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Ephrin regulation of synapse formation, function and plasticity

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Abstract

Synapses enable the transmission of information within neural circuits and allow the brain to change in response to experience. During the last decade numerous proteins that can induce synapse formation have been identified. Many of these synaptic inducers rely on trans-synaptic cell–cell interactions to generate functional contacts. Moreover, evidence now suggests that the same proteins that function early in development to regulate synapse formation may help to maintain and/or regulate the function and plasticity of mature synapses. One set of receptors and ligands that appear to impact both the development and the mature function of synapses are Eph receptors (erythropoietin-producing human hepatocellular carcinoma cell line) and their surface associated ligands, ephrins (Eph family receptor interacting proteins). Ephs can initiate new synaptic contacts, recruit and stabilize glutamate receptors at nascent synapses and regulate dendritic spine morphology. Recent evidence demonstrates that ephrin ligands also play major roles at synapses. Activation of ephrins by Eph receptors can induce synapse formation and spine morphogenesis, whereas in the mature nervous system ephrin signaling modulates synaptic function and long-term changes in synaptic strength. In this review we will summarize the recent progress in understanding the role of ephrins in presynaptic and postsynaptic differentiation, and synapse development, function and plasticity.

Keywords

Eph; Synapse development; Synaptic maturation; Glutamate receptor; LTP; Competition

Introduction

The trillions of synaptic connections found in the brain enable neuronal activity to flow between neurons. However, neuronal activity is not the only signal transmitted at synaptic sites. Pre- and postsynaptic specializations contain a host of transmembrane molecules that interact with one another to enable signals to pass between neurons. Trans-cellular signaling between sets of pre- and post-synaptic proteins can induce synaptic formation, regulate synapse function and modulate synaptic plasticity (Biederer and Stagi, 2008; Robbins et al., 2010; Yamada and Nelson, 2007). The Eph receptor tyrosine kinases (RTKs) and their ligands, the ephrins, mediate many important signals across synapses. Signaling by both Ephs and ephrins can control a diverse set of synaptic events from the initiation of new synaptic sites to the modulation of synaptic function.

Ephrins fall into two subfamilies: ephrin-As and ephrin-Bs. The ephrin-A subfamily consists of five members that bind and activate EphA RTKs (Fig. 1A). Ephrin-As (ephrin-A1–5) lack a cytoplasmic domain, but are capable of triggering downstream signaling by recruiting Src family and phosphoinositide-3 (PI3) kinases (Davy et al., 1999; San Miguel et al., 2011). The ephrin-B subfamily consists of three proteins that bind and activate the EphB class of Ephs (Fig. 1A). Binding of ephrin-Bs to EphB receptors initiates bidirectional signaling that involves activation of the catalytically active kinase domain on EphBs and phosphorylation of the cytoplasmic tail of ephrin-Bs. Specific functional domains within the intracellular portion of ephrin-Bs enable their signaling (Fig. 1B) (Pasquale, 2008). The known domains within ephrin-Bs include three tyrosine residues enabling the recruitment of Src Homology 2/3 (SH2/SH3) adaptor proteins (Bruckner and Klein, 1998; Holland et al., 1996; Xu and Henkemeyer, 2009), a PDZ binding domain enabling interaction with adaptor-proteins such as glutamate receptor interacting protein 1 (GRIP1) (Aoto et al., 2007; Bruckner et al., 1999), a D-domain for interaction with Erk/MAPK (McClelland et al., 2010), and a Grb4 domain enabling interactions with the G protein-coupled receptor kinase-interacting protein 1 (GIT1) (Cowan and Henkemeyer, 2001; Segura et al., 2007).

Both EphA and EphB RTKs play prominent and well-described roles in the formation and function of excitatory synapses (Dalva et al., 2000; Henkemeyer et al., 2003; Kayser et al., 2006, 2008; Murai et al., 2003). EphA4 is critical for dendritic spine morphogenesis in the hippocampus (Murai et al., 2003) and regulates synaptic plasticity (Filosa et al., 2009; Grunwald et al., 2004). EphBs are required for the formation of many dendritic spine synapses (Henkemeyer et al., 2003; Kayser et al., 2006) and control the formation of excitatory synapses that require the motility of dendritic filopodia (Kayser et al., 2008). EphBs are also important for the formation of spines with normal morphology (Irie and Yamaguchi, 2002; Margolis et al., 2010; Penzes et al., 2003; Tolia et al., 2007) and synaptic plasticity (Grunwald et al., 2001; Henderson et al., 2001). In addition, EphBs regulate the recruitment, localization, and function of NMDA receptors (Dalva et al., 2000; Nolt et al., 2011; Takasu et al., 2002). The role of Eph RTKs in synaptogenesis, spine formation and synaptic function is well documented (Klein, 2009) but it is becoming clear that ephrin ligands play similar roles. In this review we will discuss recent evidence linking ephrins to the formation, maturation, and function of excitatory synapses.

Expression of Ephs and ephrins in CNS

The pattern of expression of ephrins and Ephs is complex and reflects the diverse functions these proteins play in the developing and mature brain (Flenniken et al., 1996; Liebl et al., 2003; Migani et al., 2007, 2009; Mori et al., 1995). Ephrins and Ephs are expressed pre-and/or post-synaptically at both the developing and mature excitatory synapse (Aoto et al., 2007; Contractor et al., 2002; Grunwald et al., 2001, 2004; Henderson et al., 2001; Kayser et al., 2006, 2008; McClelland et al., 2009, 2010; Segura et al., 2007). Expression pattern of Ephs and ephrins is well defined in the hippocampus (Fig. 1C) (Buchert et al., 1999; Grunwald et al., 2001, 2004; Henderson et al., 2001; Torres et al., 1998). Immunostaining and transgene expression studies reveal that the patterns of expression of particular EphBs and ephrin-Bs are restricted to specific regions of the hippocampus. While EphB2 and EphA4 are expressed throughout the hippocampus, EphB1 is enriched in the CA3 region, and EphB3 is expressed in the CA1 region (Buchert et al., 1999; Grunwald et al., 2001, 2004; Henderson et al., 2001). Looking more closely at the localization of Eph protein expression in CA1 neurons, EphB2, EphB3, and EphA7 are enriched in the postsynaptic densities (PSD) of dendritic spines in hippocampus (Grunwald et al., 2004; Henderson et al., 2001; Torres et al., 1998). Recent studies examining the synaptic localization of EphB2 and EphA4 indicate that these proteins are localized to both the pre- and post-synaptic compartments (Bouvier et al., 2008). The pattern of ephrin-B expression is also complex. Ephrin-B1 and ephrin-B3 are

expressed in the mossy fiber terminals of dentate gyrus neurons (Antion et al., 2010; Contractor et al., 2002; Grunwald et al., 2001, 2004; Henderson et al., 2001; Rodenas-Ruano et al., 2006). Ephrin-B2 and ephrin-B3 are enriched in PSDs of CA1 neurons (Grunwald et al., 2001, 2004; Rodenas-Ruano et al., 2006; Torres et al., 1998). Thus, Ephs and ephrins are expressed in overlapping and specific patterns in the hippocampus.

In the cortex the expression patterns of only a few Eph and ephrin family members have been well characterized. However, what is known suggests that Ephs and ephrins are selectively expressed in distinct populations of cortical neurons. EphB3 expression is enriched in layer V pyramidal cells of the somatosensory cortex (Willson et al., 2006). Ephrin-B2 is localized mainly to layers II/III of cortex (Migani et al., 2007) and astrocytes (McClelland et al., 2009). In situ data (GENSAT) indicate that ephrin-B3 is expressed in layers II/III and V only during development. In contrast, ephrin-B1 immunoreactivity is diffuse within the neuropil (Migani et al., 2009). While their patterns of expression suggest that Ephs and ephrins may function in specific neurons or at specific times in development, a complete understanding of Eph and ephrin function in the cortex will require a more detailed study.

Ephrin-B signaling in presynaptic differentiation

EphBs are required for dendritic filopodial motility, spine synapse formation and for the recruitment and clustering of glutamate receptors to synapses (Dalva et al., 2000; Kayser et al., 2006, 2008; Nolt et al., 2011; Takasu et al., 2002). Here we discuss evidence that suggests that ephrin-Bs function as the presynaptic partners for postsynaptic EphBs and mediate EphB-dependent presynaptic differentiation (Table 1).

