

## REVIEW

# The Role of Ephs and Ephrins in Memory Formation

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## Abstract

The ability to efficiently store memories in the brain is a fundamental process and its impairment is associated with multiple human mental disorders. Evidence indicates that long-term memory formation involves alterations of synaptic efficacy produced by modifications in neural transmission and morphology. The Eph receptors and their cognate ephrin ligands have been shown to be involved in these key neuronal processes by regulating events such as presynaptic transmitter release, postsynaptic glutamate receptor conductance and trafficking, synaptic glutamate reuptake, and dendritic spine morphogenesis. Recent findings show that Ephs and ephrins are needed for memory formation in different organisms. These proteins participate in the formation of various types of memories that are subserved by different neurons and brain regions. Ephs and ephrins are involved in brain disorders and diseases with memory impairment symptoms, including Alzheimer's disease and anxiety. Drugs that agonize or antagonize Ephs/ephrins signaling have been developed and could serve as therapeutic agents to treat such diseases. Ephs and ephrins may therefore induce cellular alterations mandatory for memory formation and serve as a target for pharmacological intervention for treatment of memory-related brain diseases.

**Keywords:** Eph receptor, ephrin, synaptic plasticity, memory formation

## Introduction

Much evidence indicates that long-term memory formation involves alterations of synaptic efficacy (Konorski, 1948; Hebb, 1949; Dudai, 1989; Bliss and Collingridge, 1993; Martin et al., 2000; Tsien, 2000; Kandel, 2001; Lamprecht and LeDoux, 2004). These changes can be mediated by modifying synaptic release of transmitters or synaptic responses to these transmitters. In addition, changes in neuronal morphology regulating synaptic contacts and signal transduction near the synapse can affect synaptic efficacy. A key challenge is to identify molecules involved in initiating and maintaining synaptic alterations and memory formation. Eph receptors and their cognate ephrin ligands are attractive candidates to play a central role in memory formation, as they induce cellular events that underlie changes in synaptic efficacy, such as synaptic transmission and morphology (Klein, 2009; Lai and Ip, 2009; Hruska and Dalva, 2012).

The mammalian Eph receptors family are transmembrane tyrosine kinase receptors that are divided into 2 subgroups: the

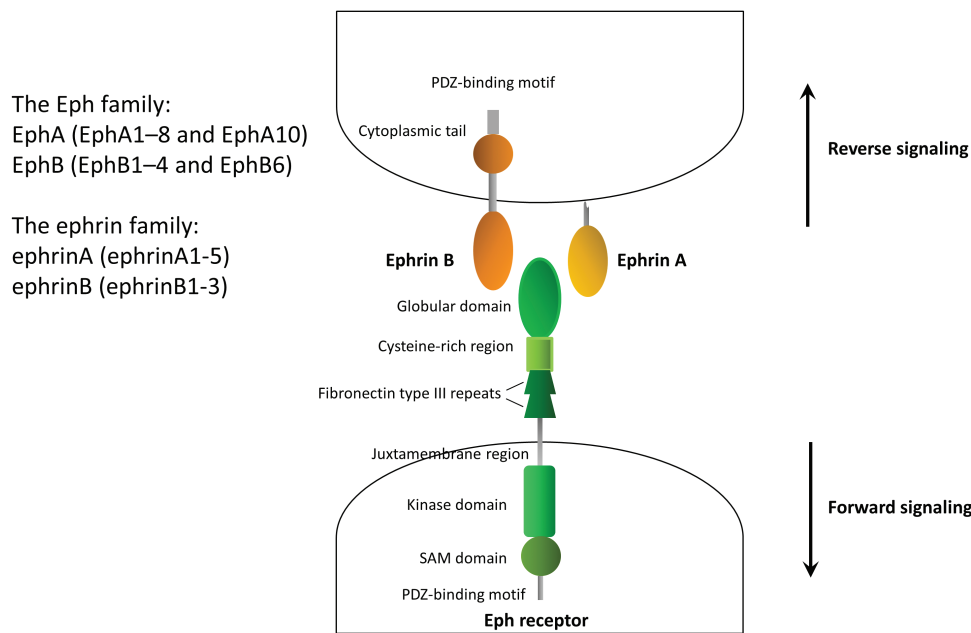
EphA receptors composed of 9 members (Ephrin type-A receptor 1 [EphA1]-8 and EphA10) and the 5 EphB receptors (EphB1-4 and EphB6) (Klein, 2009; Lai and Ip, 2009; Hruska and Dalva, 2012) (Figure 1). EphA receptors typically bind most or all types of ephrinA ligands (Ephrin-A 1-5), and EphB receptors typically bind most or all ephrinB ligands (Ephrin-B1-3). One exception is the EphA4 that can bind to both ephrinA and most ephrinB ligands. Ephrins are also membrane proteins. EphrinA is tethered to the membrane by a glycosylphosphatidylinositol anchor, and ephrinB has a transmembrane domain that is followed by a short cytoplasmic region (Figure 1).

Intracellular signaling can be transferred bidirectionally upon binding of the Eph receptor to its cognate ephrin ligand. Such binding leads to forward signaling through the Eph receptors, which mainly requires catalytic activation of its intracellular kinase. Signaling can also be transferred in a reverse direction through the ligand. EphrinB mediates reverse signaling through modifications of its cytoplasmic tail, such as

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**Figure 1.** Structure of ephrins and Ephs and the possibility for bidirectional signaling. EphrinA and ephrinB proteins are structurally different. EphrinA binds the cell membrane by a glycosylphosphatidylinositol anchor, and ephrinB contains a transmembrane domain that is followed by a short cytoplasmic domain. EphA and EphB receptors have similar regions composition. The Eph receptor globular domain is the ephrin-binding site. Ephrin-Eph interactions can lead to reverse signaling into the ephrin-expressing cell and/or forward signaling into the Eph-expressing cell. EphrinA-mediated reverse signaling requires an association with transmembrane proteins (eg, p75), whereas ephrinB can interact with intracellular proteins to transduce the signals. Binding of ephrin to its cognate Eph receptor can initiate the clustering, tyrosine phosphorylation, and transduction of Eph forward signaling through activation of intracellular signaling pathways.

phosphorylation, that recruits signaling molecules and induce intracellular signaling. EphrinA transfers the reverse signaling by interaction with transmembrane signaling molecules.

Ephrins and Ephs are expressed pre- and/or postsynaptically at the developing excitatory synapse (eg, [Hruska and Dalva, 2012](#)). The roles of Eph/ephrin receptor signaling during development were studied extensively especially in the nervous system (eg, [Egea and Klein 2007](#); [Lai and Ip, 2009](#); [Xu and Henkemeyer, 2012](#)). For example, directing of axons to their distant target tissues is mediated in several instances by the Eph/ephrin reverse or forward signaling that can lead to repulsion or attraction of the axons. Eph/ephrin signals can also regulate the formation and morphology of presynapse, dendrites, and dendritic spines.

Ephs and ephrins are also active in the adult brain. They can be found in presynaptic and postsynaptic sites and act to regulate central neuronal processes such as changes in synaptic transmission and morphology and are intimately involved in synaptic plasticity (eg, [Murai and Pasquale, 2002](#); [Klein, 2009](#); and see below). Changes in synaptic transmission and neuronal morphology are involved in memory formation ([Lamprecht and LeDoux, 2004](#)).

