Reelin functions beyond neuronal migration: from synaptogenesis to network activity modulation

Giulia Faini, Filippo del Bene, Shahad Albadri

Reelin functions beyond neuronal migration: from synaptogenesis to network activity modulation

Giulia Faini[1], Filippo Del Bene[1#], Shahad Albadri[1]

1. Institut de la Vision, Sorbonne Université, INSERM, CNRS, Paris, France.
#. Corresponding author: filippo.del-bene@inserm.fr

Abstract

Reelin, a glycoprotein of the extracellular matrix, has been the focus of several studies over the years, mostly for its role in cell migration. Here we report the role of this molecule and of its downstream pathways in post-mitotic neurons and how they contribute to neural circuit assembly, refinement and function. Accumulating evidence has pointed at a major role for Reelin in axonal guidance, synaptogenesis and dendritic spine formation. In particular new evidence points at a direct role in axonal targeting and refinement at the target site. In addition, recent advances highlight new functions of Reelin in the modulation of synaptic activity, plasticity and behavior and it directly regulates GABA receptors expression and stability. We discuss these findings in the context of neurodevelopmental disorders.

Highlights

- Reelin signaling is required for axonal targeting and refinement at the target site.
- Reelin signaling regulates dendritogenesis and spine formation in mature neurons.
- Reelin modulates GABA receptors expression and stability, pre-and-post-synaptically.
- Reelin is important for hippocampal integrity, synaptic plasticity and behavior.

Introduction

Functional neural circuits arise through a series of sequential steps including neurogenesis, cell migration, axonal navigation and synaptogenesis. Perturbations of these events, which start during early embryogenesis, have been linked with the etiology of neurodevelopmental disorders. Reelin is an extracellular matrix (ECM) glycoprotein that acts as a key regulator of different steps of brain wiring. In the mammalian neo-cortex, Reelin regulates neuronal migration and layer formation, dendritic arborization and synaptogenesis[1]. It regulates these distinct processes by acting through two receptors, ApoER2 and VLDLR, which are differentially expressed throughout the nervous system and display specific functions[2,3]. Although it is well established that Reelin and its pathway play an essential role in neuronal migration, accumulating evidence support additional functions in multiple other distinct biological processes such as axon guidance, dendritogenesis and synaptogenesis. In addition, recent work shows that Reelin signaling and its interactors are essential for the modulation of synaptic function and network activity as well as synaptic plasticity. This short review will focus on these recent findings and highlight the importance of Reelin signaling in the regulation of learning, memory and behavior abilities in adulthood, all implicated in Reelin-associated neurological disorders.

Reelin role in axon guidance

Until recently, the role of Reelin in axon guidance was dismissed as early reports failed to detect any involvement in this process[4]. However, accumulating evidence point at a role for Reelin in axon growth and synaptic targeting, essentially analyzing the role of this ECM protein in the entorhinal cortex and the visual system.

In the entorhino-hippocampal and commissural projections of *reeler* mice, several alterations including reduced axonal branching, decreased number of synapses, and abnormal topography of synapses were observed[5,6]. Other defects such as the presence of misrouted fibers and the formation of ectopic termination patches were also reported. These early studies therefore hinted at new role for Reelin in axon guidance. However a parallel study rather suggested that the observed abnormalities of hippocampal cells projections onto the entorhinal cortex were instead primarily independent of the Reelin pathway[4]. Nevertheless, later work performed in *dab1*-deficient mice revealed a similar phenotype characterized by entorhinal afferents targeting inappropriate layers in the hippocampus, thus indicating that Reelin can in fact act as guidance factor via Dab1[7]. Moreover, by using combinations of entorhinal and hippocampal organotypic co-cultures from wild type and mutants, this same study showed that Dab1 is required for both ingrowing axons and for the target tissue to ensure a normal projection pattern[7]. In addition, exposure to Reelin of entorhinal and dorsal root ganglia axons lead to the reduced axonal growth in explant cultures[7].

In the visual system, several studies have implicated Reelin and its pathway in the proper patterning of synaptic connectivity of the visual circuits. In 2001, Rice and colleagues showed the first evidence of an involvement of Reelin pathway in the regulation of the visual circuits anatomy and function by analyzing the effect of Reelin and Dab1a loss of functions[8,9]. In this work, the authors observed alterations in the projection patterns of rod bipolar cells and AII amacrine cells in the retinal inner plexiform layer of *reelin* and *dab1* mutant mice. Later studies performed in the *reeler* mouse and *reelin* mutant rat reported defects in the trajectories of retinal afferents to the *superior colliculus* but could not link these effects directly to the loss of Reelin function as the architecture of this tissue is itself affected in these animals[10,11]. However a subsequent study reported misrouted axonal projections of intrinsically photosensitive retinal ganglion cells to their appropriate partners in the lateral geniculate nucleus, which rather project to other non-retino-recipient thalamic regions of the *reeler* and *dab1* mice brains[12]. Unlike the previous observations made in the superior colliculus, these defects and the mis-targeting phenotype observed in these mutants are unlikely to arise from lamination defects or neural mis-positioning. More recently, a study in zebrafish showed that *reelin* is expressed as a gradient in the fish brain retino-recipient, the optic tectum, to differentiate the laminae where RGC axons project[13]. This work elucidated the role of Reelin in the proper establishment of synaptic stratification in the tectal neuropil, where RGCs axons and dendrites from periventricular neurons form both synaptic contacts. Through loss-of-function analysis of components of the Reelin pathway, the authors demonstrated that alterations of Reelin, Dab1a and Vldlr in zebrafish lead to the disruption of RGCs targeting in the optic tectum as well as the stratification of periventricular neurons in the tectal neuropil, both in an independent manner[13] (Fig 1). Reelin signaling is required in this context in a cell autonomous manner to guide precise axonal lamination. The general similarity in the global organization of the teleost optic tectum and the mammalian cortex, where incoming axonal inputs contact apical dendrites or neuropils of recipient neurons, raises the intriguing possibility that Reelin acts, after migration, in the orchestrated wiring of layer 1 of the mammalian neocortex (Fig 2). In this structure, Reelin is produced by two neuronal types: the superficially located Cajal-Retzius cells that secrete Reelin throughout embryogenesis and early postnatal life in mouse and a subset of interneurons that start expressing Reelin from birth. In this brain region Reelin could act similarly to what observed in the zebrafish optic tectum to regulate the precise stratification of incoming thalamocortical axons that project into layer 1.

