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SYMPOSIUM REVIEW

Guiding synaptic plasticity: Novel roles for netrin-1 in synaptic plasticity and memory formation in the adult brain

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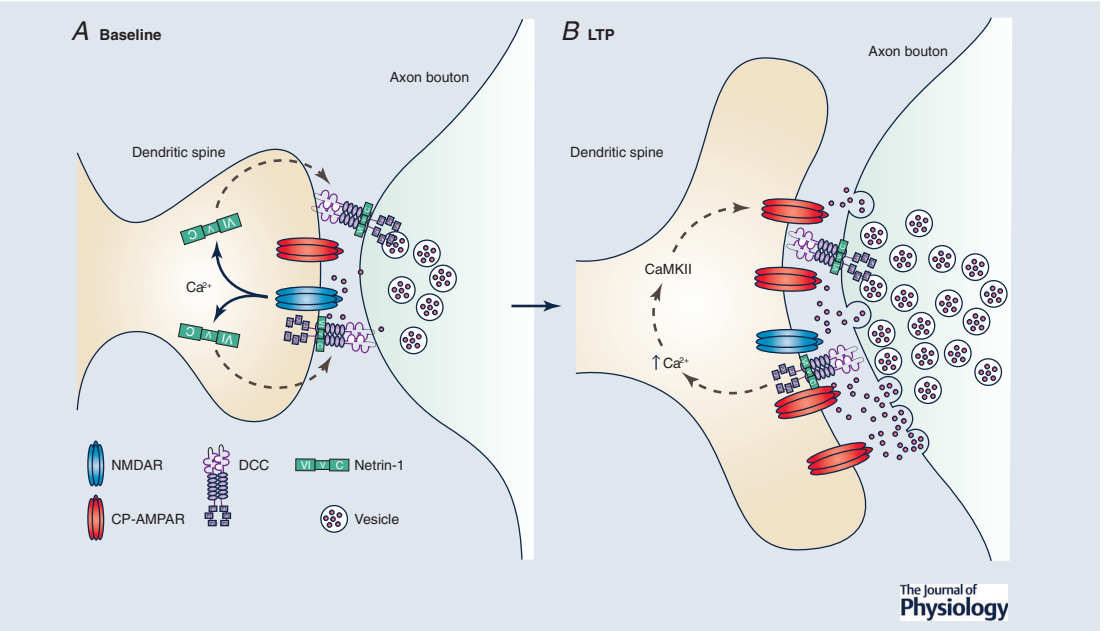

Abstract Adult neural plasticity engages mechanisms that change synapse structure and function, yet many of the underlying events bear a striking similarity to processes that occur during the

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initial establishment of neural circuits during development. It is a long-standing hypothesis that the molecular mechanisms critical for neural development may also regulate synaptic plasticity related to learning and memory in adults. Netrins were initially described as chemoattractant guidance cues that direct cell and axon migration during embryonic development, yet they continue to be expressed by neurons in the adult brain. Recent findings have identified roles for netrin-1 in synaptogenesis during postnatal maturation, and in synaptic plasticity in the adult mammalian brain, regulating AMPA glutamate receptor trafficking at excitatory synapses. These findings provide an example of a conserved developmental guidance cue that is expressed by neurons in the adult brain and functions as a key regulator of activity-dependent synaptic plasticity. Notably, in humans, genetic polymorphisms in netrin-1 and its receptors have been linked to neurodevelopmental and neurodegenerative disorders. The molecular mechanisms associated with the synaptic function of netrin-1 therefore present new therapeutic targets for neuropathologies associated with memory dysfunction. Here, we summarize recent findings that link netrin-1 signalling to synaptic plasticity, and discuss the implications of these discoveries for the neurobiological basis of memory consolidation.

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Abstract figure legend Netrin-1 release during long-term potentiation (LTP) mediates structural and functional plasticity. *A*, repeated presynaptic release of glutamate triggers postsynaptic depolarization and activation of NMDA receptors, which in turn facilitates Ca^{2+} influx. This results in postsynaptic secretion of netrin-1, which binds to pre- and postsynaptic deleted-in-colorectal cancer (DCC). *B*, binding of netrin-1 to DCC triggers further Ca^{2+} increases and activation of CaMKII, which results in postsynaptic insertion of GluA1-containing AMPA receptors and may increase the number or density of presynaptic glutamate vesicles.

Memory, the ability of organisms to store and recall information, is a fundamental adaptive process that defines experience, directs future behaviour, and ultimately serves as a central component of self-identity. Consolidation of memory is associated with changes in the relative strength of synapses, highly specialized structures that permit electrical and chemical communication between interconnected cells within neuronal circuits (Bailey *et al.* 2015). Synapse formation and stabilization, and the selective pruning of synapses, are necessary for the development and initial refinement of neural circuits (Katz & Shatz, 1996; Paolicelli *et al.* 2011). How memories form remains incompletely understood; however, the mechanisms that govern synapse formation and pruning during development have long been hypothesized to contribute to the synaptic modifications associated with plasticity in the mature nervous system (Ramón y Cajal, 1899).

