriate target muscle(s). The current view is that highly reproducible regulatory regimes during birth and development give rise to individually identifiable neurons and muscles, both of which follow individual genetic programmes to achieve, eventually, a perfect match in which a particular muscle is always innervated by the same identifiable motor neuron(s) [57,86].

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4.3. Connectivity in the visual system

In contrast to NMJs, synapse assembly at photoreceptor contacts occurs mainly at one stage of development, i.e. during pupal life, although certain plastic properties persist into adult life [8,93]. Every R1–R6 photoreceptor terminal establishes about 50 presynaptic sites, each arranging into tetrads with a predictable blend of the same postsynaptic cells, L1, L2, L3 or amacrine cells (Fig. 3) [73]. Conversely, each L1 or L2 lamina cell has to establish synaptic contacts with the six different photoreceptor terminals of its lamina module, or cartridge (Fig. 3D), in order to pool information from all photoreceptors with the same field of view. Hence, developmental mechanisms must ensure that these neurons regulate not only the number but also the specificity of synapses assembled, in order to establish reproducible and functional microcircuits in the lamina (Fig. 3C).

4.4. Formation of photoreceptor contacts

Pathfinding and target recognition in the visual system are reviewed elsewhere [20]. In brief, pathfinding initiates when successive waves of photoreceptor axons grow out, and these distinct waves are a function of the stepwise process in which the eye disc develops and differentiates [111]. Each wave of axons fasciculates into bundles which grow out rapidly and in a guided manner. They approach the exit point of the eye disc, funnel through the optic stalk to the brain, and continue towards the appropriate position in the distal optic lobe. From there the
axons grow in centripetally at the leading edge of newly proliferated optic lobe cells to establish the first retinotopic map. This map is an initial array of columns (Fig. 3A) which proliferated optic lobe cells to establish the first retinotopic map. axons grow in centripetally at the leading edge of newly proliferated optic lobe cells to establish the first retinotopic map.

4.5. Developmental mechanisms establishing synaptic ultrastructure

Thus, principal mechanisms governing temporal, spatial and quantitative aspects of synapse assembly clearly differ between photoreceptor and neuromuscular contacts, but also between de novo formation of NMJs in the embryo and the later plastic growth phase in the larva. However, the structural appearance of individual synapses is indistinguishable between embryonic and larval NMJs (Fig. 1H, I), and both share the same T-bar ribbon structure with photoreceptor tetrads (Fig. 3F, G). This observation suggests that various synapse components and mechanisms directly involved in synapse assembly might be identical or similar in different developmental or neuronal contexts in Drosophila. What are these mechanisms and components?

A number of mutants have been described which display aberrations in synaptic specialisations (listed in Table 1A of supplementary information), but few give us insights into the mechanisms that underlie the assembly of synapse structure. For instance, loss-of-function mutations of the muscle differentiation factor MEF2 abolish all neuromuscular adhesion [87]. However, even in the absence of neuromuscular adhesion, normal presynaptic specialisations form. Such orphan release sites have similarly been reported in vivo for photoreceptor terminals congenitally deprived of synaptic targets [108] and, in culture, for Drosophila interneurons [55] and mammalian hippocampal neurons [52]; these examples suggest that the assembly of normal presynaptic specialisations does not essentially depend on the postsynaptic partner. Furthermore, developmental studies have taught us that each synapse seems to assemble on-site in a progressive manner. Thus, during NMJ development, the diameter of synapses increases progressively [90] in parallel with their physiological maturation [11]. T-bar ribbons also grow gradually during the development of neuromuscular and of photoreceptor contacts [33,86], and the platform of the synaptic ribbon at adult fly photoreceptor terminals widens with light exposure in a manner suggesting that these structures are assembled locally [93]. However, we do not yet know whether the structural and functional proteins of fly synapses assemble on-site, or whether they are already pre-assembled and delivered via specialised vesicles, as is the case at vertebrate release sites [117]. In contrast to presynaptic organelles, hardly any ultrastructural data are so far available for the development of postsynaptic specialisations. However, by using immunocytochemical methods, considerable insights have been gained into mechanisms underlying the clustering of glutamate receptors at postsynaptic sites opposite active zones at NMJs (summarised in supplementary information).