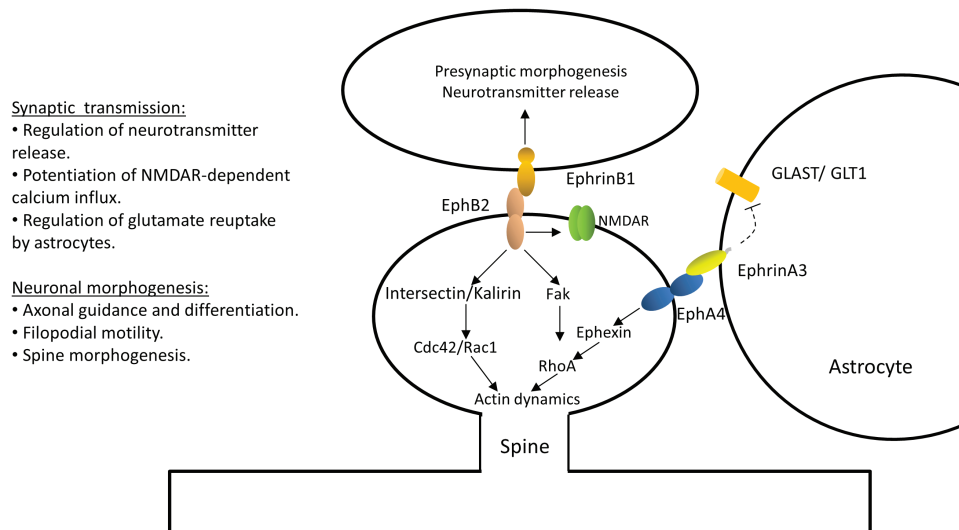
In addition to their action in normal development and cellular functions in adults, Ephs and ephrins are also involved in diseases such as cancer (eg, [Pasquale, 2008](#) and [2010](#)) and brain-related diseases (see below). In cancer, Ephs and/or ephrins are present, frequently with changes of normal expression, in essentially all types of cancer cells. Ephs and ephrins have been shown to affect the growth, migration, and invasion of cancer cells in culture as well as tumor growth, invasiveness, angiogenesis, and metastasis in vivo. Tumor suppressor activities have been reported for Eph signaling. However, reduced tumorigenicity of cancer cells in which Eph receptor expression was decreased implicates that Ephs can also have tumor-promoting effects. Eph gene mutations probably also contribute to cancer

pathogenesis. Ephs and ephrins are involved in brain tumor glioma that arises from glial cells (eg, [Nakada et al., 2011](#)).

This review is focused on the roles of Ephs and ephrins in memory formation. We present observations showing that Ephs and ephrins are needed for memory formation and insights into the functional cross-talk between the molecular and cellular mechanisms of memory and Eph/ephrin signaling. In addition, we describe the involvement of Ephs and ephrins in memory-related brain diseases and the possible pharmacological intervention targeting the Eph binding site for treatment of such diseases.

## Eph Receptors in Memory Formation

Ephs and ephrins are intimately involved in cellular events, such as neuronal morphogenesis and synaptic transmission, believed to be involved in memory formation and retention (see below and [Lamprecht and LeDoux, 2004](#)) ([Figure 2](#)). However, their roles in memory formation were not explored until recent years. Eph receptors are expressed in various brain regions that have been shown to be involved in memory formation such as the hippocampus (eg, mouse: [Grunwald et al., 2001](#); primates: [Xiao et al., 2006](#); human: [Rosenberger et al., 2014](#)), amygdala (eg, mouse: [Grunwald et al., 2001](#); primates: [Xiao et al., 2006](#)), and cortex (eg, rat: [Martone et al., 1997](#); primates: [Xiao et al., 2006](#)). It is therefore possible that Ephs that are involved in synaptic transmission, plasticity, and neuronal morphogenesis (see below), cellular events intimately involved in memory formation, mediate memory formation in these brain regions. Most of the studies focused on the roles of Eph receptors in hippocampal-dependent learning. Studies explored the roles of members of both EphA and EphB receptor families in memory formation. The role of EphA5 in memory formation in hippocampus was tested in mice ([Gerlai et al., 1999](#)). In this study, infusion of EphA5



**Figure 2.** Ephs and ephrins mediate molecular events that may be involved in memory formation. Evidence shows that memory formation involves alterations of presynaptic neurotransmitter release, activation of glutamate receptors, and neuronal morphogenesis. Eph receptors regulate synaptic transmission by regulating synaptic release, glutamate reuptake from the synapse (via astrocytes), and glutamate receptor conductance and trafficking. Ephs and ephrins also regulate neuronal morphogenesis of axons and dendritic spines through controlling the actin cytoskeleton structure and dynamics.

antagonist (EphA5-IgG) bilaterally into the hippocampus of mice for 8 days before training impaired the performance of T-maze continuous spontaneous alternation task compared with control animals injected with a control immunoadhesin CD4-IgG (Gerlai, 1998a). EphA5-IgG-injected mice were also impaired in contextual but not auditory fear conditioning memory. In addition, mice that express a truncated EphA5 receptor lacking a functional tyrosine kinase domain and serve as dominant negative are impaired in 2-way active avoidance learning and a transient deficit in spatial water maze performance (Halladay et al., 2004). Short-term spatial recognition memory examined by a spatial novelty preference task in the Y-maze (Vuilleumot et al., 2011) is impaired in EphA4 knockout mice (EphA4<sup>-/-</sup>) (Willi et al., 2012). These EphA4<sup>-/-</sup> mice are also impaired in spontaneous alternation in the T-maze test (Deacon and Rawlins, 2006). Long-term contextual fear conditioning memory is attenuated in CaMKII-cre;EphA4<sup>lox/-</sup> mice where EphA4 is removed from all pyramidal neurons of the forebrain (Dines et al., 2015). Mutant mice with targeted kinase-dead EphA4 exhibit intact long-term contextual fear conditioning memory, showing that EphA4 kinase-mediated forward signaling is not needed for contextual fear memory formation (Dines et al., 2015). EphA6 KO mice are impaired in long-term contextual fear conditioning memory (Savelieva et al., 2008). Wild-type and EphA6 KO mice did not differ significantly in preconditioning stimulus freezing (before the onset of the tone). Freezing post-CS and difference-CS freezing (differences between pre- and post-CS) were significantly lower in the KO mice. In the hidden platform phase of the Morris water maze (MWM) task, the KO mice did not reach the same level of skill as did wild-type mice. EphB2 knockout mice (EphB2<sup>-/-</sup>) are impaired in the hippocampal-dependent MWM task (Grunwald et al., 2001). EphB2<sup>lacZ/lacZ</sup> mice (Henkemeyer et al., 1996) with targeted expression of a carboxy-terminally truncated form of EphB2 rescued the EphB2 null phenotype indicating that formation of MWM memory is independent of EphB2 kinase signaling (Grunwald et al., 2001). However, it should be noted that several additional observations of the EphB2<sup>-/-</sup> mice, such as impairments in the very first trial and slightly reduced swim speed, make it difficult to distinguish between a mild impairment in learning and a more general performance deficit.