Discoveries over the past 30 years have identified and characterized highly conserved secreted chemoattractant and chemorepellent axon guidance cues that are essential for normal embryonic neural development (Kolodkin & Tessier-Lavigne, 2011). In addition to directing axon extension, several of these protein families have more recently been implicated in synaptogenesis (Kolodkin & Tessier-Lavigne, 2011; Goldman *et al.* 2013; Koropouli & Kolodkin, 2014). Similar to axon guidance, signal transduction by these cues during synaptogenesis results in the activation of intracellular mechanisms that direct cytoskeletal reorganization, while also promoting synaptic trafficking of glutamate receptors to rapidly influence excitatory synaptic transmission (Goldman *et al.* 2013; Yamashita *et al.* 2014; Goshima *et al.* 2016; Wang *et al.* 2017; Glasgow *et al.* 2018). While it was apparent early on that a number of these guidance cues and receptors are expressed in the adult nervous system, their contribution to functional and structural synaptic plasticity in the adult brain is only now beginning to be investigated. This review highlights recent findings that reveal novel roles for the chemotropic attractive guidance cue netrin-1 and netrin receptors in synaptic plasticity and memory formation in the mammalian brain.

Molecular mechanisms underlying synaptic plasticity

Synapses exhibit an immense propensity for plasticity, and can be modified in receptor composition, morphology, and density. High-frequency stimulation of excitatory synapses triggers a form of synaptic plasticity termed long-term potentiation (LTP) (Bliss & Collingridge, 1993). Presynaptic glutamate release results in postsynaptic activation of α-amino-3-hydroxy-

5-methyl-4-isoxazolepropionic acid type receptors (AMPARs) and *N*-methyl-D-aspartate type receptors (NMDARs). Strong activation of AMPARs through glutamate binding results in membrane potential depolarization and subsequent voltage-dependent removal of Mg^{2+}, which typically blocks the NMDAR ionophore under resting conditions. Glutamate binding to NMDARs, along with co-agonist, D-serine or glycine, results in transmembrane cationic flux that increases intracellular Ca^{2+} (MacDermott *et al.* 1986; Johnson & Ascher, 1987; Mothet *et al.* 2000). Ca^{2+} influx through NMDARs critically governs plasticity by activating Ca^{2+}/calmodulin-dependent kinase II (CaMKII) (Bayer *et al.* 2001). However, it should be noted that forms of LTP that are not dependent on NMDAR activation have also been reported, including a plasticity mechanism that emerges following constitutive deletion of GluA2 (Jia *et al.* 1996).

Basal glutamatergic synaptic transmission is primarily mediated through activation of GluA2-containing heteromeric AMPARs, which flux monovalent cations, such as Na^{+}. However, GluA1-containing, GluA2-lacking AMPARs, which are Ca^{2+}-permeable, have been implicated in learning and plasticity in the hippocampus and other brain regions, including the ventral tegmental area, nucleus accumbens, and neocortex (Argilli *et al.* 2008; Tukey *et al.* 2013; Zhang *et al.* 2015; Diering & Huganir, 2018; Zhou *et al.* 2018; Benke & Traynelis, 2019). GluA2-lacking AMPARs are typically composed of either GluA1 homomers or GluA1/3 heteromers with permeability to K^{+}, Na^{+} and Ca^{2+}, and exhibit higher single channel conductance compared with GluA2-containing AMPARs (Liu & Zukin, 2007; Kristensen *et al.* 2011). While GluA1-containing AMPARs are present at low levels at hippocampal synapses under basal conditions, LTP induction via chemical NMDAR activation or electrical stimulation can result in increased synaptic plasma membrane recruitment, through both lateral diffusion and synaptic insertion into highly organized synaptic densities or 'nanodomains'. This effectively renders postsynaptic sites more sensitive to presynaptic release of glutamate (Kopec *et al.* 2006; Choquet, 2018). Notably, a recent study demonstrated that acute whisker stimulation results in NMDAR-dependent plasma membrane insertion of GluA1-containing AMPARs *in vivo*, suggesting that this may be an essential mechanism for synaptic plasticity underlying neural processing of sensory information (Zhang *et al.* 2015).

Over 90% of glutamatergic synapses in the mammalian brain are localized to dendritic spines, small filamentous actin (F-actin) rich membrane protrusions that emanate from the dendritic shaft (Bourne & Harris, 2007; Bosch & Hayashi, 2012). Structural changes in spines generated during LTP require F-actin reorganization, and the strength of a synapse correlates with spine size (Fukazawa *et al.* 2003; Bosch *et al.* 2014). Importantly, LTP induction not only influences postsynaptic structure, but can also result in persistent changes in the size of the presynaptic terminal (Meyer *et al.* 2014). The molecular mechanisms underlying the structural reorganization of dendritic spines have been reviewed extensively elsewhere (Bosch & Hayashi, 2012; Segal, 2017; Nakahata & Yasuda, 2018). In short, NMDAR-mediated Ca^{2+} entry triggers multiple intracellular signalling pathways, including activation of Cdc42, Rac1, and RhoA, that direct the reorganization of F-actin within a spine (Fig. 1*A*) (Scott *et al.* 2003; Tashiro & Yuste, 2004; Kang *et al.* 2009; Murakoshi *et al.* 2011; Koleske, 2013). Remarkably, similar signalling cascades are activated in neurons by chemotropic axon guidance cues (Kolodkin & Tessier-Lavigne, 2011; Lai Wing Sun *et al.* 2011).

