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Looking Forward to EphB Signaling in Synapses

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Abstract

Eph receptors and their ligands ephrins comprise a complex signaling system with diverse functions that span a wide range of tissues and developmental stages. The variety of Eph receptor functions stems from their ability to mediate bidirectional signaling through trans-cellular Eph/ephrin interactions. Initially thought to act by directing repulsion between cells, Ephs have also been demonstrated to induce and maintain cell adhesive responses at excitatory synapses in the central nervous system. EphB receptors are essential to the development and maintenance of dendritic spines, which accommodate the postsynaptic sites of most glutamatergic excitatory synapses in the brain. Functions of EphB receptors are not limited to control of the actin cytoskeleton in dendritic spines, as EphB receptors are also involved in the formation of functional synaptic specializations through the regulation of glutamate receptor trafficking and functions. In addition, EphB receptors have recently been linked to the pathophysiology of Alzheimer's disease and neuropathic pain, thus becoming promising targets for therapeutic interventions. In this review, we discuss recent findings on EphB receptor functions in synapses, as well as the mechanisms of bidirectional trans-synaptic ephrin-B/EphB receptor signaling that shape dendritic spines and influence post-synaptic differentiation.

Keywords

Eph; ephrin; signaling; synapse; actin; dendritic spine

1. Introduction

Erythropoietin-producing hepatocellular carcinoma (Eph) receptors form the largest family of receptor tyrosine kinases (RTKs) with unique characteristics that add complexity to their signaling capacity [1,2]. Eph receptors are usually activated by their ligands, Eph receptor interacting proteins (ephrins), expressed on the neighboring cells through *trans* interactions, although *cis* interactions between ephrins and Eph receptors (Ephs) located on the same cell have been recently reported as well [3,4]. Ephs and ephrins are subdivided into two distinct classes, based on their structural similarity and affinity. Class A ephrins are anchored to the cell surface via a glycosylphosphatidylinositol (GPI) motif and preferentially interact with EphA receptors, whereas class B ephrins are type I transmembrane proteins that show high affinity to EphB receptors [5,6]. In mammals 14 Ephs (EphA1-A8, EphA10, EphB1-4, EphB6) and 9 ephrins (ephrin-A1-6, ephrin-B1-3) are identified [6]. Eph--ephrin

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interactions are highly promiscuous in each group and even across A- and B-classes, such that EphA4 can bind ephrin-B2 and ephrin-B3, while EphB2 interacts with ephrin-A5 [7]. Another distinguishing quality of Ephs and ephrins is their capacity for bidirectional signaling – ephrin-induced EphB receptor activation initiates ‘forward’ signaling in the cell expressing the receptor, while “reverse” signaling is activated in the cell expressing the ephrin ligand. The evolutionary expansion of Ephs into the largest subfamily of RTKs from only a few ancestral genes suggests the importance of Eph signaling in vertebrates [8,9].

The growing array of functions that are performed by Ephs and ephrins includes cell proliferation, cell migration, axon guidance, synaptogenesis and synaptic plasticity within the central nervous system (CNS), as well as angiogenesis, bone morphogenesis, laminar patterning and oncogenesis [10]. While role of Ephs and ephrins in the topographic mapping of retinotectal projections during embryonic development was well studied over the years [11,12], their control of stem cell proliferation and differentiation was most recently added to the list of their functions in the CNS [13,14]. Ephs and ephrins usually act as chemorepellents, utilizing complex multidimensional expression gradients to precisely guide cells or axons to their final destinations [15]. However, recent reports have suggested that Ephs can also regulate adhesive cellular events that underlie the formation of excitatory synaptic connections. Synapses are highly specialized contacts between neurons that mediate neuronal communications [16]. The vast majority of excitatory synapses in the adult mammalian brain are located on dendritic spines, micron-sized protrusions from the surface of dendrites that contain postsynaptic specializations and act primarily to generate postsynaptic responses [17]. Ephs are shown to be involved in spine and synapse formation as well as synaptic plasticity through their ability to control actin cytoskeleton [17] and to regulate glutamate receptor trafficking and function [18,19].

