Published in final edited form as:
Trends Neurosci. 2014 October ; 37(10): 539–550. doi:10.1016/j.tins.2014.07.004.

Control of Neural Circuit Formation by Leucine-Rich Repeat Proteins

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Abstract

The function of neural circuits depends on the precise connectivity between populations of neurons. Increasing evidence indicates that disruptions in excitatory or inhibitory synapse formation or function lead to excitation/inhibition (E/I) imbalances and contribute to neurodevelopmental and psychiatric disorders. Leucine-rich repeat (LRR)-containing surface proteins have emerged as key organizers of excitatory and inhibitory synapses. Distinct LRR proteins are expressed in different cell types and interact with key pre- and postsynaptic proteins. These protein interaction networks allow LRR proteins to coordinate pre- and postsynaptic elements during synapse formation and differentiation, pathway-specific synapse development, and synaptic plasticity. LRR proteins thus play a critical role in organizing synaptic connections into functional neural circuits, and their dysfunction may contribute to neuropsychiatric disorders.

Keywords

Synaptogenesis; Synaptic Adhesion; Excitation/Inhibition Balance; Connectivity; Synaptic Transmission; Glutamate Receptor

LRR proteins and the organization of functional neural circuits

The function of neural circuits depends on the precise connectivity between populations of neurons. In the central nervous system (CNS) this is mediated by glutamatergic and GABAergic synapses, and there is emerging evidence that disruptions in the formation or function of excitatory or inhibitory synapses lead to excitation/inhibition (E/I) imbalances, which characterize several psychiatric and neurodevelopmental disorders [1–8]. These considerations underscore the importance of understanding the molecular control of

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excitatory and inhibitory synapse formation, and the signals that allow cell type-specific control of E/I balance.

Here, we review recent evidence indicating that cell surface proteins containing an extracellular leucine-rich repeat (LRR) domain [9] are key organizers of excitatory and inhibitory synapses in the CNS. Distinct LRR proteins are expressed in different cell types, and are generally localized to the postsynaptic membrane, from where they exert a strong influence on the development of synaptic connections. LRR proteins interact with key components of the postsynaptic machinery and trans-synaptically couple to essential presynaptic receptors, which places them in an ideal position to coordinate pre- and postsynaptic differentiation during circuit formation. New insights indicate that the function of LRR proteins extends beyond the initial formation of synaptic contacts and point to a role in regulating functional properties and activity-dependent plasticity of synapses. These findings indicate that LRR proteins play a critical role in the organization and function of neural circuits.

LRR proteins as regulators of synapse development

Recent studies have identified several closely related LRR protein families as regulators of synapse development. A simple in vitro assay, which tests the ability of neurons to form synapses onto cocultured heterologous cells expressing candidate genes [10, 11], has been instrumental in identifying synapse-organizing or synaptogenic LRR proteins. The elucidation of their trans-synaptic interactions has highlighted a common theme of diverse postsynaptic ligands coupling to a limited repertoire of presynaptic receptors. Below, we provide an inventory of these LRR proteins and discuss their roles in synapse development and function, focusing on the vertebrate system.

LRRTMs control excitatory synapse development via distinct presynaptic partners

Since their original identification as synaptogenic proteins in a coculture assay-based expression screen [12], Leucine-Rich Repeat Transmembrane Neuronal proteins (LRRTM1-4) have rapidly become the most intensively studied family of LRR-containing synaptic organizers. LRRTMs are type I transmembrane proteins with an extracellular LRR domain and a C-terminal PDZ interaction site (Fig. 1A), and distinct, partially overlapping expression patterns in the brain [13] (Fig. 4). LRRTM2, the best characterized family member, localizes to the postsynaptic density of excitatory synapses and regulates postsynaptic differentiation by recruiting key elements of the synaptic machinery, including the scaffolding protein PSD-95 and glutamate receptor subunits [12, 14]. LRRTM2 expressed on the surface of non-neuronal cells induces presynaptic differentiation in contacting axons of co-cultured neurons, a property shared with LRRTM1, LRRTM4, and to a lesser degree, LRRTM3 [12, 14, 15]. LRRTM1 and LRRTM2 induce presynaptic differentiation by trans-synaptically binding to neurexins [14–16], a family of alternatively spliced receptors that organize presynaptic development and function [17, 18]. Neurexins interact with a multitude of postsynaptic ligands, including the neuroligins and the cerebellin-glutamate receptor δ complex [19–21]. LRRTM binding to neurexin is regulated

by alternative splicing: LRRTM1 and -2 only bind to neurexins lacking a small insert at splice site 4 (S4) [15, 16]. Surprisingly, LRRTM4 uses a different mechanism to induce presynaptic differentiation. LRRTM4 binds to heparan sulfate proteoglycans (HSPGs), most prominently glypicans, and requires HS on the presynaptic cell surface to induce synapse formation [22, 23] (Fig. 1A). Unlike neurexins, GPI-anchored glypicans lack a cytoplasmic domain, raising the possibility of a transmembrane co-receptor to trigger presynaptic differentiation. The receptor protein tyrosine phosphatase LAR (leukocyte common antigen-related) binds glypican in *Drosophila* [24], and is a candidate for such a co-receptor.

Overexpression of LRRTM2 or LRRTM4 in cultured hippocampal neurons increases the density of excitatory, but not inhibitory, synapses [14, 15, 22, 23]. LRRTMs most likely promote excitatory synapse development by interacting with their respective presynaptic binding partners, since overexpression of an LRRTM2 mutant lacking the LRR domain fails to increase the density of excitatory synapses [14], and enzymatic removal of HS abolishes the increase in presynaptic input density following LRRTM4 overexpression in hippocampal neurons [23]. In agreement with a role in regulating excitatory synapses, *LRRTM2* knockdown [14, but see 25] and *LRRTM4* knockout or knockdown [22, 23] selectively decrease excitatory synapse density in cultured neurons.

In vivo analyses of *LRRTM* function have revealed subtle pre- and postsynaptic anatomical defects. *LRRTM1* knockout mice display increased VGluT1 immunofluorescence and an altered, more dispersed, distribution of synaptic vesicles [12, 26]. Postsynaptically, Golgi stained hippocampal CA1 pyramidal neurons in *LRRTM1* knockout mice have longer, possibly immature, dendritic spines, but no overall change in spine density, although EM analysis indicates a small decrease in asymmetric synapse density [26]. Knockout or knockdown of *LRRTM4* decreases spine density in dentate gyrus and cortex, respectively [22, 23]. Functionally, *LRRTM2* knockdown decreases evoked AMPA receptor (AMPAR)- and NMDAR-mediated glutamatergic synaptic transmission in dentate granule cells [14], and combined *LRRTM1*/*LRRTM2* knockdown in early postnatal CA1 neurons selectively reduces evoked AMPAR-mediated synaptic transmission [27]. Despite the altered organization of synaptic vesicles in *LRRTM1* knockout mice, presynaptic functional defects have not been detected following *LRRTM* knockout or knockdown [14, 23, 27]. Together, these anatomical and functional changes are consistent with a role for LRRTMs in regulating synapse formation, maturation or maintenance, or possibly all of these, in vivo.