At the cell intrinsic level, other recent studies have also implicated Reelin in axonal wiring via the identification of its crucial regulatory interaction with Cofilin[14,15], a central hub for axon elongation and axonal growth cone steering[16-18]. In this context, Reelin's role in the stabilization of the Actin cytoskeleton through Cofilin phosphorylation was shown to be an important prerequisite for the stability of pyramidal cells leading process. Another recent study further implicated Reelin-Cofilin interaction in cortical neuron dendritic growth dynamic and branching by evaluating the effect of alcohol exposure on Reelin-Dab1 signaling[19]. Time-lapse imaging revealed that cortical neurons exposed to ethanol displayed similar phenotypic defects as observed in the case of Reelin-deficiency (through the blockade of the Reelin-Dab1 tyrosine kinase signaling pathway). This phenotype is mediated by the sustained dephosphorylation and activation of Cofilin.

Altogether these results indicate that Reelin signaling plays an important role both in the targeting and refining of axonal connections at the target site.

Reelin physiological function in synaptogenesis and dendritic spine formation

During development, Reelin mediates its neuronal guidance action through the binding to the lipoprotein receptors VLDLR and ApoER2 that results in cytoplasmic phosphorylation of Dab1 via the Src family of tyrosine kinases (reviewed in[20]). Once neurons have reached their destination, Reelin continues to modulate synaptic signaling pathways, regulates synaptic plasticity as well as axonal and dendritic outgrowth. However, since these anatomical studies were performed on mice in which Reelin signaling components were disrupted from the early embryonic stages, it could not be determined if Reelin signaling regulates the formation, the maturation and/or the maintenance of dendrites or spines in mature neurons.
To test these hypotheses, *in vitro* and *in vivo* analyses of juvenile and adult *reeler* mutant mice revealed a reduction in dendritic tree complexity and dendritic spine density in neurons[21-23]. These studies together indicate that Reelin is important for circuit establishment, thus impacting dendrite and spine development (reviewed in[24]). Indeed, interference with Reelin signaling was shown to strongly perturb dendritogenesis and reduce spine density in mature hippocampal neurons. In particular, the expression of a mutant *ApoER2* form, which blocks its interaction with postsynaptic density protein 95 (PSD-95) and hence cannot transduce Reelin signaling, resulted in the reactivation of dendritogenesis in mature hippocampal neurons[25]. By immunofluorescence, the authors observed that Reelin-signaling impairment reduced synaptic PSD-95 levels. Together these results indicate that Reelin/ApoER2/PSD-95 signaling is important for neuronal structure maintenance in mature neurons.
A concurrent study demonstrated the impact of the Reelin/Dab1 pathway on the synaptogenesis of newborn granule cells (GCs) in the young-adult mouse hippocampus[26]. The authors showed that neither *reelin* overexpression nor the inactivation of its intracellular adapter Dab1 substantially altered dendritic spine numbers in these neurons. In contrast, by 3D-electron microscopy, they revealed that the mis-regulation of the Reelin/Dab1 pathway leads to both transient and permanent changes in the types and morphology of dendritic spines, mainly altering mushroom, filopodial, and branched GC spines. Furthermore, they found that the Reelin/Dab1 pathway controls synaptic configuration of presynaptic boutons in the dentate gyrus and that its deregulation leads to a substantial decrease in multi-synaptic boutons innervation. In addition, in astroglia cells, Reelin/Dab1 pathway was shown to control ensheathment of synapses[26]. Other work also pointed at a possible role for Reelin in dendrites and spine formation in the neocortex by comparing the subcellular localization of Reelin in the cortex and hippocampus in wild-type mice and in heterozygous Reeler mice[27]. Thus, the Reelin pathway is a key regulator of adult-generated GC integration, by controlling dendritic spine types and shapes, their synaptic innervation patterns, and glial ensheathment. These findings reveal a new mode for reactivating dendritogenesis in neurological disorders where dendritic arbor complexity is limited, such as in depression, Alzheimer's disease (AD), and stroke.

Interestingly it should be known that in recent years, mounting evidence has linked the Reelin pathway to AD. For instance, Reelin levels are elevated in the brain of AD patients, but a lower amount of intracellular ApoER2 fragments is detected, suggesting that Reelin signaling is altered in AD[28,29]. Furthermore, the amyloid-β peptide 1–42 alters Reelin glycosylation and compromises its capacity to bind to ApoER2 and the γ-secretase component Presenilin-1 can modulate Reelin signaling by directly processing ApoER2 intracellular domain[30-32].

Reelin modulates synaptic function and network activity

Increasing evidence suggest that Reelin signaling is involved in the regulation of neuronal activity and synaptic function, both at the level of single synapses and network activity.

At the glutamatergic synapses, it is well established that Reelin signaling mediates the composition, the number and the trafficking of both AMPA and NMDA receptors (AMPAR and NMDAR respectively), both pre- and -post synaptically, thus playing an important role in the maturation, organization, stabilization, functionality and plasticity of excitatory synapses[33-35]. Although these roles for Reelin at the excitatory glutamatergic synapse have been extensively identified, less attention has been devoted to shed light on the implication of the glycoprotein in modulating GABAergic inhibitory synaptic activity. Recently, several studies have unraveled a novel synaptic function of Reelin on GABA synapses and circuits. It was suggested for the first time a potential interaction between Reelin and GABA receptors ($GABA_AR$ and $GABA_BR$)[36]. In the prefrontal cortex of *reeler* haplo-insufficient mice, postsynaptic GABA-currents into pyramidal neurons were measured and it was shown that GABAergic transmission mediated by ionotropic $GABA_AR$ is altered in absence of *reelin* and is associated with a disruption of the Excitation/Inhibition (E/I) balance, which in turn affects information processing. Another study demonstrated the link between Reelin and postsynaptic $GABA_AR$ by performing an accurate and high-resolution analysis of $GABA_AR$ subunits expressed specifically in different cell types including *reelin*-expressing neurons[37]. This study indicated that Reelin-positive cells, that are also grid cell candidates, express selectively the α3 subunit of the $GABA_AR$ and that this selective pattern is responsible for both tonic and phasic inhibition. In addition to its involvement in inhibitory postsynaptic activity, a new role of Reelin in modulating presynaptic GABA function has been identified[38]. In this study, the conditional absence of *reelin* (RelncKO) induced an alteration of spontaneous release in glutamatergic neurons via pre-synaptic $GABA_BRs$, leading to hyper-excitable network. The main function of metabotropic $GABA_BR$ at presynaptic sites is to inhibit Ca^{2+} channels, which in turn blocks neurotransmitter release. Accordingly, Ca^{2+} spiking activity in RelncKO were increased. Interestingly, these results indicate that an absence of Reelin is responsible for a down-regulation of $GABA_BRs$ at the presynaptic cell surface, due to alteration of their phosphorylation status as well as proteolytic receptor processing. This in turn leads to a possible disruption of an interaction between the non-receptor receptor type tyrosine kinase Src and the G-protein of the $GABA_BR$, $G\alpha_0$ (Fig 3A). This is in agreement with a previous study reporting that Reelin signaling induces a crosstalk between Src and G protein-coupled receptors[39]. Taken together, these findings argue for a novel function for Reelin signaling in controlling presynaptic activity and excitability through the modulation of $GABA_BRs$ expression and their stability at the neuronal surface.