2. The bidirectional nature of ephrin/Eph signaling

Ephs are transmembrane proteins, which contain a globular N-terminal “ephrins-binding” domain, a cysteine-rich region and two fibronectin type III repeats at the extracellular space (Fig. 1). The intracellular portion is composed of a juxtamembrane region containing two autophosphorylation sites, a kinase domain (inactive in EphA10 and EphB6), a sterile α -motif (SAM) domain and a PDZ domain binding motif at its C-terminal end [20]. Ephs undergo homo- and heterodimerizations directly via receptor-receptor binding interfaces residing at the cysteine-rich region, the fibronectin III repeats, SAM-domain and indirectly via the interactions with PDZ domain containing proteins [21,22]. Eph phosphorylation enables binding with Src homology 2 (SH2) domain containing proteins, while other interactions are kinase independent. Principal Eph binding partners include guanine nucleotide exchange factors (GEFs), Src family non-receptor tyrosine kinases and several adaptor proteins. Ephs are negatively regulated by several protein tyrosine phosphatases [23] and by ubiquitin ligase-mediated internalization and degradation [24]. Extracellular portion of ephrins contains receptor binding domain that interacts with Ephs. While A-class ephrins are attached to the cell membrane through a GPI linker, B-class ephrins have a complete transmembrane domain with a PDZ domain binding motif at the carboxyl terminus, and can interact with Src non-receptor tyrosine kinases, group I metabotropic glutamate receptors (mGluRs), GRIP1 and Grb4 adaptor protein [21]. Although ephrin-As cannot directly initiate intracellular signaling, they can mediate their responses through the interactions with Trk receptors and heparan sulfate [25,26].

Since both Ephs and ephrins are exclusively membrane-bound, cell to cell contact is necessary for the initiation of their signaling. Eph/ephrin interactions can trigger the formation of heterodimers with nanomolar affinity, which assemble into circular tetramers with both receptors interacting with both ligands [27]. This heterotetrameric complex is

considered the smallest functional signaling unit of the receptor, however, the tetramers can further oligomerize into higher order signaling clusters in a ligand-independent manner. The size of a cluster, and therefore its signaling capacity, could potentially reflect the abundance of receptor on the cell surface, allowing for graded responses of the signaling. [28,29]. Heterotetramer formation is accompanied by conformational changes that allow for the receptor autophosphorylation. This in turn relieves the inhibition of kinase domain imposed by the juxtamembrane segment and sets off signaling events through the phosphorylation of intracellular proteins [10]. Ephrin-B “reverse” signaling can be activated by the phosphorylation of receptor-bound ephrin-Bs involving Src family of non-receptor tyrosine kinases, PDGF and FGF receptors, or can be negatively regulated by PTP-BL phosphatase [21,30]. In addition to the *trans*-cellular receptor-ligand interactions that occur between opposing cells, Ephs and ephrins can interact in *cis*, when expressed within the same cell. These interactions have been described to inhibit receptor activity and may modulate the formation of functional Eph receptor gradients [3] and EphB receptor selectivity in glutamatergic synapses [4].

3. Repulsion versus adhesion

Ephs and ephrins are capable of producing both adhesive and repulsive responses between interacting cells. For example, Ephs and ephrins are known to be involved in axon guidance by triggering repulsive responses, and to induce synaptogenesis by enhancing adhesive interactions between pre-synaptic terminals and postsynaptic sites [31]. Cellular context determines the outcome of Eph signaling, as opposite effects can be generated in the same cell by the same Eph receptor [10]. For example, the level of the EphA receptor expression determines topographic targeting of retinotectal projections [32,33], whereas a specific subtype of the EphB receptor, but not the level of EphB receptor expression, directs the repulsion of ipsilateral retinal projections at the optic chiasm [34]. Although cell adhesion is required for the interactions between membrane-bound Ephs and ephrins, these high affinity *trans*-cellular interactions are subsequently broken to convert cell adhesion into repulsion. One possibility is that ephrin/Eph interactions are disrupted by proteolytic cleavage of the ligand or receptor. Transmembrane metalloproteinase ADAM10 has been reported to process ephrin-A ligand upon its binding to EphA receptor through either *cis* or *trans* interactions [35,36], while ephrin-Bs can be cleaved by the rhomboid family proteases [37]. In addition, proteolytic cleavage of EphB receptors by matrix metalloproteinases and γ -secretase has also been demonstrated [38,39]. Interestingly, the entire receptor-ligand complex can be removed from the cell membrane by *trans*-endocytosis, which can occur in either the cell expressing the receptor or the ligand, resulting in internalization of the entire receptor-ligand complex [35]. The internalized complex can potentially contain other ligand/receptor associated molecules and may retain its signaling capacity [40]. Astrocytes are also capable of *trans*-endocytosis and have been shown to uptake full length EphB2 receptors from neuronal cell surface [41]. In summary, ephrins/Eph interactions can trigger both adhesive and repulsive responses depending on the levels of receptor expression, its interacting ligand and a signaling state of cell at the time of contact.