Consistent with the functions of ephrins at synapses, ephrin-Bs colocalize with excitatory synaptic markers in vitro and in vivo (Kayser et al., 2006; Lim et al., 2008; McClelland et al., 2009; Xu et al., 2011). Evidence for the functional significance of ephrin-Bs in presynaptic differentiation comes from a number of studies that take advantage of a widely used co-culture assay (Biederer et al., 2002; Scheiffele et al., 2000). In this assay, a putative postsynaptic inducer is transfected into non-neuronal cells that are then plated onto a neuronal culture. After several hours of co-culture the ability of the putative inducer to form and organize hemisynapses is tested using a variety of assays (Linhoff et al., 2009). Using this method, EphB2 was shown to induce functional presynaptic release sites on axons that contact transfected non-neuronal cells, suggesting a presynaptic role for ephrin-Bs (Kayser et al., 2006). The ephrin-Bs responsible for EphB2-dependent induction of presynaptic sites were then identified using a modified version of the co-culture assay in which the expression of each ephrin-B molecule was knocked down in axons with ephrin-B specific shRNAs. These results revealed that reducing the expression level of either ephrin-B1 or ephrin-B2, but not ephrin-B3, prevented EphB2 expressing non-neuronal cells to induce clustering of the presynaptic marker proteins (McClelland et al., 2009). These data suggest that ephrin-B1 and ephrin-B2 are each required for normal EphB2-dependent presynaptic formation.

The mechanism that ephrin-Bs use to initiate presynaptic formation appears similar to that used by other trans-synaptic signaling systems. Ephrins contain a PDZ-binding domain (Bruckner et al., 1999; Torres et al., 1998) that allows them to associate with PDZ-domain containing proteins, such as syntenin-1 (McClelland et al., 2009; Torres et al., 1998). Deletion of this domain on ephrin-B1, or knockdown of syntenin-1, prevents EphB-dependent presynaptic differentiation (McClelland et al., 2009). These results suggest a model whereby postsynaptic EphB2 induces presynaptic differentiation by binding ephrin-B1 and ephrin-B2, which then recruit syntenin-1 via PDZ interactions (Fig. 2A) (Grembecka et al., 2006; Lin et al., 1999). Syntenin-1 then enables the clustering of synaptic

vesicles by organizing a multiprotein complex that likely consists of ERC2/CAST1, RIM and Rab synaptic vesicle proteins (Ko et al., 2003, 2006; Ohtsuka et al., 2002; Takao-Rikitsu et al., 2004). Thus, similar to other presynaptic organizers, ephrin-Bs associate with a protein complex that promotes the formation and maturation of presynaptic active zones.

Ephrin-B1 and ephrin-B2 null mice die well before birth making it difficult to determine whether these proteins function to control presynaptic differentiation in vivo. The best in vivo evidence for the function of ephrin-Bs in organizing presynaptic terminals comes from elegant experiments in the *Xenopus* retinotectal system (Lim et al., 2008). In retinal ganglion cells (RGCs), ephrin-B1 is expressed in axons and localizes near the active zone. Consistent with in vitro findings, disruption of ephrin-B signaling by the expression of a dominant negative ephrin-B1 lacking its cytoplasmic domain reduces the density of excitatory synapses (Lim et al., 2008). Acute elevation of ephrin-B signaling by the infusion of extracellular domain of EphB2 into the tectum leads to an increased number of presynaptic neurotransmitter release sites (Fig. 2A) without detectable effects on PSD-95 density (Lim et al., 2008). Thus, both in vitro and in vivo data indicate that ephrin-Bs can induce functional excitatory presynaptic differentiation.

Presynaptic ephrin-B signaling in synaptic function and plasticity

In addition to the regulation of presynaptic development, ephrin-Bs also appear to control presynaptic function at mature synapses in vivo. In the mature hippocampus at the mossy fiber-CA3 synapse, presynaptic ephrin-Bs appear to modulate the induction of long-term potentiation (LTP) that is thought to be independent of N-methyl-D-aspartate (NMDA) receptors (Armstrong et al., 2006; Castillo et al., 1997, 2002; Contractor et al., 2002; Weisskopf and Nicoll, 1995). At this synapse, activation of ephrin-Bs by treatment of brain slices with EphB2 receptor bodies enhances mossy fiber synaptic transmission and prevents further synaptic potentiation (Contractor et al., 2002). The specific ephrin-B protein responsible for these effects is not known. However, the enrichment of ephrin-B3 in the mossy fiber terminals (Grunwald et al., 2001, 2004; Rodenas-Ruano et al., 2006) suggests that signaling downstream of ephrin-B3 could recruit more neurotransmitter vesicles to presynaptic active zones, leading to increased synaptic transmission and induction of LTP. Consistent with this possibility, mice expressing ephrin-B3 proteins lacking their intracellular signaling domain have impaired induction of mossy fiber LTP (Armstrong et al., 2006). Thus, signaling by presynaptic ephrin-Bs appears to play an important role in the induction of the NMDA receptor-independent form of synaptic plasticity.

In addition to their role in regulating presynaptic function, a series of experiments in the tectum of *Xenopus* demonstrate that presynaptic ephrin-Bs can modulate neural circuit function. In the developing optic tectum of *Xenopus*, a crude topographical map is initially established by the extensive arborization of RGCs within the tectum. The precise mature map is then generated by the elimination and refinement of axonal arbors into topographically ordered synaptic connections between RGCs and the tectal cells (O'Leary and McLaughlin, 2005). The refinement of these connections occurs during a specific critical period and is dependent on visually driven neuronal activity (Luo and Flanagan, 2007). While the role of ephrin-As and ephrin-Bs in establishment of the coarse map is well characterized and the subject of numerous reviews (Flanagan, 2006; Lemke and Reber, 2005; Scicolone et al., 2009), it appears that ephrin-Bs also play a role in the activity dependent fine-tuning that leads to generation of the mature retinotectal map (Luo and Flanagan, 2007). For instance, overexpression of ephrin-B1 in RGC axons causes enhanced presynaptic neurotransmitter release and induction of LTP in the tadpole tectum (Lim et al., 2008). These findings suggest that ephrin-Bs signaling may provide a link between cues that guide axons to the appropriate locations within the tectum and activity dependent plasticity.

Consistent with their importance during activity-dependent refinement, the highest level of expression of ephrin-Bs is found in the axons that project to the region of the tectum where plasticity and synaptic refinement are the highest (Lim et al., 2010). To test whether the level of ephrin-Bs might impact activity dependent changes in receptive fields, the ability of visual activity to shift the location of receptive fields (RFs) was monitored. Overexpression of presynaptic ephrin-B1 in the axons of RGCs increased RF shifts in the ventral, but not the dorsal, tectum (Fig. 2B). Consistent with a presynaptic function for ephrin-B1, overexpression of ephrin-B1 in the tectal neurons had no effect on the receptive field shifts (Lim et al., 2010) while conditional expression of ephrin-B1 lacking a PDZ binding domain in RGC axons reduced a shift in receptive fields (Fig. 2B). Thus ephrin-B1 appears to regulate both presynaptic formation and plasticity through similar mechanisms.

Ephrin-Bs in postsynaptic development

Ephrin-Bs are expressed both pre- and postsynaptically, suggesting that in addition to their presynaptic role, they may also regulate aspects of postsynaptic function (Grunwald et al., 2001, 2004; Henderson et al., 2001; Torres et al., 1998). Indeed, we will discuss evidence linking each ephrin-B to specific aspects of postsynaptic maturation and function. Postsynaptic ephrin-Bs regulate synaptic plasticity, spinogenesis, glutamate receptor recruitment, and synapse density through diverse molecular mechanisms that involve both ephrin-B signaling and protein–protein interactions (Contractor et al., 2002; Grunwald et al., 2004; Henkemeyer et al., 2003; McClelland et al., 2010; Palmer and Klein, 2003; Rodenas-Ruano et al., 2006; Xu et al., 2011). Although the fact that they function in both the pre- and postsynaptic compartments complicates our understanding of the roles that ephrin-Bs play, it highlights their diverse roles and importance at synapses (Table 1).

Ephrin-Bs signal through a variety of pathways to control numerous postsynaptic functions. For example, in cultured hippocampal neurons, ephrin-Bs regulate maturation of dendritic spines and synapses by interacting with presynaptic EphB receptors (Fig. 3C) (Segura et al., 2007). Ephrin-Bs appear to control the transition from filopodia to spines by recruiting GIT1 to sites of ephrin-B signaling (Segura et al., 2007). Recruitment of GIT1 to ephrin-Bs then enables the activation of Rac, a Rho GTPase (Segura et al., 2007; Zhang et al., 2003) (Fig. 3C) which in turn could recruit the machinery necessary to regulate remodeling of the actin cytoskeleton that is thought to underlie spine morphogenesis (Dunaevsky et al., 2001; Fischer et al., 1998; Lendvai et al., 2000; Zhang et al., 2003, 2005). Spine development and GIT1 recruitment are initiated by EphB binding to ephrin-B that results in the selective phosphorylation of specific tyrosine residues in ephrin-B Grb4 domain and association with SH2/SH3 adaptor protein Grb4 (Fig. 3C) (Cowan and Henkemeyer, 2001; Holland et al., 1996; Segura et al., 2007).