In addition to the modulation of presynaptic GABA transmission mentioned above, another Reelin-mediated presynaptic mechanism has been reported in hippocampal slices *in vitro*, where Reelin regulates presynaptic spontaneous neurotransmission. Here, Reelin signaling through both ApoER2 and VLDLR receptors, present at the presynaptic membrane, induces an elevation of intracellular Ca^{2+} which specifically increases the fusion of vesicles containing vesicle-associated membrane protein 7 (VAMP7), and is associated with activity of the PI3 kinase[33,34,40] (Fig 3B). Importantly, presynaptic neurotransmission regulation mediated by VAMP7 has been shown to modulate synaptic strength and plasticity[41].

Different studies investigated how Reelin controls and modulates brain network activity, integrity and function. In particular, a recent study showed a link between hippocampal oscillations, dopamine and Reelin[42]. This work revealed that dopamine influences oscillations in the γ-band, and that their modulation, mediated by NMDAR–PI3K signaling, is altered in *reelin* haplo-insufficient mice. These results as such highlight an important implication of Reelin in Schizophrenia where γ oscillations and NMDA transmission are affected[1]. Finally, a role for Reelin signaling in promoting network integrity and activity, especially in the hippocampus was described[43,44]. *NDEL1* mutant mice, a gene encoding for a cytoskeleton protein, showed Reelin depletion together with a loss of both excitatory and inhibitory synapses, leading to marked alteration in integrity and connectivity within the CA1 area of the hippocampus. Importantly, a single application of Reelin in CA1 improved ultra-structural, cellular, morphological, and anatomical defects as well as spatial learning and memory.

Altogether these recent findings illustrate that Reelin signaling is crucial for the proper E/I balance of brain networks, acting both on glutamatergic and GABAergic neurotransmission, and at both pre-and-post synaptic sites. Importantly, an imbalance between the E/I ratio leads to neurological disorders such as epilepsy, autism, schizophrenia and AD, pathologies where Reelin has been shown to be affected[1]. In addition, these results highlight the role of Reelin in controlling and regulating the integrity of neuronal networks, especially in a highly plastic region like the hippocampus.

Reelin modulates synaptic plasticity, learning and behavior

A variety of studies demonstrated the importance of Reelin signaling in synaptic plasticity, memory formation and cognitive function. Indeed, it is well documented that Reelin regulates the insertion of AMPAR and the phosphorylation of NMDAR, resulting in enhancement of long-term potentiation in the cortex and hippocampus (reviewed in[33,45]). Nevertheless, synaptic plasticity also involves the interaction between Reelin and other partners as described below.

Recently, an interesting component of Reelin signaling involved in the modulation of hippocampal synaptic plasticity was identified[46]. The multi-scaffold protein Intersectin-1 (ITSN1) was indeed shown to be directly associated with elements of the Reelin canonical pathway, and mice lacking ITSN1 revealed alterations of NMDA-long-term potentiation in response to Reelin stimulation. Molecularly, it has been suggested that ITSN1 acts as a molecular bridge that facilitates the interaction between VLDLR and its intracellular adaptor Dab1, favoring its phosphorylation and downstream signaling, with the potential help of other factors such as Ephrin B, known to be associated with both ITSN1 and Reelin signaling (Fig 3C). In accordance with these results, ITSN1 was shown to be involved in hippocampal-dependent functions such as learning and memory[47].

It was recently shown that Notch1, a transmembrane receptor and substrate of the γ-secretase, contributes to Reelin-mediated synaptic potentiation via a non-canonical pathway which stimulates Erk1/2 activity and CREB-dependent transcription[48]. In the CA region of the hippocampus, Notch1 functionally interacts with ApoER2 and NMDAR at the postsynaptic sites, and the targeted loss of *Notch1* resulted in an impairment of Reelin signaling. To date, it is not clear how the two functional complexes (Notch1/ApoER2/Dab1 and Notch1/NMDAR) are connected or if they are part of the same synaptic super-complex. Furthermore, Reelin bath application in these mutants failed to promote long-term potentiation, in contrast to wild-type animals. These results highlight a crosstalk of Notch1 and Reelin pathways in regulating mechanisms involved in synaptic plasticity and memory formation (Fig 3D).

Components of the extracellular matrix, and in particular Chondroitine-and-Heparan-sulfate proteoglycans (CSPG and HSPG respectively), are also known to be involved in brain stability and plasticity[49,50]. Specialized extracellular matrix structures called Perineuronal Nets (PNNs), which enwrap *parvalbumin*-expressing interneurons, play a crucial role in visual plasticity. As such, a disruption of PNNs reactivates visual plasticity[51,52]. Importantly, Reelin interacts and co-localizes with both CSPGs and HSPGs, as shown in two recent papers[13,53]. Along this line, an interesting recent study demonstrated a new role for Reelin in modulating cortical plasticity[54]. The lack of *reelin* prolonged experience-dependent plasticity in the visual cortex into adulthood, similarly to the effect of PNN enzymatic degradation on visual plasticity. This work, once again, puts Reelin at the center of brain plasticity modulation in the postnatal brain.

Finally, accordingly with previous and more recent studies[55,56-58], the contribution of Reelin signaling to the genetic and behavioral expression of learning and memory has been confirmed[59,60]. Indeed, an interesting work using epigenomic analysis revealed that the binding of Reelin to its receptor results in the expressions of immediate early genes involved in synaptic plasticity, and that these epigenomic changes possibly involve nuclear translocation of the intracellular domain of ApoER2[61].