EphB2<sup>-/-</sup> knockout mice are impaired in short- and long-term contextual fear conditioning memory. EphB2 forward signaling was found to be required for long-term, but not short-term, contextual fear conditioning memory formation (Dines et al., 2015).

The aforementioned studies show a role for EphA and EphB receptors in memory formation. In some instances, the Eph receptors (eg, EphB2) regulate memory formation in a kinase-independent manner, suggesting that reverse signaling through ephrins may subserve memory formation. Below are studies exhibiting a role for ephrins in memory formation.

## Ephrins in Memory Formation

Ephrins are expressed in brain regions that have been shown to be involved in memory formation such as the hippocampus (eg, mouse: Carmona et al., 2009; primates: Xiao et al., 2006), amygdala (eg, mouse: Trabalza et al., 2012; primates: Xiao et al., 2006), and cortex (eg, mouse: Theus et al., 2014; primates: Xiao et al., 2006). It is therefore possible that ephrins that are involved in synaptic transmission, plasticity, and neuronal morphogenesis (see below), cellular processes involved in memory formation, mediate memory formation in these brain regions. Both ephrinAs and ephrinBs were shown to be needed for memory formation. EphrinA3 KO mice were tested for cued and contextual fear memory formation (Carmona et al., 2009). The freezing responses to the tone 2 days after conditioning were similar between wild-type and ephrinA3 knockout mice, indicating normal long-term auditory fear conditioning memory. However, the freezing responses to the contextual cues 1 day after training were significantly reduced in the ephrinA3 knockout mice, showing that long-term contextual fear conditioning memory is impaired. The ephrinA3 knockout mice were also impaired in an object placement test. The effects of the ephrinA5-IgG, an agonist immunoadhesin (Winslow et al., 1995; Meima et al., 1997), in hippocampus on memory formation was examined in DBA/2 mice (Gerlai et al., 1999). This mouse strain displays deficits in hippocampal learning tasks and other measures of hippocampal function (Crusio et al., 1990; Matsuyama et al., 1997; Gerlai, 1998b). DBA/2 mice infused with ephrinA5-IgG exhibited an improvement in performance in both T-maze continuous

spontaneous alternation task and contextual fear conditioning paradigms. EphrinB3 was shown to be needed for spatial learning and memory (Rodenas-Ruano et al., 2006). EphrinB3 knockout mice were tested in the MWM task. It took significantly longer for the ephrinB3<sup>KO</sup> mice to locate the hidden platform than wild type mice, suggesting a reduced learning behavior. The performance of ephrinB3<sup>KO</sup> mice differed from both wild type mice and ephrinB3<sup>lacZ</sup> mice with a deletion of the ephrinB3 intracellular region. There was no difference between wild type and ephrinB3<sup>lacZ</sup> mice. These data show a role for ephrinB3 in performing a hippocampus-dependent learning task, independent of its cytoplasmic domain. Mice trained for contextual and auditory fear conditioning showed changes in dendritic morphology in hippocampus and lateral amygdala 24 hours after training compared with naïve or pseudoconditioned mice (Trabalza et al., 2012). Conditioned but not pseudoconditioned or naïve mice showed a specific increase in the amount of ephrinB2 in the hippocampus CA1 region but not cortex or amygdala (Trabalza et al., 2012). These results suggest that an increase in ephrinB2 levels in hippocampal CA1 neurons is involved in the behavioral and neuronal changes induced by contextual fear conditioning.

EphrinA4 is involved in regulation of neuronal morphogenesis (Moss et al., 2005). It was shown that EphA4, involved in synaptic plasticity in amygdala (Deininger et al., 2008), an area that mediates fear memory formation, has a very high (in the range of nmolars) affinity to ephrinA4 (Bowden et al., 2009). To assess possible roles of ephrinA4 in fear memory formation, a specific inhibitory ephrinA4 mimetic peptide (pep-ephrinA4) targeted to EphA binding site was used (Dines and Lamprecht, 2014). This peptide, composed of the ephrinA4 binding domain, interacts with EphA4 and inhibits ephrinA4-induced phosphorylation of EphA4. Microinjection of the pep-ephrinA4 into rat lateral amygdala (LA) 30 minutes before training impaired long- but not short-term fear conditioning memory. Microinjection of a control peptide derived from a nonbinding E helix site of ephrinA4, which does not interact with EphA, had no effect on fear memory formation. Acute systemic administration of pep-ephrinA4 1 hour after training also impaired long-term fear conditioning memory formation. These results demonstrate that ephrinA4 binding sites in LA are essential for long-term fear memory formation.

The aforementioned observations show that ephrins are needed for memory formation. Observations have also suggested that memory in certain instances is mediated by reverse signaling (eg, EphB2; Grunwald et al., 2001). On the other hand, studies have indicated that forward signaling is needed for memory formation (eg, ephrinB3; Rodenas-Ruano et al., 2006). Since ephrins can bind multiple Eph receptors, further studies are essential to identify Ephs-ephrins pairs involved in memory formation. This would advance considerably our understanding of the mechanisms underlying memory, as combinations of different pairs of Eph/ephrin could have different roles in alteration and maintenance of synaptic efficacy after learning since the various Ephs and ephrins have diverse functions at the synapse.

Cumulatively, the aforementioned studies show that Eph receptors and ephrins are intimately involved in memory formation. Several Eph receptors and ephrins have been shown by several studies to be involved in different behavioral paradigms (eg, EphB2 and EphA4), and the roles of other Eph receptors and ephrins were confined to specific behavioral paradigms in single studies. In the later cases, more studies are needed to evaluate whether these receptors have additional roles in different behaviors and types of memories. To further elucidate possible roles of Ephs and ephrins in memory formation, their roles in

controlling synaptic morphology, transmission, and plasticity in neurons are discussed below.

## Ephs and Ephrins Regulate Cellular Processes Involved in Memory Formation

The formation and storage of long-term memory is suggested to be subserved by a sequence of cellular and molecular events (eg, Lamprecht and LeDoux, 2004). During learning, activity-dependent release of glutamate from presynaptic neurons leads to the activation of AMPA receptors (AMPA) and to the depolarization of the postsynaptic neuron. Depolarization occurs locally at the synapse and/or by back-propagating action potentials. Depolarization of the postsynaptic neuron leads to removal of NMDA receptor (NMDAR) inhibition, by Mg<sup>2+</sup>, and to Ca<sup>2+</sup> influx through the receptor. Depolarization also activates voltage-gated calcium channels, another source of synaptic calcium. Calcium influx into the synapse activates kinases, which in turn modulate the activity of their substrates. These substrates contribute to local changes at the synapse, such as morphological alteration through cytoskeletal regulation, trafficking of glutamate receptors, or the transcription of RNA in the nucleus by regulating transcription factors. Transcribed mRNA is translated into proteins that are captured by activated synapses and contribute to stabilization of synaptic changes. Learning may also induce changes in ephrin/Eph functions to regulate these cellular events. As can be seen below, Eph receptors and ephrins may intervene to mediate and regulate each step in these processes regulating synaptic neurotransmitter release, AMPAR and NMDAR function and trafficking, or/and regulating neuronal morphogenesis through the cytoskeleton. Memory consolidation may also recruit Ephs and ephrins that can contribute to the stabilization of molecular changes in synapses, such as at the AMPAR level, and of the morphology of structural-altered and newly formed synapses.