Slitrks regulate excitatory and inhibitory synapse development via RPTPs

Based on their structural similarity to LRRTMs (Fig. 1B), Slit- and Trk-like proteins (Slitrk1-6) were predicted to have synaptogenic properties in a bioinformatics search [12]. When tested in the coculture assay, Slitrk2 expressed on the surface of fibroblasts indeed induced presynaptic differentiation in contacting axons [12]. Subsequent studies showed that all Slitrk family members have similar activity [28]. *Slitrks* are broadly expressed in the hippocampus (Fig. 4), and are postsynaptically localized [28–30]. Whereas Slitrk1, -2, -4, -5 and -6 induce both excitatory and inhibitory presynaptic differentiation in cocultures [28], overexpression of Slitrk1, -2, -4, or -5 in cultured hippocampal neurons increases the density of excitatory, but not inhibitory, synapses [29]. Conversely, knockdown of *Slitrk1*, *-2*, *-4*, or

-5 selectively decreases excitatory synapse density [29], indicating that these Slitrk family members regulate excitatory synapse development in cultured neurons. In vivo, the role of Slitrks in excitatory synapse development has been best characterized for Slitrk5. Loss of *Slitrk5* decreases dendritic arbor complexity of striatal neurons, reduces AMPAR and NMDAR levels, and decreases synaptic transmission in corticostriatal circuits without affecting presynaptic properties, indicating a role for *Slitrk5* in the development of corticostriatal excitatory synapses [30]. *Slitrk6* mutants display a reduction in the synaptic marker Ribeye in the retina during postnatal development, but apparently normal levels in adult, suggesting a developmental delay in synaptogenesis in the absence of *Slitrk6* [31].

Slitrk3 is unique in that it is the only LRR protein identified thus far to function exclusively at inhibitory synapses. Slitrk3 selectively induces inhibitory presynaptic differentiation in cocultures as well as in cultured neurons, and *Slitrk3* knockdown decreases inhibitory, but not excitatory, synapse density [28, 29]. Consistent with a role in regulating inhibitory synapse development, loss of *Slitrk3* in vivo results in a decreased numbers of functional inhibitory, but not excitatory hippocampal synapses [28]. Anatomically, there is a select reduction in inhibitory synapse density in specific hippocampal laminae or even subregions of laminae in *Slitrk3* knockout mice [28], suggesting the loss of subsets of inhibitory inputs, possibly belonging to distinct interneuron types.

The effects of Slitrks on excitatory or inhibitory synapse development depend on their interactions with presynaptic receptors. Candidate-based approaches and affinity chromatography of brain extract with recombinant Slitrk1 identified the LAR family receptor protein tyrosine phophatase δ (RPTPδ) as a Slitrk interactor, and subsequent experiments confirmed that Slitrk1, -2 and -3 interact with RPTPδ and RPTPσ, but not with LAR itself [28, 29] (Fig. 1B). RPTPs interact with a host of postsynaptic receptors, including the LRR proteins NGL-3 and TrkC (see below), interleukin-1-receptor accessory protein-like 1 (IL1RAPL1) and interleukin-1 receptor accessory protein (IL1RAcP) [32–36]. Thus, analogous to LRRTMs and their presynaptic partners neurexins, Slitrks bind to presynaptic receptors that interact with multiple postsynaptic ligands [reviewed in 37].

Although Slitrks are capable of interacting with both RPTPδ and RPTPσ, their functional effects on excitatory and inhibitory synapse development are mediated by more selective interactions. Slitrk2 and -3 require RPTPδ but not RPTPσ, to induce inhibitory presynaptic differentiation, and conversely, excitatory presynaptic differentiation induced by Slitrk1 and -2 requires RPTPσ, but not RPTPδ [28, 29]. Endogenous RPTPσ appears to be selectively expressed on glutamatergic axons [32], which would explain the functional requirement for RPTPσ in Slitrk-mediated excitatory synapse development. Less clear is why Slitrk1 and -2 interact with both RPTPδ and RPTPσ receptors and induce both excitatory and inhibitory synapse formation in fibroblast-neuron cocultures, but selectively promote excitatory synapse development in neurons. Possibly, RPTP-Slitrk1 or -2 interactions activate postsynaptic signaling pathways that only stabilize excitatory synapses, but little is known about Slitrk signaling [38]. Like Slitrk1 and -2, Slitrk3 interacts with both RPTPδ and RPTPσ, yet Slitrk3 only induces inhibitory presynaptic differentiation in cocultures and in neurons. Slitrk3 may require additional receptors to induce inhibitory synaptic differentiation, or glutamatergic axons may express cues that specifically inhibit Slitrk3. To

complicate things further, the RPTP ligand IL1RAPL1 induces excitatory synapse formation via RPTPδ [34, 36], indicating that binding of postsynaptic partners to presynaptic RPTPδ does not always trigger inhibitory synapse formation. Binding of IL1RAPL1 to RPTPs is modulated by alternative splicing [34], which could be a mechanism to differentially regulate ligand-receptor interactions in glutamatergic and GABAergic axons. Clearly, determining how the interactions of Slitrks with their RPTP receptors shape excitatory and inhibitory synapses constitutes a major challenge.

NGLs regulate input-specific synapse development

Netrin-G Ligands (NGL1-3) are structurally related to LRRTMs and Slitrks, but contain an additional Ig domain in their extracellular region (Fig. 1C). Their identification as PSD-95 interactors in yeast two hybrid screens suggested a role at synapses, and biochemical fractionation and immuno-EM confirmed that NGL-2 and -3 localize to the postsynaptic density [33, 39]. All NGLs induce presynaptic differentiation in coculture assays, with NGL-3 having markedly stronger effects than NGL-1 or -2 [33, 39]. NGL-3 binds to all LAR family RPTPs [33, 40]. NGL-1 and -2 interact with the GPI-anchored proteins Netrin-G1 and Netrin G2, respectively [39, 41, 42] (Fig. 1C). The lack of a cytoplasmic domain in Netrin-Gs suggests the existence of a transmembrane co-receptor to mediate NGL-1- and NGL-2-induced presynaptic differentiation. Indeed, NGL-1 binding to presynaptic Netrin-G1 induces a *cis* interaction of Netrin-G1 with LAR to promote presynaptic differentiation [43]. Netrin-G1 binding to LAR is modulated by alternative splicing of Netrin-G1 [43], which can generate at least 10 isoforms [44–46]. Alternative splicing of Netrin-G2 does not affect binding to NGL-2 [47], suggesting that Netrin-G splicing serves to regulate *cis* interactions. Thus, like LRRTMs, different NGL family members interact with transmembrane or GPI-anchored presynaptic partners, and as suggested for the LRRTM4-glypican interaction, the recruitment of additional *cis* co-receptors is required for Netrin-G-mediated presynaptic differentiation. It will be of interest to determine whether such *trans*-induced formation of *cis* protein complexes constitutes a general theme in synapse development.

What might be the advantage of the indirect interaction of NGL-1 with LAR via Netrin-G1, as opposed to NGL-3's direct interaction with LAR RPTPs? One possibility could be that through alternative splicing, Netrin-Gs may couple trans-synaptic interaction with NGLs to a network of multiple *cis* receptors, providing additional layers of control over synapse development. Remarkably, NGL-2 binding to Netrin-G2 does not recruit LAR to the complex [43], suggesting the existence of additional *cis* interactors. Whether these are other RPTPs or unrelated synaptic receptors remains to be determined.