Altogether, these results indicate that Reelin signaling regulates synaptic plasticity, learning and memory - mainly in the adult hippocampus - and that alterations of Reelin pathways can drastically affect these neural processes and behavior, leading to irreversible neurological diseases.

Funding

This work has received funding from French National Research Agency (ANR-18-CE16-0017-01), the Fondation Simone and Cino del Duca and UNADEV/AVIESAN (UNADEV-19UU51-DEL BENE). The Del Bene laboratory is supported by the Programme Investissements d'Avenir IHU FOReSIGHT (ANR-18-IAHU-01).

Conflict of interest statement

Declarations of interest: none

References

[1] Ishii, K., Kubo, K.-I. & Nakajima, K. **Reelin and Neuropsychiatric Disorders.** *Front. Cell. Neurosci.* **10,** 229 (2016).

[2] Hirota, Y. *et al.* **Reelin receptors ApoER2 and VLDLR are expressed in distinct spatiotemporal patterns in developing mouse cerebral cortex.** *J. Comp. Neurol.* **523,** 463–478 (2014).

[3] Hirota, Y., Kubo, K.-I., Fujino, T., Yamamoto, T. T. & Nakajima, K. **ApoER2 Controls Not Only Neuronal Migration in the Intermediate Zone But Also Termination of Migration in the Developing Cerebral Cortex.** *Cereb. Cortex* **28,** 223–235 (2018).

[4] Jossin, Y. & Goffinet, A. M. **Reelin does not directly influence axonal growth.** *J. Neurosci.* **21,** RC183–RC183 (2001).

[5] Del Río, J. A. *et al.* **A role for Cajal-Retzius cells and reelin in the development of hippocampal connections.** *Nature* **385,** 70–74 (1997).

[6] Borrell, V. *et al.* **Reelin Regulates the Development and Synaptogenesis of the Layer-Specific Entorhino-Hippocampal Connections.** *J. Neurosci.* **19,** 1345–1358 (1999).

[7] Borrell, V. *et al.* **Reelin and mDab1 regulate the development of hippocampal connections.** *Mol. Cell. Neurosci.* **36,** 158–173 (2007).
[8] Rice, D. S. *et al.* **The reelin pathway modulates the structure and function of retinal synaptic circuitry.** *Neuron* **31,** 929–941 (2001).
[9] Rice, D. S. & Curran, T. **Role of the reelin signaling pathway in central nervous system development.** *Annu. Rev. Neurosci.* **24,** 1005–1039 (2001).
[10] Baba, K., Sakakibara, S., Setsu, T. & Terashima, T. **The superficial layers of the superior colliculus are cytoarchitectually and myeloarchitectually disorganized in the reelin-deficient mouse, reeler.** *Brain Res.* **1140,** 205–215 (2007).
[11] Sakakibara, S., Misaki, K. & Terashima, T. **Cytoarchitecture and fiber pattern of the superior colliculus are disrupted in the Shaking Rat Kawasaki.** *Developmental Brain Research* **141,** 1–13 (2003).
[12] Su, J. *et al.* **Reelin is required for class-specific retinogeniculate targeting.** *J. Neurosci.* **31,** 575–586 (2011).
[13] Di Donato, V. *et al.* **An Attractive Reelin Gradient Establishes Synaptic Lamination in the Vertebrate Visual System.** *Neuron* **97,** 1049–1062.e6 (2018).
In this study the authors report a role for Reelin signaling in the lamina-specific targeting of RGC axons into the neuropil. In Zebrafish, Reelin is gradually distributed and stabilized by heparan sulfate proteoglycans in the neuropil. This study shows that it acts as an important chemico-attractant molecule for the proper laminar targeting of RGC axons in single laminae of the neuropil. Together with Reelin, the authors demonstrate that VLDLR and Dab1a transmit Reelin signaling to RGCs, thereby contributing to this mechanism.

[14] Chai, X. & Frotscher, M. **How does Reelin signaling regulate the neuronal cytoskeleton during migration?** *Neurogenesis (Austin)* **3,** e1242455 (2016).
[15] Frotscher, M., Zhao, S., Wang, S. & Chai, X. **Reelin Signaling Inactivates Cofilin to Stabilize the Cytoskeleton of Migrating Cortical Neurons.** *Front. Cell. Neurosci.* **11,** 148 (2017).
[16] Aizawa, H. *et al.* **Phosphorylation of cofilin by LIM-kinase is necessary for semaphorin 3A-induced growth cone collapse.** *Nature Neuroscience* **4,** 367–373 (2001).
[17] Tilve, S., Difato, F. & Chieregatti, E. **Cofilin 1 activation prevents the defects in axon elongation and guidance induced by extracellular alpha-synuclein.** *Sci. Rep.* **5,** 16524 (2015).
[18] Choi, J.-H. *et al.* **IRES-mediated translation of cofilin regulates axonal growth cone extension and turning.** *The EMBO Journal* **37,** 62 (2018).
[19] Wang, D., Enck, J., Howell, B. W. & Olson, E. C. **Ethanol Exposure Transiently Elevates but Persistently Inhibits Tyrosine Kinase Activity and Impairs the Growth of the Nascent Apical Dendrite.** *Mol. Neurobiol.* **56,** 5749–5762 (2019).
[20] Bock, H. H. & May, P. **Canonical and Non-canonical Reelin Signaling.** *Front. Cell. Neurosci.* **10,** 166 (2016).
[21] Liu, W. S. *et al.* **Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse.** *Proceedings of the National Academy of Sciences* **98,** 3477–3482 (2001).
[22] Niu, S., Renfro, A., Quattrocchi, C. C., Sheldon, M. & D'Arcangelo, G. **Reelin Promotes Hippocampal Dendrite Development through the VLDLR/ApoER2-Dab1 Pathway.** *Neuron* **41,** 71–84 (2004).
[23] Niu, S., Yabut, O. & D'Arcangelo, G. **The Reelin signaling pathway promotes dendritic spine development in hippocampal neurons.** *J. Neurosci.* **28,** 10339–10348 (2008).
[24] Lee, G. H. & D'Arcangelo, G. **New Insights into Reelin-Mediated Signaling Pathways.** *Front. Cell. Neurosci.* **10,** 122 (2016).