## Eph and Ephrin Regulation of Synaptic Transmission

Memory formation and synaptic plasticity are subserved by changes of synaptic strength. This could be achieved by affecting synaptic release of neurotransmitters and their content at the synapse and/or the level and conductance of synaptic receptors for neurotransmitters. Learning leads to changes in synaptic efficacy. For example, at the presynapse, it was shown that conditioned fear is accompanied by an enhancement of transmitter release at cortico-amygdala synapses (Tsvetkov et al., 2002) and leads to presynaptic facilitation of AMPAR-mediated transmission in LA neurons (McKernan and Shinnick-Gallagher, 1997). At the postsynapse, olfactory discrimination learning induces enhancement in the averaged amplitude of AMPA or GABA receptor-mediated miniature synaptic events in piriform cortex pyramidal neurons (Saar et al., 2012). Learning also leads to alteration in the number of postsynaptic AMPARs (Rumpel et al., 2005; Yeh et al., 2006; Whitlock et al., 2006; Matsuo et al., 2008; Nedelescu et al., 2010). These changes in synaptic AMPAR levels were shown to be essential for memory formation (eg, Rumpel et al., 2005). The NMDAR is also involved in memory formation. For example, the NR2B subunit of the NMDAR was shown to be involved in different types of memory formation (eg, Rosenblum et al., 1997; Tang et al., 1999; Rodrigues et al., 2001).

Ephs and ephrins have been shown to be involved in regulating neurotransmitter release from presynapse, neurotransmitter content at the synapse, and responses to the neurotransmitters at the postsynapse. Ephs and ephrins can affect synaptic

release and neurotransmitter content at the synapse. It has been shown that in the *Xenopus* retinotectal system, activation of ephrinB signaling by EphB2-Fc leads to an enhanced evoked transmission by an early increase in the presynaptic transmitter release and a delayed enhancement of the postsynaptic glutamate responses (Lim et al., 2008). In mice, hippocampus dendritic Ephs and ephrins in astrocytes may control glutamate concentrations near synapses. For example, astrocytes receive a signal from dendritic EphA4 receptors through ephrinA3 at their membrane, which prevents them from upregulating glial glutamate transporter expression and thus regulating the glutamate concentration at the synapse (Filosa et al., 2009). EphA4-mediated ephrin-A3 reverse signaling in astrocytes control glial glutamate transporters and protect rat hippocampal neurons from glutamate excitotoxicity under ischemic conditions (Yang et al., 2014). EphB2 may also be involved in neuroprotection by mediating TNF- $\alpha$  functions (Pozniak et al., 2014).

Eph receptors can also affect synaptic transmission by regulating glutamate receptor conductance and trafficking. Ephs/ephrins may regulate NMDAR functions. EphB2 directly interacts with and promotes tyrosine phosphorylation of NMDA receptor subunit by Src family kinases in dissociated neurons (Dalva et al., 2000; Takasu et al., 2002). Moreover, ephrinB stimulation of EphBs in rat cortical neurons enhances the influx of Ca<sup>2+</sup> through NMDARs by activating a Src family member, which in turn phosphorylates NR2B (Takasu et al., 2002). Another study has shown that Slap, a SH2-SH3 adapter protein, is recruited to synaptic contacts through signaling cascades activated by Eph receptors and can protect cells from aberrant NMDAR activation and can regulate the levels of NMDARs in an activity- and proteasome-dependent manner (Semerdjieva et al., 2013). Ephs/ephrins can also regulate AMPAR functions. EphrinB2 is essential for the stabilization of AMPARs at the cellular membrane (Essmann et al., 2008). Mouse hippocampal neurons from conditional ephrinB2 knockouts showed enhanced constitutive internalization of AMPARs and reduced synaptic transmission. Furthermore, the authors identified the molecular mechanism for the stabilization of AMPARs by ephrinBs, which involves GRIP proteins.

In addition, Ephs and ephrins may affect synaptic transmission through their regulation of gliotransmitters such as serine (that affects NMDAR transmission; eg, Kleckner and Dingledine, 1998) and glutamine (precursor of transmitters glutamate and GABA; Albrecht et al., 2007) that can affect presynaptic and postsynaptic transmission (Zhuang et al., 2010, 2011).

Cumulatively, the aforementioned studies show that Ephs and ephrins are involved in regulating synaptic transmission by affecting pre- and postsynaptic molecular and cellular events that are also involved in synaptic plasticity and memory formation. Additional research is warranted to determine whether Ephs and ephrins are needed for such presynaptic or postsynaptic alterations during and following learning.

### Ephs and Ephrins and Neuronal Morphogenesis

It has been shown that changes in neuronal morphology are associated with memory formation and may be required to modulate neuronal connectivity to form or alter memory (Bailey and Kandel, 1993; Lamprecht and LeDoux, 2004; Holtmaat and Svoboda, 2009). Most excitatory synapses in the brain terminate on spines, which have been the focus of recent studies in mammalian brain. Spines receive most of the excitatory synaptic inputs in the brain, compartmentalize local synaptic signaling pathways, and restrict the diffusion of postsynaptic molecules (Nimchinsky et al., 2002; Lamprecht and LeDoux, 2004; Newpher and Ehlers, 2009). Alteration of the number of spines and/or

their morphology has been proposed to contribute to changes in excitatory synaptic transmission following learning (Lamprecht and LeDoux, 2004). Changes in number and shape of spines were observed after learning. For example, contextual fear conditioning leads to an increase in spine density in the CA1 hippocampal area and in the anterior cingulate cortex (Restivo et al., 2009; Vetere et al., 2011), and auditory fear conditioning leads to an increase in spinophilin-immunoreactive spines in LA (Radley et al., 2006). Postsynaptic density area on a smooth endoplasmic reticulum-free spines increases with fear conditioning, whereas the head volume of these spines decreases (Ostroff et al., 2010). An increase in spine number (density) was detected in the hippocampus 24 hours after trace eyeblink conditioning (Leuner et al., 2003), and an increase in the number of multiple synaptic boutons that formed synapses on spines was also detected in the hippocampus 24 hours after trace eyeblink conditioning (Geinisman et al., 2001). The number of synapses increases in the cerebellum after eyeblink conditioning (Kleim et al., 2002) and in the piriform cortex following olfactory learning (Knafo et al., 2001). Repetitive motor learning leads to coordinated formation of clustered spines (Fu et al., 2012).