Regulation of synapse maturation by postsynaptic ephrin-B3 signaling

Ephrin-B3 is emerging as an important regulator of synapse and spine formation in the hippocampus and cortex. Consistent with a postsynaptic function, ephrin-B3 protein is enriched in postsynaptic densities from brain (Grunwald et al., 2004; Torres et al., 1998) and colocalizes with PSD-95 at synapses in cultured neurons (McClelland et al., 2009, 2010; Rodenas-Ruano et al., 2006), and in vivo (Xu et al., 2011). Ephrin-B3 has been shown to regulate both synapse and spine formation in the hippocampus (Aoto et al., 2007; Rodenas-Ruano et al., 2006; Xu et al., 2011). In the cortex, rather than regulating the formation of individual synapses and dendritic spines, ephrin-B3 appears to control how many spine and shaft synapses a neuron receives relative to other neurons (McClelland et al., 2010). Thus, ephrin-B3 may play different roles in the regulation of synapse development in different neuronal cell types.

Competitive control of synapse density in the cortex by ephrin-B3

The density of synapses a neuron forms depends on the type of neuron and the level of its activity (Rao and Craig, 1997; Turrigiano et al., 1998), but little is known about the molecular mechanisms that control synapse density. Ephrin-B3 is well suited to function as an activity-independent regulator of synaptic density due to its high synaptic localization and its ability to coordinate events on both sides of the synapse (Grunwald et al., 2004; Torres et al., 1998). Interestingly, in the cortex ephrin-B3 appears to control synapse number by a competitive mechanism that relies on the relative ephrin-B3 expression levels between neurons. Much of our understanding of how competitive signals work to control events comes from classic studies on the development of the ocular dominance columns (ODC) in visual cortex (Crowley and Katz, 2000; Horton and Hocking, 1996; Hubel and Wiesel, 1970; Wiesel and Hubel, 1965). When the competitive signal is unequal, like in monocularly deprived animals, the open eye has a competitive advantage and expands its area of influence within the primary visual cortex (Hubel and Wiesel, 1970). However, in the absence of a competitive signal, like in binocularly deprived animals, the area of influence of each eye in the cortex remains relatively equal (Wiesel and Hubel, 1965).

Similarly, ephrin-B3 appears to function as a competitive signal that gives neurons with more ephrin-B3 an advantage enabling them to receive more synapses (McClelland et al., 2010). This was demonstrated using a heterogenotypic co-culture assay, in which wild-type neurons were cultured with neurons from ephrin-B3 knockout mice (Fig. 3A). When a few (10% of the culture) wild type neurons were cultured with many ephrin-B3 null neurons (90% of the culture), wild-type neurons made more synapses than normal. In contrast, culturing a few ephrin-B3 null neurons with many wild-type neurons resulted in a reduction in synapse density on ephrin-B3 null neurons (McClelland et al., 2010). Heterogenotypic experiments indicate that the presynaptic protein mediating these events is likely to be EphB2 (McClelland et al., 2010). The current model which still needs to be tested is that neurons may compete for the limiting amount of EphB2 in axons. Neurons having higher levels of ephrin-B3 could then generate more synaptic contacts than their counterparts that express less ephrin-B3. The development of tools such as Mosaic Analysis with Double Markers (MADM) mice will enable the generation of mouse models to test whether similar competitive mechanisms that establish synapse density function in vivo (Espinosa et al., 2009; Hippenmeyer et al., 2010; Zong et al., 2005).

Ephrin-B3 signaling in the control of synapse density

Ephrin-B3 appears to have specialized protein interaction domains that allow it to control synapse density. In addition to domains homologous to those found on ephrin-B1 and ephrin-B2 (Aoto et al., 2007; Bruckner et al., 1999; Cowan and Henkemeyer, 2001; Xu et al., 2011), ephrin-B3 also has an Erk binding D-domain, centered around leucine 308 (Fig. 1B) (McClelland et al., 2010). In vitro rescue experiments in cultured cortical neurons indicate that Erk2 binding activity of ephrin-B3's D-domain is necessary for ephrin-B3 dependent control of synapse density. While some studies suggest that Erk1/2 activation causes increased synapse density (Alonso et al., 2004), results from heterogenotypic cell culture and rescue experiments indicate that Erk2 activation negatively regulates synapse density (Fig. 3B) (McClelland et al., 2010). Thus, by inhibiting Erk2 function, ephrin-B3 can increase synapse density in cortical neurons (McClelland et al., 2010). There is ample evidence from other systems for a negative role of Erk/MAPK pathway in the control of synapse development (Wairkar et al., 2009; Yan et al., 2009). Given the importance of synapse number for normal circuit function, determining how Erk2 signals to negatively regulate synapse density will be informative.

Regulation of synapse development by ephrin-B3 in the hippocampus

Loss-of-function studies in the hippocampus established a postsynaptic role for ephrin-B3 (Aoto et al., 2007; Rodenas-Ruano et al., 2006; Xu et al., 2011). However, the literature on how ephrin-B3 regulates synapse development in the hippocampus is still controversial. Ephrin-B3 loss-of-function has been shown to reduce (Xu et al., 2011), to have little or no effect on (Aoto et al., 2007) or even to increase (Rodenas-Ruano et al., 2006) the total number of synaptic contacts formed onto CA1 neurons in vivo. Similar to immunohistochemical analyses, some studies using neurons and slices from ephrin-B3 null mice report a decrease in the frequency, but not amplitude, of mEPSCs (Xu et al., 2011), while others observe reductions in the amplitude but not the frequency of mEPSCs (see section on synaptic plasticity) (Antion et al., 2010; Aoto et al., 2007). The inconsistencies in these results are puzzling, especially since each of these studies used the same ephrin-B3 loss of function mutants (Yokoyama et al., 2001). However, a recent report on the importance of ephrin-B3 for the formation of synaptic contacts in a specific region of the hippocampus in vivo may begin to resolve some of these inconsistencies (Xu et al., 2011). In these studies genetic knock-out and rescue experiments that allowed for the temporal control of ephrin-B3 expression, demonstrated that ephrin-B3 is required and sufficient for synaptic formation and function in the stratum oriens of the CA1 hippocampal region (Xu et al., 2011). Turning on ephrin-B3 expression in ephrin-B3 knockout mice by application of tamoxifen at P5 rescued the number of functional synapses to control levels. Based on these results, there appear to be regional differences that influence how ephrin-B3 promotes synapse formation and function that may begin to explain some of the differences seen in the previous studies.

Ephrin-B3 signaling in the hippocampus

Given the complexity of function for ephrin-B3 in the hippocampus, it is not surprising that the signaling downstream of ephrin-B3 is also diverse (Aoto et al., 2007; McClelland et al., 2010; Rodenas-Ruano et al., 2006; Xu et al., 2011). Genetic evidence indicates that ephrin-B3 dependent regulation of synapse development relies on protein–protein interactions through ephrin-B3's PDZ binding domain (Bruckner et al., 1999; Torres et al., 1998). Ephrin-B3 has been shown to promote synapse and spine formation by interacting with several PDZ containing proteins, such as GRIP1, PICK1 and syntenin-1 (Figs. 3B, C) (Aoto et al., 2007; Xu et al., 2011). Interaction with different scaffolding proteins might affect distinct aspects of synapse formation. For example, ephrin-B3–GRIP1 interactions appear essential for the development of shaft synapses in cultured hippocampal neurons (Fig. 3B) (Aoto et al., 2007). In contrast, genetic experiments showed that the association of ephrin-B3 with PICK1 and/or syntenin-1 is needed for the generation of both shaft and spine synapses (Figs. 3B, C) (Xu et al., 2011). Importantly, these results demonstrate that ephrin-B3 controls synapse formation by clustering PDZ-containing scaffolding proteins at the sites of axo-dendritic contact. In the future, it will be interesting to determine whether regional differences in the interactions between ephrin-B3 and these scaffolding proteins might explain the different effects that ephrin-B3 appears to have on synapse development.

