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Review

Live imaging of inhibitory axons: Synapse formation as a dynamic trial-and-error process

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ABSTRACT

In this review I discuss recent live imaging studies that demonstrate that synapses, and in particular inhibitory synapses, are highly dynamic structures. The ongoing changes of presynaptic boutons within axons emphasize the stochastic aspect of inhibitory synapse formation and paint a picture of a dynamic trial-and-error process. Furthermore, I discuss recent and previous insights in the molecular and mechanistic pathways that underlie synapse formation, with a specific focus on the formation of inhibitory presynaptic boutons.

Contents

1. Introduction

Recent developments in microscopy have had a great impact in neuroscience. Live imaging in brain tissue has revealed a dynamic world of ongoing changes of molecular complexes and subcellular structures in dendrites and axons within the brain. In particular, live imaging studies have demonstrated that synapses are incredibly dynamic structures. Both pre- and postsynaptic sites undergo structural changes in both shape and size spontaneously, and in response to environmental signals (Bonhoeffer and Yuste, 2002; Bury and Sabo, 2015; Frias and Wierenga, 2013). At the molecular level synaptic proteins show fast turnover and movement in and out of synapses (Alvarez-Castelao and Schuman, 2015; Matz et al., 2010; Ziv and Fisher-Lavie, 2014). Synaptic dynamics presumably reflect ongoing adjustments of synaptic strength and connectivity of the neuronal circuitry and are thought to be very important for experience-dependent circuit adaptations. Inhibitory axons appear particularly dynamic (Dobie and Craig, 2011; Kuriu et al., 2012; Schuemann et al., 2013; Villa et al., 2016; Wierenga et al., 2008). Rapid adaptation of inhibitory synapses has been suggested to serve as a gating mechanism for plasticity at nearby excitatory synapses, occurring at a slower time scale (Chen et al., 2015; Froemke et al., 2007; Keck et al., 2011; Villa et al., 2016), and may be a general feature during circuit development and adaptation (Froemke, 2015; Hensch, 2005). In this review I will focus on dynamic changes in presynaptic structures during synapse formation, and specifically focus on bouton formation in inhibitory axons which reflects the initial stage of inhibitory synapse formation.

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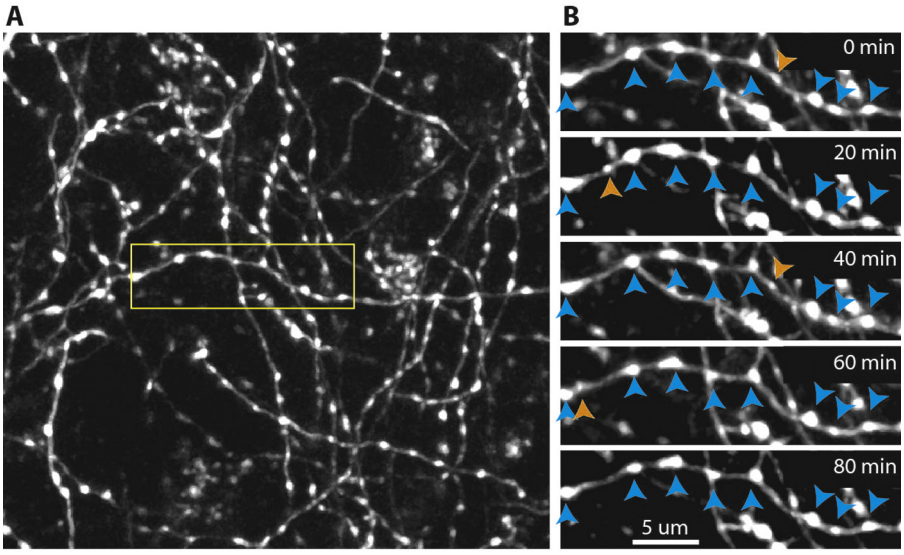

Fig. 1. Time-lapse two-photon microscopy of inhibitory axons.
A. Overview of a large field of view showing multiple GFP-labeled inhibitory axons in an organotypic slice from a GAD65-GFP mouse. B. Individual axon imaged repeatedly every 10 min (only every 2nd image is shown here). The majority of inhibitory boutons are present at all time points. These persistent boutons (blue arrow heads) reflect inhibitory synapses (see main text for references). Their shape and size can change substantially between time points. In addition, some boutons are only present at some, but not all time points. These non-persistent boutons (orange arrow heads) reflect ongoing processes of inhibitory synapse formation and disassembly. The axon in B is indicated with the box in A.

2. Live imaging of inhibitory axons in brain slices

In labeled presynaptic axons, synaptic terminals forming *en passant* boutons along the axon are visible under a microscope as local swellings of the axonal shaft. In our lab, we are using time-lapse two-photon microscopy to visualize presynaptic structures of inhibitory synapses in organotypic slices of mouse hippocampus. We make use of a transgenic mouse line, the GAD65-GFP line, in which a known subset of inhibitory neurons express GFP (López-Bendito et al., 2004; Wierenga et al., 2010). Only ~20% of inhibitory neurons are labeled and the bright labeling of all processes, including axons, makes this mouse line ideally suited to follow changes in individual GFP-labeled inhibitory axons over time. We study inhibitory synapse formation and plasticity by monitoring individual inhibitory boutons by repeated imaging (typically every 10 min) using two-photon microscopy (Fig. 1). Using this method we generally distinguish two classes of inhibitory boutons, defined by their dynamics. *Persistent* boutons (blue arrow heads in Fig. 1) are generally large and continuously present at a fixed location throughout the imaging period (2–6 h). They represent inhibitory synapses, and contain pre-and postsynaptic structural specializations such as synaptic vesicles and scaffolding proteins, which can be revealed by post-hoc immunostaining (Schuemann et al., 2013; Wierenga et al., 2008), or by electron microscopy (Müllner et al., 2015). Even though these boutons are stable in the sense that they are continuously present during the imaging period, their shape and size varies significantly over time, presumably reflecting ongoing changes in their molecular content (Matz et al., 2010; Minerbi et al., 2009). In addition to persistent boutons, there are many boutons which appear, disappear and reappear during the imaging period (orange arrow heads in Fig. 1). In addition, neighboring boutons occasionally merge or split. We generally refer to these boutons as *non-persistent* boutons. They are usually smaller than persistent boutons and most likely reflect incomplete synapses that are in the process of being formed or disassembled (Schuemann et al., 2013; Wierenga et al., 2008).