4. EphB receptor synaptic localization

Consistent with their diverse functions in both developing and mature nervous systems, Ephs and ephrins exhibit highly complex spatial and temporal patterns of expression throughout the CNS [42,43]. Over the course of CNS development, expression levels of Ephs and ephrins vary greatly [44], while displaying precise expression gradients and sharp boundaries, reflecting their roles in topographical mapping and tissue patterning [45,46,47]. On a subcellular level, Ephs are differentially expressed and can be found in axons, dendrites, presynaptic terminals or dendritic spines, depending on the brain region or

developmental stage [48,49]. For instance, EphB2 receptors are preferentially expressed in dendrites and dendritic spines in the hippocampus, whereas they are primarily detected in axon terminals of the ventrobasal thalamus [50]. The same study showed an enrichment of EphA4 at the postsynaptic density and also detected EphA4 positive immunoreactivity on astrocytes [51]. Ephrin-A3 was also found to be expressed in the fine astrocytic processes enveloping synapses, where it interacts with EphA receptors located on dendritic spines [52]. In addition, EphA receptors and ephrin-A ligands are expressed at neuromuscular junctions [53], where they participate in synapse formation and homeostatic plasticity [54,55]. The expression of ephrins and their receptors within the same subcellular microdomain may enhance or inhibit *trans*-cellular ephrin/Eph receptor signaling. Therefore, the regulation of their expression level and specific membrane targeting may potentially determine the signaling outcome. Although the mechanisms regulating the targeting of Ephs and ephrins to specific cellular domains are still under investigation, a recent study demonstrates a role of GRIP1 in EphB2 receptor targeting to the dendritic surface [56]. Since Eph receptors and ephrins can be expressed pre- and postsynaptically and also within the same membrane, it would be interesting to know the biological significance of different combinations of receptors and/or ligands and how they interact to produce specific signaling outcomes. Future work will provide new insights into spatial and temporal control of Eph and ephrin expression, which can potentially determine the selectivity of their signaling.

5. EphB receptors shape dendritic spines

Formation of excitatory synaptic connections directly coincides with dendritic spine morphogenesis that involves actin cytoskeleton reorganization and assembly of post-synaptic specializations. EphB receptors provide for both of these key functions in the developing and mature nervous system.

5.1. Spine formation and maturation

The first evidence of the involvement of EphB receptor signaling in dendritic spine formation demonstrated that inhibition of EphB receptor “forward” signaling by the expression of a kinase-inactive, dominant-negative form of EphB2 in cultured hippocampal neurons impaired dendritic spine formation [57]. This study has also shown that EphB2 can trigger phosphorylation and clustering of heparan sulfate proteoglycan syndecan-2 at postsynaptic sites, which was previously reported to induce dendritic spine maturation in rat hippocampal neurons [58]. Although the events downstream of syndecan-2 activation leading to dendritic spine maturation remain to be elucidated, its interactions with PDZ domain containing adaptor proteins may contribute to postsynaptic differentiation. Further evidence of the involvement of EphB receptors in spinogenesis came from a study of EphB1/B2/B3 receptor knockout mice, showing the effects of single, double and triple EphB receptor knockouts on dendritic spines [59]. Cultured hippocampal neurons from triple EphB1/B2/B3 receptor knockout mice failed to develop mature spines. The *in vitro* results were confirmed by *in vivo* observations showing that dendritic spines exhibited immature morphologies and reduced numbers of post-synaptic specialization in the hippocampus and cerebral cortex of EphB1/B2/B3 triple knockout mice but not in those of single or double knockout mice [59,31]. In contrast, stimulation of endogenous EphB receptors with ephrin-B1 or ephrin-B2 in hippocampal cultures triggered a rapid growth of mature spines that was prevented by the inhibition of EphB kinase activity with a kinase inactive EphB2 [59,60]. An *in vitro* study seeking to further specify the roles of EphB receptors in dendritic spine and synapse development demonstrated that EphB synaptogenic activity is restricted to a period of active synaptogenesis when dendritic filopodia are most abundant. Synapse formation was rescued in cultured cortical neurons derived from EphB1/B2/B3 triple knockout mice by expressing EphB2 at 7 days in vitro (DIV), but not at 14 DIV [61]. The

synaptogenic effects of EphB2 were attributed to its ability to induce filopodial motility through the regulation of actin cytoskeletal organization [61].