An additional advantage of the Netrin-G/NGL interaction is increased specificity. Netrin-Gs are expressed on the surface of distinct axonal populations [41, 44, 45, 48], and determine dendritic clustering of their respective postsynaptic NGLs in a pathway-specific manner [41]. In the hippocampal CA1 region, Netrin-G1 is expressed on entorhinal cortex-derived temporoammonic fibers in the stratum lacunosum moleculare (SLM), and NGL-1 distribution is restricted to the corresponding distal dendritic segment of the CA1 neuron (Fig. 2A, B). Loss of axonal Netrin-G1 in *Ntng1* knockout mice results in a diffuse

distribution of NGL-1 along the entire CA1 dendrite. Similarly, loss of *Ntng2* in Schaffer collateral axons in the stratum radiatum (SR) results in dispersal of NGL-2, which is normally restricted to the proximal CA1 dendritic segment [41] (Fig. 2A, B). In agreement with the pathway-specific distribution of NGL-2, knockout or knockdown of *NGL-2* in CA1 neurons decreases the density of dendritic spines and the strength of glutamatergic transmission in SR, but not in SLM [49]. The decrease in SR spine density following *NGL-2* knockdown can be rescued by restoring the interaction with presynaptic Netrin-G2, indicating that the Netrin-G2/NGL-2 complex regulates synapse development in an input-specific manner. Furthermore, loss of *NGL-2* impairs the ability of CA1 neurons to functionally integrate coincident subthreshold SR and SLM stimuli, resulting in reduced CA1 spiking probability and thus affecting CA1 output in the hippocampal circuit [49]. A similar pathway-specific role for NGL-2 was discovered in the horizontal cell (HC) axon – rod photoreceptor connection in the retina [50]. NGL-2 localizes to horizontal cell axon tips, which form postsynaptic specializations onto *Ntng2*-expressing rod photoreceptor cells (Fig. 2C). In *NGL-2* knockout mice, HC axons overshoot into neighboring laminae and form fewer synapses with rods. Presynaptic release sites in rod photoreceptors display immature characteristics, suggesting that NGL-2/Netrin-G2 signaling regulates presynaptic maturation. Loss of *NGL-2* does not affect HC dendrite – cone photoreceptor connectivity, and impairs visual function in a pathway-specific manner [50]. Together, these studies suggest that the Netrin-G/NGL trans-synaptic interaction may broadly regulate pathway-specific synapse development.

The mechanisms by which NGLs regulate postsynaptic differentiation are not well understood. Cyclin-dependent kinase-like 5 (Cdkl5) interacts with NGL-1 and phosphorylates NGL-1 on a serine residue close to the PDZ interaction site. This phosphorylation promotes NGL-1 binding to PSD-95 and formation of dendritic spines in cultured hippocampal neurons [51]. The PDZ interaction site in NGL-2 was previously shown to recruit PSD-95 to dendritic protrusions in cultured neurons [39] and is also required for NGL-2-mediated regulation of dendritic spines in vivo [49]. Thus, interactions of NGL with synaptic scaffolding proteins regulate spine morphogenesis in vitro and in vivo.

FLRTs interact with latrophilin, a presynaptic adhesion GPCR

Similar to LRRTMs, Slitrks, and NGLs, Fibronectin Leucine-Rich Repeat Transmembrane proteins (FLRT1-3) bind to a presynaptic receptor that interacts with multiple postsynaptic ligands. In the hippocampus, *FLRT2* and *FLRT3* show complementary expression patterns (Fig. 4), whereas *FLRT1* is only weakly expressed. The role of FLRTs in synaptic development is best characterized for FLRT3, an adhesion molecule present at excitatory synapses and in postsynaptic density fractions [52]. Affinity chromatography on brain extracts using the recombinant FLRT3 extracellular domain identified the G protein-coupled receptor (GPCR) latrophilin 3 (LPHN3) [52] (Fig. 1D). Latrophilins (LPHN1-3) are adhesion-GPCRs [53] containing large extracellular domains with multiple interaction motifs [54]. They were originally identified, together with neurexins, as receptors for the neurotransmitter release-inducing black widow spider venom α-latrotoxin [55–59]. Affinity chromatography with recombinant LPHN1 or LPHN3 ectodomains also identified FLRTs

and an additional family of surface receptors, the Teneurins, as the main latrophilin interactors [52, 60, 61]. Teneurins (Ten1-4) are cell adhesion molecules that regulate connectivity in invertebrate and vertebrate olfactory, neuromuscular and visual systems [62–64]. Immuno-EM on purified synaptosomes indicates that endogenous LPHN1 is mostly presynaptically localized, and that LPHN1 binding activity is largely postsynaptic [60]. Thus, like the neurexins and RPTPs, latrophilins interact with multiple postsynaptic ligands. Furthermore, these interactions can be modulated by alternative splicing, as the presence of a small insert between the LPHN1 lectin and olfactomedin domains reduces the affinity for Teneurins without affecting binding to FLRT3 [61].

FLRTs differ from LRRTMs, Slitrks, and NGLs in that they do not induce presynaptic differentiation in coculture assays [52]. Remarkably, Teneurins do induce presynaptic differentiation in cocultures [60], suggesting that FLRT3 or Teneurin binding to LPHN mediates differential effects. The two LPHN ligands may bind to different domains in LPHN, activate different intracellular signaling pathways, or induce recruitment of additional receptors in *cis*, all of which could account for their differential effects. FLRT3 binds to the LPHN3 olfactomedin domain [65], whereas Teneurins bind to both olfactomedin and lectin domains and minimally require the lectin domain [61, 65], suggesting that the two ligands bind to different regions of the LPHN ligand-binding domain. Little is known about downstream effectors of LPHN, although the addition of a soluble extracellular fragment of Ten-2 increases Ca^{2+} signaling in cultured neurons, which could indicate activation of G protein signaling [60]. LPHN1 also binds to S4-lacking neurexins [66], raising the possibility that Teneurins could induce a *cis* complex of LPHN and neurexin to trigger presynaptic differentiation.

Although FLRTs do not induce presynaptic differentiation in cocultures, manipulating FLRT3 and LPHN3 levels does affect glutamatergic synapse development. Knockdown of *FLRT3* in cultured hippocampal granule cells reduces excitatory synapse density, and competition experiments with excess soluble LPHN3 ectodomains or culture-wide knockdown of *LPHN3* similarly reduces the density of excitatory synapses [52]. In vivo, knockdown of *FLRT3* reduces dendritic spine density and evoked perforant path synaptic transmission onto hippocampal granule cells [52]. Knockdown of *LPHN3* in cortical layer 2/3 (L2/3) neurons reduces the density of synapses formed by L2/3 axons in L5 [65]. This defect can be rescued by co-expressing shRNA-resistant mutant LPHN3 lacking the lectin domain, but not the olfactomedin domain [65], suggesting that the interaction with FLRTs, but not Teneurins, is required for synapse formation by L2/3 axons. Thus, FLRT3 regulates glutamatergic synapse development in cultured neurons and in vivo, likely via a trans-synaptic interaction with LPHN3.