Postsynaptic ephrin-B signaling in synaptic function and plasticity

Genetic loss-of-function studies indicate that postsynaptic ephrin-Bs are important regulators of synaptic function and plasticity (Antion et al., 2010; Armstrong et al., 2006; Grunwald et al., 2004; Rodenas-Ruano et al., 2006). Conditional knockouts of ephrin-B2 in CA1 pyramidal neurons display strong reduction in LTP (Grunwald et al., 2004). The effects of ephrin-B2 on LTP appear to rely on its interaction with presynaptic EphA4. In fact, ablation of EphA4 results in a reduction in the early phase of LTP that resembles the effects in ephrin-B2 loss-of-function mutants (Grunwald et al., 2004). In contrast to ephrin-B2, the

role of ephrin-B3 at the CA3/CA1 synapse in the hippocampus is still debated with some groups reporting defects in LTP (Grunwald et al., 2004; Rodenas-Ruano et al., 2006) and others observing no effects in ephrin-B3 null mice (Antion et al., 2010; Armstrong et al., 2006). Although LTP was induced in a similar manner, in these studies differences in outcomes might be attributed to different genetic backgrounds among the ephrin-B3 knockout mice used (Kullander et al., 2001; Yokoyama et al., 2001). However, ephrin-B3 is still an important regulator of synaptic function. Consistent with its role in regulating AMPA receptors at synapses in vitro (Aoto et al., 2007; Essmann et al., 2008; McClelland et al., 2010), ephrin-B3 knockout mice display reduced AMPAR synaptic transmission, without a change in frequency of mEPSCs (Antion et al., 2010; Rodenas-Ruano et al., 2006). Therefore, these results indicate that in mature synapses, ephrin-B family members are important regulators of synaptic function and modulators of synaptic plasticity (Table 1).

Modulation of synaptic plasticity by ephrin-Bs

Ephrin-B modulation of synaptic strength likely depends on the stabilization of surface AMPA receptors (Aoto et al., 2007; Essmann et al., 2008; McClelland et al., 2010). Activation of ephrin-B2 signaling reduces AMPA receptor internalization in cultured neurons (Essmann et al., 2008). This retention of surface AMPA receptors requires the association between the AMPA receptor the PDZ binding domain of ephrin-B2 and GRIP1 (Fig. 3D) (Bruckner et al., 1999; Dong et al., 1997; Essmann et al., 2008; Torres et al., 1998). The ability of the ephrin-B2 to interact with GRIP1 is dependent on phosphorylation of a serine near the PDZ-binding domain of ephrin-B2. Phosphorylation of this serine (328) residue on ephrin-B2 strengthens the interaction between GRIP1 and the ephrin-B2 and results in an increase in surface AMPA receptors retained at synapses (Essmann et al., 2008). Interestingly, ephrin-B signaling may also antagonize internalization of the GluR2 subunit by inhibiting PKC function (Chung et al., 2003; Essmann et al., 2008). Consistent with this, activation of ephrin-B2 in cultured hippocampal neurons with EphB2 receptor bodies decreased phosphorylation of the GluR2 subunit at a serine known to be phosphorylated by PKC (Ser-880), while ephrin-B2 null neurons exhibited higher levels of GluR2 phosphorylation (Essmann et al., 2008). Thus, ephrin-Bs appear to regulate synaptic strength through the modulation of surface AMPA receptor expression. Future experiments will be needed to identify the kinases phosphorylating ephrin-B2 and how they are controlled by activity.

Ephrin-B3 may regulate synaptic function through a mechanism that is distinct from that of ephrin-B2 (Antion et al., 2010; Rodenas-Ruano et al., 2006). Ephrin-B3 regulation of synaptic strength in CA1 pyramidal neurons appears to depend on a cis interaction with EphB2, which inhibits EphB2 kinase activity (Fig. 3D) (Antion et al., 2010). Because EphB2 is an important regulator of NMDA receptor function (Takasu et al., 2002), ephrin-B3 dependent inhibition of EphB2 activity should result in decreased NMDAR currents. Indeed, ephrin-B3 knockout neurons have higher levels of EphB2 activation, increased phosphorylation of the NR2B subunit, and enhancement of the NMDA receptor component of synaptic transmission than control hippocampal neurons (Antion et al., 2010). These results suggest that ephrin-B3 may regulate glutamate receptor function in CA1 pyramidal neurons by inhibiting the EphB2–NMDAR interaction or by inhibiting EphB2 receptor signaling (Fig. 3D). In addition these findings support several lines of evidence that point to the ability of Ephs and ephrins to interact both within and between cells (Carvalho et al., 2006; Dudanova and Klein, 2011; Hornberger et al., 1999).

Ephrin-As in synapse development and plasticity

EphA receptors are important regulators of spine morphology in the hippocampus (Murai et al., 2003). However, the role of ephrin-A ligands in synapse formation and plasticity is less

well understood. Emerging evidence suggests that the ephrin-A family of proteins also plays important roles in the regulation of both synapse development and function (Table 1).

Ephrin-A5 has been linked to the regulation of both spine density and synapse function in cortex and hippocampus. In cortex, layer IV stellate neurons of ephrin-A5 knockout mice have increased numbers of filopodia-like protrusions early in development, at a time when thalamic afferents are beginning to establish their connections with cortical targets (Guellmar et al., 2009). After completion of synaptogenesis these cells have significantly fewer dendritic spines (Guellmar et al., 2009) suggesting that they may have defects in synapse formation.

In the hippocampus, ephrin-A5 is expressed in CA1–CA3 pyramidal axons and appears to regulate synapse development and function by modulating activation of the PI3 kinase/AKT pathway (Fig. 4A). Presynaptically, ephrin-A5 promotes BDNF-induced synapse formation by potentiating TrkB signaling, which augments the activation of the PI3 kinase/AKT pathway (Gottschalk et al., 1999; Marler et al., 2008), while BDNF-dependent induction of synapse formation is significantly reduced in neurons cultured from ephrin-A5 knockout mice (Marler et al., 2008). In contrast, the binding of postsynaptic EphA receptors to presynaptic ephrin-A5 disrupts the association between TrkB and ephrin-A5, leading to suppression of BDNF-induced synaptogenesis (Fig. 4A). These results suggest a model in which local ephrin-A/EphA interactions work together with global signals, such as BDNF, to fine-tune the formation of synaptic connections.

Neuron–glia communication is an important regulator of synapse development and function (Eroglu and Barres, 2010). Hippocampal astrocytes express high levels of ephrin-A3, suggesting that ephrin-A3 expressing glial cells could also regulate synapse function through ephrin/Eph interactions (Carmona et al., 2009; Murai et al., 2003). Indeed ephrin-A3 expressing astrocytes appear to regulate spine morphogenesis, glutamate transport and LTP at CA1–CA3 synapses by interacting with EphA4 receptors on dendrites of CA1 pyramidal neurons (Fig. 4B) (Carmona et al., 2009; Filosa et al., 2009). The CA1 pyramidal neurons from ephrin-A3 knockout mice and EphA4 conditional knockout mice that lack the expression of EphA4 in the CA1 pyramidal neurons display abnormally shaped dendritic spines and reduced LTP (Carmona et al., 2009; Filosa et al., 2009). Not only do ephrin-A3 knockout mice have reduced LTP, but they also have impaired acquisition of hippocampus-dependent contextual fear memory (Carmona et al., 2009). Detailed analysis of these mice suggests that the post-transcriptional upregulation of the glial excitatory amino acid transporters (EAAT) GLAST/EAAT1 and GLT-1/EAAT2, in both EphA4 and ephrin-A3 knockout mice is the likely mechanism for these functional and behavioral deficits (Carmona et al., 2009; Filosa et al., 2009). These data suggest that in the ephrin-A3 knockout mice glutamate is more efficiently removed from the synaptic cleft resulting in reduced activation of glutamate receptors and impaired induction of LTP (Fig. 4B). Altogether, several lines of evidence link ephrin-A activation in neurons and in glial cells to the control of synaptic maturation, function, and plasticity.

Conclusions and perspectives

Ephrins are localized both pre- and post-synaptically and are also expressed by glia. These patterns of expression permit them to coordinate signaling with their cognate Eph receptors between neighboring axons and dendrites and neighboring neurons and glia. In addition, the synaptic localization of ephrins enables them to regulate synapse formation, function, and long-term changes in synaptic strength (Table 1). While it is clear that ephrins play important roles at synapses, much work will be needed to determine the signaling pathways downstream of ephrin activation. Intriguing results suggesting that ephrins long known to

regulate activity-independent events like axon guidance (Flanagan, 2006; Luo and Flanagan, 2007; O'Leary and McLaughlin, 2005) can regulate glutamate receptor function and impact synaptic plasticity (Antion et al., 2010; Armstrong et al., 2006; Contractor et al., 2002; Essmann et al., 2008; Filosa et al., 2009; Grunwald et al., 2004; Lim et al., 2008; Xu et al., 2011) suggest that understanding the function of these proteins may provide insights into how activity-dependent and -independent processes are coordinated.