Similar bouton dynamics have been observed in many studies by others in excitatory (Becker et al., 2008; Bury and Sabo, 2011; Sabo et al., 2006) and inhibitory (Fu et al., 2012; Kuriu et al., 2012) axons. These dynamics are not limited to *in vitro* preparations, but also occur *in vivo* (Chen et al., 2015; Grillo et al., 2013; Keck et al., 2011), indicating that dynamic boutons are a general feature of many, if not all, axons.

3. Dynamic boutons

The formation of inhibitory synapses along the axon is not random, but boutons appear, disappear and reappear repeatedly at specific axonal locations (Schuemann et al., 2013; Wierenga et al., 2008), suggesting multiple attempts to form new synapses at these spots. Indeed, when we performed post-hoc immunostaining at locations where boutons had appeared and reappeared, we noticed that these locations often contained pre- and/or postsynaptic proteins, suggesting that synapses were being built or being disassembled at these locations (Schuemann et al., 2013). We also found that newly formed boutons which stabilize, acquire pre- and postsynaptic markers over the course of several hours (Wierenga et al., 2008), with the presynaptic marker VGAT generally arriving before the postsynaptic gephyrin (Dobie and Craig, 2011; Schuemann et al., 2013). This indicates that persistent and non-persistent boutons are not separate categories of inhibitory boutons, but reflect inhibitory synapses at different stages of their life cycle. Indeed, non-persistent boutons that appeared *de novo* and then stabilized at an axon-dendrite crossings during an imaging session were found back at the same position on the next day, suggesting they had transformed into persistent boutons over time (Wierenga et al., 2008). Vice versa, some persistent boutons may destabilize and become non-persistent when imaged longer.

Our observations of inhibitory bouton dynamics build upon previous studies of axons in primary cultures, which showed that new boutons emerge not randomly along an axon, but at specific, apparently predefined, locations (Sabo et al., 2006). It was shown that clusters of synaptic vesicles continuously travel up and down the axonal shaft (Bury and Sabo, 2011; Darcy et al., 2006; Staras et al., 2010; Wu et al., 2013), and that new boutons are formed by clusters of synaptic vesicles stopping and pausing at these specific locations.

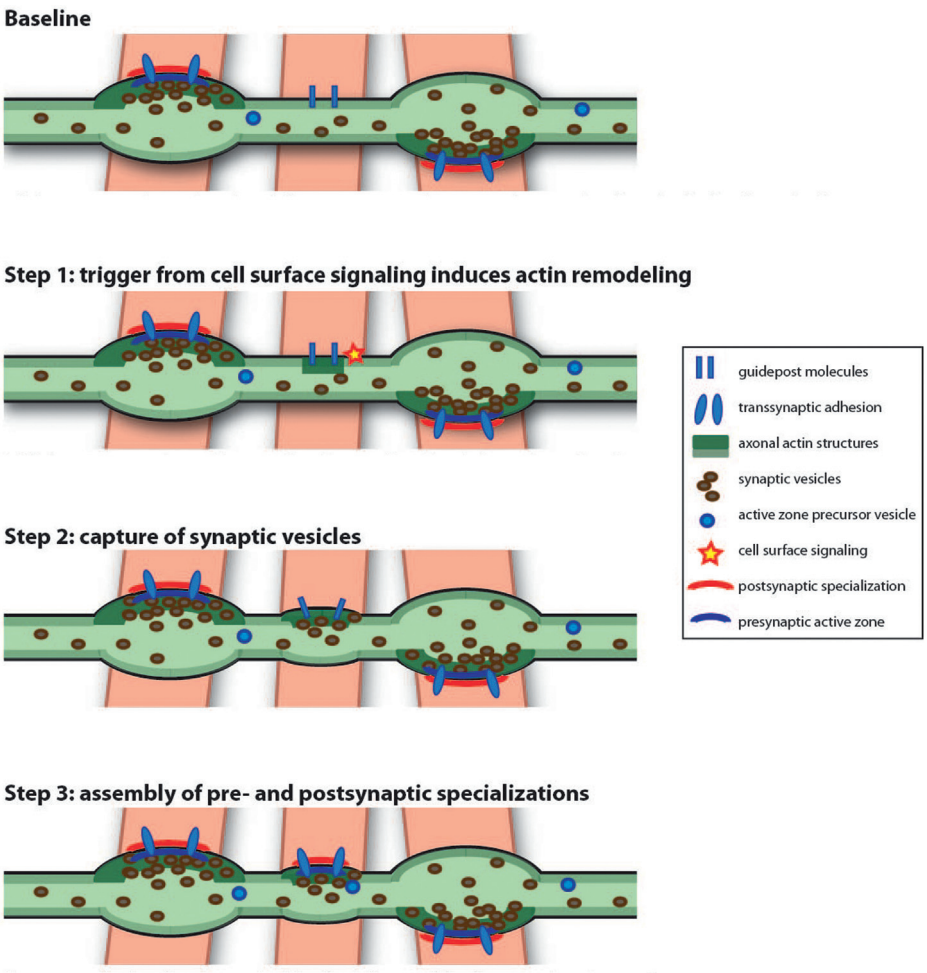

Fig. 2. Working model for inhibitory synapse formation.
Baseline
Inhibitory axons (green) make inhibitory synapses (persistent boutons) with multiple dendrites (red). Synaptic vesicles and larger vesicles containing active zone components are transported along the axon. Synaptic vesicles are continuously exchanged between neighboring boutons. Even though actin is prominently present at mature synapses, their molecular structure is independent of actin. At axon-dendrite crossings without inhibitory synapses transient clustering of synaptic vesicles occur (non-persistent boutons). These locations are presumably somehow marked, for instance by guidepost molecules, or by other membrane specializations.
Step 1: trigger from cell surface signaling induces actin remodeling
Inhibitory synapse formation may be triggered by signals from the cell surface, for instance from the postsynaptic dendrite or through extracellular (secreted) factors. This results in remodeling of intracellular actin structures within the local axon. Presumably, different signaling pathways will trigger specific actin remodeling.
Step 2: capture of synaptic vesicles
Local actin structures recruit presynaptic proteins such as liprinα, synapsin and neurabin, which induces stabilization of synaptic vesicle clusters. The immature inhibitory synapse does not have an active zone, but spontaneous vesicle release already occurs. Additional signaling proteins required for full maturation may be delivered by local vesicle fusion.
Step 3: assembly of pre- and postsynaptic specializations
Transsynaptic signaling is required for the full assembly of pre- and postsynaptic specializations. Neurotransmitter release from the presynaptic terminal may be instructive for the assembly of the postsynaptic specialization, and vice versa, retrograde signaling from the dendrite to the axon may be required to induce maturation of the presynaptic active zone. Acquiring of mature synaptic morphology and function will take several hours to days.