The molecular mechanisms by which FLRT3 regulates postsynaptic development are unclear. Unlike LRRTMs, Slitrks, and NGLs, FLRT proteins lack a PDZ interaction site, suggesting that they do not recruit synaptic scaffolding proteins. Work in other systems indicates that FLRT3 acts as a modulator of cell surface receptors regulating various developmental processes including growth factor signaling, adhesion and axon pathfinding. In *Xenopus*, FLRT3 is required for FGF signaling, and interacts in *cis* with cotransfected FGF receptors [67–69]. FLRT3 further interacts with cadherin and protocadherin in

coexpression experiments, and interferes with cadherin-mediated cell adhesion via the small GTPase RndI, possibly by modulating cadherin surface levels [70–72]. In mouse rostral thalamic neurons, FLRT3 acts as a co-receptor of the Robo1 receptor and, in the presence of the Robo1 ligand Slit1, determines attraction to the axon guidance cue Netrin-1 via its DCC receptor [73]. Intermediate thalamic neurons expressing Robo1 and DCC, but are not attracted by Netrin-1 because they lack FLRT3. Thus, FLRT3 can engage in *cis* interactions with other surface receptors and thereby modulate cellular responsiveness, depending on context. Whether similar interactions occur at the synapse, and whether they might be regulated by *trans* interactions of FLRT3 with LPHN, or with Unc5, receptors that also interact with FLRT3 in *trans* [74], remains to be determined.

Other LRR proteins regulating excitatory and inhibitory synapse development

A surprising discovery from a coculture-based expression screen is TrkC [32], the tyrosine kinase receptor for the neurotrophin NT-3, which broadly regulates neural development [75]. TrkC is the only Trk receptor that induces presynaptic differentiation in cocultures, and this requires the LRR and first Ig domain, which are not involved in NT-3 binding. TrkC trans-synaptically interacts with RPTPσ, but not with RPTPδ or LAR, and this interaction is required for excitatory synapse development, independent of TrkC kinase activity [32].

Leucine-Rich Repeat and Fibronectin Type III Domain Containing proteins (also known as Synaptic Cell Adhesion-Like proteins; LRFN/SALM1-5) were identified as PSD-family-interacting proteins [76–78]. LRFN4/SALM3 and LRFN5/SALM5 are the only LRFN/SALM family members with synaptogenic activity in coculture assays and promote both excitatory and inhibitory synapse formation [79]. LRFN1/SALM2 regulates excitatory synapse maturation [76]. The LRFN/SALM trans-synaptic binding partners have not been identified, although LRFN3/SALM4 and LRFN5/SALM5 members can interact homophilically in *trans* [80].

LRR proteins as regulators of synaptic function and plasticity

The LRR proteins discussed thus far all have synaptogenic effects in cultured neurons. An exception is Elfn1, an LRR protein with a domain organization similar to FLRTs, but containing a longer cytoplasmic tail. Elfn1 does not induce presynaptic differentiation in coculture assays or affect synapse number when overexpressed in cultured neurons [81]. Rather, Elfn1 trans-synaptically instructs presynaptic neurotransmitter release properties. *Elfn1* is expressed in somatostatin-positive hippocampal interneurons called oriens-lacunosum moleculare (O-LM) cells [9, 81] (Fig. 4). O-LM cells receive excitatory input from CA1 axons, and Elfn1 localizes to the postsynaptic density of these inputs [81] (Fig. 3A, B). CA1 axons target a second population of interneurons, which are parvalbumin (PV)-positive but do not express *Elfn1*. The two classes of synapses made by the same axon, the CA1 - O-LM synapse and the CA1 - PV synapse, have strikingly different functional characteristics: CA1 - O-LM synapses are strongly facilitating with a low initial probability of release, whereas CA1 - PV synapses are depressing, with a high probability of release (Fig. 3A, B). Knockdown of *Elfn1* in O-LM cells reduces facilitation and increases release

probability, suggesting that postsynaptic Elfn1 controls presynaptic release probability at CA1 – O-LM synapses. Indeed, expressing Elfn1 at CA1 - PV synapses turns these normally depressing synapses into mildly facilitating ones [81]. Thus, Elfn1 trans-synaptically regulates target-specific release properties and contributes to the functional diversity of synapses. Elfn1-mediated facilitation requires presynaptic GluK6 kainate receptors [81], but whether Elfn1 interacts with a presynaptic receptor like other LRR proteins, requires additional receptors in *cis*, or activates a retrograde signal is unknown.

The LRRTMs are another class of LRR proteins with a role in shaping synaptic properties. Combined knockdown of *LRRTM1*/*LRRTM2* at both developing and mature CA1 synapses blocks LTP at Schaffer collateral synapses [82]. The block in LTP can be rescued by re-expressing the extracellular domain of LRRTM2, but not the extracellular domain in which two key residues that mediate the interaction with presynaptic neurexin [16] have been mutated. *LRRTM1*/*LRRTM2* knockdown decreases GluA1 AMPAR surface expression under basal conditions and prevents the increase in synaptic GluA1 surface levels following chemical induction of LTP (cLTP) in cultured neurons. Intriguingly, the initial delivery of surface GluA1 upon cLTP is not impaired by *LRRTM1*/*LRRTM2* knockdown, suggesting that LRRTM1 and -2 are mainly required for maintaining AMPARs at synapses during LTP [82]. LRRTMs can directly interact with AMPARs [14, 83], which could be a mechanism for stabilizing AMPARs at synaptic sites. LRRTM4 has similar, though not identical, effects to LRRTM1 and -2. Knockout of *LRRTM4* also prevents the cLTP-induced increase in GluA1 synaptic surface levels in dentate granule cells, but does not affect basal GluA1 synaptic surface levels [23]. Together, these results suggest that LRRTMs, and possibly other LRR proteins, are important regulators of plasticity-induced changes in synaptic properties.

Concluding Remarks

There has been tremendous progress in the past few years in the identification of novel LRR-containing synaptic adhesion molecules, the elucidation of their trans-synaptic interactions, and the understanding of their role in the development and plasticity of synapses. Several families of postsynaptic LRR proteins, with similar domain organization, but with distinct cell type-specific expression, have now been identified. The significance of such diversity of LRR proteins, especially at glutamatergic synapses, is not well understood, but an attractive possibility is that they may provide the underlying basis of structural and functional diversity of CNS synapses. Since individual neurons likely express several LRR proteins, it will be important to determine how LRR proteins, possibly in combination, control the development and plasticity of functional neural circuits. Many LRR proteins have multiple binding partners, both across the synaptic cleft as well as in *cis*, and it will be important to fully characterize these protein interaction networks and determine how they are modulated by alternative splicing and activity. Furthermore, very little is known about the downstream signaling pathways to which LRR proteins and their binding partners couple. The role of LRR proteins in the plasticity and functional properties of synapses is only beginning to be understood. Addressing these questions should enhance our understanding of this important family of synaptic proteins (Box 1).