Distinct ephrin-B family members can regulate synapse formation and function through diverse mechanisms. Future studies will be needed to determine the molecular pathways that are shared and those that are unique to specific ephrin proteins. Development of in vivo tools will be critical to determine how, when, and where distinct Eph/ephrin interactions result in activation of unique signaling pathways that are important for the development and function of specific synapses within defined neuronal circuits. Recent work in the study of axon guidance has begun to challenge the traditional view that transcellular interactions between ephrins and Ephs alone mediate Eph/ephrin signaling and suggests that Ephs and ephrins can also interact with each other in the same cellular compartment, in cis (Antion et al., 2010; Carvalho et al., 2006; Dudanova and Klein, 2011). While this type of Eph–ephrin signaling has only begun to be linked to the ephrin/Eph regulation of synaptic function, it may provide for additional levels of complexity.

There is a growing appreciation that a number of human diseases affecting the brain may have defects in synaptic function at their core. As important regulators of synapse formation and function, it is not surprising that defects in Eph/ephrin signaling are found in a number of neurological disorders including schizophrenia (ephrin-B2 locus), neuropathic pain (ephrin-Bs and EphBs), Alzheimer's diseases (EphBs) and anxiety disorders (EphBs) (Attwood et al., 2011; Cisse et al., 2011; Ruan et al., 2010; Zhang et al., 2010). Given their importance at synapses, an increased understanding of ephrin/Eph signaling will provide outstanding potential for therapeutic targets that could eventually lead to the treatment of these disorders.

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References

- Alonso M, Medina JH, Pozzo-Miller L. ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons. *Learn Mem.* 2004; 11:172–178. [PubMed: 15054132]
- Antion MD, Christie LA, Bond AM, Dalva MB, Contractor A. Ephrin-B3 regulates glutamate receptor signaling at hippocampal synapses. *Mol Cell Neurosci.* 2010; 45:378–388. [PubMed: 20678574]
- Aoto J, Ting P, Maghsoodi B, Xu N, Henkemeyer M, Chen L. Postsynaptic ephrinB3 promotes shaft glutamatergic synapse formation. *J Neurosci.* 2007; 27:7508–7519. [PubMed: 17626212]
- Armstrong JN, Saganich MJ, Xu NJ, Henkemeyer M, Heinemann SF, Contractor A. B-ephrin reverse signaling is required for NMDA-independent long-term potentiation of mossy fibers in the hippocampus. *J Neurosci.* 2006; 26:3474–3481. [PubMed: 16571754]
- Attwood BK, Bourgognon JM, Patel S, Mucha M, Schiavon E, Skrzypiec AE, Young KW, Shiosaka S, Korostynski M, Piechota M, Przewlocki R, Pawlak R. Neuropsin cleaves EphB2 in the amygdala to control anxiety. *Nature.* 2011; 473:375–375.
- Biederer T, Stagi M. Signaling by synaptogenic molecules. *Curr Opin Neurobiol.* 2008; 18:261–269. [PubMed: 18725297]

- Biederer T, Sara Y, Mozhayeva M, Atasoy D, Liu X, Kavalali ET, Sudhof TC. SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science*. 2002; 297:1525–1531. [PubMed: 12202822]
- Bouvier D, Corera AT, Tremblay ME, Riad M, Chagnon M, Murai KK, Pasquale EB, Fon EA, Doucet G. Pre-synaptic and post-synaptic localization of EphA4 and EphB2 in adult mouse forebrain. *J Neurochem*. 2008; 106:682–695. [PubMed: 18410519]
- Bruckner K, Klein R. Signaling by Eph receptors and their ephrin ligands. *Curr Opin Neurobiol*. 1998; 8:375–382. [PubMed: 9687349]
- Bruckner K, Pablo Labrador J, Scheiffele P, Herb A, Seeburg PH, Klein R. EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains. *Neuron*. 1999; 22:511–524. [PubMed: 10197531]
- Buchert M, Schneider S, Meskenaite V, Adams MT, Canaani E, Baechi T, Moelling K, Hovens CM. The junction-associated protein AF-6 interacts and clusters with specific Eph receptor tyrosine kinases at specialized sites of cell–cell contact in the brain. *J Cell Biol*. 1999; 144:361–371. [PubMed: 9922461]
- Carmona MA, Murai KK, Wang L, Roberts AJ, Pasquale EB. Glial ephrin-A3 regulates hippocampal dendritic spine morphology and glutamate transport. *Proc Natl Acad Sci U S A*. 2009; 106:12524–12529. [PubMed: 19592509]
- Carvalho RF, Beutler M, Marler KJ, Knoll B, Becker-Barroso E, Heintzmann R, Ng T, Drescher U. Silencing of EphA3 through a cis interaction with ephrinA5. *Nat Neurosci*. 2006; 9:322–330. [PubMed: 16491080]
- Castillo PE, Janz R, Sudhof TC, Tzounopoulos T, Malenka RC, Nicoll RA. Rab3A is essential for mossy fibre long-term potentiation in the hippocampus. *Nature*. 1997; 388:590–593. [PubMed: 9252190]
- Castillo PE, Schoch S, Schmitz F, Sudhof TC, Malenka RC. RIM1alpha is required for presynaptic long-term potentiation. *Nature*. 2002; 415:327–330. [PubMed: 11797010]
- Chung HJ, Steinberg JP, Haganir RL, Linden DJ. Requirement of AMPA receptor GluR2 phosphorylation for cerebellar long-term depression. *Science*. 2003; 300:1751–1755. [PubMed: 12805550]
- Cisse M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, Orr A, Lotz G, Kim DH, Hamto P, Ho K, Yu GQ, Mucke L. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature*. 2011; 469:47–52. [PubMed: 21113149]
- Contractor A, Rogers C, Maron C, Henkemeyer M, Swanson GT, Heinemann SF. Trans-synaptic Eph receptor-ephrin signaling in hippocampal mossy fiber LTP. *Science*. 2002; 296:1864–1869. [PubMed: 12052960]
- Cowan CA, Henkemeyer M. The SH2/SH3 adaptor Grb4 transduces B-ephrin reverse signals. *Nature*. 2001; 413:174–179. [PubMed: 11557983]
- Crowley JC, Katz LC. Early development of ocular dominance columns. *Science*. 2000; 290:1321–1324. [PubMed: 11082053]
- Dalva MB, Takasu MA, Lin MZ, Shamah SM, Hu L, Gale NW, Greenberg ME. EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell*. 2000; 103:945–956. [PubMed: 11136979]
- Davy A, Gale NW, Murray EW, Klinghoffer RA, Soriano P, Feuerstein C, Robbins SM. Compartmentalized signaling by GPI-anchored ephrin-A5 requires the Fyn tyrosine kinase to regulate cellular adhesion. *Genes Dev*. 1999; 13:3125–3135. [PubMed: 10601038]
- Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Haganir RL. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature*. 1997; 386:279–284. [PubMed: 9069286]
- Dudanova I, Klein RD. The axon's balancing act: cis- and trans-interactions between Ephs and ephrins. *Neuron*. 2011; 71:1–4. [PubMed: 21745632]
- Dunaevsky A, Blazeski R, Yuste R, Mason C. Spine motility with synaptic contact. *Nat Neurosci*. 2001; 4:685–686. [PubMed: 11426220]
- Eroglu C, Barres B. Regulation of synaptic connectivity by glia. *Nature*. 2010; 468:223–254. [PubMed: 21068831]