Ephs and ephrins are intimately involved in regulating spine morphology (Irie and Yamaguchi, 2004; Klein, 2009; Lai and Ip, 2009; Hruska and Dalva, 2012). Mice that lack EphB receptors exhibit defective dendritic spine formations that are abnormally headless or with small-headed morphology in hippocampus (Henkemeyer et al., 2003). The study further shows that neurons with a kinase-defective truncated EphB2 also exhibit abnormal spine development and that ephrinB2-mediated activation of the EphB receptors facilitates dendritic spine development. The results demonstrate the need for EphB2 forward signaling in spine development. EphAs are also involved in regulating spine morphology. For example, EphA4, which is enriched in dendritic spines of pyramidal neurons in the adult mouse hippocampus, is critical in regulating spine morphology. Activation of EphA4 in hippocampal slices by ephrinA3-Fc decreases spine length and density, and EphA4 inactivation results in spine shape abnormalities (Murai et al., 2003). In addition, this study indicates that forward signaling through EphA4 is critical for maintaining dendritic spine length. Interestingly, ephrinA3 is localized in astrocytes, suggesting that interactions between astrocytic ephrinA3 and neuronal EphA4 are required for regulating spine morphology. Astrocytes may also regulate neurogenesis through Eph/ephrin signaling. For example, it was shown that ephrinB2 in hippocampal astrocytes regulate neurogenesis in vivo (Ashton et al., 2012). Neurogenesis has been suggested to be involved in memory formation (eg, Deng et al., 2010; Gu et al., 2013).

Eph and ephrins were also shown to be involved in dendritic morphogenesis. Knockdown of GRIP1, an AMPAR interacting protein, in cultured hippocampal neurons caused a loss of dendrites (Hoogenraad et al., 2005). The loss of dendrites by GRIP1 knockdown neurons was rescued by overexpression of the extracellular domain of EphB2. Loss of dendrites is detected after overexpression of the intracellular domain of EphB2 and extracellular application of ephrinB-Fc. Neurons from EphB1-EphB2-EphB3 triple knockout mice exhibited abnormal dendrite morphogenesis. Interfering with KIF5-GRIP1 interaction inhibited EphB2 trafficking and impaired dendritic growth. These results indicate an important role for GRIP1 in dendrite morphogenesis functioning as an adaptor protein for kinesin-dependent transport of EphB receptors to dendrites. Another study shows that ephrinB3 in hippocampus functions as a postsynaptic receptor to transduce reverse signals that are required for both long-scale dendrite pruning and short-scale spine maturation (Xu et al., 2011).

Regulation of spine morphology is mediated by actin cytoskeleton enriched in spines (Matus, 2000; Luo, 2002; Ethell and Pasquale, 2005; Tada and Sheng, 2006; Schubert and Dotti, 2007; Honkura et al., 2008; Hotulainen and Hoogenraad, 2010). Indeed, Ephs control signaling molecules in spines that are key regulators of the actin cytoskeleton. Filopodial motility and synapse formation need EphB activation of PAK, a serine/threonine kinase that regulates actin dynamics (Kayser et al., 2008). Thus, EphB-mediated PAK activation may facilitate the filopodial exploration for synaptic partners. EphBs also signal to phosphorylate guanine exchange factors such as Tiam1, kalirin-7, and intersectin, which catalyze the Rho family GTPases Rac1 and Cdc42 into the active state (Irie and Yamaguchi, 2002; Penzes et al., 2003 and Tolia et al., 2007). These GTPases are needed for actin cytoskeleton reorganization and spine morphogenesis (Irie and Yamaguchi, 2002; Penzes et al., 2003, Tolia et al., 2005; Tolia et al., 2007). It was shown that Reelin binds to and activates EphB proteins, thereby inducing receptor forward signaling and functional reorganization of the cytoskeleton in responsive cells (Bouché et al., 2013). Reelin was shown to be needed for memory formation (Levenson et al., 2008). EphA downstream signaling mediating spine morphology also involves in many cases signaling pathways that regulate the actin cytoskeleton. EphrinA stimulation of hippocampal brain slices leads to the recruitment of cyclin-dependent kinase (Cdk5) to EphA4 and to increased Cdk5 kinase activity by tyrosine phosphorylation. Cdk5 is required for ephrinA-induced spine retraction. Cdk5 phosphorylates ephexin1, a Rho GTPase guanine nucleotide exchange factor, which regulates actin reorganization in spines (Fu et al., 2007). EphA4 activation can also modulate spine morphology through inhibiting  $\beta$ 1-integrin activity (Bourgin et al., 2007). In addition, EphA activation leads to activation of phospholipase C $\gamma$ 1 (Zhou et al., 2007). Phospholipase C activity is required for the maintenance of spine morphology and ephrin-induced spine retraction. Furthermore, EphA4 can regulate neuronal morphology through RapGAP (Richter et al., 2007).

Interestingly, the actin cytoskeleton is essential for memory formation (Lamprecht, 2014). Further investigation is needed to elucidate whether the regulation of actin cytoskeleton by Ephs/ephrins is required for memory formation.

### Ephs and Ephrins Are Required for Synaptic Plasticity Leading to LTP and LTD

The roles of Ephs and ephrins were studied in long-term potentiation (LTP) and long-term depression (LTD), physiological models of memory (eg, Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Martin et al., 2000; Gerlai, 2001). Findings relate LTP and LTD with learning and memory (eg, Lynch, 2004; Collingridge et al., 2010). For example, studies suggest that LTP occurs in the LA and hippocampus during fear conditioning. LTP induction at thalamic auditory inputs to the LA facilitates auditory-induced responses in the LA similarly to the increase of CS-evoked responses observed during auditory fear conditioning (Rogan and LeDoux, 1995). In addition, fear conditioning alters auditory CS-evoked responses in LA in the same way as LTP induction (Rogan et al., 1997). Thalamic inputs to the LA were enhanced in brain slices from trained animals compared with naive or unpaired animal groups (McKernan and Shinnick-Gallagher, 1997). Moreover, fear conditioning inhibits the induction of LTP at cortical inputs, suggesting that LA synapses that have already undergone potentiation by training are less capable of inducing LTP (Tsvetkov et al., 2002; Schroeder and Shinnick-Gallagher, 2004; Schroeder and Shinnick-Gallagher, 2005). Inhibitors and

genetic manipulation that impair hippocampal LTP also block hippocampal learning and memory retention. Intraventricular injection of the NMDAR antagonist AP-5 impaired hippocampal LTP and MWM memory formation (Morris et al., 1986), and mice in which the deletion of the NMDAR1 gene was restricted to the hippocampal CA1 pyramidal cells were impaired in LTP in the CA1 synapses and in spatial memory (Tsien et al., 1996). Studies have shown that contextual fear conditioning increased synaptic responses in hippocampal CA1 (eg, Sacchetti et al., 2001) and that contextual fear conditioning altered the ability to induce LTP in hippocampus (Sacchetti et al., 2002). LTD is also implicated in learning and memory (eg, Collingridge et al., 2010). For example, blocking the interactions between GluR2 and AP2 impaired LTD in perirhinal cortex in vitro and produced striking deficits in visual object recognition memory (Griffiths et al., 2008).