Chemotropic guidance cues as effectors of structural and functional plasticity

During embryonic neural development, axons extend along well-organized stereotypical trajectories to ultimately connect with their synaptic targets. Canonical chemotropic axon guidance cues, such as netrins, semaphorins, ephrins and slits, are secreted or plasma membrane proteins that guide axon growth by directing cytoskeletal reorganization within axonal growth cones (Kalil & Dent, 2005; Kolodkin & Tessier-Lavigne, 2011). Although these families of cues were first identified due to essential contributions to neural development, they are all also expressed in the adult nervous system, with some demonstrated to be enriched at synapses. Similar to growth cone motility, dendritic spines also exhibit locally regulated, rapid, assembly and disassembly of F-actin, with many common molecular regulatory mechanisms implicated (Bosch & Hayashi, 2012; Murakoshi & Yasuda, 2012). Together, these findings raise the possibility that guidance cues present at synapses in the mature nervous system may impact synaptic structure and function.

Roles for netrin-1 and DCC regulating synaptogenesis and plasticity in the developing and adult brain

The netrin orthologue UNC-6 was first described in *Caenorhabditis elegans* and shortly after, netrins were identified as a conserved family of secreted axon guidance proteins in the developing vertebrate nervous system (Ishii *et al.* 1992; Kennedy *et al.* 1994; Serafini *et al.* 1994). Receptors for netrin-1 in mammals include Down's syndrome cell adhesion molecule (DSCAM), four UNC-5 homologues, deleted-in-colorectal cancer (DCC) and the DCC paralogue neogenin (Lai Wing Sun *et al.* 2011). The canonical netrin-1 receptor DCC is a single-pass transmembrane protein with four extracellular Ig domains

and six fibronectin type III domains that was originally described as a tumour suppressor associated with colon cancer (Vogelstein *et al.* 1988; Keino-Masu *et al.* 1996). Netrin-1 binds the fifth and sixth fibronectin repeats to initiate a number of intracellular signalling pathways, including activating phospholipase Cγ, regulating the Rho-GTPases Cdc42, Rac1, and RhoA, increasing intracellular Ca^{2+}, and triggering local protein synthesis (Fig. 1*B*) (Hong *et al.* 2000; Xie *et al.* 2006; Lai Wing Sun *et al.* 2011; Finci *et al.* 2014; Kim & Martin, 2015). Notably, recent studies have identified polymorphisms in the genes encoding human netrin-1 and netrin receptors that are correlated with the susceptibility to develop neurodegenerative diseases, including Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease (Lesnick *et al.* 2007; Lesnick *et al.* 2008; Lin *et al.* 2009; Wetzel-Smith *et al.* 2014), as well as developmental neuropsychiatric disorders such as autism spectrum disorder and schizophrenia (Grant *et al.* 2012; Wang *et al.* 2013; Siu *et al.* 2016). The mechanisms underlying the contribution of netrins to these disorders are not known.

Evidence for netrin function in synapse formation during development originated with findings in *Drosophila* and *C. elegans*. Initial studies in *Drosophila* indicated that netrins made by developing muscle cells are not essential to direct motor neuron axon extension but, along with neuronal expression of the DCC homologue *frazzled*, are required for nerve–muscle synaptogenesis (Kolodziej *et al.* 1996; Mitchell *et al.* 1996; Winberg *et al.* 1998; Kennedy, 2000). Subsequent studies in *C. elegans* demonstrated that the binding of UNC-6/Netrin to the DCC orthologue UNC-40 promotes presynaptic terminal assembly (Colon-Ramos *et al.* 2007). In contrast, injection of exogenous netrin-1 in the optic tectum of *Xenopus laevis* alters dendrite growth and promotes the formation of postsynaptic densities (Nagel *et al.* 2015). In the mature mammalian hippocampus, punctate distributions of DCC and netrin-1 immunoreactivity can be detected throughout the dendritic arbor of pyramidal neurons and enriched in dendritic spines (Horn *et al.* 2013; Glasgow *et al.* 2018). Focal application of netrin-1 bound to the surface of a microbead rapidly recruited the