Box 1

Outstanding questions

- Why are there so many LRR proteins involved in regulating excitatory synapses? Do they act at specific synapses, regulate different aspects of glutamatergic synapse development, or act in combination to define synaptic identity?
- What is the cellular and subcellular localization of LRR proteins in vivo? Do individual synapses contain more than one LRR protein?
- Are there other LRR proteins that selectively function at GABAergic synapses?
- How are LRR protein interaction networks modulated by activity and alternative splicing?
- How can LRR proteins interact with multiple receptors yet selectively regulate one class of synapses?
- How do mutations in LRR proteins affect neural circuit development and function?

Finally, it is worth noting that the central role of LRR proteins in regulating excitatory and inhibitory connections may provide insight into processes that are disrupted in neurodevelopmental and psychiatric disorders. Some LRR genes have been directly linked to human brain disorders, including autism and schizophrenia (Box 2 and Table 1) [84], and we expect that deep sequencing approaches will likely reveal additional associations that are not detected by common variant (SNP) analysis. More generally, the fact that LRR proteins can regulate excitatory and inhibitory synapses in a cell type-specific manner should enhance our understanding of how the synaptic substructure contributes to circuit function and behavior, and that understanding could provide a framework for developing modulators of LRR interactions to correct synaptic dysfunction associated with mental illness.

Box 2

LRR genes in neurodevelopmental and psychiatric disorders

Recent genetic studies of disorders such as autism, schizophrenia, and attention deficit hyperactivity disorder (ADHD) suggest that LRR proteins and their binding partners are important for the organization and function of neural circuits in humans as well. Table 1 gives an overview of mutations associated with neurodevelopmental and psychiatric disorders in genes encoding LRR proteins and binding partners discussed in this review. A recent, comprehensive overview of the extensive literature on mutations in *NRXN1* associated with neurodevelopmental disorders is given in [85]. A recent, detailed overview of mutations in *PTPR*, *SLITRK* and *NTRK3* (TrkC) genes associated with neuropsychiatric disorders is provided in [37].

The mutations in genes encoding LRR proteins and binding partners summarized in Table 1 are rare, often affecting only a few individuals in that study's patient cohort, and

are not always reproduced in other studies. Several of the LRR genes discussed in this review have been linked to multiple disorders; mutations in *LRRTM3* for instance have been associated with disorders as diverse as autism and late onset Alzheimer's disease. Conversely, a disorder often involves mutations in multiple LRR genes and their binding partners (as well as other, unrelated genes). Given their critical role in the organization and function of neural circuits in vertebrate model systems, it seems likely that human mutations in LRR genes or their binding partners could compromise synapse development or function, perturb E/I balance, and lead to a range of neurodevelopmental and psychiatric disorders. However, the effects of these mutations may be subtle. In many cases, patients still carry a functional copy of the gene, and for many of the mutations described in Table 1, such as single nucleotide polymorphisms (SNPs) in introns, it is not yet clear how they affect gene expression. In the case of the *LRRTM* genes (with the exception of *LRRTM4*), which are nested in the introns of α-catenin genes [13], mutations in *LRRTMs* might not only affect expression of *LRRTM* genes, but could conceivably also affect *CTNNA* gene expression.

In a few cases, the effects of disease-associated mutations on gene expression levels have been determined. A rare disruption in *NTNG1* in a single patient with Rett syndrome was reported to decrease the abundance of a specific *NTNG1* splice variant [86]. Decreased expression of specific *NTNG1* splice variants has also been observed in patients with schizophrenia and bipolar disorder [87, 88]. This could conceivably affect binding of Netrin-G1 to LAR, which is modulated by alternative splicing of Netrin-G1 [43], and thus perturb synapse development. Rare missense mutations in *NTNG1* and *LRRTM1* (Table 1) do not seem to affect critical regions of the proteins. A rare missense mutation (A247S) has been identified in the *LPHN3* olfactomedin domain [89], the domain required for FLRT3 binding [65], but whether this mutation is ADHD-specific and affects the interaction with FLRT3 is not clear. Thus, more work is needed to obtain a better understanding of the impact of disease-associated mutations on gene expression, gene function, and the function of neural circuits.

Acknowledgments

Work in the authors' labs on LRR proteins is supported by an ERC Starting Grant (#311083) and FWO Odysseus Grant (JdW), and NIH grant R01NS067216 (AG).

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Highlights

- LRR proteins regulate the development, function and plasticity of excitatory and inhibitory synapses
- Postsynaptic LRR proteins interact with key presynaptic receptors to promote pre- and postsynaptic differentiation
- Distinct LRR proteins are expressed in different cell types in the CNS
- LRR protein dysfunction may disrupt the excitation/inhibition balance and contribute to neuropsychiatric disorders