5.2. EphB signaling to RhoGTPases

Several members of the Rho family of small GTPases have been identified as primary targets of the signaling pathways that mediate EphB receptor effects on filopodial motility and dendritic spine morphology (Fig. 2). RhoGTPases exist in either a GTP-bound (active) or GDP-bound (inactive) state, and possess intrinsic GTPase activity that can be enhanced by Rho-GEFs (Guanine Nucleotide Exchange Factors) to regulate both the polymerization and the dynamics of filamentous (F)-actin [62]. It was shown that EphB2 phosphorylates the Rho-GEF kalirin-7, which in turn activates Rac1 and p21-activated kinase (PAK) in hippocampal neurons [60]. This pathway was implicated in EphB2-mediated maturation of dendritic spines, which was blocked by inhibition of kalirin-7 with an inactive kalirin-7 mutant, inhibition of Rac1 activity with a dominant-negative form of Rac1 or pharmacological inhibition of PAK activity [60]. EphB2 receptor can also regulate dendritic spine formation through the recruitment and activation of another Rac1 specific GEF Tiam1 [63]. Tiam1 knockdown with specific RNAi or its inhibition with a dominant-negative Tiam1 mutant was shown to inhibit the ability of ephrin-B1 to induce dendritic spine formation in rat hippocampal cultures [63]. Synaptogenic effects of EphB receptor were also attributed to its ability to recruit the RhoGEF intersectin-1 and to activate it through the association with neural Wiskott-Aldrich syndrome protein (N-WASP) [64]. Intersectin-1 activates Cdc42, another member of the RhoGTPases, which can regulate F-actin polymerization and assembly of branched filaments via N-WASP and the Arp2/3 complex [17]. To confirm the relevance of this pathway to spine development, the authors demonstrated that inhibition of intersectin-1, N-WASP or Cdc42 activity impaired the development of mature dendritic spines in cultured hippocampal neurons [64]. In addition to their ability to directly influence RhoGTPase activity through the recruitment and activation of RhoGEFs, EphB receptors were also shown to indirectly affect RhoGTPases through recruitment and assembly of focal adhesion-like complexes [65]. In mouse hippocampal cultures, EphB2 activation by ephrin-B1 or ephrin-B2 was demonstrated to trigger dendritic spine morphogenesis through the assembly of an EphB-associated protein complex that includes focal adhesion kinase (FAK), Src, Grb2, and paxillin and their subsequent activations, potentially initiating a number of downstream signaling pathways [65]. This downstream signaling involved FAK-mediated activation of RhoA as EphB-mediated spine morphogenesis was blocked by the disruption of FAK expression through either Cre-mediated deletion of *loxP*-flanked *fak* or siRNA-mediated knockdown of FAK and RhoA inhibition by overexpression of dominant negative RhoA. These studies demonstrate that EphB-induced spine morphogenesis also depends on FAK-mediated RhoA activation, potentially via p190RhoGEF. EphB dependent filopodia motility was also shown to be regulated through the activation of PAK as impaired filopodia motility was rescued in EphB2 deficient cortical neurons by expression of constitutively active PAK [61]. However, PAK expression was insufficient to restore synapse formation. These studies show that EphB receptor interaction with its ephrins-B ligand was critical for synapse formation, as an EphB2 mutant lacking the ligand binding domain failed to rescue synapse formation. The EphB synaptogenic effects were also shown to be negatively regulated by a RhoA GEF ephexin5, which is expressed on filopodia where it may limit their motility and growth by activating RhoA [66]. In hippocampal cultures, ephexin5 limits excitatory synapse number without influencing spine morphology by restricting synapse-promoting effects of EphB2, as an increase in excitatory synapse density that follows ephexin5 knockdown requires EphB2 kinase activity. In contrast, ephrin-B1-induced EphB2 activation promotes phosphorylation of ephexin5, which triggers ephexin5 degradation mediated by the ubiquitin ligase Ube3A [66]. Although several RhoGTPases and their effectors have been shown to mediate EphB

receptor spinogenic effects in various ways and contexts, the spatial and temporal integration of these different and sometimes opposing pathways is still unknown.