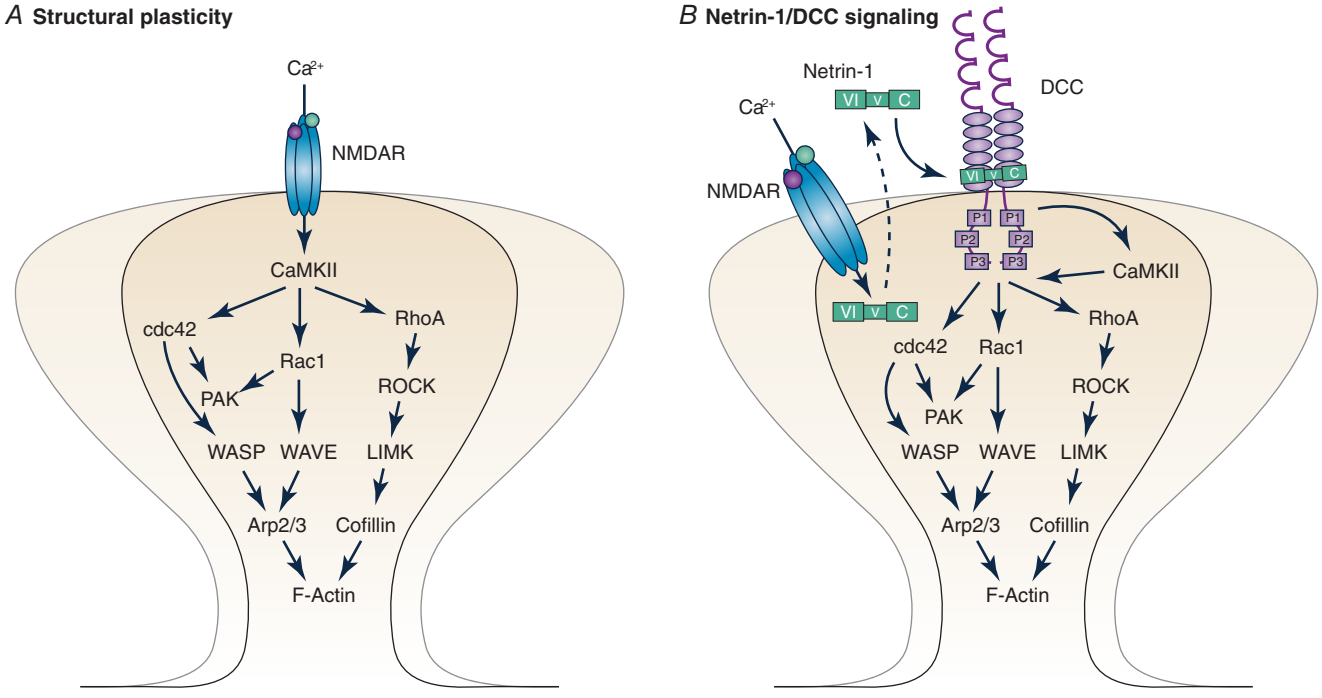

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Figure 1. Structural reorganization underlying plasticity and netrin-1 share common signalling pathways
A, concomitant postsynaptic depolarization and glutamate binding to NMDARs results in Ca^{2+} influx, which triggers a number of intracellular signalling cascades through activation of CaMKII, including Cdc42, RhoA, and Rac1. These pathways lead to phosphorylation of Arp2/3 and ADP/cofilin which, in turn, regulates actin polymerization underlying increased dendritic spine volume. *B*, Netrin-1 secretion from intracellular vesicles, triggered by NMDAR activation, results in extracellular netrin-1 binding to the fifth and sixth fibronectin-III domains of DCC to regulate intracellular signalling mechanisms that similarly converge on the reorganization of F-actin.

key postsynaptic adaptor PSD-95 and GluA1-containing AMPARs. Netrin-1 coated beads also triggered a slower accumulation of the presynaptic marker synaptophysin, suggesting that local netrin-1 is sufficient to induce synaptic differentiation and promote the recruitment of both pre- and postsynaptic proteins during development (Goldman *et al.* 2013).

Analysis of synaptosomal fractions from adult rat brain revealed that netrin-1 and DCC are highly enriched in synaptic intracellular vesicles (Horn *et al.* 2013), suggesting that activity-dependent exocytosis of netrin-1 and DCC might influence synaptic differentiation. Regulated exocytosis of cargo vesicles can be triggered by increased intracellular Ca^{2+} (Neher, 1998; Kreutzberger *et al.* 2017), yet little was known regarding the spatiotemporal dynamics of DCC plasma membrane insertion and netrin-1 release at synapses. We had previously reported that depolarization of cultured cortical neurons with high KCl resulted in neuronal plasma membrane recruitment of DCC (Bouchard *et al.* 2008). More recently, we provided initial evidence for activity-dependent release of netrin-1 from neurons, demonstrating that depolarization of cultured hippocampal neurons either using high extracellular KCl, or by activation of neurons expressing a modified muscarinic type 3 receptor using a Designer Receptor Exclusively Activated by Designer Drug (DREADD) pharmacogenetic paradigm, increased netrin-1 protein in the culture medium (Glasgow *et al.* 2018). To directly visualize netrin-1 release from hippocampal dendrites, we engineered a cDNA encoding full-length netrin-1 fused to an enhanced pH-sensitive GFP (super-ecliptic pHluorin; NTN1-SEP). Driving NMDAR activation using a chemical LTP protocol, we detected a rapid increase in exocytotic release of netrin-1 at both synaptic and perisynaptic sites along the dendrites of hippocampal neurons. These findings provide strong evidence for the activity-induced secretion of netrin-1, at or near synapses (Glasgow *et al.* 2018).

The application of genetic models to investigate the contributions made by chemotropic guidance cues to plasticity in the mature central nervous system has been limited by the essential role of these proteins during embryonic development. For example, full genetic deletion of netrin-1 in mice is embryonic lethal at ∼E13 (Bin *et al.* 2015). To circumvent the embryonic lethality of null mice, conditional floxed alleles of netrin-1 and DCC were developed, which, when combined with Cre driver mouse lines that express postnatally, or virus-based approaches, have provided valuable insights into the roles of these proteins at synapses in the adult brain (Horn *et al.* 2013; Bin *et al.* 2015; Glasgow *et al.* 2018; Glasgow *et al.* 2019*b*; Wong *et al.* 2019).