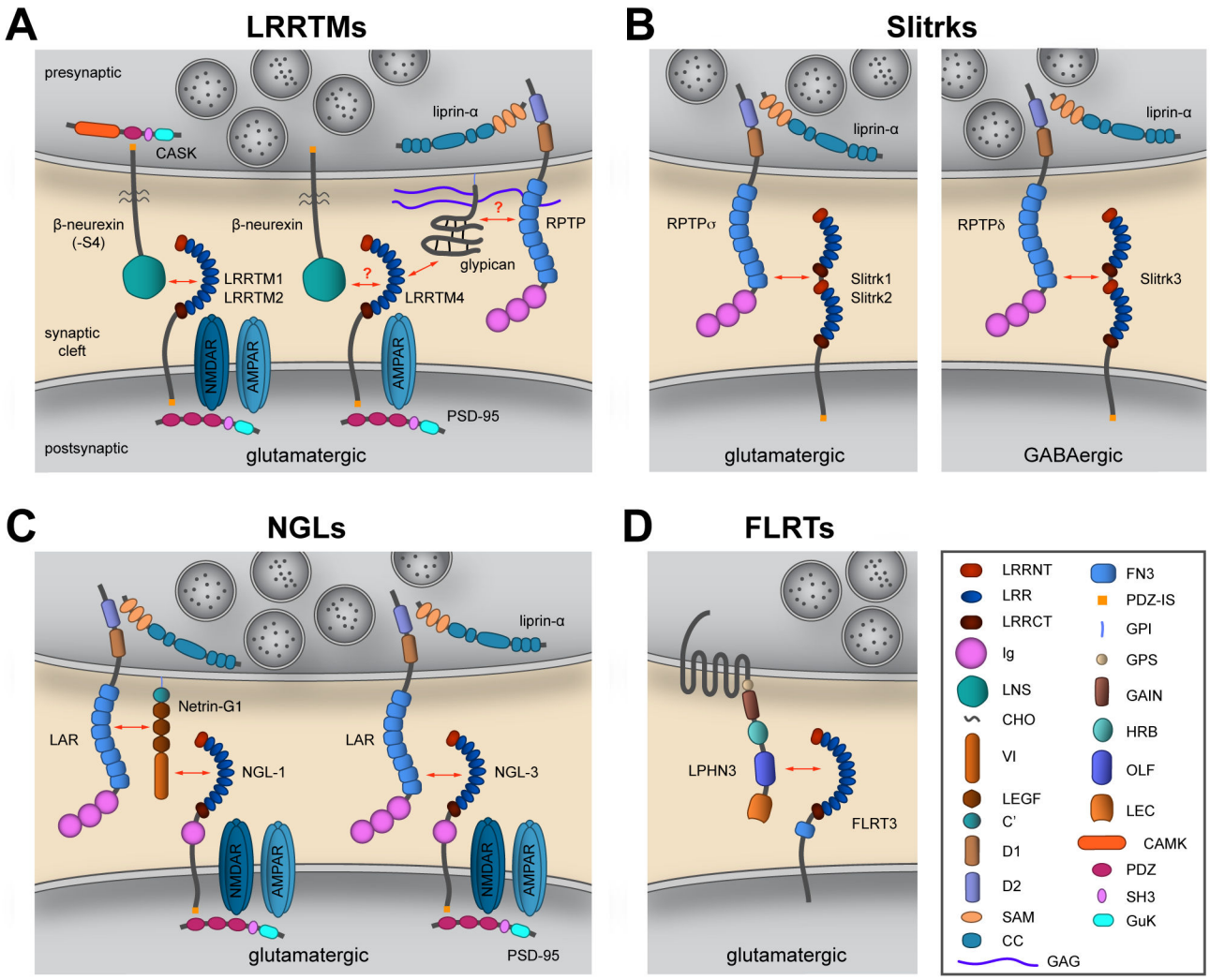

Figure 1. Synaptic LRR proteins and their interactions

Postsynaptic LRR proteins and their presynaptic partners form a trans-synaptic complex that bridges the synaptic cleft and recruits essential scaffolding molecules and neurotransmitter receptors to the synapse. A, Regulation of excitatory synapse development by LRRTMs. LRRTM1 and LRRTM2 bind to presynaptic α- (long) and β- (short) neurexins that lack a small insert at splice site 4 (S4) in the LNS domain. Only β-neurexin is shown. LRRTM4 binds to presynaptic HSPGs, including glypican. Glypican may act via a co-receptor such as LAR family RPTPs to induce presynaptic differentiation. LRRTM4 can also bind neurexin under certain conditions, but the functional significance of this interaction is not clear. The cytoplasmic tails of LRRTMs, neurexins and RPTPs couple to the scaffolding proteins PSD-95, CASK and liprin-α, respectively. B, Slitrk1 and Slitrk2 bind to presynaptic RPTPσ and regulate excitatory synapse formation in cultured neurons. Slitrk3 binds to presynaptic RPTPδ and regulates inhibitory synapse formation. C, NGL-1 binds to presynaptic Netrin-G1, and this interaction induces recruitment of LAR to the complex. NGL-2 binds to presynaptic Netrin-G2 (not shown). NGL-3 binds to the first two FN3 repeats in LAR. D, FLRT3 binds to the adhesion GPCR latrophilin.

Domain abbreviations: LRRNT, LRRCT, LRR N- and C-terminal flanking domains; Ig, immunoglobulin-like; LNS, laminin-α/neurexin/sex-hormone-binding globulin (also known as Laminin-G domain); CHO, carbohydrate attachment; VI, laminin N-terminal; LEGF, laminin EGF-like motifs 1–3; C′, C-terminal domain; D1, D2, membrane-proximal (catalytically active) and -distal (inactive) tyrosine phophatase domains; SAM, sterile alpha motif; CC, coiled coil; GAG, glycosaminoglycan (heparan sulfate); FN3, fibronectin type III; PDZ-IS, post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1) (PDZ) interaction site; GPI, glycosylphosphatidylinositol; GPS, GPCR proteolytic site; GAIN, GPCR autoproteolysis-inducing domain; HRB, hormone-binding domain; OLF, olfactomedin; LEC, lectin domain; CaMK, Ca^{2+}/calmodulin-dependent kinase; SH3, Src homology 3; GuK, guanylate kinase domain.

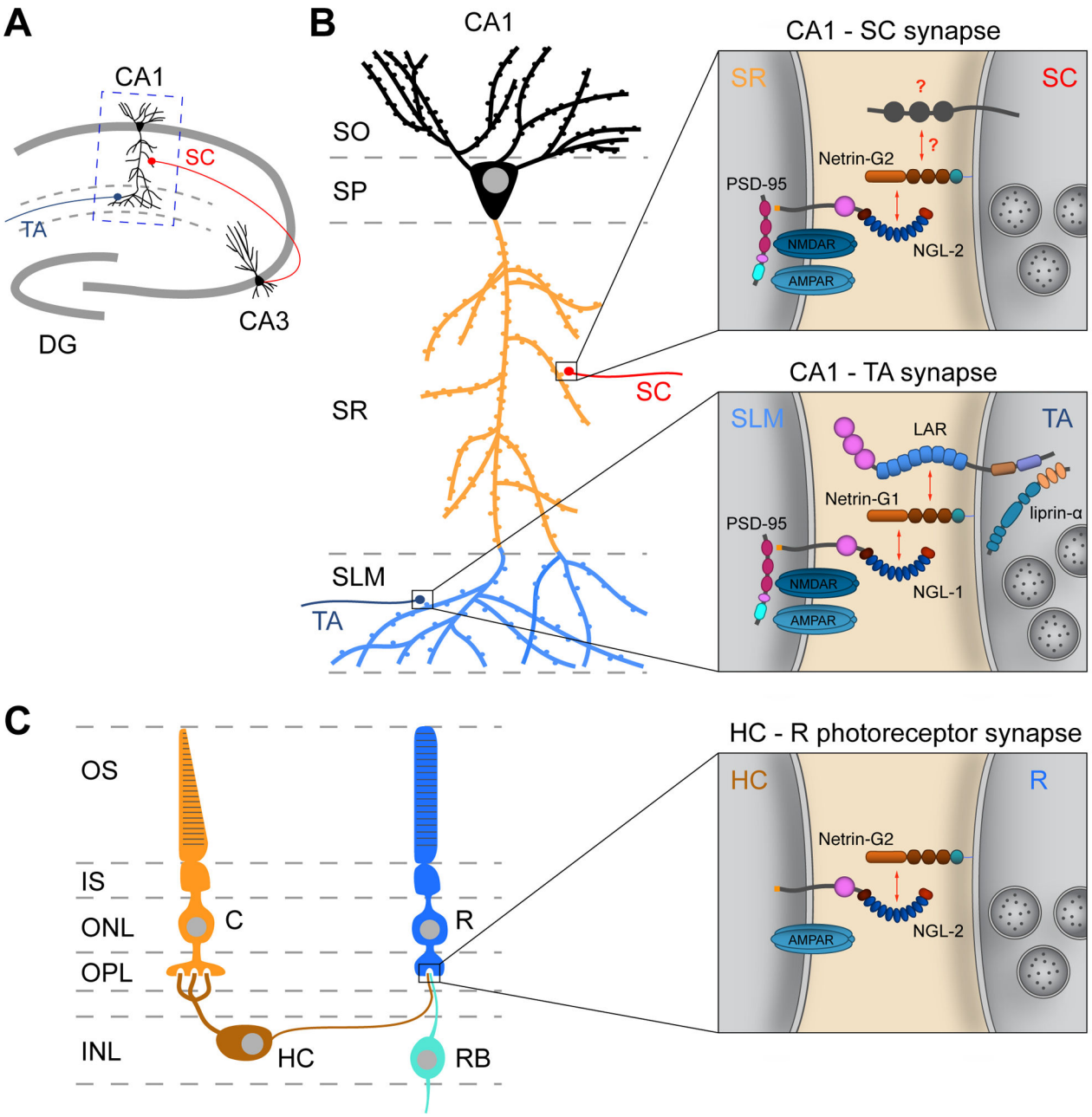

Figure 2. Regulation of pathway-specific synapse development by NGLs

A, Schematic representation of CA1 hippocampal circuits. CA1 pyramidal neurons receive input on their proximal dendrites from hippocampal CA3 axons (the Schaffer collaterals, SC), and input on their distal dendrites from temporoammonic (TA) axons originating from entorhinal cortex. B, Schematic representation of a CA1 neuron receiving SC and TA inputs. SC axons are confined to the stratum radiatum (SR) and TA axons to the stratum lacunosum moleculare (SLM). Netrin-G2 and NGL-2 localize to the pre- and postsynaptic side, respectively, of the SC inputs in SR, and NGL-2 is functionally required for the development of these inputs. Netrin-G1 and NGL-1 localize to the pre- and postsynaptic side, respectively, of the TA inputs in SLM. Analogous to the NGL-1/Netrin-G1/LAR