5. Conclusions

Many insights have been gained into the structure and development of synaptic contacts in Drosophila. However, essential gaps in our understanding have still to be addressed. The most obvious of these concern the mechanisms of presynaptic assembly and of selective adhesion between presumptive synaptic partners, and the molecular composition and function of T-bar
we know surprisingly little about CNS synapses, of which the large cholinergic projection neuron terminals of the mushroom body calyx (Fig. 2E) are promising candidates for further study. In addition to gaining new insights, we also need to integrate the different lines of research on each type of synapse and form a comprehensive model of synapse formation.

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Appendix A. Supplementary data


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Mechanisms of postsynaptic development

During recent years, considerable insights have been gained into the development of the postsynaptic organisation of the NMJ, in particular the formation of ionotropic glutamate receptor (GluR) fields opposite presynaptic release sites. Ionotropic glutamate receptors at the NMJ are hetero-tetrameric and contain each of the three subunits GluRIID, D and E together with either GluRIIA or GluRIIB. GluRIIA and B exclude each other, and define two GluR subtypes which localise in partially overlapping patterns and display distinct properties [20, 49, 62]. At newly forming embryonic NMJs, postsynaptic GluR clustering depends on signals from presynaptic terminals [10, 13]. The organisation of this machinery depends on presynaptic action potentials [8, 70] in ways not yet understood. Vesicular transmitter release may or may not be involved [7, 48, 69], but certainly levels of the neurotransmitter glutamate are important for GluR clustering [25, 26]: enzymatic up-regulation of glutamate contents in the presynaptic terminal negatively influences clustering of functional GluRs at the postsynaptic membrane. The corresponding glutamate release might occur via non-vesicular neurotransmitter transporters [25, 26]. Therefore, given that the amount of presynaptic glutamate released is instructive for GluR clustering, we would expect that GluRs themselves should trigger their own localisation. Calcium signalling events might mediate such processes, since it has been shown that calcium/calmodulin-dependent kinase (CaMKII) phosphorylates and removes Discs large (Dlg) from the postsynaptic site at larval NMJs [38] - although this might differ during embryogenesis [36]. Dlg is a PDZ-domain containing scaffolding protein, which becomes enriched at postsynaptic sites in response to innervation [13]. Its homologues are involved in GluR clustering at synapses in the CNS of mammals [75]. In the absence of Dlg, GluRIIB but not GluRIIA receptors, fail to localise synaptically, but this role of Dlg is likely to be indirect [13]. Conversely, GluRIIA but not GluRIIB localisation depends on the cytoskeletal linker 4.1 protein Coracle [14], whereas both Dlg and GluRIIA localisation can be regulated through muscular functions of Pix and Pak kinases and the adaptor protein Dreadlocks [2, 56]. In addition, postsynaptic GluR levels are adjusted through expression regulation of GluR-encoding genes, e.g. via translational control [77, 78]. Control of GluR expression is already observed at late embryonic stages, induced by presynaptic innervation [9]. Additional genes required for clustering at postsynaptic sites have been identified [44] and will provide us with the genetic tools to test to what degree the regular cleft material at neuromuscular synapses (Fig. 1H,I) represents the extracellular domains of GluRs.

Compared with these advances at the NMJ, the postsynaptic organisation at photoreceptor tetrad synapses awaits investigations based on the recently reported histamine receptor gene, DmHisCl1 [27, 28, 100].
Table 1. Factors underlying the regulation of NMJ structure

A) Genetic factors mutations or manipulations of which have been reported to cause defects at the EM level of synaptic structures reviewed here. B) Genetic factors mutations or manipulations of which have been reported to cause structural defects of larval NMJs at any microscopic level; factors have been assigned to only one group of proteins (underlined in B), although for some of the factors different assignments would have been possible. Abbreviations indicate the structural feature regulated by the respective genetic factor: bs, bouton size; bn, bouton number; sd, synapse density; tl, terminal length; gr, glutamate receptor mislocalisation; sa, structural aberrations (shape aberrations, altered numbers of satellite boutons, cytoskeletal defects, synaptic defects, abnormal inclusions, abnormal distribution of organelles, SSR aberrations etc.). A source of further mutations potentially affecting NMJ morphology is provided by an over-expression screen published previously [40].
A) Ultrastructural mutant NMJ phenotypes:
- alteration of synapse size: Liprin-alpha, DLar [34]

B) Genetic factors involved in structural larval NMJ development:
- metabolism: Stress-sensitive B & Atpα [32, 88]
Supplementary References


the MAP1B homolog Futsch to control synaptic structure and function. Cell 2001; 107: 591-603.