Do DCC and netrin-1 impact synaptic plasticity in the adult brain? Having determined that netrin-1 is released from dendrites in response to neuronal excitability and NMDAR activation, we speculated that activity-dependent LTP might be impaired in these animals. Indeed, attempts to induce NMDAR-dependent LTP in neurons lacking either netrin-1 or DCC revealed significant attenuation of plasticity (Horn *et al.* 2013; Glasgow *et al.* 2018). Moreover, selective deletion of netrin-1 from principal excitatory neurons blocked high-frequency stimulation (HFS)-induced increases in the AMPAR-to-NMDAR ratio, suggesting a critical role for netrin-1 in AMPAR trafficking following LTP induction (Glasgow *et al.* 2018). Remarkably, we found that bath application of netrin-1 alone to hippocampal slices from wild-type mice was sufficient, without HFS, to induce long-lasting, dose-dependent potentiation of AMPAR-mediated synaptic responses at Schaffer collateral-CA1 (SC-CA1) synapses. We found that netrin-1 potentiation of synaptic responses does not require NMDAR activation, suggesting that netrin-1 may serve as a downstream effector in HFS-induced LTP. Further, we determined that netrin-1, through a DCC-dependent mechanism, promotes the insertion of Ca^{2+}-permeable GluA1-containing AMPARs downstream of NMDAR activation, thereby enhancing postsynaptic sensitivity to presynaptic glutamate release (Glasgow *et al.* 2018) (Fig. 2). Together, these findings indicate that netrin-1 binding to DCC can trigger an intracellular signalling cascade downstream of NMDARs that potently modulates the surface distribution of AMPARs at hippocampal synapses.

While netrin-1 impacts postsynaptic glutamatergic synaptic transmission, it remains unclear as to whether presynaptic mechanisms may also be altered by netrin-1. Indeed, work in the developing nervous system implicated axonal DCC, and other receptors for guidance cues, such as the Wnt receptor Frizzled, in synapse formation at both central and peripheral synapses (Miech *et al.* 2008; Manitt *et al.* 2009; Goldman *et al.* 2013). We recently reported that presynaptic DCC also contributes to basal synaptic transmission and LTP at the SC-CA1 synapse in the adult hippocampus (Glasgow *et al.* 2019*b*). Using CA3- and CA1-selective *Cre*-mediated DCC deletion, we examined the specific pre- and postsynaptic contributions of DCC to synaptic transmission at the SC-CA1 synapse. Surprisingly, and in contrast to a purely postsynaptic site of action, selective deletion of DCC from presynaptic CA3 neurons resulted in significant attenuation of basal synaptic transmission as well as impaired HFS-induced LTP. Moreover, while changes in synaptic transmission following HFS-induced LTP are primarily mediated by altering postsynaptic insertion of AMPARs, selective postsynaptic deletion of DCC did not impair LTP. These surprising findings provide evidence that DCC governs distinct pre- and postsynaptic elements of LTP expression.

Many of the signalling mechanisms associated with DCC activation are well-positioned to influence post-synaptic architecture and AMPAR trafficking. However, it remains unclear as to how presynaptic DCC modulates presynaptic glutamate release. DCC is localized to the leading edge of developing axonal growth cones and netrin-1 application can lead to increases in local Ca^{2+} that promote vesicle insertion to the plasma membrane (Bouchard *et al.* 2004; Tang & Kalil, 2005; Manitt *et al.* 2009; Urbina *et al.* 2018). The DCC intracellular domain interacts with the E3 ubiquitin ligase, TRIM9, which binds synaptic vesicle SNARE proteins, such as the target (t)-SNARE SNAP25 (Li *et al.* 2001; Winkle *et al.* 2014; Plooster *et al.* 2017). Netrin-1/DCC signalling also activates the t-SNARE, syntaxin-1 (Ros *et al.* 2018), which forms a complex with SNAP25 and synaptobrevin-2 that is required for depolarization-mediated vesicular fusion at synapses (Sudhof & Rothman, 2009). These findings raise the intriguing possibility that presynaptic loss of DCC may impair neurotransmitter release, thereby attenuating basal synaptic transmission to the extent that it may not be sufficient to induce LTP (Isaac *et al.* 1995; Emptage *et al.* 2003; Glasgow *et al.* 2019*a*).

While LTP is closely associated with altered post-synaptic receptor composition and structure, changes in activity also impact the structure and function of presynaptic terminals. Following LTP induction via postsynaptic photolysis of caged glutamate, presynaptic boutons show considerable growth in volume, albeit more slowly than changes in postsynaptic dendritic spine structure (Meyer *et al.* 2014). This suggests that the postsynaptic neuron releases a retrograde signal to initiate changes in presynaptic structure. Although little is known regarding the underlying mechanism, post-synaptic activity-dependent release of netrin-1 binding to presynaptic receptors such as DCC presents an intriguing candidate interaction to modify the presynaptic terminal.