[25] Ampuero, E., Jury, N., Härtel, S., Marzolo, M.-P. & van Zundert, B. **Interfering of the Reelin/ApoER2/PSD95 Signaling Axis Reactivates Dendritogenesis of Mature Hippocampal Neurons.** *J. Cell. Physiol.* **232,** 1187–1199 (2017).
In this work, the authors reveal a new role for Reelin signaling in the maintenance of adult network stability and dendritogenesis by interfering with its function in vivo and in vitro in hippocampal neurons. The overexpression of a dominant-negative form of ApoER2, unable to interact with PSD95, led to increased dendritogenesis and reduced spine density in mature hippocampal neurons. This study therefore shows an important role for Reelin/ApoER2/PSD95 signaling in dendritogenesis and network maintenance.

[26] Bosch, C. *et al.* **Reelin Regulates the Maturation of Dendritic Spines, Synaptogenesis and Glial Ensheathment of Newborn Granule Cells.** *Cereb. Cortex* **26,** 4282–4298 (2016).

[27] Pappas, G. D., Kriho, V. & Pesold, C. **Reelin in the extracellular matrix and dendritic spines of the cortex and hippocampus: a comparison between wild type and heterozygous reeler mice by immunoelectron microscopy.** *J. Neurocytol.* **30,** 413–425 (2001).

[28] Botella-López, A. *et al.* **Reelin expression and glycosylation patterns are altered in Alzheimer's disease.** *Proceedings of the National Academy of Sciences* **103,** 5573–5578 (2006).

[29] Mata-Balaguer, T., Cuchillo-Ibáñez, I., Calero, M., Ferrer, I. & Sáez-Valero, J. **Decreased generation of C-terminal fragments of ApoER2 and increased reelin expression in Alzheimer's disease.** *FASEB J* **32,** 3536–3546 (2018).

[30] Cuchillo-Ibáñez, I. *et al.* **Beta-amyloid impairs reelin signaling.** *PLoS ONE* **8,** e72297 (2013).

[31] Cuchillo-Ibáñez, I. *et al.* **The β-amyloid peptide compromises Reelin signaling in Alzheimer's disease.** *Sci. Rep.* **6,** 31646–11 (2016).

[32] Balmaceda, V. *et al.* **ApoER2 processing by presenilin-1 modulates reelin expression.** *The FASEB Journal* **28,** 1543–1554 (2014).

[33] Wasser, C. R. & Herz, J. **Reelin: Neurodevelopmental Architect and Homeostatic Regulator of Excitatory Synapses.** *Journal of Biological Chemistry* **292,** 1330–1338 (2017).

[34] Bosch, C., Muhaisen, A., Pujadas, L., Soriano, E. & Martínez, A. **Reelin Exerts Structural, Biochemical and Transcriptional Regulation Over Presynaptic and Postsynaptic Elements in the Adult Hippocampus.** *Front. Cell. Neurosci.* **10,** 7779 (2016).

[35] Armstrong, N. C., Anderson, R. C. & McDermott, K. W. **Reelin: Diverse roles in central nervous system development, health and disease.** *Int. J. Biochem. Cell Biol.* **112,** 72–75 (2019).

[36] Bouamrane, L. *et al.* **Reelin-Haploinsufficiency Disrupts the Developmental Trajectory of the E/I Balance in the Prefrontal Cortex.** *Front. Cell. Neurosci.* **10,** 308 (2016).

[37] Berggaard, N., Seifi, M., van der Want, J. J. L. & Swinny, J. D. **Spatiotemporal Distribution of GABAA Receptor Subunits Within Layer II of Mouse Medial Entorhinal Cortex: Implications for Grid Cell Excitability.** *Front Neuroanat* **12,** 46 (2018).

[38] Hamad, M. I. K. *et al.* **Reelin signaling modulates GABAB receptor function in the neocortex.** *Journal of Neurochemistry* (2020). doi:10.1111/jnc.14990

[39] Cho, S.-K. *et al.* **AKT-independent Reelin signaling requires interactions of heterotrimeric Go and Src.** *Biochemical and Biophysical Research Communications* **467,** 1063–1069 (2015).

[40] Bal, M. *et al.* **Reelin mobilizes a VAMP7-dependent synaptic vesicle pool and selectively augments spontaneous neurotransmission.** *Neuron* **80,** 934–946 (2013).

[41] Crawford, D. C., Ramirez, D. M. O., Trauterman, B., Monteggia, L. M. & Kavalali, E. T. **Selective molecular impairment of spontaneous neurotransmission modulates synaptic efficacy.** *Nat Commun* **8,** 14436–14 (2017).

[42] Wang, L. *et al.* **Modulation of Hippocampal Gamma Oscillations by Dopamine in Heterozygous Reeler Mice in vitro.** *Front. Cell. Neurosci.* **13,** 586 (2019).

[43] Jiang, Y. *et al.* **Ndel1 and Reelin Maintain Postnatal CA1 Hippocampus Integrity.** *J. Neurosci.* **36,** 6538–6552 (2016).

[44] Kiroski, I. *et al.* **Reelin Improves Cognition and Extends the Lifespan of Mutant Ndel1 Mice with Postnatal CA1 Hippocampus Deterioration.** *Cereb. Cortex* **154,** 75 (2020).

The authors of this study showed that reelin is lost in mice where the gene Ndel1, encoding for a protein of the cytoskeleton, is knock-out. This is associated with a loss of both excitatory and inhibitory neurons, as shown by electrophysiological recordings, and results in the disruption of hippocampal integrity as well as deficits in learning and memory. Importantly, KO mice treated with a single injection of reelin showed considerable improvement in their anatomical and behavioral deficits.

[45] Herz, J. & Chen, Y. **Reelin, lipoprotein receptors and synaptic plasticity.** *Nat. Rev. Neurosci.* **7,** 850–859 (2006).

[46] Jakob, B. *et al.* **Intersectin 1 is a component of the Reelin pathway to regulate neuronal migration and synaptic plasticity in the hippocampus.** *Proc. Natl. Acad. Sci. U.S.A.* **114,** 5533–5538 (2017).

By combining genetic, biochemical, cell biological and electrophysiological methods, this paper identified Intersectin 1, a multi-scaffold protein involved in Down Syndrome, to be associated with reelin signaling via its interaction with ApoER2 receptor. Importantly, the protein regulates neuronal migration and synaptic plasticity in the hippocampus, as shown by deficits of these aspects in mice knocked-out for Intersectin 1. Molecularly, the protein acts as a scaffold between VLDLR and Dab1 and facilitates Dab1 phosphorylation. These results indicate that a manipulation of reelin signaling, may be helpful for the treatment of neurodevelopmental disorders such as Down syndrome.