5.3. Spine maintenance and plasticity

While our knowledge of the mechanisms underlying synaptogenic activity of EphB receptors has significantly advanced in recent years, the role of trans-synaptic ephrinB-EphB receptor signaling in maintaining mature dendritic spines and synapses remains unclear. Both ephrin-Bs and EphB receptors are expressed in mature synapses and may function to maintain mature synapses, or alternatively to regulate synaptic plasticity. Recent studies indicate that EphB receptor signaling in synapses is important for the stabilization of mature dendritic spines and inactivation of EphB receptor forward signaling in mature spines leads to their disassembly and remodeling [67]. One potential way how EphB2 could accomplish stabilization of mature spine morphology is by suppressing the activity of the F-actin severing protein cofilin [67]. Cofilin is an actin binding protein that twists and severs F-actin and its activity is negatively regulated by phosphorylation. Low levels of cofilin activity are associated with F-actin turnover in resting cells, whereas motile cells exhibit high levels of cofilin activity [68]. EphB-mediated stabilization of mature spines and synapses relies on the ability of EphB receptors to activate downstream mechanisms that work to discourage cofilin-mediated remodeling of existing dendritic spines. These studies report that the RhoA-ROCK-LIMK-1 pathway becomes active following EphB2 activation, and that LIMK-1 down-regulates cofilin activity thus securing dendritic spine stability. The activation of RhoA-ROCK-LIMK-1 pathway depends on FAK recruitment to the EphB receptor complex and its activation, which is impaired in FAK-deficient hippocampal neurons. The regulation of cofilin activity was also implicated in synapse and spine remodeling [69] indicating that the ability of the EphB receptor to regulate cofilin activity may also underlie its effects on synaptic plasticity.

6. EphB receptors and post-synaptic glutamate receptors

EphB receptors participate not only in the regulation of spine structure and synapse number, but also in establishing functional synaptic specializations. EphB2 directly interacts with NMDA receptors through the NR1 subunit [18]. Activation of EphB2 with ephrin-B1-Fc in cultured cortical neurons was shown to induce EphB2 receptor association with the NMDAR and the assembly of a complex including EphB2, NMDAR, CaMKII and Grb10. The interaction between EphB2 and NR1 occurs through their extracellular domains and does not require EphB2 kinase activity. Long-term stimulation of cultured neurons with ephrin-B1 led to an increase in the number of NR1 postsynaptic sites, but also affected the number of presynaptic release sites. These effects were dependent on EphB2 kinase activity and were abolished by the overexpression of a kinase inactive EphB2 mutant demonstrating the role of EphB2 in the development of functional synaptic specializations [18]. Recent studies have also shown that EphB2 regulates NMDAR trafficking to synapses in a subunit specific manner [70]. EphB receptor activation decreases Ca^{2+} -dependent desensitization of NR2B-containing NMDARs, resulting in an overall increase in NR2B phosphorylation, its surface localization and potentiation of NMDAR currents. Cell surface and total expression of NMDAR subunits was not altered in EphB1/B3 double knockout mice, suggesting that the EphB2 receptor was sufficient for their cell surface expression. Interestingly, triple EphB1/B2/B3 knockout mice exhibited a dramatic increase in total and surface expression of NR2B, whereas synaptic expression of NR2B was significantly reduced [70]. These studies suggest that the EphB receptor may be responsible for synaptic targeting of the NR2B subunit, and the ablation of EphB receptors leads to non-specific trafficking of the NR2B, resulting in overall increased cell surface expression. In agreement with this observation, the authors reported an increase of NR2B synaptic expression in cultured cortical neurons upon ephrin-B2-induced activation of EphB receptors at 14DIV and 21DIV

[70]. Besides the ability to cluster NMDARs and modulate their functions, the EphB2 receptor was also shown to associate with the AMPARs through PDZ domain containing proteins [31]. While overexpression of the EphB2 receptor in cultured cortical neurons enhanced AMPAR clustering, an EphB2 deletion mutant lacking PDZ domain binding motif, which failed to associate with the AMPAR subunits GluR2 and GluR3, did not affect AMPAR clustering or its association with dendritic spines [31]. The EphB2 receptor was also shown to regulate clathrin-mediated endocytosis of AMPARs in a synaptojanin 1 dependent fashion [71]. Stimulation of hippocampal cultures with AMPA or NMDA promoted GluR1 subunit internalization, which was dependent on EphB2-mediated phosphorylation of synaptojanin 1 as overexpression of a kinase inactive dominant-negative EphB2 or a non-phosphorylatable synaptojanin 1 mutant blocked glutamate-induced GluR1 endocytosis [71]. Together, these results indicate that EphB receptors play a role in post-synaptic differentiation by regulating NMDAR and AMPAR trafficking and clustering at the synapse.