complex, NGL-2 binding to Netrin-G2 may also induce recruitment of a presynaptic co-receptor (indicated with '?'). Cartoon CA1 neuron based on [123]. DG, dentate gyrus; CA, cornu ammonis; SO, stratum oriens; SP, stratum pyramidale. C, Schematic representation of B-type horizontal cell (HC) connectivity in the retina. Photoreceptors form triad synapses with HCs and bipolar cells (RB) in the outer plexiform layer (OPL). HC axon terminals synapse with rod (R) photoreceptors, and HC dendrites with cone (C) photoreceptors. NGL-2 selectively localizes to the tips of HC axons, which form a postsynaptic specialization at the HC-R photoreceptor synapse. Loss of *NGL-2* results in fewer HC-R synapses, but does not affect HC-C connectivity. OS, photoreceptor outer segment; IS, inner segment; ONL, outer nuclear layer; INL, inner nuclear layer; RB, rod bipolar cell.

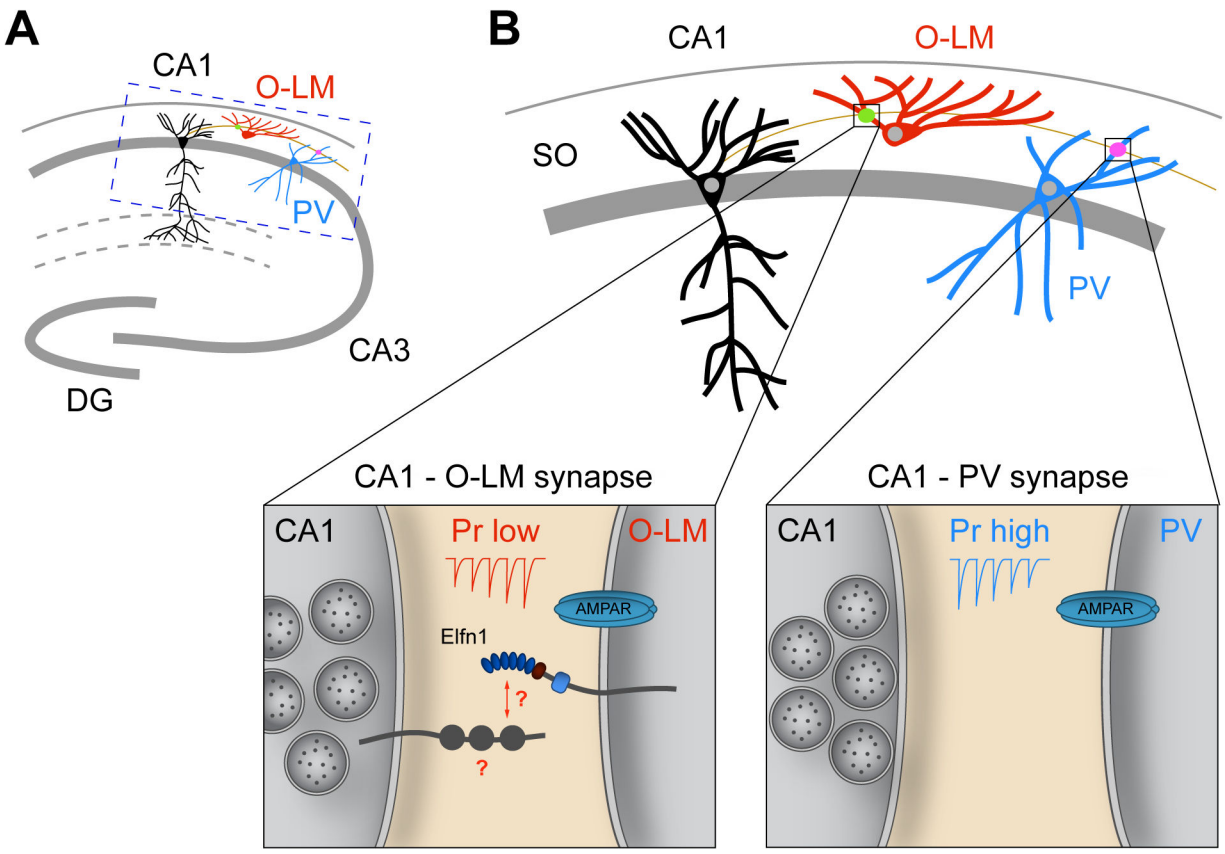

Figure 3. Elfn1 regulates release properties at a specific synapse

A, CA1 axons synapse on two interneuron populations in the stratum oriens (SO): somatostatin-positive O-LM (oriens-lacunosum moleculare) interneurons and parvalbumin (PV)-positive interneurons. B, CA1 – O-LM synapses have a low probability of release (Pr) and are facilitating; CA1 – PV synapses have a high Pr and are depressing. Elfn1 specifically localizes to the postsynaptic side of the CA1 – O-LM synapse and controls release probability in CA1 axons in a target cell-specific manner. Elfn1 may interact with a presynaptic receptor (indicated with '?'), require additional receptors in *cis*, or activate a retrograde signal to regulate presynaptic release properties.