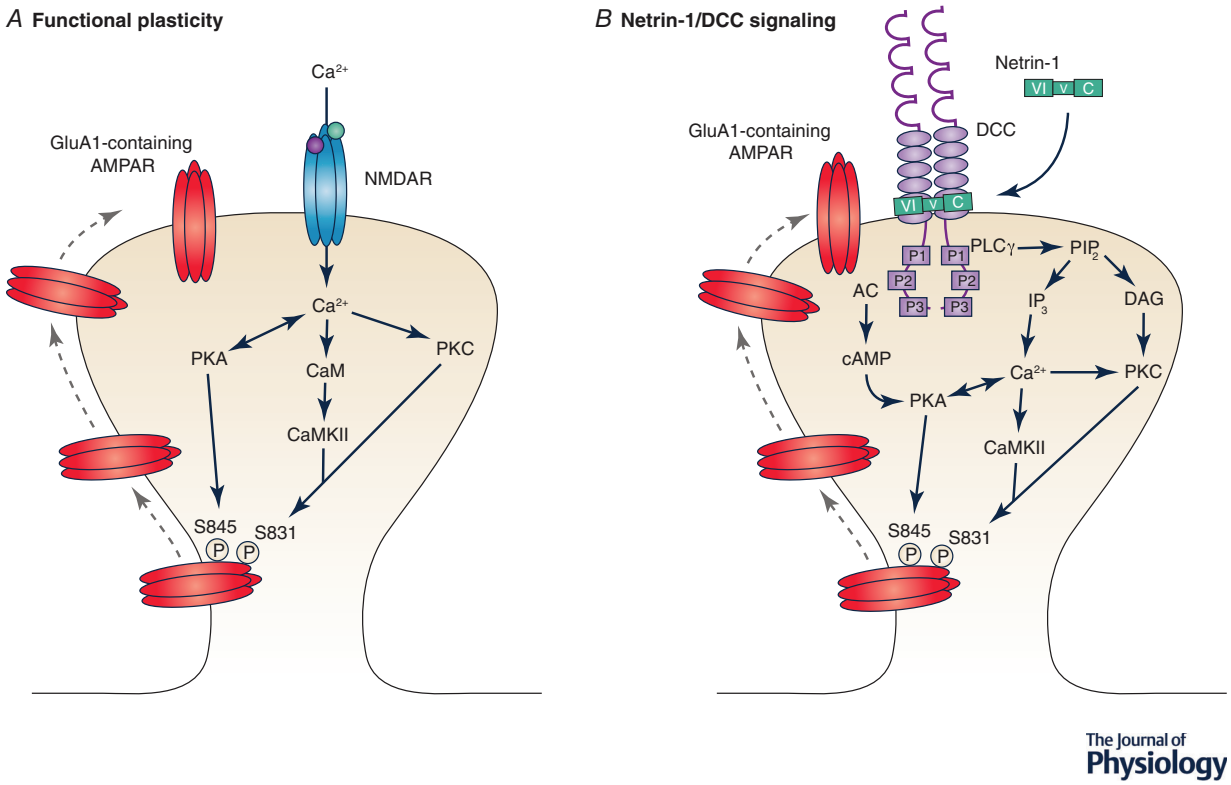

Figure 2. Functional reorganization of excitatory synapses during plasticity mirrors molecular cascades underlying netrin-1 potentiation
A, Ca^{2+} influx through the NMDAR results in activation of CaMKII, PKC, and PKA, which converge on phosphorylation of GluA1 at serine residues 831 and 845. GluA1-containing AMPARs are inserted into the plasma membrane and trafficked to synaptic sites. *B*, Netrin-1 release following NMDAR activation binds to post-synaptic DCC, which triggers adenylyl cyclase (AC) to activate PKA, as well as activating phospholipase Cγ (PLCγ), leading to hydrolysis of membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP_2). In turn, PIP_2 leads to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), stimulating the release of intracellular Ca^{2+} stores and activation of PKC, respectively. Increased intracellular Ca^{2+} and activation of PKC likely leads to phosphorylation of serine 831, whereas increased PKA leads to phosphorylation of serine 845 on GluA1-containing AMPARs. Phosphorylation of S831 and S845 on GluA1 promote the trapping and trafficking of GluA1-containing AMPARs.

Local protein synthesis mediated by netrin-1 at synapses?

Extracellular signals regulate local protein synthesis at synapses, which in turn can modify synaptic strength through local *de novo* translation of AMPARs and other proteins involved in synaptic stabilization (Wang *et al.* 2010). Although this mechanism provides high spatiotemporal precision for synaptic modifications, it is unclear as to how RNA translation is regulated locally at synapses. Both rRNA and mRNA are ubiquitous throughout the dendritic arbor of neurons and show no specific enrichment at synapses, yet translation occurs at synaptic contacts or sites of synaptic stimulation, suggesting that an activity-dependent secreted factor may trigger local protein synthesis (Costa-Mattioli *et al.* 2009; Wang *et al.* 2009; Kim & Martin, 2015). Netrin-1 signalling via DCC is linked to the activation of protein translation machinery in neurons (Campbell & Holt, 2001; Tcherkezian *et al.* 2010) and, consistent with a role for translational control of long-lasting synaptic plasticity, netrin-1 binding to DCC results in increased synapse-specific local translation in *Aplysia* neurons following 5-HT induced long-term facilitation (Kim & Martin, 2015). Additionally, netrin-1 signalling can regulate local translation of DSCAM in developing murine growth cones, and DSCAM has been implicated in synapse function in the mature nervous system (Jain & Welshhans, 2016). Together with our studies of hippocampal neurons (Goldman *et al.* 2013; Horn *et al.* 2013; Glasgow *et al.* 2018), these findings suggest that autocrine release of netrin-1 from dendrites not only rapidly recruits AMPAR insertion to excitatory synapses, but may also promote synaptic stabilization and structural remodelling following LTP by locally activating protein synthesis. However, it remains to be determined how netrin-1 may influence the expression of late phase, *de novo* protein synthesis-dependent LTP.