Although it is not clear how these pausing sites are determined or 'marked' within the axonal shaft, these observations suggest that some structural change in the axon precedes or even induces subsequent molecular specialization. The formation of new boutons followed by subsequent recruitment of pre- and postsynaptic proteins is reminiscent of bouton formation at *Drosophila* neuromuscular junction (NMJ). During development or in response to elevated activity levels, the NMJ undergoes a rapid growth of many new presynaptic boutons. These newly formed boutons are called ghost boutons, as they do not contain pre- or postsynaptic membrane specializations (Ataman et al., 2008). Ghost boutons are small in size and their active zones are immature, but they already contain synaptic vesicles (Ataman et al., 2008). One of the first proteins to be transported into the ghost boutons is synapsin (Vasin et al., 2014), which is a known presynaptic organizer of synaptic vesicles (Chi et al., 2003). After their initial formation, the new boutons rapidly grow in size and acquire the pre- and postsynaptic molecular specializations of mature synapses. It was shown that ankyrin is required for this second growth phase (Koch et al., 2008; Pielage et al., 2008), providing structural stability as well as stabilization of microtubules, which guide the transport of building blocks for further bouton growth. This demonstrates that a structural change in the axon may be an early event during the formation of new synapses.

4. Location of future synapses

In primary cultures, predefined locations for bouton formation can be present in the absence of contact with any postsynaptic partners (either neuron or glia cell), illustrating the self-organizing

power of axons and their boutons (Sabo et al., 2006). However, in a more physiological situation within neuronal tissue, axonal locations for presynaptic terminal assembly will generally be determined by external cues, such as physical contact with dendrites of other neurons (Gerrow et al., 2006; Wierenga et al., 2008). Not all contacts will result in bouton formation and whether or not boutons will be formed is likely determined by the presence of specific recognition or signaling molecules, such as cell adhesion molecules or other surface molecules, at the pre- and postsynaptic membranes. In this way specificity of synaptic contacts is achieved (de Wit and Ghosh, 2016), something which is of crucial importance in the complex neuronal circuits within the brain. An adhesive event *per se* can be sufficient to trigger assembly of presynaptic specializations within axons (Gokce and Südhof, 2013; Lucido et al., 2009). A positive adhesive trigger at axon-dendrite crossings will instruct stabilization of boutons at these axonal locations and promote the synapse assembly process. In *C. elegans*, it was shown that future locations of synapses are decorated with adhesion molecules that are present at the membrane before synapse formation (Shen and Bargmann, 2003). In this case, boutons were temporarily formed at future synapse locations with guidepost cells (Shen et al., 2004). Guidepost cells also exist in the developing mammalian brain (Squarzoni et al., 2015), but molecules marking future synapses have not been identified so far in mammalian systems. In inhibitory axons, in which new synapses are formed at pre-existing axon-dendrite crossings (Wierenga et al., 2008), crossings without synapses may be somehow marked as potential synapses where new boutons preferentially would emerge (Sabo et al., 2006). It is currently not clear if such locations are marked by specific guidepost molecules, or if they are defined by local membrane properties.

5. Synaptic maturation

Spontaneous fusion of synaptic vesicles and neurotransmitter release can occur at the axonal membrane in the absence of any presynaptic specialization (Krueger et al., 2003; Ratnayaka et al., 2011), but for fast action potential-triggered neurotransmitter release at nascent presynapses development of an active zone is required (Gundelfinger and Fejtova, 2012; Südhof, 2012). Interestingly, it was shown that spontaneous (miniature) synaptic transmission is required for the maturation of ghost boutons into larger, more mature, boutons and for maturation of the active zone (Cho et al., 2015; Choi et al., 2014). Active zone assembly at nascent synapses may be achieved by the fusion of large vesicles, containing preassembled active zone protein complexes (Petzoldt et al., 2016; Ziv and Garner, 2004). Fusion of a few of these large vesicles allows active zone assembly within 1–2 h after initial bouton stabilization (Ahmari et al., 2000; Friedman et al., 2000; Zhai et al., 2001; Ziv and Garner, 2004). Postsynaptic scaffolding proteins and receptors are also recruited within the first hours, probably at slightly slower pace (Bresler et al., 2004; Garner et al., 2006; Gerrow et al., 2006). Full maturation of ultrastructural morphology and electrophysiological properties of a newly formed synapse continues during the following 1–2 days (Ahmari et al., 2000; McAllister, 2007; Nägerl et al., 2007). The requirement for vesicle release for completing the transition from ghost bouton to functional presynaptic terminal in Drosophila (Cho et al., 2015; Choi et al., 2014) indicates the involvement of bidirectional transsynaptic signaling during a crucial step of synapse development. It is currently not known if vesicle release is also required for development of pre- and postsynaptic specializations in mammalian synapses, but different transsynaptic signaling pathways have been identified (see below).

6. Exchange of synaptic vesicles between neighboring synapses

Intra-axonal transport of synaptic vesicles and presynaptic proteins does not only occur during axonal development. In fact, it has been shown that exchange of synaptic vesicles and presynaptic proteins is still ongoing in mature synapses between neighboring presynaptic terminals (Staras, 2007). Presynaptic dynamics are often monitored with epifluorescence and confocal microscopy, in which labeled synaptic proteins or morphological structures are monitored in live primary cultures. Such studies have revealed that the molecular machinery in presynaptic terminals is dynamic and continuously changing. For instance, use-dependent and photoactivatable labeling of synaptic vesicles has shown that synaptic vesicles are shared and continuously exchanged between neighboring synapses within tens of minutes (Fernandez-Alfonso and Ryan, 2008; Krueger et al., 2003; Staras et al., 2010). In addition, FRAP (Fluorescence Recovery After Photobleaching) experiments showed that proteins at synapses are continuously replaced and that turn-over rates are different for different proteins and can change with activity (Darcy et al., 2006; Staras, 2007). This demonstrates that presynaptic boutons, even in mature axons, are in continuous interaction with neighboring boutons through sharing of presynaptic components and synaptic vesicles. New boutons emerging along the axon recruit presynaptic material from neighboring mature boutons and presynaptic material is recycled and used for strengthening of neighboring synapses when synapses are being disassembled (Park et al., 2011; Wu et al., 2013). In case of limited availability, sharing may turn into competition. The balance between capture and dissociation of packages of presynaptic proteins and synaptic vesicles from the axonal pool determines success or failure to form a new synapse (Wu et al., 2013). Intracellular factors can interfere with this recruitment process and may directly or indirectly affect synapse formation. For instance, synapse formation can be hindered by promoting interactions between presynaptic assembly packages and motor proteins, or can be promoted by strengthening local interactions with active zone proteins (Bamji et al., 2006; Klassen et al., 2010; Park et al., 2011; Wu et al., 2013). In this way, the local intracellular environment at and near newly formed boutons can influence bouton stabilization and subsequent synapse formation.