7. EphB receptors and synaptic plasticity

Several studies investigated the functional consequences of EphB signaling on synaptic plasticity. It was shown that activation of EphB receptors with ephrin-B2-Fc potentiates Ca^{2+} influx through NMDARs in cortical cultures by stimulating NR2B phosphorylation in a Src-dependent manner [19]. EphB-mediated increase in intracellular Ca^{2+} also leads to phosphorylation of Ca^{2+} /cAMP-responsive element binding protein (CREB), induction of the immediate early gene *c-fos* and increased transcription of two genes, namely *BDNF* and *cpg15*, which are relevant to long-term synaptic plasticity [19]. In a later study, EphB2 was shown to increase Ca^{2+} influx by reducing desensitization of NR2B but not NR2A containing NMDARs resulting in prolonged Ca^{2+} dependent currents [70]. Additionally, functional overexpression of EphB2 was demonstrated to increase both NMDAR and AMPAR components of glutamatergic mEPSCs, which was attributed to the EphB2 ability to target glutamate receptors to synapses. EphB receptors have also been implicated in hippocampal synaptic plasticity, as EphB2 null mice express impaired long term potentiation (LTP) [45,46]. EphB2 dependent plasticity did not require its kinase activity and could be rescued with a kinase deficient EphB2 mutant. Upon further investigation it was revealed that both LTP and long term depression (LTD) rely on ephrin-B reverse signaling and that EphBs only serve as their “ligands” [72,73]. Lastly, Eph receptors share their binding partners, GRIP1 and PICK1 with AMPARs, but whether these interactions have a functional significance remains unclear [74].

8. Ephrin-B “reverse” signaling in spines and synapses

Accumulating evidence indicates the importance of ephrin-B reverse signaling in spine and synapse formation. In primary rat hippocampal cultures, activation of ephrin-B signaling with soluble pre-clustered EphB2-Fc promotes spine morphogenesis, which is achieved through the recruitment of an SH2-SH3 adaptor protein Grb4, and its association with the G protein-coupled receptor kinase-interacting protein 1 (GIT1) [75]. Manipulations that disrupt this signaling, including overexpression of an ephrin-B1 mutant lacking the cytoplasmic domain, treatment with a small ephrin-B interfering peptide or SH2 domain of adaptor protein Grb4, lead to impaired spine morphogenesis and synapse formation [75]. Ephrin-B3 has also been demonstrated to promote formation of glutamatergic synapses in cultured hippocampal neurons by interacting with GRIP1 through its PDZ domain binding motif [21]. However, the density of spine synapses was normal in CA1 hippocampus of ephrin-B3 knockout mice with fewer CA1 dendritic shaft synapses [76]. In contrast, different studies using electron microscopy analysis demonstrated a significant increase in the overall number of CA1 excitatory synapses in both ephrin-B3 KO mice and knock in mice expressing

ephrin-B3lacZ mutant, which lacks the ephrin-B3 intracellular domain, suggesting that these effects were mediated through forward EphB receptor signaling [77]. Interestingly, while there was a significant increase in the overall number of CA1 excitatory synapses, pre- and post-synaptic areas were significantly reduced in the CA1 hippocampus of ephrin-B3 KO as compared to wild type controls. The reduction in the size of pre- and postsynaptic specializations coincided with a decrease in number of NMDAR receptors and the pre-synaptic protein synapsin I in synaptosome fractions isolated from the ephrin-B3 KO hippocampal neurons [77]. It seems that the effects of ephrin-B3 on dendritic spines and synapses are highly influenced by ephrin-B3 expression level, its localization and Eph receptor complement. Recent studies suggest that the level of ephrin-B3 expressed by neighboring neurons may determine synaptic selectivity showing that loss of ephrin-B3 in cortical neurons *in vitro* and *in vivo* does not reduce synapse density, but mixing ephrin-B3 null and wild type neurons causes the null neurons to have fewer synapses. These data demonstrate that the ephrin-B3 expression level provides a signal that enables neurons to control synaptic density in a competitive manner [78]. These interesting findings suggest that synapse development may occur through competitive intermolecular interactions.

In addition, reverse signaling through the ephrin-B ligand can regulate pre-synaptic differentiation through the pre-synaptic adaptor protein syntenin-1 [79]. Significance of ephrin-B reverse signaling in pre-synaptic differentiation was also investigated in an *in vivo* study of the developing *Xenopus laevis* optic tectum [80]. Activation of ephrin-B reverse signaling by EphB2-Fc infusion promoted formation and maturation of pre-synaptic specializations while increasing stability of axon branches. Both effects were blocked by competitive inhibition of ephrin-B signaling with soluble ephrin-B1-Fc [80]. In addition to synaptogenesis, several forms of synaptic plasticity have been attributed to ephrin-B reverse signaling. LTP at CA3 synapses is associated with increased neurotransmitter release and is mediated by ephrin-B3 expressed at pre-synaptic terminals of mossy fibers [72]. In contrast, synaptic plasticity at CA1 synapses depends on NMDA receptors and appears to involve reverse signaling of post-synaptically expressed ephrin-B2 and B3 [73]. Several other studies also reported an involvement of ephrin-B2 and ephrin-B3 reverse signaling in LTP and LTD at the hippocampus, supporting both pre- and post-synaptic ephrin-B signaling [73,81,77].