Eph and ephrin were shown to mediate synaptic plasticity through LTP and LTD studies. At the CA3-CA1 synapse, EphB2 mediates long-lasting LTP and LTD in a kinase-independent fashion (Grunwald et al., 2001). EphB2 interacts and regulates the NMDA receptor through its kinase activity (Dalva et al., 2000; Takasu et al., 2002). It is therefore possible that the interaction of EphB2 with NMDA receptors mediates changes in synapses underlying LTP and LTD formation. Mossy fiber-CA3 LTP is impaired after perfusion of postsynaptic neurons with peptides and antibodies that interfere with binding of EphB receptor to GRIP and by application of ephrinB that increased basal excitatory transmission and occluded both tetanus-induced synaptic potentiation (Contractor et al., 2002). It is therefore possible that EphB2 clustering of AMPARs via PDZ interaction with GRIP mediates LTP. EphA4 was shown to be required for the early stages of LTP at the CA3-CA1 synapse in a kinase-independent fashion (Grunwald et al., 2004). EphA4 receptor is also required for synaptic plasticity in the amygdala (Deininger et al., 2008). EphA4 may mediate LTP through astrocytic ephrinA3. EphrinA3 is needed for the formation of hippocampal LTP (Filosa et al., 2009). Furthermore, EphA4 restricts the expression of glial glutamate transporters, presumably by interacting with ephrinA3 (Carmona Carmona et al., 2009; Filosa et al., 2009). Moreover, glutamate uptake by astrocytes in response to stimuli leading to LTP was significantly elevated in *EfnA3*<sup>-/-</sup> mice, and clearance of glutamate is more efficient in the EphA4 mutants, possibly because of the upregulation of glial glutamate transporters in astrocytes (Filosa et al., 2009). The authors performed further studies, suggesting that the impaired LTP observed in EphA4 and ephrinA3 KO mice is due to the reduced levels of glutamate near synapses, caused by increased glutamate transport in astrocytes.

EphrinB2 is enriched on the postsynaptic side in hippocampus (Grunwald et al., 2004). EphrinB2 and ephrinB3 are required for LTP and LTD (Grunwald et al., 2004; Rodenas-Ruano et al., 2006). Of note, however, is that another study found no impairment in LTP in ephrinB3 KO but impairment in mice where the ephrinB3 cytoplasmic C-terminal is replaced with beta-galactosidase (Armstrong et al., 2006). EphrinB2, when bound with its cognate Ephs, becomes tyrosine phosphorylated by Src family kinases (Palmer et al., 2002). It was shown that tyrosine phosphorylation sites in ephrinB2 are required for hippocampal LTP, but not LTD and that ephrinB2 lacking the C-terminal PDZ interaction site, but that can undergo tyrosine phosphorylation, cannot mediate either form of plasticity (Bouzioukh et al., 2007). In the *Xenopus* retinotectal system, ephrinB1 is enriched in axon terminals. EphB2-Fc application triggers ephrinB1-mediated enhanced transmitter release and facilitates theta burst stimulation-induced LTP (Lim et al., 2008).

EphB receptors are also involved in synaptic transmission and plasticity related to hyperalgesia (Sheffler-Collins and Dalva, 2012). For example, ephrinB2-Fc injected intrathecal (i.t.) to the lumbar spinal cord resulted in a short latency and prolonged thermal hyperalgesia, suggesting that EphB activation in dorsal horn (DH) neurons induces thermal hyperalgesia (Battaglia et al., 2003). The effect of ephrinB2-Fc-induced hyperalgesia was abolished in animals pretreated with the NMDA receptor antagonist MK-801. EphB1-Fc, an EphB1 blocking molecule, injected i.t. before formalin injection significantly reduced pain-related behavior. Other studies using EphB1<sup>-/-</sup> knockout mice show that EphB1 mediates neuropathic pain responses (Han et al., 2008) and the development and/or maintenance of thermal and mechanical hyperalgesia and spontaneous pain in a variety of pain models (Cibert-Goton et al., 2013). Zhao et al. (2010) examined ephrin-B2-mediated signaling in pain pathways by deleting ephrin-B2 from Nav1.8-expressing nociceptors with the Cre-recombinase-loxP system. They show that inflammatory pain was attenuated in ephrin-B2 mutant mice. Thermal hyperalgesia and mechanical allodynia were significantly reduced in the Seltzer model of neuropathic pain. Song et al. (2008) showed that i.t. injection of blocking reagents for EphB-receptors, EphB1-Fc and EphB2-Fc, inhibited the induction and maintenance of nerve injury-induced thermal hyperalgesia and mechanical allodynia. These blockers also inhibited the nerve injury-induced hyperexcitability of nociceptive small dorsal root ganglia neurons, sensitization of DH neurons, and LTP of synapses between C fibers and DH neurons. In uninjured animals, i.t. injection of EphB receptor activators induced thermal hypersensitivity and lowered the threshold for LTP, while EphB1-Fc prevented induction of the LTP. Liu et al. (2009) studied LTP at synapses of C-fibers onto neurons in the superficial DH following high-frequency stimulation of a peripheral nerve at an intensity that activates C-fibers. They show that intrathecal pretreatment of EphB2-Fc or targeted mutation of EphB1 receptor prevented LTP.

Cumulatively, the aforementioned studies show that the Ephs and ephrins serve as regulators of synaptic plasticity, possibly by affecting synaptic morphology and transmission and thereby tuning synaptic efficacy. Furthermore, Ephs and ephrins are intimately involved in synaptic plasticity in brain regions needed for memory formation such as the amygdala and hippocampus.

## Conclusions and Insights From Behavioral and Cellular Studies of Ephs and Ephrins

Several conclusions and insights can be drawn from the above observations: 1) Eph receptors and ephrins subserve the formation of different types of memory (eg, spatial memory such as MWM and aversive memory such as fear conditioning). These observations suggest that different types of memory are mediated by similar cellular events subserved by Ephs/ephrins. This is not unique to Ephs/ephrins, as other pathways are found to underlie various behaviors and memories in different organisms (eg, CREB-mediated transcription; Lamprecht, 1999). 2) Studies show that different Ephs or ephrins are involved in the same behavioral paradigms subserved by defined brain regions. The need for multiple Eph receptors in a particular memory (eg, contextual fear conditioning) may stem from the different roles they play in neuronal functions and plasticity, including effects on different neurons or parts of the neuron (eg, Bouvier et al., 2008), different effects on synaptic transmission, and intracellular signaling (Klein 2009) (see below). Thus,

multiple cellular events mediated by Ephs and ephrins are probably needed for encoding memory. A better spatiotemporal control of Ephs/ephrins activities in adult brain is needed to shed additional key insights into the mechanisms of Eph receptors and ephrins in memory formation. For example, the use of cre/lox system (eg, ephrinB2 in Zhao et al., 2010; EphA4 in Filosa et al., 2009) to manipulate the expression of Ephs and ephrins in distinct neuronal population within a brain region could contribute to such research. 3) Both reverse and forward signaling are needed for memory formation. The identity of the Ephs and ephrins pairs involved in particular memory remain to be clarified to gain insights into their synaptic function. 4) Eph receptors and ephrin ligands are involved in basic fundamental functions such as synaptic transmission and neuronal morphogenesis. However, studies show that interfering with Ephs/ephrins functions has no effect on basal synaptic transmission but specifically on synaptic plasticity and memory formation (eg, Gerlai et al., 1999; Grunwald et al., 2001). A tenable hypothesis is that Ephs/ephrins affect synaptic properties needed specifically for alterations in synaptic efficacy in a stimuli-dependent manner. These stimuli may be related to learning or stimuli leading to synaptic plasticity. 5) Interruption of Ephs/ephrins mediated functions is sufficient for disruption of memory formation. Other cellular functions shown to be essential for memory formation, such as protein synthesis, can be mediated by Ephs/ephrins or are needed for memory formation in addition to Ephs/ephrins (at same or different time points).