[47] Malakooti, N. *et al.* **The Long Isoform of Intersectin-1 Has a Role in Learning and Memory.** *Front Behav Neurosci* **14,** 24 (2020).

[48] Brai, E. *et al.* **Notch1 Regulates Hippocampal Plasticity Through Interaction with the Reelin Pathway, Glutamatergic Transmission and CREB Signaling.** *Front. Cell. Neurosci.* **9,** 447 (2015).

[49] Minge, D. *et al.* **Heparan Sulfates Support Pyramidal Cell Excitability, Synaptic Plasticity, and Context Discrimination.** *Cereb. Cortex* **27,** 903–918 (2017).

[50] Yang, X. **Chondroitin sulfate proteoglycans: key modulators of neuronal plasticity, long-term memory, neurodegenerative, and psychiatric disorders.** *Rev Neurosci* **31,** 555–568 (2020).

[51] Pizzorusso, T. *et al.* **Reactivation of ocular dominance plasticity in the adult visual cortex.** *Science* **298,** 1248–1251 (2002).

[52] Faini, G. *et al.* **Perineuronal nets control visual input via thalamic recruitment of cortical PV interneurons.** *Elife* **7,** 1968 (2018).

[53] Zluhan, E., Enck, J., Matthews, R. T. & Olson, E. C. **Reelin counteracts chondroitin sulfate proteoglycan-mediated cortical dendrite growth inhibition.** *eNeuro* ENEURO.0168–20.2020 (2020).

[54] Pielecka-Fortuna, J. *et al.* **The disorganized visual cortex in reelin-deficient mice is functional and allows for enhanced plasticity.** *Brain Struct Funct* **220,** 3449–3467 (2015).

[55] Wang, R.-H. *et al.* **Maternal Deprivation Enhances Contextual Fear Memory via Epigenetically Programming Second-Hit Stress-Induced Reelin Expression in Adult Rats.** *Int. J. Neuropsychopharmacol.* **21,** 1037–1048 (2018).

[56] Dalla Vecchia, E., Di Donato, V., Young, A. M. J., Del Bene, F. & Norton, W. H. J. **Reelin Signaling Controls the Preference for Social Novelty in Zebrafish.** *Front Behav Neurosci* **13,** 214 (2019).
[57] Fraley, E. R. *et al.* **Mice with Dab1 or Vldlr insufficiency exhibit abnormal neonatal vocalization patterns.** *Sci. Rep.* **6,** 719–12 (2016).
[58] Romano, E., Michetti, C., Caruso, A., Laviola, G. & Scattoni, M. L. **Characterization of Neonatal Vocal and Motor Repertoire of Reelin Mutant Mice.** *PLoS ONE* **8,** e64407 (2013).
[59] Rogers, J. T. *et al.* **Reelin supplementation enhances cognitive ability, synaptic plasticity, and dendritic spine density.** *Learn. Mem.* **18,** 558–564 (2011).
[60] Xu, S., Zhu, J., Mi, K., Shen, Y. & Zhang, X. **Functional Role of SIL1 in Neurodevelopment and Learning.** *Neural Plasticity* **2019,** 9653024–12 (2019).
[61] Telese, F. *et al.* **LRP8-Reelin-Regulated Neuronal Enhancer Signature Underlying Learning and Memory Formation.** *Neuron* **86,** 696–710 (2015).
In this epigenetic study, the authors identified a signature of Reelin-dependent enhancers required for the process of learning and memory involving Reelin-dependent LRP8 signaling. These LRN enhancers were found present in the promoter region of genes implicated in synaptic plasticity and were shown to be activated by Reelin that mediates the delivery of the LRP8 intracellular domain which take parts in the activation of these enhancers.