9. EphA receptors and ephrin-As also contribute to post-synaptic differentiation

EphB forward signaling has been established to be a major player in synapse formation and plasticity, particularly in the context of excitatory spine synapses. Nonetheless, EphA forward signaling and ephrin-A reverse signaling also participate in these functions and deserve mentioning in this otherwise EphB-oriented review. The EphA4 receptor is highly expressed in the dendritic spines of hippocampal neurons, where it plays critical roles in regulating spine morphology, particularly through its interactions with glial ephrin-A3 [6]. EphA4 knockout mice display spine shape distortions, while activation of forward EphA4 signaling induces spine shortening and elimination [52]. The mechanics of EphA4 forward signaling have been investigated in hippocampal slices, where its activation has been shown to induce spine elimination via the serine/threonine cyclin-dependent kinase 5 (Cdk5) in an ephrin-A1 dependent manner [82]. Ephrin-A1-induced activation of EphA4 triggers phosphorylation of Cdk5, which in turn phosphorylates and activates the RhoA-specific GEF ephexin1. Consequently, RhoA activation triggers actin cytoskeletal remodeling and spine retraction [82]. EphA4 has also been shown to regulate spine morphology via the phospholipase C γ 1 (PLC γ 1)-dependent regulation of cofilin [69]. Upon activation with ephrin-A3-Fc, EphA4 recruits and activates PLC γ 1, which releases the actin severing protein cofilin from the plasma membrane, allowing it to depolymerize actin filaments,

resulting in spine shortening and retraction. Ephrin-A3-Fc activation of the EphA4 receptor has been also demonstrated to negatively regulate β 1-integrin signaling by decreasing phosphorylation of the scaffolding protein Crk-associated substrate (Cas), focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2), while also reducing the association of Cas with the Src family kinase Fyn and the adaptor protein Crk [83]. In addition, adhesion of neurons to a substrate coated with the integrin ligand fibronectin was reduced as a result of EphA4 activation [83]. Therefore, the inhibition of integrins, which are known to induce spine growth and elongation [84], may also contribute to EphA4-mediated spine shortening and elimination. Interestingly, synaptic activity can regulate EphA4 receptor signaling through the proteolytic processing of EphA4 by γ -secretase [85]. EphA4 receptor proteolytic cleavage leads to the release of its intracellular portion (EphA4-ICD) into the cytosol, where it activates the Rac1 signaling pathway, enhancing the formation and maintenance of dendritic spines.

In addition to the EphA4 receptor, synaptic functions of other EphA receptors have been also investigated. Most recently, the EphA5 receptor, which is highly expressed during hippocampal development, has been shown to contribute to the early stages of synaptogenesis [86]. Ephrin-A5-induced activation of the EphA5 receptor potentiates voltage sensitive Ca^{2+} channels (VSCCs), leading to an elevation of cAMP levels and a PKA-dependent induction of the expression of NMDAR subunits and PSD-95 via CREB mediated transcription. Furthermore, PKA acts through PI3K to activate cdc42 which leads to filopodia formation, whereas Ca^{2+} dependent CaMKII activation causes the filopodia to mature into dendritic spines. Moreover, P6 hippocampal slices from EphA5 knockout mice failed to produce NMDAR currents [86]. Initially overshadowed by the interest in EphA receptors, the studies of ephrin-A reverse signaling have only recently begun to emerge. Reverse signaling through glial ephrin-A3 activated by the postsynaptic EphA4 receptor was shown to regulate LTP by modulating the expression of glutamate transporters GLAST and GLT-1, thus regulating astrocytic glutamate uptake and perisynaptic glutamate levels [87,88].

10. EphB receptors in neuropathologies

Taking into account their wide distribution and functional diversity both within and outside of the CNS it is not surprising that Ephs and ephrins are considered as potential therapeutic targets for several neurological diseases and cancer [89]. Ephs and ephrins were linked to CNS regeneration primarily due to their role in axon guidance and cell migration [90]. Their ability to regulate synaptic plasticity was recently suggested to underlie the mechanisms of neuropathic pain induced by nerve injury, which triggers hyperexcitability of nociceptive dorsal root ganglion (DRG) neurons and sensitization of dorsal horn (DH) neurons [91]. Ephrin-B1 and EphB1 were found to be upregulated in DRG and the spinal cord in rats following nerve injury. While activation of the EphB receptor with ephrin-B1-Fc lowered the threshold for LTP in synapses between C-fibers and DH neurons and evoked thermal hyperalgesia, blocking EphB receptor activation prevented hyperexcitability of DRG neurons and inhibited sensitization of DH neurons [91]. Decrease in EphB2 and EphA4 expression was also observed in hA β PPswe-ind mice, a mouse model for an Alzheimer's disease (AD), which coincided with the onset of memory decline [92]. Impaired synaptic plasticity, learning and memory deficits were reversed by expressing EphB2 in the dentate gyrus of hAPP mice [93]. These studies also demonstrate that amyloid- β oligomers can bind to the fibronectin repeats of EphB2 and induce its proteasomal degradation, suggesting EphB2 as an excellent therapeutic target for AD.