7. Transsynaptic signaling pathways

The picture that emerges from live imaging studies expose synaptic assembly as a nonlinear process with many stochastic elements in which synapses are built and rebuilt several times before stabilization and eventually maturation takes place. At first sight these trial-and-error dynamics seem at odds with studies using co-culturing assays in which the formation of presynaptic terminals can be directly induced by so-called 'synaptogenic' signaling molecules (Scheiffele et al., 2000). In this assay, axons get into contact with non-neuronal cells expressing a strong synaptic adhesion molecule, which induces the formation of presynaptic terminals on the surface of the non-neuronal cells (Krueger et al., 2012; Siddiqui and Craig, 2011). For example, transsynaptic interactions between neuroligin-2 and neurexin (Poulopoulos et al., 2009; Varoqueaux et al., 2004), or between slitrk3 and PTPδ (Takahashi et al., 2012; Yim et al., 2013) are sufficient to induce the formation of inhibitory presynaptic terminals by themselves. Co-culturing experiments are well-suited to study synapse formation in a controlled manner, but the dynamics of the process are often lost. Presynaptic assembly appears as a sequential process, in which the local adhesion between the non-neuronal cell and the axon simply triggers the subsequent recruitment of presynaptic proteins and synaptic vesi-

cles. However, trial-and-error bouton dynamics have also been observed at adhesion sites with non-neuronal cells (Sabo et al., 2006), suggesting that strong adhesion signals may reduce bouton dynamics by simply speeding up synapse formation. It is currently not clear if synaptogenic signals form the initial trigger for synapse formation, or if synaptogenic molecules get recruited to nascent synapses in response to a separate triggering signal.

For inhibitory synapses, many additional proteins have been identified that are involved in synapse formation, but that cannot induce inhibitory synapses by themselves. Some of these proteins interfere directly or indirectly with synaptogenic signaling pathways (e.g. IgSF9b and MDGA1 both interact with neuroligin-2, but with opposite effects (Lee et al., 2013; Pettem et al., 2013; Woo et al., 2013)), while other signaling proteins, such as Sema4D (Kuzirian et al., 2013; Paradis et al., 2007), or FGF7 (Terauchi et al., 2010) seem to signal through independent pathways. It is currently not known if these different pathways can occur in parallel or sequentially in the same synapses, or to what extent differences between cell-types or brain regions exist. For an overview of signaling pathways involved in inhibitory synapse formation, I refer the reader to recent reviews (Frias and Wierenga, 2013; Ko et al., 2015; Lu et al., 2017). It will be important to understand how different regulatory pathways interact. The involvement of specific pathways at specific stages of synapse formation shows that synapse formation is a process that involves multiple steps with modulation and regulation at each level, allowing (activity-dependent) fine-tuning of synaptic connections. The existence of multi-level signaling pathways indicates that the formation of inhibitory synapses, perhaps even more so than excitatory synapses, is a highly regulated process.

8. Actin dynamics

During formation of inhibitory synapses, signals from the extracellular environment (for instance from the postsynaptic neuron) induce intracellular changes in the inhibitory axon to form a new bouton. Many studies have indicated a direct or indirect link between adhesion molecule signaling and intracellular cytoskeleton rearrangements. Indeed, in *C. elegans* it was shown that synapse formation actually starts with changes in the local actin scaffold that subsequently induce recruitment of synapse organizing molecules to the nascent presynaptic zone (Chia et al., 2014, 2012). Also the formation of ghost boutons in *Drosophila* was shown to require rearrangements of the actin cytoskeleton by cofilin (Piccioli and Littleton, 2014). These studies show a clear involvement of actin at an early stage of bouton formation. The important structural role of actin in maintaining molecular components at immature presynaptic terminals has also been shown by experiments in which depolymerization of actin structures results in the complete loss of synapses in young axons (Zhang and Benson, 2001). In mature synapses, actin is still important in regulating and modulating several aspects of synaptic transmission (Cingolani and Goda, 2008; Sankaranarayanan et al., 2003), but maintenance of synaptic composition or transmission at mature synapses no longer depends on actin (Morales et al., 2000; Zhang and Benson, 2001). It is currently not known what determines the transition from immature, actin-dependent, to mature, actin-independent, synapses. The shape and dynamics of the actin cytoskeleton are determined by a large number of actin-binding proteins and other actin regulators inside the cell. There are many different actin structures present within axons which presumably compartmentalize presynaptic terminals and axonal shaft (Bleckert et al., 2012; Roy, 2016). Intracellular actin structures can be highly dynamic and interact and compete amongst each other (Burke et al., 2014; Suarez et al., 2015). Indeed, filamentous actin in presynaptic boutons is continuously assembled and disassembled under baseline conditions (Bleckert et al., 2012; Colicos et al., 2001), and actin can be recruited from the axonal shaft to presynaptic boutons after a period of enhanced synaptic activity (Colicos et al., 2001; Sankaranarayanan et al., 2003). It will be important for future studies to unravel the changes in intra-axonal actin structures that occur when new presynaptic boutons are formed and how these structures differ between immature and mature synapses.