- Espinosa JS, Wheeler DG, Tsien RW, Luo L. Uncoupling dendrite growth and patterning: single-cell knockout analysis of NMDA receptor 2B. *Neuron*. 2009; 62:205–217. [PubMed: 19409266]
- Essmann CL, Martinez E, Geiger JC, Zimmer M, Traut MH, Stein V, Klein R, Acker-Palmer A. Serine phosphorylation of ephrinB2 regulates trafficking of synaptic AMPA receptors. *Nat Neurosci*. 2008; 11:1035–1043. [PubMed: 19160501]
- Filosa A, Paixao S, Honsek SD, Carmona MA, Becker L, Feddersen B, Gaitanos L, Rudhard Y, Schoepfer R, Klopstock T, Kullander K, Rose CR, Pasquale EB, Klein R. Neuron–glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. *Nat Neurosci*. 2009; 12:1285–1292. [PubMed: 19734893]
- Fischer M, Kaech S, Knutti D, Matus A. Rapid actin-based plasticity in dendritic spines. *Neuron*. 1998; 20:847–854. [PubMed: 9620690]
- Flanagan JG. Neural map specification by gradients. *Curr Opin Neurobiol*. 2006; 16:59–66. [PubMed: 16417998]
- Flenniken AM, Gale NW, Yancopoulos GD, Wilkinson DG. Distinct and overlapping expression patterns of ligands for Eph-related receptor tyrosine kinases during mouse embryogenesis. *Dev Biol*. 1996; 179:382–401. [PubMed: 8903354]
- Gottschalk WA, Jiang H, Tartaglia N, Feng L, Figurov A, Lu B. Signaling mechanisms mediating BDNF modulation of synaptic plasticity in the hippocampus. *Learn Mem*. 1999; 6:243–256. [PubMed: 10492006]
- Grembecka J, Cierpicki T, Devedjiev Y, Derewenda U, Kang BS, Bushweller JH, Derewenda ZS. The binding of the PDZ tandem of syntenin to target proteins. *Biochemistry*. 2006; 45:3674–3683. [PubMed: 16533050]
- Grunwald IC, Korte M, Wolfer D, Wilkinson GA, Unsicker K, Lipp HP, Bonhoeffer T, Klein R. Kinase-independent requirement of EphB2 receptors in hippocampal synaptic plasticity. *Neuron*. 2001; 32:1027–1040. [PubMed: 11754835]
- Grunwald IC, Korte M, Adelmann G, Plueck A, Kullander K, Adams RH, Frotscher M, Bonhoeffer T, Klein R. Hippocampal plasticity requires postsynaptic ephrinBs. *Nat Neurosci*. 2004; 7:33–40. [PubMed: 14699416]
- Guellmar A, Rudolph J, Bolz J. Structural alterations of spiny stellate cells in the somatosensory cortex in ephrin-A5-deficient mice. *J Comp Neurol*. 2009; 517:645–654. [PubMed: 19827157]
- Henderson JT, Georgiou J, Jia Z, Robertson J, Elowe S, Roder JC, Pawson T. The receptor tyrosine kinase EphB2 regulates NMDA-dependent synaptic function. *Neuron*. 2001; 32:1041–1056. [PubMed: 11754836]
- Henkemeyer M, Itkis OS, Ngo M, Hickmott PW, Ethell IM. Multiple EphB receptor tyrosine kinases shape dendritic spines in the hippocampus. *J Cell Biol*. 2003; 163:1313–1326. [PubMed: 14691139]
- Hippenmeyer S, Youn YH, Moon HM, Miyamichi K, Zong H, Wynshaw-Boris A, Luo L. Genetic mosaic dissection of Lis1 and Ndel1 in neuronal migration. *Neuron*. 2010; 68:695–709. [PubMed: 21092859]
- Holland SJ, Gale NW, Mbamalu G, Yancopoulos GD, Henkemeyer M, Pawson T. Bidirectional signalling through the EPH-family receptor Nuk and its transmembrane ligands. *Nature*. 1996; 383:722–725. [PubMed: 8878483]
- Hornberger M, Dütting D, Ciossek T, Yamada T, Handwerker C, Lang S, Weth F, Huf J, Wessel R, Logan C, Tanaka H, Drescher U. Modulation of EphA receptor function by coexpressed ephrinA ligands on retinal ganglion cell axons. *Neuron*. 1999; 22:731–773. [PubMed: 10230793]
- Horton JC, Hocking DR. An adult-like pattern of ocular dominance columns in striate cortex of newborn monkeys prior to visual experience. *J Neurosci*. 1996; 16:1791–1807. [PubMed: 8774447]
- Hubel DH, Wiesel TN. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J Physiol*. 1970; 206:419–436. [PubMed: 5498493]
- Irie F, Yamaguchi Y. EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP. *Nat Neurosci*. 2002; 5:1117–1118. [PubMed: 12389031]

- Kayser MS, McClelland AC, Hughes EG, Dalva MB. Intracellular and trans-synaptic regulation of glutamatergic synaptogenesis by EphB receptors. *J Neurosci*. 2006; 26:12152–12164. [PubMed: 17122040]
- Kayser MS, Nolt MJ, Dalva MB. EphB receptors couple dendritic filopodia motility to synapse formation. *Neuron*. 2008; 59:56–69. [PubMed: 18614029]
- Klein R. Bidirectional modulation of synaptic functions by Eph/ephrin signaling. *Nat Neurosci*. 2009; 12:15–20. [PubMed: 19029886]
- Ko J, Na M, Kim S, Lee JR, Kim E. Interaction of the ERC family of RIM-binding proteins with the liprin-alpha family of multidomain proteins. *J Biol Chem*. 2003; 278:42377–42385. [PubMed: 12923177]
- Ko J, Yoon C, Piccoli G, Chung HS, Kim K, Lee JR, Lee HW, Kim H, Sala C, Kim E. Organization of the presynaptic active zone by ERC2/CAST1-dependent clustering of the tandem PDZ protein syntenin-1. *J Neurosci*. 2006; 26:963–970. [PubMed: 16421316]
- Kullander K, Croll SD, Zimmer M, Pan L, McClain J, Hughes V, Zabski S, DeChiara TM, Klein R, Yancopoulos GD, Gale NW. Ephrin-B3 is the midline barrier that prevents corticospinal tract axons from recrossing, allowing for unilateral motor control. *Genes Dev*. 2001; 15:877–888. [PubMed: 11297511]
- Lemke G, Reber M. Retinotectal mapping: new insights from molecular genetics. *Annu Rev Cell Dev Biol*. 2005; 21:551–580. [PubMed: 16212507]
- Lendvai B, Stern EA, Chen B, Svoboda K. Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. *Nature*. 2000; 404:876–881. [PubMed: 10786794]
- Liebl DJ, Morris CJ, Henkemeyer M, Parada LF. mRNA expression of ephrins and Eph receptor tyrosine kinases in the neonatal and adult mouse central nervous system. *J Neurosci Res*. 2003; 71:7–22. [PubMed: 12478610]
- Lim BK, Matsuda N, Poo MM. Ephrin-B reverse signaling promotes structural and functional synaptic maturation in vivo. *Nat Neurosci*. 2008; 11:160–169. [PubMed: 18193042]
- Lim BK, Cho SJ, Sumbre G, Poo MM. Region-specific contribution of ephrin-B and Wnt signaling to receptive field plasticity in developing optic tectum. *Neuron*. 2010; 65:899–911. [PubMed: 20346764]
- Lin D, Gish GD, Songyang Z, Pawson T. The carboxyl terminus of B class ephrins constitutes a PDZ domain binding motif. *J Biol Chem*. 1999; 274:3726–3733. [PubMed: 9920925]
- Linhoff MW, Lauren J, Cassidy RM, Dobie FA, Takahashi H, Nygaard HB, Airaksinen MS, Strittmatter SM, Craig AM. An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers. *Neuron*. 2009; 61:734–749. [PubMed: 19285470]
- Luo L, Flanagan J. Development of continuous and discrete neural maps. *Neuron*. 2007; 56:284–284. [PubMed: 17964246]
- Margolis SS, Salogiannis J, Lipton DM, Mandel-Brehm C, Wills ZP, Mardinly AR, Hu L, Greer PL, Bikoff JB, Ho HY, Soskis MJ, Sahin M, Greenberg ME. EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation. *Cell*. 2010; 143:442–455. [PubMed: 21029865]
- Marler KJ, Becker-Barroso E, Martinez A, Llovera M, Wentzel C, Poopalasundaram S, Hindges R, Soriano E, Comella J, Drescher U. A TrkB/EphrinA interaction controls retinal axon branching and synaptogenesis. *J Neurosci*. 2008; 28:12700–12712. [PubMed: 19036963]
- McClelland AC, Sheffler-Collins SI, Kayser MS, Dalva MB. Ephrin-B1 and ephrin-B2 mediate EphB-dependent presynaptic development via syntenin-1. *Proc Natl Acad Sci U S A*. 2009; 106:20487–20492. [PubMed: 19915143]
- McClelland AC, Hruska M, Coenen AJ, Henkemeyer M, Dalva MB. Trans-synaptic EphB2–ephrin-B3 interaction regulates excitatory synapse density by inhibition of postsynaptic MAPK signaling. *Proc Natl Acad Sci U S A*. 2010; 107:8830–8835. [PubMed: 20410461]
- Migani P, Bartlett C, Dunlop S, Beazley L, Rodger J. Ephrin-B2 immunoreactivity distribution in adult mouse brain. *Brain Res*. 2007; 1182:60–72. [PubMed: 17945206]
- Migani P, Bartlett C, Dunlop S, Beazley L, Rodger J. Regional and cellular distribution of ephrin-B1 in adult mouse brain. *Brain Res*. 2009; 1247:50–61. [PubMed: 18973746]