The complexity that permeates the Ephs and ephrins is not surprising, considering their diverse functions and the intricacies of their signaling mechanisms. The role of Ephs and

ephrins as potential targets for the treatment of neuropathic pain, AD and other neurologic diseases has not yet been explored to its full potential. It is likely that future work will reveal even more functions of Ephs and ephrins in the normal brain, and may shed light on their role in the diseased brain, providing the groundwork for exciting new therapeutic discoveries.

Abbreviations

ADAM10	A disintegrin and metallopeptidase domain 10
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Arp2/3	Actin related protein 2/3
BDNF	Brain-derived neurotrophic factor
CaMKII	Calcium/calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
Cas	Crk-associated substrate
Cdc42	Cell division cycle 42
Cdk5	cyclin-dependent kinase 5
Cpg15	Candidate plasticity gene 15
CREB	Ca ²⁺ /cAMP-responsive element binding protein
Crk	v-crk avian sarcoma virus CT10 oncogene homolog
Eph	Erythropoietin-producing hepatocellular carcinoma
Ephrin	Eph receptor interacting protein
FAK	Focal adhesion kinase
FGF	Fibroblast growth factor
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
GLAST	Glutamate aspartate transporter
GLT-1	Glutamate transporter 1
GPI	Glycosylphosphatidylinositol
Grb	Growth factor receptor-bound protein
GRIP1	Glutamate receptor interacting protein
GTP	Guanosine triphosphate
LIMK	LIM domain kinase 1
LTP	Long term potentiation
LTD	Long term depression
mEPSC	Miniature excitatory postsynaptic current
mGluR	Metabotropic glutamate receptor
MMP9	Matrix metalloprotease 9
N-WASP	Neural Wiskott-Aldrich syndrome protein

NMDA	N-methyl D-aspartate
PAK	p21-activated kinase
PDGF	Platelet-derived growth factor
PDZ	Post synaptic density protein (PSD95), drosophila disc large tumor suppressor (DlgA), zonula occludens-1 protein (zo-1)
PI3K	Phosphatidylinositol 3-kinase
PICK1	protein interacting with PRKCA 1
PKA	Protein kinase A
PLCγ1	phospholipase C γ 1
PSD-95	postsynaptic density-95 kDa
PTP-BL	Protein tyrosine phosphatase BL
Pyk2	proline-rich tyrosine kinase 2
Rac1	Ras-related C3 botulinum toxin substrate 1
ROCK	Rho-associated, coiled-coil containing protein kinase
RTK	Receptor tyrosine kinase
SAM	Sterile α -motif
SH2	Src homology 2 domain
TIAM1	T-cell lymphoma invasion and metastasis 1
Trk	Tropomyosin receptor kinase

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Highlights

Synaptogenic effects of EphB receptors are mediated through cell adhesion

EphB receptors target RhoGTPases to control dendritic spine morphology

EphB receptors participate in establishing functional post-synaptic specializations

New role of ephrin-B/EphB signaling in pathophysiology of Alzheimer's disease and neuropathic pain

Figure 1. EphB receptor structure and interacting proteins

A schematic diagram showing structural organization of ephrin-A, ephrin-B and EphB receptors. EphB receptor interacting partners in synapses include intersectin-1, kalirin-7, Tiam1, Src non-receptor tyrosine kinases, SH2-domain containing proteins and PDZ-domain containing proteins. Sites of EphB receptor cleavage are shown by arrows. Abbreviations: GEF – guanine nucleotide exchange factor; GPI – glycosylphosphatidylinositol; Grb2 – growth factor receptor-bound protein 2; MMP9 – matrix metalloprotease 9; PDZ – post synaptic density protein (PSD95), drosophila disc large tumor suppressor (DlgA), zonula occludens-1 protein (zo-1); SAM – sterile α -motif; SH2- Src homology 2 domain.

Figure 2. EphB forward signaling in postsynaptic sites

A schematic diagram depicts molecular targets and synaptic effects of EphB signaling in postsynaptic sites.