Beyond DCC

Do netrin receptors other than DCC contribute to the regulation of synaptic transmission? Notably, the DCC paralogue neogenin also appears to impact netrin-1 mediated modulation of synaptic transmission in the adult brain (Sun *et al.* 2018). Neogenin is enriched at excitatory synapses in the basolateral amygdala, and following genetic deletion of neogenin, both induction and expression of activity-dependent LTP in the basolateral amygdala are compromised. Further, deletion of neogenin results in morphological and physiological abnormalities in excitatory neurons, including decreased dendritic spine density and lower frequency of spontaneous miniature excitatory postsynaptic currents (mEPSCs), but no effect on the AMPAR-to-NMDAR ratio. Interestingly, no changes were observed in inhibitory synaptic transmission, suggesting that netrin-1/neogenin modulation of amygdalar circuits may be specific to excitatory synapses. Behaviourally, animals selectively lacking neogenin in the amygdala showed impaired freezing in contextual fear conditioning, a task dependent on synaptic reorganization and plasticity within the amygdala (Johansen *et al.* 2011). Taken together, these findings suggest that neogenin contributes to the synaptic reorganization and plasticity that underlies fear memory by regulating synapse number. Moreover, while these findings provide evidence for a critical role governing the structure and function of excitatory synapses in the amygdala, neogenin is widely expressed by neurons and its precise contribution to synaptic mechanisms in other brain regions remains unclear.

Netrin-1 may also impact synapse function and structure through other receptors, including DSCAM (Schmucker *et al.* 2000; Ly *et al.* 2008). DSCAM and DCC are co-receptors in the axonal growth cone, and netrin-1 binding to DSCAM promotes axonal outgrowth (Ly *et al.* 2008). Similar to netrin-1 mediated potentiation of synapses in the adult hippocampus, DSCAM regulates synaptic transmission following learning, promoting the accumulation and clustering of AMPARs at *de novo* synapses in cultured *Aplysia* neurons (Li *et al.* 2009). While these findings suggest DSCAM may contribute to netrin-1 induced potentiation in other cellular contexts, in the developing *Xenopus laevis* visual system, DSCAM regulation of dendritic arborization appears to be independent of netrin-1, suggesting a possible modulatory relationship between netrin-1, DCC, and DSCAM (Santos *et al.* 2018). Further, analysis of spinal commissural axon outgrowth in DSCAM-null mice identified no defects in netrin-1/DCC-dependent commissural axon outgrowth, either *in vivo* or with *in vitro* assays, indicating that, at least in this cellular context, DSCAM is not required for this response to netrin-1 (Palmesino *et al.* 2012). However, whether DSCAM contributes to netrin-1 induced synaptic potentiation remains to be determined.

Netrin-1 interacts with various other receptors and transmembrane proteins that have been implicated in neurotransmission and synaptic plasticity, including integrins, amyloid precursor protein (APP), and the UNC-5 homologues (Leonardo *et al.* 1997; Nalbantoglu *et al.* 1997; Lourenco *et al.* 2009; Lai Wing Sun *et al.* 2011; Park & Goda, 2016). Notably, APP is reported to heterodimerize with DCC under non-pathological conditions, and netrin-1 suppresses amyloid-β (Aβ) production, which contributes to memory impairments in Alzheimer's disease (Lourenco *et al.* 2009). Further, intracerebroventricular administration of netrin-1 is reported to restore spatial cognition and LTP in a mouse model of Alzheimer's disease (Shabani *et al.* 2017). Together, these

findings suggest that loss of netrin-1 may contribute to synaptic dysfunction, and may present a novel therapeutic target for treatment of pathophysiologies associated with memory impairment.

Netrin-1 regulation of spatial memory function

The hippocampus is required for spatial memory consolidation; disruption of hippocampal circuits through ablation or genetic deactivation results in severe memory impairment (Knierim, 2015). Due to the essential roles of certain chemotropic guidance cues during embryonic development, many behavioural analyses have addressed the relatively subtle phenotypes exhibited by knockdown or in haploinsufficiency mouse models (Torres-Berrio *et al.* 2017). In contrast, consistent with major deficits in synaptic transmission and plasticity, selective homozygous deletion of netrin-1 or DCC from glutamatergic neurons in the forebrain, including hippocampal CA1 pyramidal neurons, result in major impairment of memory consolidation (Horn *et al.* 2013; Wong *et al.* 2019).