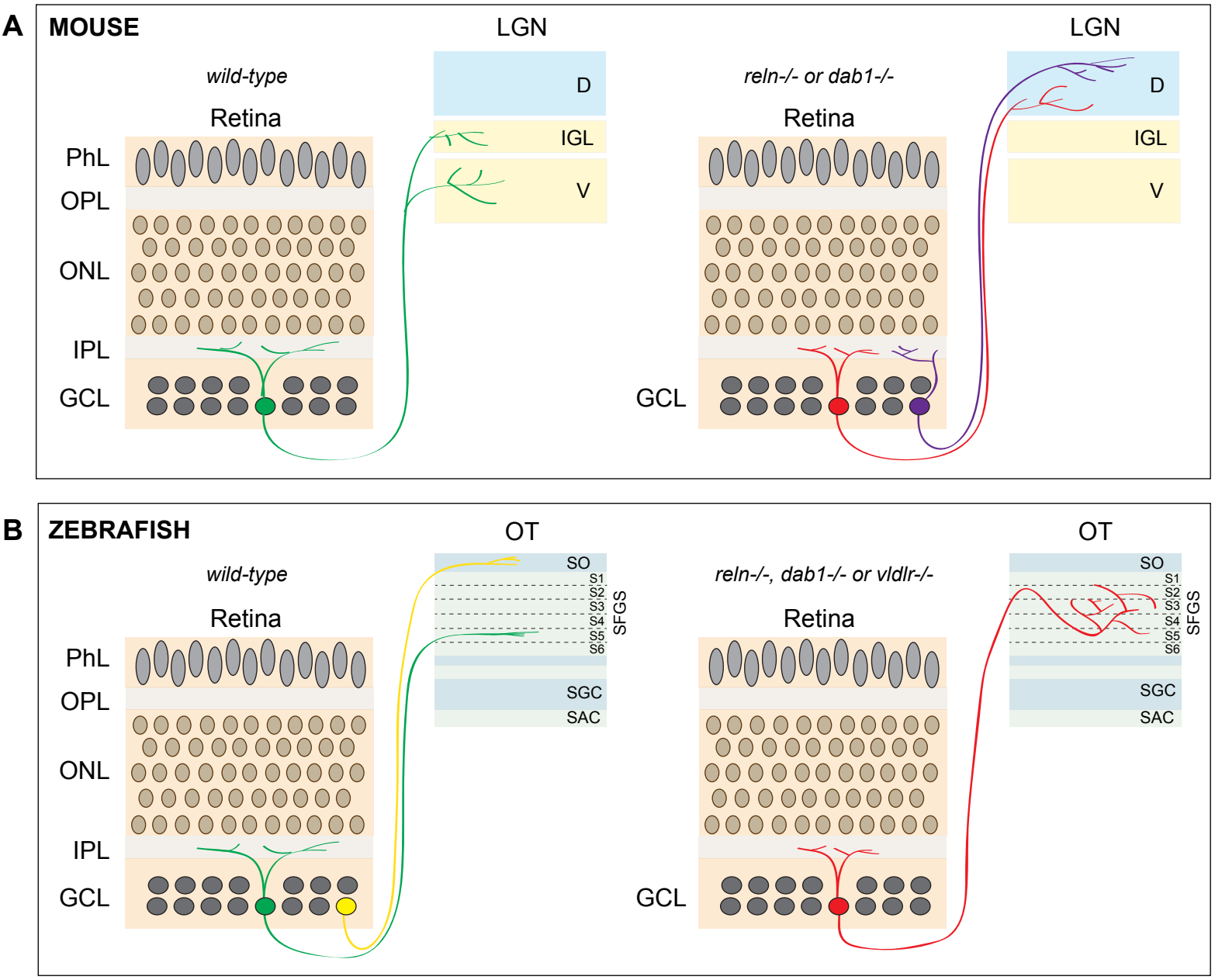
FIGURE CAPTIONS

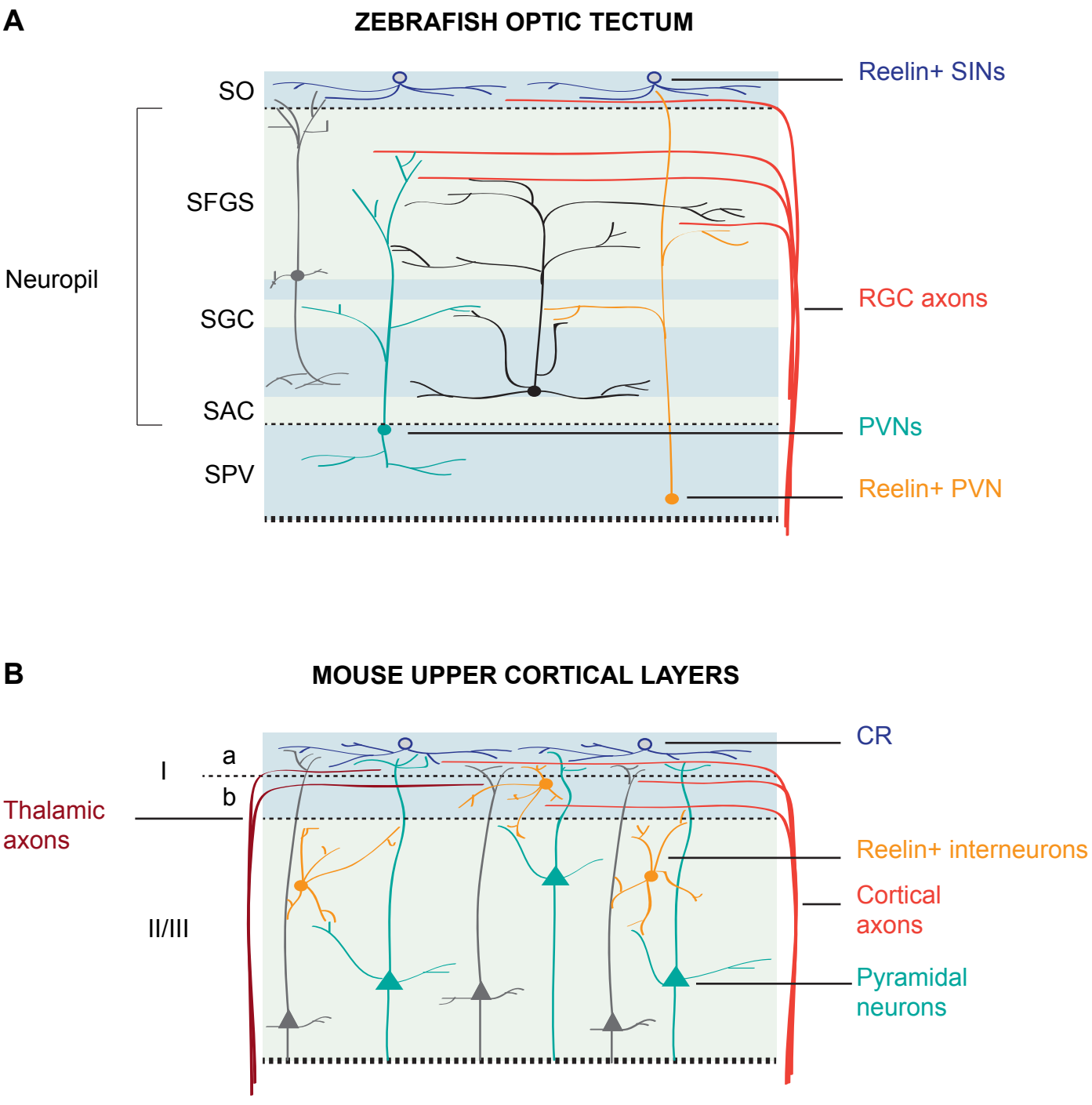
Fig 1. Reelin and its pathways are required for the proper patterning of synaptic connectivity of retinal ganglion cell axons. (A) In mouse, Reelin and Dab1 are required for the proper retinogeniculate targeting of RGC axons. In wild-type animals, RGC axons target two retino-recipient nuclei of the mouse cortex: the lateral geniculate nucleus (LGN) subdivided in the dorso (D) and ventro (V) LGN and the intergeniculate leaflet (IGL). In the *reelin* mutant (*reln*$^{-/-}$) and *dab1*$^{-/-}$ mutants, reduced pattern of innervation as well as mistargeting of the vLGN and IGL to the dLGN were observed. **(B)** In zebrafish, alterations of Reelin, Dab1a and Vldlr lead to the disruption of RGCs targeting in the optic tectum that cross over several sublaminae of the neuropil unlike in the wild-type condition where RGC axons only project to a single lamina. PhL, photoreceptor layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

Fig 2. Schematic representations of the anatomical architectures of the zebrafish optic tectum (A) and the mouse upper cortical layers, highlighting their global organization similarities (B). In blue (SIN and CR neurons) and orange (PVN and interneurons) are represented *reelin*-expressing cells in both structures. Note how in both cases RGC and cortical axons project to the most superficial layers in defined synaptic laminae. These similarities in architecture and organization raise the possibility that, like in the optic tectum, Reelin could orchestrated the proper wiring of layer 1 of the mammalian neocortex. SO, *startum opticum*; SIN, superficial inhibitory neurons; RGC, retinal ganglion cell; PVN, periventricular neuron; CR, Cajal-Retzius cells; SPV, *stratum periventriculare*; SFGS, *stratum fibrosum et griseum superficiale*; SAC, *stratum album centrale*; SGC, *stratum griseum centrale*.