- Mori T, Wanaka A, Taguchi A, Matsumoto K, Tohyama M. Differential expressions of the eph family of receptor tyrosine kinase genes (sek, elk, eck) in the developing nervous system of the mouse. *Brain Res Mol Brain Res*. 1995; 29:325–335. [PubMed: 7609620]
- Murai KK, Nguyen LN, Irie F, Yamaguchi Y, Pasquale EB. Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. *Nat Neurosci*. 2003; 6:153–160. [PubMed: 12496762]
- Nolt MJ, Lin Y, Hruska M, Murphy J, Sheffler-Colins SI, Kayser MS, Passer J, Bennett MV, Zukin RS, Dalva MB. EphB controls NMDA receptor function and synaptic targeting in a subunit-specific manner. *J Neurosci*. 2011; 31:5353–5364. [PubMed: 21471370]
- Ohtsuka T, Takao-Rikitsu E, Inoue E, Inoue M, Takeuchi M, Matsubara K, Deguchi-Tawarada M, Satoh K, Morimoto K, Nakanishi H, Takai Y. Cast: a novel protein of the cytomatrix at the active zone of synapses that forms a ternary complex with RIM1 and munc13-1. *J Cell Biol*. 2002; 158:577–590. [PubMed: 12163476]
- O’Leary DD, McLaughlin T. Mechanisms of retinotopic map development: Ephs, ephrins, and spontaneous correlated retinal activity. *Prog Brain Res*. 2005; 147:43–65. [PubMed: 15581697]
- Palmer A, Klein R. Multiple roles of ephrins in morphogenesis, neuronal networking, and brain function. *Genes Dev*. 2003; 17:1429–1450. [PubMed: 12815065]
- Pasquale EB. Eph–ephrin bidirectional signaling in physiology and disease. *Cell*. 2008; 133:38–52. [PubMed: 18394988]
- Penzes P, Beeser A, Chernoff J, Schiller MR, Eipper BA, Mains RE, Huganir RL. Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB–EphB receptor activation of the Rho-GEF kalirin. *Neuron*. 2003; 37:263–274. [PubMed: 12546821]
- Rao A, Craig AM. Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. *Neuron*. 1997; 19:801–812. [PubMed: 9354327]
- Robbins EM, Krupp AJ, Perez de Arce K, Ghosh AK, Fogel AI, Boucard A, Sudhof TC, Stein V, Biederer T. SynCAM 1 adhesion dynamically regulates synapse number and impacts plasticity and learning. *Neuron*. 2010; 68:894–906. [PubMed: 21145003]
- Rodenas-Ruano A, Perez-Pinzon MA, Green EJ, Henkemeyer M, Liebl DJ. Distinct roles for ephrinB3 in the formation and function of hippocampal synapses. *Dev Biol*. 2006; 292:34–45. [PubMed: 16466709]
- Ruan JP, Zhang HX, Lu XF, Liu YP, Cao JL. EphrinBs/EphBs signaling is involved in modulation of spinal nociceptive processing through a mitogen-activated protein kinases-dependent mechanism. *Anesthesiology*. 2010; 112:1234–1249. [PubMed: 20395829]
- San Miguel S, Serrano MJ, Sachar A, Henkemeyer M, Svoboda KK, Benson MD. Ephrin reverse signaling controls palate fusion via a PI3 kinase-dependent mechanism. *Dev Dyn*. 2011; 240:357–364. [PubMed: 21246652]
- Scheiffele P, Fan J, Choih J, Fetter R, Serafini T. Neuroligin expressed in non-neuronal cells triggers presynaptic development in contacting axons. *Cell*. 2000; 101:657–669. [PubMed: 10892652]
- Scicolone G, Ortalli AL, Carri NG. Key roles of Ephs and ephrins in retinotectal topographic map formation. *Brain Res Bull*. 2009; 79:227–247. [PubMed: 19480983]
- Segura I, Essmann CL, Weinges S, Acker-Palmer A. Grb4 and GIT1 transduce ephrinB reverse signals modulating spine morphogenesis and synapse formation. *Nat Neurosci*. 2007; 10:301–310. [PubMed: 17310244]
- Takao-Rikitsu E, Mochida S, Inoue E, Deguchi-Tawarada M, Inoue M, Ohtsuka T, Takai Y. Physical and functional interaction of the active zone proteins, CAST, RIM1, and Bassoon, in neurotransmitter release. *J Cell Biol*. 2004; 164:301–311. [PubMed: 14734538]
- Takasu MA, Dalva MB, Zigmond RE, Greenberg ME. Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. *Science*. 2002; 295:491–495. [PubMed: 11799243]
- Tolias KF, Bikoff JB, Kane CG, Tolias CS, Hu L, Greenberg ME. The Rac1 guanine nucleotide exchange factor Tiam1 mediates EphB receptor-dependent dendritic spine development. *Proc Natl Acad Sci U S A*. 2007; 104:7265–7270. [PubMed: 17440041]

- Torres R, Firestein BL, Dong H, Staudinger J, Olson EN, Huganir RL, Bredt DS, Gale NW, Yancopoulos GD. PDZ proteins bind, cluster, and synaptically colocalize with Eph receptors and their ephrin ligands. *Neuron*. 1998; 21:1453–1463. [PubMed: 9883737]
- Turriano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature*. 1998; 391:892–896. [PubMed: 9495341]
- Wairkar YP, Toda H, Mochizuki H, Furukubo-Tokunaga K, Tomoda T, Diantonio A. Unc-51 controls active zone density and protein composition by downregulating ERK signaling. *J Neurosci*. 2009; 29:517–528. [PubMed: 19144852]
- Weisskopf MG, Nicoll RA. Presynaptic changes during mossy fibre LTP revealed by NMDA receptor-mediated synaptic responses. *Nature*. 1995; 376:256–259. [PubMed: 7617037]
- Wiesel TN, Hubel DH. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *J Neurophysiol*. 1965; 28:1029–1040. [PubMed: 5883730]
- Willson CA, Foster RD, Onifer SM, Whitemore SR, Miranda JD. EphB3 receptor and ligand expression in the adult rat brain. *J Mol Histol*. 2006; 37:369–380. [PubMed: 17103029]
- Xu NJ, Henkemeyer M. Ephrin-B3 reverse signaling through Grb4 and cytoskeletal regulators mediates axon pruning. *Nat Neurosci*. 2009; 12:268–276. [PubMed: 19182796]
- Xu NJ, Sun S, Gibson JR, Henkemeyer M. A dual shaping mechanism for postsynaptic ephrin-B3 as a receptor that sculpts dendrites and synapses. *Nat Neurosci*. 2011; 14:1421–1429. [PubMed: 21964490]
- Yamada S, Nelson WJ. Synapses: sites of cell recognition, adhesion, and functional specification. *Annu Rev Biochem*. 2007; 76:267–294. [PubMed: 17506641]
- Yan D, Wu Z, Chisholm AD, Jin Y. The DLK-1 kinase promotes mRNA stability and local translation in *C. elegans* synapses and axon regeneration. *Cell*. 2009; 138:1005–1018. [PubMed: 19737525]
- Yokoyama N, Romero MI, Cowan CA, Galvan P, Helmbacher F, Charnay P, Parada LF, Henkemeyer M. Forward signaling mediated by ephrin-B3 prevents contralateral corticospinal axons from recrossing the spinal cord midline. *Neuron*. 2001; 29:85–97. [PubMed: 11182083]
- Zhang H, Webb DJ, Asmussen H, Horwitz AF. Synapse formation is regulated by the signaling adaptor GIT1. *J Cell Biol*. 2003; 161:131–142. [PubMed: 12695502]
- Zhang H, Webb DJ, Asmussen H, Niu S, Horwitz AF. A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. *J Neurosci*. 2005; 25:3379–3388. [PubMed: 15800193]
- Zhang R, Zhong NN, Liu XG, Yan H, Qiu C, Han Y, Wang W, Hou WK, Liu Y, Gao CG, Guo TW, Lu SM, Deng HW, Ma J. Is the EFNB2 locus associated with schizophrenia? Single nucleotide polymorphisms and haplotypes analysis. *Psychiatry Res*. 2010; 180:5–9. [PubMed: 20483485]
- Zong H, Espinosa JS, Su HH, Muzumdar MD, Luo L. Mosaic analysis with double markers in mice. *Cell*. 2005; 121:479–492. [PubMed: 15882628]

Fig. 1.