Does synaptic potentiation correlate with learning in the intact animal? In the mature brain, behavioural states linked to memory formation are associated with the formation of new dendritic spines, suggesting that synaptogenesis plays a critical role in learning and memory consolidation (Xu *et al.* 2009; Peters *et al.* 2014). Consistent with a critical role in memory formation, conditional deletion of either netrin-1 or DCC from principal excitatory neurons in the forebrain and hippocampus results in significant impairments in spatial memory tasks, including novel object place recognition and the Morris water maze (Horn *et al.* 2013; Wong *et al.* 2019). These deficits appear to selectively impact spatial memory, as mice lacking netrin-1 show no measurable impairment of exploratory behaviour, motor activity, or non-hippocampal-dependent object recognition (Wong *et al.* 2019). Further, animals lacking DCC show altered dendritic spines, potentially signalling a deficit in spine maturation (Horn *et al.* 2013; Glasgow *et al.* 2019*b*). Together, these findings indicate that netrin-1 and DCC expressed by hippocampal neurons play critical roles in the consolidation of spatial information, likely through changes in the ability to induce long-lasting changes in synaptic strength and modification of dendritic spine structure.

However, it is important to note that the ability to induce synaptic plasticity is not consistently associated with spatial memory performance, with some transgenic animal models showing normal plasticity despite pronounced behavioural learning deficits (Kim *et al.* 2016). While many of the molecular effectors that regulate synaptic formation and pruning during development continue to be expressed at high levels across the lifespan, further behavioural characterization in the mature central nervous system is required.

Yin-and-Yang of chemotropic guidance cues in adult plasticity

It is clear that other chemotropic guidance cues, in addition to netrin-1, contribute to the ongoing modification of synaptic transmission. Netrins were initially characterized due to their chemoattractive function. In contrast, the family of extracellular and membrane-bound semaphorins were originally identified as chemorepellents that promote growth cone collapse and retraction during development (Kolodkin *et al.* 1993; Tran *et al.* 2007). Similar to netrins, the study of semaphorin function has largely focused on their roles during embryogenesis, but these proteins also are expressed in the adult brain (Giger *et al.* 1998; Holtmaat *et al.* 2002; Bagri *et al.* 2003). Moreover, semaphorin signalling mechanisms have been implicated in changes in synaptic physiology, suggesting possible roles in influencing synaptic structure and synaptic transmission in the adult nervous system.

Reorganization of the dendritic cytoskeleton during memory formation suggests that both chemoattractive and chemorepellent guidance cues influence synaptic consolidation and elimination during activity-dependent plasticity (Nakamura *et al.* 2009; Tran *et al.* 2009; Zhang *et al.* 2015). The repulsive guidance cue, semaphorin IIIA (Sema3A), mediates growth cone collapse during development, and is also expressed in the adult brain (Lein *et al.* 2007; Cembrowski *et al.* 2016). Some semaphorins are membrane-bound, but Sema3s are secreted and bind a transmembrane holoreceptor complex consisting of neuropilin-1 (Nrp1) and Plexin A1-4 to regulate neuronal morphology, including dendritic spine structure (Takahashi *et al.* 1999; Polleux *et al.* 2000; Pasterkamp & Giger, 2009). In the developing nervous system, Sema3A triggers dendritic spine collapse through a Nrp1/Plexin A1-4 mediated mechanism that activates collapsin response mediator protein 2 (CRMP2) (Uchida *et al.* 2005). Notably, CRMP2 signalling is linked to synapse function and neurodegenerative disorders, including Alzheimer's disease (Yoshida *et al.* 1998; Cole *et al.* 2007). Phosphorylation of CRMP2 regulates microtubule disassembly, cytoskeletal reorganization, and dendritic spine collapse, which, in turn, may promote the consolidation of other existing synapses (Schubert *et al.* 2006; Gu *et al.* 2008). Recent work in *Drosophila* has demonstrated that interactions between the invertebrate orthologue of Sema3A, Sema2b, and its receptor Plexin B promotes axonal attraction and refinement of neural processes underlying sleep–wake cycling, suggesting a key role for Sema2b in synaptic modification (Xie *et al.* 2019). These findings indicate that semaphorins are critically

involved in the neural mechanisms underlying typical behavioural processes, including sleep and arousal.

Secreted developmental morphogens that also function as axon guidance cues, including Wnts, sonic hedgehog (Shh), and bone morphogenic proteins (BMPs), have also been shown to regulate synaptic transmission and structure via regulation of the Rho-GTPases, including Cdc42, RhoA, and Rac1 (Nahm *et al.* 2010). Further, recent findings have implicated Wnt signalling via post-synaptic Frizzled-7 in activity-dependent functional and structural synaptic plasticity (Oliva *et al.* 2013; McLeod *et al.* 2018). Future studies will determine the mechanisms that underly the contribution of secreted morphogens to ongoing synaptic modification during learning and memory consolidation.

Together, the findings described reveal central roles for multiple families of chemotropic axon guidance cues regulating synapse function and activity-dependent synaptic plasticity during learning and memory formation in the adult mammalian brain. Of critical importance, chemotropic guidance cues provide new targets for therapeutic intervention in neurodevelopmental and neurodegenerative disorders characterized by disruption of memory function, including Alzheimer's disease and autism spectrum disorder.

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Additional information

Competing interests

The authors state that they have no competing interests.

Author contributions

SDG, ESR and TEK contributed to the conception, drafting, and revising of the work. All authors approved the final version of the manuscript, agree to be accountable for all aspects of the work, agree that all persons designated as authors qualify for authorship, and that all those who qualify for authorship are listed.

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