9. Conclusions

In conclusion, live imaging studies have revealed that inhibitory synapse formation is a dynamic and often non-linear process. Presynaptic terminals forming boutons along inhibitory axons are themselves highly dynamic structures that can rapidly be assembled and disassembled. The major advantage of a system in which synaptic connections are constantly being updated and compete with neighbors for resources is that it can quickly adapt to changing circumstances. In this review I have tried to put our work on inhibitory axons in the context of what is known from previous studies. In many studies excitatory axons are used as model systems and much of our current knowledge of molecular mechanisms underlying synapse formation comes from studies in invertebrates, in which intracellular pathways can be more easily studied. One might argue that presynaptic terminals are very different in excitatory and inhibitory synapses and different species. However, there are currently no indications that bouton formation is fundamentally different in different types of axons. In fact, the similarities between different axons and preparations are more remarkable than the differences, suggesting that different presynaptic terminals undergo a common assembly process and only diverge at a later developmental stage.

Together, the available data indicate that synapse formation involves a series of events with many stochastic elements, and suggest that multiple signals need to coincide to successfully assemble a new presynaptic terminal. In an attempt to reconcile available data from literature with our own observations, I have constructed a working model for inhibitory synapse formation, illustrating the several mechanistic steps of the process (Fig. 2). The particular proteins and molecular pathways involved will likely be synapse-specific and there may be a wide variety of signaling pathways that regulate and modulate different steps of inhibitory synapse formation, allowing synapse-specific and activity-dependent adjustments of synaptic connections. Characterization of these regulatory pathways will help our understanding of experience-dependent circuit adaptation in the healthy brain and eventually may enhance insight in neurodevelopmental diseases in which neuronal circuitry is disturbed.

Competing interests

The author declares to have no competing interests.

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References

Ahmari, S.E., Buchanan, J., Smith, S.J., 2000. Assembly of presynaptic active zones from cytoplasmic transport packets. Nat. Neurosci. 3, 445–451, http://dx.doi.org/10.1038/74814.

Alvarez-Castelao, B., Schuman, E.M., 2015. The regulation of synaptic protein turnover. J. Biol. Chem. 290, 28623–28630, http://dx.doi.org/10.1074/jbc.R115.657130.

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

Bamji, S.X., Rico, B., Kimes, N., Reichardt, L.F., 2006. BDNF mobilizes synaptic vesicles and enhances synapse formation by disrupting cadherin-beta-catenin interactions. J. Cell Biol. 174, 289–299, http://dx.doi.org/10.1083/jcb.200601087.

Becker, N., Nägerl, U.V., Wierenga, C.J., Fonseca, R., Bonhoeffer, T., 2008. LTD induction causes morphological changes of presynaptic boutons and reduces their contacts with spines. Neuron 60, 590–597, http://dx.doi.org/10.1016/j.neuron.2008.09.018.

Bleckert, A., Photowala, H., Alford, S., 2012. Dual pools of actin at presynaptic terminals. J. Neurophysiol. 107, 3479–3492, http://dx.doi.org/10.1152/jn.00789.2011.

Bonhoeffer, T., Yuste, R., 2002. Spine motility. Phenomenology mechanisms, and function. Neuron 35, 1019–1027.

Bresler, T., Shapira, M., Boeckers, T., Dresbach, T., Futter, M., Garner, C.C., Rosenblum, K., Gundelfinger, E.D., Ziv, N.E., 2004. Postsynaptic density assembly is fundamentally different from presynaptic active zone assembly. J. Neurosci. 24, 1507–1520, http://dx.doi.org/10.1523/JNEUROSCI.3819-03.2004.

Burke, T.A., Christensen, J.R., Barone, E., Suarez, C., Sirotkin, V., Kovar, D.R., 2014. Homeostatic actin cytoskeleton networks are regulated by assembly factor competition for monomers. Curr. Biol. 24, 579–585, http://dx.doi.org/10.1016/j.cub.2014.01.072.

Bury, L.A., Sabo, S.L., 2011. Coordinated trafficking of synaptic vesicle and active zone proteins prior to synapse formation. Neural Dev. 6, 24, http://dx.doi.org/10.1186/1749-8104-6-24.

Bury, L.A., Sabo, S.L., 2015. Building a terminal: mechanisms of presynaptic development in the CNS. Neuroscience 20, 1–20, http://dx.doi.org/10.1177/1073858415596131.

Chen, S.X., Kim, A.N., Peters, A.J., Komiyama, T., 2015. Subtype-specific plasticity of inhibitory circuits in motor cortex during motor learning. Nat. Neurosci. 18, 1109–1115, http://dx.doi.org/10.1038/nn.4049.

Chi, P., Greengard, P., Ryan, T.A., 2003. Synaptic vesicle mobilization is regulated by distinct synapsin I phosphorylation pathways at different frequencies. Neuron 38, 69–78.

Chia, P.H., Patel, M.R., Shen, K., 2012. NAB-1 instructs synapse assembly by linking adhesion molecules and F-actin to active zone proteins. Nat. Neurosci. 15, 234–242, http://dx.doi.org/10.1038/nn.2991.

Chia, P.H., Chen, B., Li, P., Rosen, M.K., Shen, K., 2014. Local F-actin network links synapse formation and axon branching. Cell 156, 208–220, http://dx.doi.org/10.1016/j.cell.2013.12.009.

Cho, R.W., Buhl, L.K., Volfson, D., Tran, A., Li, F., Akbergenova, Y., Littleton, J.T., 2015. Phosphorylation of complexin by PKA regulates activity-dependent spontaneous neurotransmitter release and structural synaptic plasticity. Neuron 88, 749–761, http://dx.doi.org/10.1016/j.neuron.2015.10.011.

Choi, B.J., Imlach, W.L., Jiao, W., Wolfram, V., Wu, Y., Grbic, M., Cela, C., Baines, R.A., Nitabach, M.N., McCabe, B.D., 2014. Miniature neurotransmission regulates drosophila synaptic structural maturation. Neuron 82, 618–634, http://dx.doi.org/10.1016/j.neuron.2014.03.012.

Cingolani, L.A., Goda, Y., 2008. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nat. Rev. Neurosci. 9, 344–356, http://dx.doi.org/10.1038/nrn2373.

Colicos, M.A., Collins, B.E., Sailor, M.J., Goda, Y., 2001. Remodeling of synaptic actin induced by photoconductive stimulation. Cell 107, 605–616, http://dx.doi.org/10.1016/S0092-8674(01)00579-7.

Darcy, K.J., Staras, K., Collinson, L.M., Goda, Y., 2006. Constitutive sharing of recycling synaptic vesicles between presynaptic boutons. Nat. Neurosci. 9, 315–321, http://dx.doi.org/10.1038/nn1640.

de Wit, J., Ghosh, A., 2016. Specification of synaptic connectivity by cell surface interactions. Nat. Rev. Neurosci. 17, 4, http://dx.doi.org/10.1038/nrn.2015.3.