## Ephrins and Ephs in Brain Diseases Affecting Memory and Cognition

Ephs and ephrins malfunctions in brain are associated with the development of brain diseases that are associated with memory dysfunction. In this review, we focus on the roles of Ephs and ephrins in Alzheimer's disease and stress. Drug addiction and the CranioFrontoNasal syndrome also involve alterations in memory formation and are associated with Ephs and ephrins dysfunction. Consumption of drugs of abuse can form or alter existing memory (Hyman et al., 2006) and may involve Ephs and ephrins. In rodents, upregulation of Ephs and ephrins is observed in the ventral tegmental area and in the nucleus accumbens and nigrostriatal and mesolimbic pathways after drug treatments (cocaine and amphetamine: Yue et al., 1999; cocaine: Bahi and Dreyer, 2005). Pups injected either chronically or acutely with cocaine significantly increased the expression of EphB1 in the cortex, striatum, and substantia nigra (Halladay et al., 2000). In addition, disruption of EphA/ephrinA signaling in the nigrostriatal system dissociates behavioral responses to amphetamine and cocaine (Sieber et al., 2004). In monkeys, EphA4 level is denser in dorsal putamen than in ventral putamen in the chronic cocaine group compared with the acute cocaine-injected group (Xiao et al., 2006). In CranioFrontoNasal Syndrome, ephrinB1 is mutated (Twigg et al., 2004; Wieland et al., 2004; Wieland et al., 2007). This syndrome is characterized by severe hypertelorism, frontonasal dysplasia, craniosynostosis, and developmental delays. A mouse model of CranioFrontoNasal syndrome deficient of ephrinB1 is impaired in nonspatial learning and memory tasks (Arvanitis et al., 2014).

## Alzheimer's Disease

Alzheimer's disease (AD) is a progressive brain disease that slowly destroys memory and thinking skills and eventually even the ability to carry out the simplest tasks. Amyloid- $\beta$  (A $\beta$ ) is the

major component of senile plaques (Glennner et al., 1984), a hallmark of AD, and is generated from proteolysis of the  $\beta$ -amyloid precursor protein by  $\beta$ -secretase and  $\gamma$ -secretase activities (Checler, 1995; Thinakaran and Koo, 2008). The mechanisms of  $A\beta$ -dependent neural dysfunction and degeneration, including impairment of synapses and cellular toxicity, remain to be clarified. Several studies have shown that  $A\beta$  may induce Eph receptor reduction and that Eph receptors may be involved in cellular processes underlying neuronal dysfunctions observed in AD. Moreover, these studies show that regaining Eph receptor function may protect against  $A\beta$  oligomer neurotoxicity and serve as potential therapeutic targets in AD pathogenesis. Below, we provide several examples for the role of Eph receptors in AD, suggest cellular mechanisms for their involvement in AD, and provide evidence that regulation of Eph receptors may rescue AD symptoms (for a recent review, see Cissé and Checler, 2015).

Several studies have shown that Eph receptor levels are reduced in mouse models for this disease. For example, reduction in Eph receptors EphA4 and EphB2 in the hippocampus was shown to occur before the development of impaired object recognition and spatial memory in AD mouse model overexpressing human amyloid-beta protein precursor (hA beta PP) with familial mutations (hA beta PP swe-ind mice) and similarly in transgenic A beta PP mice, Tg2576 (Simon et al., 2009). A mild reduction in EphB2 was observed later in aged (13 months) wild-type mice in hippocampus and olfactory bulb. These results suggest that changes in Eph receptors may play a role in synaptic dysfunction in the hippocampus, leading to cognitive impairment in a model of AD. EphB2 is reduced in the olfactory bulb and the hippocampus, and its cellular localization is changed in cortex in an age-dependent manner in AD mouse model Tg2576. This reduction of EphB2 appeared earlier than that of MAP2, a dendritic cytoskeleton marker (Qu et al., 2013). Moreover, a recent study has shown that amyloid- $\beta$  oligomers bind to the fibronectin repeats domain of EphB2 and trigger EphB2 degradation in the proteasome (Cissé et al., 2011). Similar effects were shown in a cellular model of AD.  $A\beta$ 1–42 oligomer application to cultured hippocampal neurons induced neurotoxicity in a time-dependent manner and resulted in a major decrease of EphB2 (Geng et al., 2013).

Interestingly, Eph receptors and their cognate ligands can rescue neurotoxicity in neurons induced by  $A\beta$ . Increasing EphB2 expression in the dentate gyrus of human amyloid precursor protein (hAPP) mice rescued the deficits in MWM spatial memory and the nonspatial novel place recognition memory test (Cissé et al., 2011). Furthermore, the authors showed that expression of EphB2 in dentate gyrus rescued deficits in passive avoidance learning observed in hAPP mice. Moreover, it was shown in a cellular model of AD that EphB2 overexpression could prevent the neurotoxicity of hippocampal neurons from exposure to  $A\beta$ 1–42 oligomers for 1 hour (Geng et al., 2013). EphB2 inhibits  $A\beta$ 1–42 oligomer-induced decrease of synaptic NR1 and NR2B expression and prevents  $A\beta$ 1–42 oligomer-induced decreased dephospho-p38 MAPK and phospho-CREB. The authors suggest that EphB2 protects hippocampal neurons against the toxicity of  $A\beta$ 1–42 oligomers by increasing the synaptic NMDA receptor level and downstream p38 MAPK and CREB signaling in hippocampal neurons. In conclusion, these studies show that depletion of EphB2 is critical in amyloid- $\beta$ -induced neuronal dysfunction associated with AD and that increasing EphB2 levels or function could be a beneficial treatment of this disease.

Eph receptors may also protect AD from excitotoxicity due to excessive activation of glutamate receptors, a neurotoxic

mechanism implicated in the pathogenesis of neurodegeneration including AD (Greenamyre and Young, 1989). A recent study shows that ephrinB rescues primary cortical neuronal cultures from cell death induced by glutamate excitotoxicity, a function that depends on EphB (Barthet et al., 2013). This neuroprotection depends on presenilin 1 (PS1), a protein that plays key roles in AD pathology. Moreover, it was shown that absence of PS1 decreases cell surface expression of EphB2 without affecting total cellular levels of the receptors and that PS1-knockout neurons show defective ligand-dependent internalization and degradation of Eph receptors.

Another Eph receptor that can be affected during AD is EphA4. Gamma-secretase dysfunction is evident in many cases of early onset familial Alzheimer's disease. Inoue et al. (2009) identified EphA4 as a substrate of gamma-secretase and found that EphA4 processing is enhanced by synaptic activity and suggested that the processing of EphA4 by gamma-secretase can affect the pathogenesis of AD.