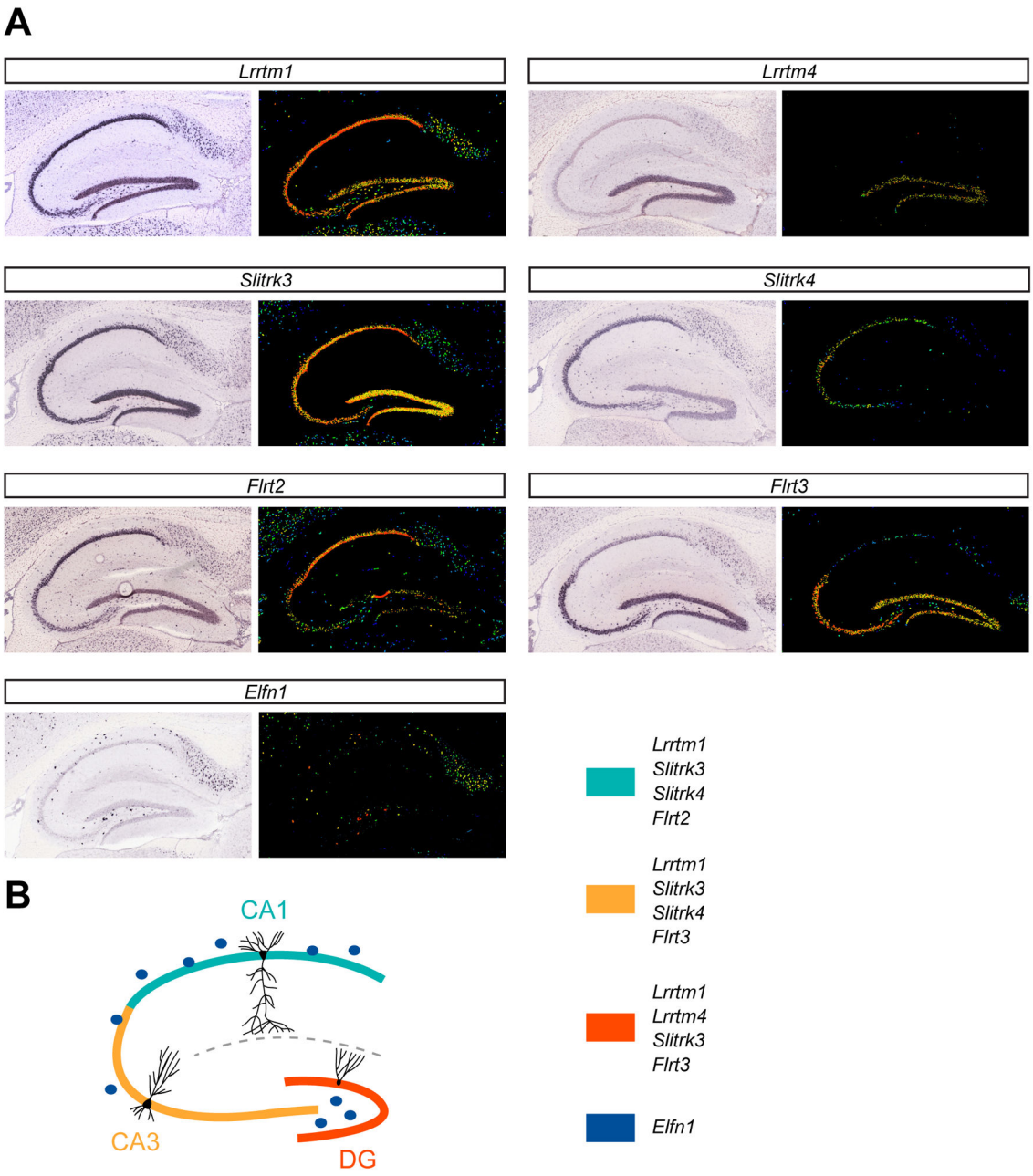

Figure 4. Cell type-specific expression patterns of synaptic LRR genes

A, *In situ* hybridizations showing gene expression patterns in P56 sagittal mouse hippocampal sections for a limited set of synaptic LRR genes discussed in this review. *Lrrtm1* (http://mouse.brain-map.org/experiment/show/68162201) is broadly expressed in the hippocampus, whereas *Lrrtm4* (http://mouse.brain-map.org/experiment/show/70437762) expression is restricted to DG. *Slitrk3* (http://mouse.brain-map.org/experiment/show/69874150) is broadly expressed in the hippocampus, whereas *Slitrk4* (http://mouse.brain-map.org/experiment/show/69874156) is expressed in the CA region, but is absent from DG. *Flrt2* (http://mouse.brain-map.org/experiment/show/70565569) and *Flrt3* (http://

mouse.brain-map.org/experiment/show/71656690) show complementary expression patterns in the hippocampus: *Flrt2* is expressed in CA1, whereas *Flrt3* is expressed in CA3 and DG. *Elfn1* (http://mouse.brain-map.org/experiment/show/77791978) is expressed in somatostatin-positive interneurons in the hippocampus. All images in (A) are obtained from the Allen Mouse Brain Atlas (http://mouse.brain-map.org) [124]. For each gene, the left panel shows the original *in situ* hybridization signal, and the right panel shows the expression mask image using a heat map color scale to indicate intensity of expression (with red signal indicating high expression and blue signal low expression). B, Schematic summary representation of gene expression patterns in the hippocampus based on (A). Different hippocampal cell types express different (combinations of) synaptic LRR genes.

Table 1

Postsynaptic LRR proteins and their presynaptic binding partners implicated in neurodevelopmental and psychiatric disorders.

Gene/Protein	Mutation	Disorder	References
***LRRTM1*/LRRTM1**	Rare missense mutation N330S; SNP (upstream region)	Schizophrenia	[90–92]
***LRRTM2*/LRRTM2**	CNV (duplication)	Bipolar disorder	[93]
	CNV (deletion)	Developmental delay	[94]
***LRRTM3*/LRRTM3**	SNP (5′UTR, promoter and intronic)	Alzheimer's disease, late onset (LOAD)	[95–103]
	SNP (intronic)	Autism	[104, 105]
	CNV (deletion and duplication)	Autism	[106, 107]
***LRRTM4*/LRRTM4**	CNV (deletion)	Autism	[107–109]
	SNP (intronic)	Autism	[105]
***GPC1*/GPC1**	CNV (deletion)	Autism	[107, 110]
***GPC4*/GPC4**	CNV (deletion)	Autism	[107]
***GPC5*/GPC5**	CNV (deletion and duplication)	Autism	[107, 109]
	CNV	Bipolar disorder	[111]
***GPC6*/GPC6**	CNV (deletion and duplication)	Autism	[108, 109]
***NTNG1*/Netrin-G1**	Rare missense mutations (Y23C, T135I)	Autism	[112, 113]
	Rare gene disruption affecting alternative splicing	Rett syndrome	[86]
	SNP (intronic)	Schizophrenia	[87, 114, 115]
***NTNG2*/Netrin-G2**	SNP (intronic)	Schizophrenia	[87]
	GWAS	Autism	[108]
***FLRT1*/FLRT1**	CNV (duplication)	Autism	[116]
***FLRT2*/FLRT2**	CNV (duplication)	Autism	[106, 108]
***LPHN1*/LPHN1**	Silent mutation	Autism	[117]
***LPHN3*/LPHN3**	SNP (intronic); Rare missense mutations	Attention-deficit/hyperactivity disorder (ADHD)	[89, 118–120]
	CNV (deletion)	Autism	[107, 108]
***LRFN2*/LRFN2 (SALM1)**	CNV (duplication)	Autism	[107]
***LRFN5*/LRFN5 (SALM5)**	SNP (intronic)	Autism	[121]
	CNV (deletion)	Autism; developmental delay	[107, 108, 122]

Sources: PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and the following disease-specific gene databases: Autism: SFARI, Simons Foundation Autism Research Initiative (http://www.sfari.org), AutismKB (http://www.autismkb.cbi.pku.edu.cn); Schizophrenia: SZGene (http://www.szgene.org); Bipolar Disorder: BDGene (http://www.bdgene.psych.ac.cn); ADHD: ADHDgene (http://www.adhd.psych.ac.cn); Alzheimer's disease: Alzgene (http://www.alzgene.org). SNP: single nucleotide polymorphism; CNV: copy number variation; GWAS: genome-wide association study.