Dobie, F.A., Craig, A.M., 2011. Inhibitory synapse dynamics: coordinated presynaptic and postsynaptic mobility and the major contribution of recycled vesicles to new synapse formation. J. Neurosci. 31, 10481–10493, http://dx.doi.org/10.1523/JNEUROSCI.6023-10.2011.

Fernandez-Alfonso, T., Ryan, T.A., 2008. A heterogeneous resting pool of synaptic vesicles that is dynamically interchanged across boutons in mammalian CNS synapses. Brain Cell Biol. 36, 87–100, http://dx.doi.org/10.1007/s11068-008-9030-y.

Frias, C.P., Wierenga, C.J., 2013. Activity-dependent adaptations in inhibitory axons. Front. Cell. Neurosci. 7, 219, http://dx.doi.org/10.3389/fncel.2013.00219.

Friedman, H.V., Bresler, T., Garner, C.C., Ziv, N.E., 2000. Assembly of new individual excitatory synapses: time course and temporal order of synaptic molecule recruitment. Neuron 27, 57–69.

Froemke, R.C., Merzenich, M.M., Schreiner, C.E., 2007. A synaptic memory trace for cortical receptive field plasticity. Nature 450, 425–429, http://dx.doi.org/10.1038/nature06289.

Froemke, R.C., 2015. Plasticity of cortical excitatory-inhibitory balance. Annu. Rev. Neurosci. 38, 195–219, http://dx.doi.org/10.1146/annurev-neuro-071714-034002.

Fu, Y., Wu, X., Lu, J., Huang, Z.J., 2012. Presynaptic GABAB receptor regulates activity-dependent maturation and patterning of inhibitory synapses through dynamic allocation of synaptic vesicles. Front. Cell. Neurosci. 6, 1–20, http://dx.doi.org/10.3389/fncel.2012.00057.

Garner, C.C., Waites, C.L., Ziv, N.E., 2006. Synapse development: still looking for the forest, still lost in the trees. Cell Tissue Res. 326, 249–262, http://dx.doi.org/10.1007/s00441-006-0278-1.

Gerrow, K., Romorini, S., Nabi, S.M., Colicos, M.A., Sala, C., El-Husseini, A., 2006. A preformed complex of postsynaptic proteins is involved in excitatory synapse development. Neuron 49, 547–562, http://dx.doi.org/10.1016/j.neuron.2006.01.015.

Gokce, O., Südhof, T.C., 2013. Membrane-tethered monomeric neurexin LNS-domain triggers synapse formation. J. Neurosci. 33, 14617–14628, http://dx.doi.org/10.1523/JNEUROSCI.1232-13.2013.

Grillo, F.W., Song, S., Teles-Grilo Ruivo, L.M., Huang, L., Gao, G., Knott, G.W., Maco, B., Ferretti, V., Thompson, D., Little, G.E., De Paola, V., 2013. Increased axonal bouton dynamics in the aging mouse cortex. Proc. Natl. Acad. Sci. U. S. A. 110, E1514–E1523, http://dx.doi.org/10.1073/pnas.1218731110.

Gundelfinger, E.D., Fejtova, A., 2012. Molecular organization and plasticity of the cytomatrix at the active zone. Curr. Opin. Neurobiol. 22, 423–430, http://dx.doi.org/10.1016/j.conb.2011.10.005.

Hensch, T.K., 2005. Critical period plasticity in local cortical circuits. Nat. Rev. Neurosci. 6, 877–888, http://dx.doi.org/10.1038/nrn1787.

Keck, T., Scheuss, V., Jacobsen, R.I., Wierenga, C.J., Eysel, U.T., Bonhoeffer, T., Hübener, M., 2011. Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. Neuron 71, 869–882, http://dx.doi.org/10.1016/j.neuron.2011.06.034.

Klassen, M.P., Wu, Y.E., Maeder, C.I., Nakae, I., Cueva, J.G., Lehrman, E.K., Tada, M., Gengyo-Ando, K., Wang, G.J., Goodman, M., Mitani, S., Kontani, K., Katada, T., Shen, K., 2010. An Arf-like small G protein, ARL-8 promotes the axonal transport of presynaptic cargoes by suppressing vesicle aggregation. Neuron 66, 710–723, http://dx.doi.org/10.1016/j.neuron.2010.04.033.

Ko, J., Choii, G., Um, J.W., 2015. The balancing act of GABAergic synapse organizers. Trends Mol. Med. 21, 256–268, http://dx.doi.org/10.1016/j.molmed.2015.01.004.

Koch, I., Schwarz, H., Beuchle, D., Goellner, B., Langegger, M., Aberle, H., 2008. Drosophila ankyrin 2 is required for synaptic stability. Neuron 58, 210–222, http://dx.doi.org/10.1016/j.neuron.2008.03.019.

Krueger, S.R., Kolar, A., Fitzsimonds, R.M., 2003. The presynaptic release apparatus is functional in the absence of dendritic contact and highly mobile within isolated axons. Neuron 40, 945–957.

Krueger, D.D., Tuffy, L.P., Papadopoulos, T., Brose, N., 2012. The role of neurexins and neuroligins in the formation, maturation, and function of vertebrate synapses. Curr. Opin. Neurobiol. 22, 412–422, http://dx.doi.org/10.1016/j.conb.2012.02.012.

Kuriu, T., Yanagawa, Y., Konishi, S., 2012. Activity-dependent coordinated mobility of hippocampal inhibitory synapses visualized with presynaptic and postsynaptic tagged-molecular markers. Mol. Cell. Neurosci. 49, 184–195, http://dx.doi.org/10.1016/j.mcn.2011.11.002.

Kuzirian, M.S., Moore, A.R., Staudenmaier, E.K., Friedel, R.H., Paradis, S., 2013. The class 4 semaphorin Sema4D promotes the rapid assembly of GABAergic synapses in rodent hippocampus. J. Neurosci. 33, 8961–8973, http://dx.doi.org/10.1523/JNEUROSCI.0989-13.2013.

López-Bendito, G., Sturgess, K., Erdélyi, F., Szabó, G., Molnár, Z., Paulsen, O., 2004. Preferential origin and layer destination of GAD65-GFP cortical interneurons. Cereb. Cortex 14, 1122–1133, http://dx.doi.org/10.1093/cercor/bhh072.