Bidirectional signaling by Eph RTKs and their cell-surface ligands, ephrins. (A) Ephrins fall into two subfamilies based on the Eph RTK they bind. The ephrin-A subfamily consists of five GPI-linked members (ephrin-A1–A5) that bind and activate EphAs (ephrin-A5 that can also bind to EphB2 RTK). The ephrin-B subfamily consists of three family members (ephrin-B1–B3). All ephrin-Bs are transmembrane proteins that bind and activate the EphB class of Ephs and are themselves capable of intracellular signaling. One notable exception to this rule is that all ephrin-B members can also bind and activate EphA4 RTK. (B) Diagrams of the domains within the intracellular portion of ephrin-Bs that enable ephrinBs to function as signaling proteins. These include three tyrosine residues enabling recruitment of Src Homology 2/3 (SH2/SH3) adaptor proteins, a PDZ-binding domain enabling interaction with PDZ domain containing adapter proteins and a Grb4 domain. In addition to the conserved domains, ephrin-B3 contains a D-domain allowing it to interact with Erk2. (C) The expression patterns of Ephs and ephrins in the hippocampus. EphB2 and EphA4 are expressed throughout the hippocampus, while EphB1, EphB3, and ephrin-Bs exhibit regional differences in their expression. In dentate gyrus (DG) ephrin-Bs have well-established pre-synaptic role, while in the CA1 region ephrin-Bs function largely postsynaptically.

Fig. 2. Ephrin-Bs in presynaptic differentiation and function. (A) Activation of ephrin-B1 and ephrin-B2 by presynaptic EphB receptors initiates presynaptic differentiation and causes increases in the density of presynaptic release sites. Ephrin-Bs recruit the dual PDZ domain containing protein, syntenin-1, through their PDZ-binding domains to nascent presynaptic sites. Recruitment of syntenin to the presynaptic terminal could initiate a cascade of events resulting in the accumulation of neurotransmitter-filled vesicles and maturation of presynaptic sites. (B) Presynaptic ephrin-Bs can impact the connectivity of neural circuits. In *Xenopus* retinotectal system visually driven neuronal activity leads to remodeling of circuitry between the retina and the tectum. Application of visual stimulus causes shifts in receptive field locations (RFs) in the tectum. After visual stimulation, the shifts in the RFs are enhanced in the ventral tectum by overexpression of ephrin-B in presynaptic RGCs. Visual stimulation fails to induce shifts in RFs when ephrin-B signaling is blocked or when ephrin-Bs are overexpressed in tectal neurons.

Fig. 3.

Ephrin-B signaling in postsynaptic differentiation and function. (A) The use of heterotypic mixed neuronal cultures demonstrated that ephrin-B3 functions as a competitive signal that regulates overall synapse density on individual cortical neurons. Under conditions of competition (wt/wt) or in the absence of competition (eB3^{-/-}/eB3^{-/-}) neurons develop normal synapse density. Creating competitive conditions by mixing 10% GFP-labeled ephrin-B3 null neurons with 90% unlabeled wild-type neurons (eB3^{-/-}/wt) results in ephrin-B3 null neurons with fewer than normal density of synapses. Mixing 10% GFP-labeled wild-type neurons with 90% unlabeled ephrin-B3 null neurons (wt/eB3^{-/-}) results in wild-type neurons with greater than normal density of synapses. (B) Synapse development: Ephrin-B3 regulation of synapse development relies on two distinct intracellular regions, a PDZ binding domain and a Erk binding D-domain. The PDZ binding domain utilizes protein-protein interactions that bring the PDZ domain-containing proteins, such as PICK1, Syntenin-1 and GRIP1 to the sites of ephrin-B3 signaling. Ephrin-B3 controls synapse density by inhibiting Erk2 signaling via a D-domain. When not bound to ephrin-B3, Erk2 functions as a negative regulator of synapse density. Binding of Erk2 to the ephrin-B3 D-domain inhibits Erk function. In this manner, by suppressing the negative regulation of Erk2, ephrin-B3 acts as a positive regulator of synapse density. (C) Spine formation: Ephrin-B regulation of the transition from filopodia to spine utilizes the ephrin-B Grb4 domain. Phosphorylation of a specific tyrosine within the Grb4 domain leads to an association with the SH2/SH3 adapter molecule, Grb4. Grb4 can then recruit GIT1. In this manner GIT1 links ephrin-B2 (likely through βPIX and Rac) to the remodeling of the actin cytoskeleton. The regulation of spine density by ephrin-B3 relies on protein-protein interactions via the ephrin-B3 PDZ binding domain. Association between ephrin-B3 and PICK1 or Syntenin-1 leads to an increase in mature dendritic spine density. (D) Synapse function: Both, ephrin-B2 and ephrin-B3 can regulate surface retention of the AMPA type glutamate receptor through their PDZ binding domains. In case of ephrin-B2, the surface retention of AMPA receptors is positively regulated by the phosphorylation of the serine 328 residue. Phosphorylation of S328 can increase the association among GRIP1, AMPARs and ephrin-B2. Ephrin-B3 can modulate synaptic transmission by indirectly regulating NMDAR

function. Ephrin-B3 may associate with EphB2 in cis. This non-canonical interaction results in inhibition of EphB2 receptor tyrosine kinase signaling and subsequently, de-phosphorylation of NR1 subunits of the NMDAR that render NMDARs less permeable to calcium.

Fig. 4.

Regulation of synapse formation and plasticity by ephrin-As. (A) Presynaptic ephrin-A5 influences early stage synaptogenesis. Synapse development: Ephrin-A5 potentiates BDNF-dependent induction of synapse formation by interacting with TrkB in cis. This interaction leads to increased PI3 kinase/AKT signaling downstream of TrkB that is likely responsible for the induction of synapse formation. Binding of postsynaptic EphAs to presynaptic ephrin-A5, leads to the suppression of BDNF-induced synapse formation. (B) Synaptic function and plasticity: Ephrin-A3 expressed in astrocytes can regulate dendritic spine morphogenesis and LTP in hippocampal neurons by interacting with EphA4 expressed in the dendrites of CA1 neurons. Activation of ephrin-A3 signaling in astrocytes results in reduced expression of excitatory amino acid transporters, GLAST/EAAT1 and GLT-1/EAAT2 through the post-transcriptional mechanisms. As a consequence, more glutamate remains in the synaptic cleft leading to increased excitatory synaptic transmission and enhanced LTP.

Table 1

Ephrin signaling in synapse development and function: evidence for regulatory roles at synapses during development and in the mature nervous system.

Treatment	Description	Functional effects at the synapse	Refs.
<i>Presynaptic ephrin-Bs</i>			
↓Ephrin- B1	siRNA knockdown in developing cultured cortical neurons (DIV10–21)	• ↓Presynaptic specializations	McClelland et al. (2009)
	Overexpression of dominant-negative ephrin-B1 lacking PDZ-binding domain in RGCs in vivo	• ↓Presynaptic specializations	Lim et al. (2008)
↑Ephrin- B1	Overexpression of wt ephrin-B1 in <i>Xenopus</i> RGCs in vivo	• ↑Neurotransmitter release • ↑RF shifts in tectum	Lim et al. (2008, 2010)
↓Ephrin- B2	siRNA knockdown in developing cultured cortical neurons (DIV21)	• ↓Presynaptic specializations	McClelland et al. (2009)
↓Ephrin- B3	Transgenic ephrin-B3 mice lacking intracellular domain	• ↓LTP at mossy fiber synapses	Armstrong et al. (2006)
<i>Postsynaptic ephrin-Bs</i>			
↑Ephrin-B (all)	Activation of ephrin-B signaling by EphB2-Fc receptor bodies	• ↓Density of filopodia-like protrusions • ↑Spine maturation	Segura et al. (2007)
↓Ephrin- B2	Conditional ephrin-B2 knockout mice	• ↓Synaptic transmission/LTP • ↑Internalization of AMPARs	Essmann et al. (2008) and Grunwald et al. (2004)
↓Ephrin- B3	Heterogenotypic mouse cortical co-cultures from wt and ephrin-B3 knockout mice (DIV10)	• Competitive regulation of synapse density	McClelland et al. (2010)
	siRNA knockdown in developing hippocampal neurons (DIV14)	• ↓Dendritic shaft synapse density	Aoto et al. (2007)
	Characterization of hypomorphic ephrin- B3 knock out mice	• ↓Synapse and spine density in stratum oriens • ↓NMDAR synaptic transmission/LTP	Antion et al. (2010), Grunwald et al. (2004), Rodenas- Ruano et al. (2006) and Xu et al. (2011)
<i>Ephrin-As</i>			
↓Ephrin- A3	In vivo characterization of ephrin-A3 knockout mice	• ↓Spine morphogenesis • ↓LTP	Carmona et al. (2009) and Filosa et al. (2009)
↓Ephrin- A5	In vivo characterization of ephrin-A5 knockout mice	• ↓BDNF-dependent synaptogenesis • ↓Spine density (layer 4, stellate neurons)	Guellmar et al. (2009) and Marler et al. (2008)