Alteration in ephrin and eph functions is also found in humans with AD. For example, EphA1 is documented to be one of the most strongly associated locus with Alzheimer's disease (AD). A recent study utilizing cerebrospinal fluid and neuroimaging biomarkers suggests that EphA1 interferes with the pathological alteration of the hippocampus and the lateral occipitotemporal and inferior temporal gyri throughout the AD process, leading to a lower risk of AD (Wang et al., 2015). In another study, a reduction in EphB2 and EphA4 receptor levels was found in postmortem hippocampal tissue from patients with incipient AD (Simon et al., 2009).

Eph receptor decrease may affect neuronal dysfunctions in AD through several cellular and molecular mechanisms. First, changes in Eph receptors may affect neuronal dysfunction by affecting excitatory transmission. Soluble  $A\beta$  oligomers may contribute to learning and memory deficits in AD by inhibiting NMDA-receptor-dependent LTP, a physiological model of memory (Kamenetz et al., 2003; Walsh and Selkoe, 2004; Shankar et al., 2008). Moreover,  $A\beta$  reduced NMDAR subunit NR1 in neuronal cell culture and hAPP transgenic mice (Snyder et al., 2005). NMDA-type glutamate receptors are regulated by EphB2 (Dalva et al., 2000; Takasu et al., 2002). Knockdown of EphB2 decreased surface levels of NMDAR subunit NR1, reduced NMDAR currents, and impaired LTP in the dentate gyrus. Increasing EphB2 expression in the dentate gyrus of hAPP transgenic mice rescued deficits in NMDAR-dependent LTP (Cissé et al., 2011). Second, Eph receptors can affect downstream effectors essential for neuronal morphogenesis, such as dendritic spine structural plasticity, needed for normal function of neurons and for learning and memory (Lamprecht and LeDoux, 2004). Dendritic spine loss represents the best pathological correlate to the cognitive impairment in AD patients (Cavallucci et al., 2012). For example, EphB receptor-dependent activation of the Rho-guanine exchange factor Kalirin induces dendritic spine morphogenesis (Penzes et al., 2003), and Kalirin7 may be involved in AD, as it is significantly diminished in the hippocampus of AD patients relative to controls (Youn et al., 2007). Another protein affected by Eph receptors that is reduced in AD model mice is the actin cytoskeletal regulatory protein cofilin, which also affects spine morphology (Zhou et al., 2012; Rust, 2015). A decrease in membrane-associated phospho-cofilin levels was observed at the time of onset of memory decline in hA beta PP swe-ind mice, mice that have a reduction in Eph receptors (Simon et al., 2009). Third, changes in Eph receptor activity may be involved in affecting neuronal neurogenesis in AD brains. Adult neurogenesis



is compromised in AD (for review, see [Ming and Song, 2005](#); [Winner et al., 2011](#)). For example, EphB1 receptors regulate different aspects of neurogenesis, such as the level of neural progenitors in the hippocampus, polarity, cell positioning, and proliferation (eg, [Chumley et al., 2007](#)).

### Anxiety Disorders

The formation and storage of fear memory is needed to adapt behavior and avoid danger during subsequent fearful events. However, severe stress can lead to neuronal alterations and impairments of fear memory formation (eg, [Conrad et al., 1999](#)), generalization of fear, and high anxiety ([Lupien et al., 2009](#)). A recent study implicated EphB2 in stress-related plasticity in amygdala and anxiety-like behavior ([Attwood et al., 2011](#)). This study shows that stress leads to a neuropsin-dependent cleavage of EphB2 in the amygdala followed by dissociation of EphB2 from the NR1 subunit of the NMDAR and membrane turnover of EphB2 receptors. Dynamic EphB2–NR1 interaction enhances NMDAR current, induces Fkbp5 gene expression, and enhances behavioral signatures of anxiety. Stress-induced decrease in the EphB2–NR1 interaction was not seen in neuropsin knockout mice. Moreover, stress caused an increase in anxiety in wild-type mice as measured by open field and elevated-plus maze tests, whereas neuropsin<sup>-/-</sup> mice did not develop anxiety after stress. The development of anxiety was hindered by blocking EphB2 in the amygdala of wild-type mice.

The above examples show that Ephs are involved in brain disorders that present memory impairments symptoms. Furthermore, changes in Eph activity in adults can contribute to the development of such disorders. It is therefore useful to develop drugs that can modulate Eph and ephrin activity for the treatment of such diseases.

### Ephs and Ephrins as Targets for Treatments of Memory Impairments

Ephs and ephrins have been implicated in brain disorders including AD ([Cissé and Checler, 2015](#)) and anxiety ([Attwood et al., 2011](#)). Developing drugs that can reverse the impairments in Eph and ephrin signaling at the critical time window could be beneficial for the treatment of such diseases. For example, blocking EphB2 functions may be beneficial for patients that suffer from anxiety, whereas activation of EphB2 could help with memory impairments in AD patients. It would therefore be very useful to develop such therapeutic drugs for the treatment of these diseases. Indeed, several approaches have been taken to develop such drugs ([Boyd et al., 2014](#); [Lamminmäki et al., 2015](#)), mostly targeting the binding sites of ephrin to Eph receptor. For treatment of brain disorder, an ideal drug should cross the blood brain barrier (BBB) for systemic application and have specific effects on the disease with minimal side effects.

A variety of small molecule inhibitors of ephrin binding have been developed, which might provide the basis for therapeutic of brain diseases. For example, 2,5-dimethylpyrrolyl benzoate ([Noberini et al., 2008](#)), disalicylic acid-furanyl derivative ([Noberini et al., 2011](#)), and lithocholic acid derivatives ([Giorgio et al., 2011](#)) compete for ephrinA binding to various EphA receptors. Small molecules are useful, as they can potentially pass the BBB more readily. Other approaches include the application of ephrin mimetic peptides that bind Eph receptors and block ephrin binding to Eph receptors (eg, [Lamberto et al., 2012](#)). For example, systemic injection of ephrinA4 mimetic peptide was shown to inhibit long-term fear conditioning memory formation

and can be a potentially useful drug for the treatment of stress-related disorders such as PTSD ([Dines and Lamprecht, 2014](#)). In addition, the use of antibodies against Eph receptors ([Mao et al., 2004](#)) is also a useful approach for the inhibition of Eph receptor activity but may exhibit a problem in crossing the BBB. Activation of Eph receptors could be achieved by application of soluble ephrins (eg, [Noren et al., 2004](#)), but here also the development of small molecules that pass the BBB easily is necessary.

### Future Research

Evidence indicates that Ephs and ephrins are involved in memory formation. However, key questions remain unresolved. For example, are the morphological changes shown to be mediated by ephrins and Ephs needed for memory formation? Such changes may include alteration of spines and axonal morphology. Do Ephs and ephrins regulate changes in synaptic transmission needed for memory formation? If so, are they related to presynaptic alterations (eg, changes in neurotransmitters release) or postsynaptic alterations (eg, changes in receptor trafficking and conductance) or to both? Will application of drugs that regulate ephrin and Eph function treat brain diseases where ephrins and ephs are involved? Studies aimed to elucidate such questions will undoubtedly provide key insights into the roles of Ephs and ephrins in memory and also on the cellular processes essential for memory formation and greatly contribute to a better understanding of the intricate molecular and cellular processes governing memory formation. Such studies may lead to the cure of Eph- and ephrin-regulated brain diseases.

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### Interest Statement

None.

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