Lee, K., Kim, Y., Lee, S.-J., Qiang, Y., Lee, D., Lee, H.W., Kim, H., Je, H.S., Südhof, T.C., Ko, J., 2013. MDGAs interact selectively with neuroligin-2 but not other neuroligins to regulate inhibitory synapse development. Proc. Natl. Acad. Sci. U. S. A. 110, 336–341, http://dx.doi.org/10.1073/pnas.1219987110.

Lu, W., Bromley-Coolidge, S., Li, J., 2017. Regulation of GABAergic synapse development by postsynaptic membrane proteins. Brain Res. Bull. 1290, 30–42, http://dx.doi.org/10.1016/j.brainresbull.2016.07.004.

Lucido, A.L., Suarez Sanchez, F., Thostrup, P., Kwiatkowski, A.v., Leal-Ortiz, S., Gopalakrishnan, G., Liazoghli, D., Belkaid, W., Lennox, R.B., Grutter, P., Garner, C.C., Colman, D.R., 2009. Rapid assembly of functional presynaptic boutons triggered by adhesive contacts. J. Neurosci. 29, 12449–12466, http://dx.doi.org/10.1523/JNEUROSCI.1381-09.2009.

Müllner, F.E., Wierenga, C.J., Bonhoeffer, T., 2015. Precision of inhibition: dendritic inhibition by individual GABAergic synapses on hippocampal pyramidal cells is confined in space and time. Neuron 87, 576–589.

Matz, J., Gilyan, A., Kolar, A., Mccarvill, T., Krueger, S.R., 2010. Rapid structural alterations of the active zone lead to sustained changes in neurotransmitter release. Proc. Natl. Acad. Sci. U. S. A. 107, 8836–8841.

McAllister, A.K., 2007. Dynamic aspects of CNS synapse formation. Annu. Rev. Neurosci. 30, 425–450, http://dx.doi.org/10.1146/annurev.neuro.29.051605.112830.

Minerbi, A., Kahana, R., Goldfeld, L., Kaufman, M., Marom, S., Ziv, N.E., 2009. Long-term relationships between synaptic tenacity, synaptic remodeling, and network activity. PLoS Biol. 7, e1000136, http://dx.doi.org/10.1371/journal.pbio.1000136.