Fig 3. Pre and post -synaptic roles of Reelin in controlling network excitability, synaptic plasticity, learning and memory. (A) In wild-type animals, Reelin controls network excitability via the modulation of presynaptic $GABA_BR$: the binding of Reelin to both receptors (1) and Dab1 phosphorylation (2) activate members of the Src family tyrosine kinases (SFK, 3) which interact with the alpha subunit of the G-protein (Gα) of $GABA_BR$ (4). This induces the maintenance of $GABA_BR$ at the cell surface, whose role is to inhibit NMDAR and thus Ca^{2+} influx (5), resulting in a decrease of excitability. In conditional mutants for *reelin* ($Reln^{cKO}$), non-activated SFK may disrupt the interaction between SFK and Gα (1) and AMPK phosphorylates

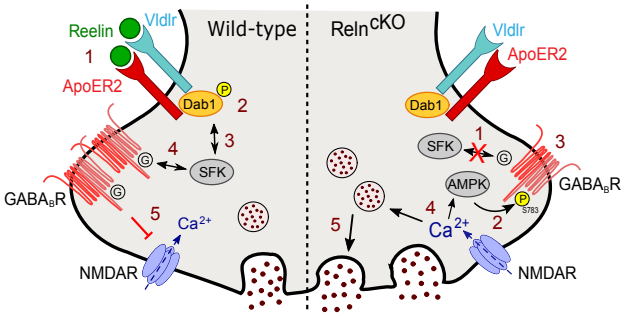
$GABA_BR$ at the Serine site S783 (2). This induces the degradation of $GABA_BR$ at the cell surface (3) which results in the dis-inhibition of NMDAR and thus in an increase in Ca2+ influx (4) and neurotransmitter release. **(B)** Reelin enhances presynaptic spontaneous neurotransmission through the mobilization of VAMP7: Reelin binds to ApoER2 (1) leading to the phosphorylation of Dab1 (2) and activation of PI3K (3) which in turn increases presynaptic Ca^{2+} influx (4). This triggers the fusion of VAMP7-containing synaptic vesicles. **(C)** Reelin signaling regulates synaptic plasticity in the hippocampus: here, the large scaffold protein Intersectin 1 (ITSN1) acts as a molecular bridge that enhances the interaction between Vldlr and Dab1 (1) after Reelin binding (2), favoring Dab1 phosphorylation and downstream signaling (3, 4) which result in an increase in long-term potentiation (LTP, 5). **(D)** Crosstalk between Reelin and Notch1 pathways to regulate synaptic plasticity and memory formation: at the postsynaptic site, Notch1 functionally interacts with both ApoER2/Dab1 (1) and NMDAR (2). These complexes activate downstream effectors (3 and 4), which induce the activation of CREB signaling (5), essential for the establishment of memories.


A
MOUSE
LGN
wild-type
Retina
PhL
OPL
ONL
IPL
GCL
D
IGL
V
reln-/- or dab1-/-
LGN
Retina
GCL
D
IGL
V
B
ZEBRAFISH
OT
wild-type
Retina
PhL
OPL
ONL
IPL
GCL
SO
S1
S2
S3
S4
S5
S6
SFGS
SGC
SAC
reln-/-, dab1-/- or vldlr-/-
OT
Retina
GCL
SO
S1
S2
S3
S4
S5
S6
SFGS
SGC
SAC


A
ZEBRAFISH OPTIC TECTUM
SO
SFGS
SGC
SAC
SPV
Neuropil
Reelin+ SINs
RGC axons
PVNs
Reelin+ PVN
B
MOUSE UPPER CORTICAL LAYERS
I
a
b
II/III
Thalamic axons
CR
Reelin+ interneurons
Cortical axons
Pyramidal neurons

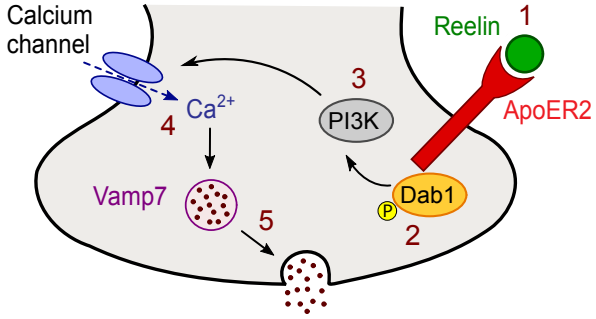
PRESYNAPTIC FUNCTIONS

A



Regulation of excitability via the stabilization of GABA $_B$R at the membrane

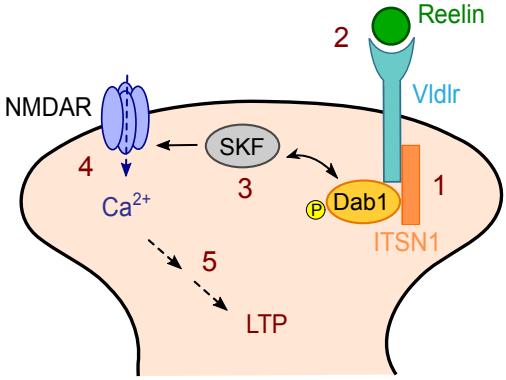
B



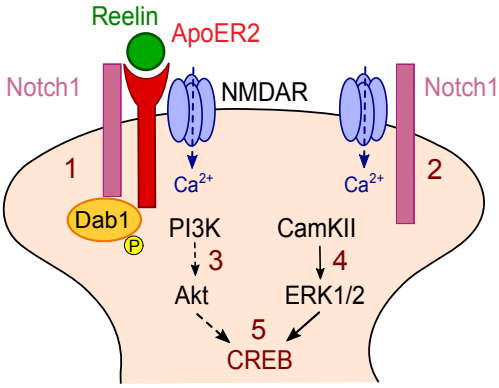
Regulation of neurotransmitter release

POSTSYNAPTIC FUNCTIONS

C



D



Regulation of synaptic plasticity, learning and memory