Morales, M., Colicos, M.A., Goda, Y., 2000. Actin-dependent regulation of neurotransmitter release at central synapses. Neuron 27, 539–550, http://dx.doi.org/10.1016/S0896-6273(00)00064-7.
Nägerl, U.V., Köstinger, G., Anderson, J.C., Martin, K.A.C., Bonhoeffer, T., 2007. Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons. J. Neurosci. 27, 8149–8156, http://dx.doi.org/10.1523/JNEUROSCI.0511-07.2007.
Paradis, S., Harrar, D.B., Lin, Y., Koon, A.C., Hauser, J.L., Griffith, E.C., Zhu, L., Brass, L.F., Chen, C., Greenberg, M.E., 2007. An RNAi-based approach identifies molecules required for glutamatergic and GABAergic synapse development. Neuron 53, 217–232, http://dx.doi.org/10.1016/j.neuron.2006.12.012.
Park, M., Watanabe, S., Poon, V.Y.N., Ou, C.-Y., Jorgensen, E.M., Shen, K., 2011. CYY-1/cyclin Y and CDK-5 differentially regulate synapse elimination and formation for rewiring neural circuits. Neuron 70, 742–757, http://dx.doi.org/10.1016/j.neuron.2011.04.002.
Pettem, K.L., Yokomaku, D., Takahashi, H., Ge, Y., Craig, A.M., 2013. Interaction between autism-linked MDGAs and neuroligins suppresses inhibitory synapse development. J. Cell Biol. 200, 321–326, http://dx.doi.org/10.1083/jcb.201206028.
Petzoldt, A.G., Lützkendorf, J., Sigrist, S.J., 2016. Mechanisms controlling assembly and plasticity of presynaptic active zone scaffolds. Curr. Opin. Neurobiol. 39, 69–76, http://dx.doi.org/10.1016/j.conb.2016.04.009.
Piccioli, Z.D., Littleton, J.T., 2014. Retrograde BMP signaling modulates rapid activity-dependent synaptic growth via presynaptic LIM kinase regulation of cofilin. J. Neurosci. 34, 4371–4381, http://dx.doi.org/10.1523/JNEUROSCI.4943-13.2014.
Pielage, J., Cheng, L., Fetter, R.D., Carlton, P.M., Sedat, J.W., Davis, G.W., 2008. A presynaptic giant ankyrin stabilizes the NMJ through regulation of presynaptic microtubules and transsynaptic cell adhesion. Neuron 58, 195–209, http://dx.doi.org/10.1016/j.neuron.2008.02.017.
Poulopoulos, A., Aramuni, G., Meyer, G., Soykan, T., Hoon, M., Harvey, K., Jedlicka, P., Zhang, M., Paarmann, I., Schwarzacher, S.W., Betz, H., Harvey, R.J., Brose, N., Zhang, W., Papadopoulos, T., 2009. Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. Neuron 63, 628–642, http://dx.doi.org/10.1016/j.neuron.2009.08.023.
Ratnayaka, A., Marra, V., Branco, T., Staras, K., 2011. Extrasynaptic vesicle recycling in mature hippocampal neurons. Nat. Commun. 2, 531, http://dx.doi.org/10.1038/ncomms1534.
Roy, S., 2016. Waves, rings, and trails: the scenic landscape of axonal actin. J. Cell Biol. 212, http://dx.doi.org/10.1083/jcb.201511016jcb.201511016, jcb.201511016.
Südhof, T.C., 2012. The presynaptic active zone. Neuron 75, 11–25, http://dx.doi.org/10.1016/j.neuron.2012.06.012.
Sabo, S.L., Gomes, R.A., McAllister, A.K., 2006. Formation of presynaptic terminals at predefined sites along axons. J. Neurosci. 26, 10813–10825, http://dx.doi.org/10.1523/JNEUROSCI.2052-06.2006.
Sankaranarayanan, S., Atluri, P.P., Ryan, T.A., 2003. Actin has a molecular scaffolding, not propulsive, role in presynaptic function. Nat. Neurosci. 6, 127–135, http://dx.doi.org/10.1038/nn1002.
Scheiffele, P., Fan, J., Choih, J., Fetter, R., Serafini, T., 2000. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell 101, 657–669.
Schuemann, A., Klawiter, A., Bonhoeffer, T., Wierenga, C.J., 2013. Structural plasticity of GABAergic axons is regulated by network activity and $GABA_A$ receptor activation. Front. Neural Circuits 7, 113, http://dx.doi.org/10.3389/fncir.2013.00113.
Shen, K., Bargmann, C.I., 2003. The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in C. elegans. Cell 112, 619–630.
Shen, K., Fetter, R.D., Bargmann, C.I., 2004. Synaptic specificity is generated by the synaptic guidepost protein SYG-2 and its receptor, SYG-1. Cell 116, 869–881.
Siddiqui, T.J., Craig, A.M., 2011. Synaptic organizing complexes. Curr. Opin. Neurobiol. 21, 132–143, http://dx.doi.org/10.1016/j.conb.2010.08.016.
Squarzoni, P., Thion, M.S., Garel, S., 2015. Neuronal and microglial regulators of cortical wiring: usual and novel guideposts. Front. Neurosci. 9, 1–16, http://dx.doi.org/10.3389/fnins.2015.00248.
Staras, K., Branco, T., Burden, J.J., Pozo, K., Darcy, K., Marra, V., Ratnayaka, A., Goda, Y., 2010. A vesicle superpool spans multiple presynaptic terminals in hippocampal neurons. Neuron 66, 37–44, http://dx.doi.org/10.1016/j.neuron.2010.03.020.
Staras, K., 2007. Share and share alike: trading of presynaptic elements between central synapses. Trends Neurosci. 30, 292–298, http://dx.doi.org/10.1016/j.tins.2007.04.005.
Suarez, C., Carroll, R.T., Burke, T.A., Christensen, J.R., Bestul, A.J., Sees, J.A., James, M.L., Sirotkin, V., Kovar, D.R., 2015. Profilin regulates F-Actin network homeostasis by favoring formin over Arp2/3 complex. Dev. Cell 32, 43–53, http://dx.doi.org/10.1016/j.devcel.2014.10.027.
Takahashi, H., Katayama, K., Sohya, K., Miyamoto, H., Prasad, T., Matsumoto, Y., Ota, M., Yasuda, H., Tsumoto, T., Aruga, J., Craig, A.M., 2012. Selective control of inhibitory synapse development by Slitrk3-PTPδ trans-synaptic interaction. Nat. Neurosci. 15, 389–398, http://dx.doi.org/10.1038/nn.3040.
Terauchi, A., Johnson-Venkatesh, E.M., Toth, A.B., Javed, D., Sutton, M.A., Umemori, H., 2010. Distinct FGFs promote differentiation of excitatory and inhibitory synapses. Nature 465, 783–787, http://dx.doi.org/10.1038/nature09041.
Varoqueaux, F., Jamain, S., Brose, N., 2004. Neuroligin 2 is exclusively localized to inhibitory synapses. Eur. J. Cell Biol. 83, 449–456.
Vasin, A., Zueva, L., Torrez, C., Volfson, D., Littleton, J.T., Bykhovskaia, M., 2014. Synapsin regulates activity-dependent outgrowth of synaptic boutons at the Drosophila neuromuscular junction. J. Neurosci. 34, 10554–10563, http://dx.doi.org/10.1523/JNEUROSCI.5074-13.2014.
Villa, K.L., Berry, K.P., Subramanian, J., Cha, J.W., Oh, W.C., Kwon, H.-B., Kubota, Y., So, P.T.C., Nedivi, E., 2016. Inhibitory synapses are repeatedly assembled and removed at persistent sites in vivo. Neuron 89, 756–769, http://dx.doi.org/10.1016/j.neuron.2016.01.010.
Wierenga, C.J., Becker, N., Bonhoeffer, T., 2008. GABAergic synapses are formed without the involvement of dendritic protrusions. Nat. Neurosci. 11, 1044–1052, http://dx.doi.org/10.1038/nn.2180.
Wierenga, C.J., Müllner, F.E., Rinke, I., Keck, T., Stein, V., Bonhoeffer, T., 2010. Molecular and electrophysiological characterization of GFP-expressing CA1 interneurons in GAD65-GFP mice. PLoS One 5, e15915, http://dx.doi.org/10.1371/journal.pone.0015915.
Woo, J., Kwon, S.-K., Nam, J., Choi, S., Takahashi, H., Krueger, D., Park, J., Lee, Y., Bae, J.Y., Lee, D., Ko, J., Kim, H., Kim, M.-H., Bae, Y.C., Chang, S., Craig, A.M., Kim, E., 2013. The adhesion protein IgSF9b is coupled to neuroligin 2 via S-SCAM to promote inhibitory synapse development. J. Cell Biol. 201, 929–944, http://dx.doi.org/10.1083/jcb.201209132.
Wu, Y.E., Huo, L., Maeder, C.I., Feng, W., Shen, K., 2013. The balance between capture and dissociation of presynaptic proteins controls the spatial distribution of synapses. Neuron 78, 994–1011, http://dx.doi.org/10.1016/j.neuron.2013.04.035.
Yim, Y.S., Kwon, Y., Nam, J., In, H., Lee, K., Goo, D., Kim, E., 2013. Slitrks control excitatory and inhibitory synapse formation with LAR receptor protein tyrosine phosphatases. Proc. Natl. Acad. Sci. U. S. A. 110, 4057–4062, http://dx.doi.org/10.1073/pnas.1209881110.
Zhai, R.G., Vardinon-Friedman, H., Cases-Langhoff, C., Becker, B., Gundelfinger, E.D., Ziv, N.E., Garner, C.C., 2001. Assembling the presynaptic active zone: a characterization of an active zone precursor vesicle. Neuron 29, 131–143.
Zhang, W., Benson, D.L., 2001. Stages of synapse development defined by dependence on F-Actin. J. Neurosci. 21, 5169–5181.
Ziv, N.E., Fisher-Lavie, A., 2014. Presynaptic and postsynaptic scaffolds: dynamics fast and slow. Neurosci 20, 439–452, http://dx.doi.org/10.1177/1073858414523321.
Ziv, N.E., Garner, C.C., 2004. Cellular and molecular mechanisms of presynaptic assembly. Nat. Rev. Neurosci. 5, 385–399, http://dx.doi.org/10.1038/nrn1370.