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Reelin signaling in development, maintenance, and plasticity of neural networks

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Abstract

The developing brain is formed through an orchestrated pattern of neuronal migration, leading to the formation of heterogeneous functional regions in the adult. Several proteins and pathways have been identified as mediators of developmental neuronal migration and cell positioning. However, these pathways do not cease to be functionally relevant after the embryonic and early postnatal period; instead, they switch from guiding cells, to guiding synapses. The outcome of synaptic guidance determines the strength and plasticity of neuronal networks by creating a scalable functional architecture that is sculpted by cues from the internal and external environment. Reelin is a multifunctional signal that coordinates cortical and subcortical morphogenesis during development and regulates structural plasticity in adulthood and ageing. Gain or loss of function in reelin or its receptors has the potential to influence synaptic strength and patterns of connectivity, with consequences for memory and cognition. The current review highlights similarities in the signaling cascades that modulate neuronal positioning during development, and synaptic plasticity in the adult, with a focus on reelin, a glycoprotein that is increasingly recognized for its dual role in the formation and maintenance of neural circuits.

Keywords

hippocampus; dendritic spine; long-term potentiation; reelin; apolipoprotein E receptor 2; disabled-1; very low density lipoprotein receptor

The formation of spatially heterogeneous structures relies on a complex series of cell autonomous and extrinsically determined signaling events. Extrinsic determinants include chemoattractants, as well as repulsive guidance cues, and positional signals that direct lamination of cortical and subcortical structures. The extracellular matrix glycoprotein reelin promotes appropriate laminar organization of cortical and subcortical regions, but its actions are not limited to the developmental period. Reelin also promotes appropriate morphogenesis of the dendritic arbor, and eventually transitions to regulating motility at spines, the predominant sites for excitatory neurotransmission, in the adult brain. The role of reelin signaling in neurons therefore undergoes a developmentally regulated switch from guiding cells, to guiding synapses, and thereby regulates plasticity over the lifespan.

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This review draws parallels between mechanisms for reelin signaling during development, maturity, and senescence, in order to better understand how reelin contributes to the underlying mechanisms for memory and cognition. The receptors and downstream signaling targets for reelin signaling during development are highlighted in terms of their potential relevance for understanding reelin actions in the adult and ageing brain. We also discuss epigenetic regulation of reelin expression, as the reelin promoter is a target for multiple modifications with potential consequences for reelin gene transcription. Given recent data indicating that interruptions in reelin signaling occur during age-related cognitive impairment and Alzheimer's disease (Stranahan et al., 2011a; Chin et al., 2007), pharmacological modulation of the reelin pathway may prove to be a therapeutically viable approach to prevent cognitive decline and neuropathology.

Binding partners for reelin in the developing brain

Reelin binds to two receptors, the very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2). Binding to either receptor induces tyrosine phosphorylation of intracellular disabled-1 (DAB1) by the Src family kinases Fyn and Src (Kuo et al., 2005; for review see Bock and Herz, 2003). Tyrosine-phosphorylated DAB1 associates with several adaptor proteins including non-catalytic region of tyrosine kinase adaptor protein 1 (Nck1), proto-oncogene Crk (Crk), and avian sarcoma virus CT10-homolog-like (CrkL) (Pramatarova et al., 2003; Huang et al., 2004). Each receptor coordinates distinct aspects of neuronal positioning (Hack et al., 2007), with a specific role of ApoER2 in stabilization of the leading process (Förster et al., 2010). Reelin signaling via ApoER2 represents an obligatory element in appropriate morphogenesis of cortical and subcortical structures.

The mutant mouse *reeler* lacks reelin, resulting in perturbation of the normal inside-out lamination of the cortex. Instead, cortical neurons are roughly layered in an outside-in birth order and the lamination of the cells in the hippocampus and cerebellum is disrupted as well (Caviness and Rakic, 1978; Caviness et al., 1978; Rakic and Caviness, 1995). Despite developmental organizational defects, cortical neurons retain normal hodological features, such as the strength and pattern of afferent and efferent connections. Homozygous *reeler* mice are viable through development although they show selective deficits in motor coordination (Caviness et al., 2008). Heterozygous *reeler* mutant mice, which exhibit a nearly fifty percent reduction in reelin expression, have normal patterns of cortical and hippocampal lamination but exhibit cognitive and synaptic impairments in adulthood (Qiu et al., 2006a), underscoring the dual roles of reelin signaling in the formation and plasticity of neural networks.

Disruption of reelin, DAB1, or both ApoER2 and VLDLR in mice leads to indistinguishable *reeler* phenotypes, as demonstrated by many loss-of-function studies with varying loci for interference in the reelin signaling pathway (D'Arcangelo et al., 1995; Howell et al., 1999; Sheldon et al., 1997; Ware et al., 1997; Trommsdorff et al., 1999). In mice, defects in ApoER2 or VLDLR by conventional gene targeting leads to less severe phenotypes with regional specificity. Mild cerebellar disturbances are characteristic of VLDLR deletion, whereas ApoER2 disruption gives rise to cortical and hippocampal lamination deficits

(Trommsdorff et al., 1999). In the human cerebral cortex, reelin deficiency due to a loss-of-function mutation leads to deficits in neuronal migration that give rise to lissencephaly, a developmental disorder characterized by impaired neuronal migration and thickening of the cerebral cortex (Hong et al., 2001). Chromosomal deletion of the region containing VLDLR leads to an autosomal recessive syndrome of nonprogressive cerebellar ataxia with mental retardation that is associated with inferior cerebellar hypoplasia and simplification of cerebral gyri (Boycott et al., 2005). Taken together, deficits in the formation of appropriate neural architecture are a shared feature of neuropathological conditions that perturb the reelin signaling pathway.

Reelin transitions from guiding cells to guiding cellular processes

The molecular mechanisms that allow reelin to function as a positional signal during development are recapitulated and conserved during ongoing regulation of neuronal morphogenesis that occurs during adolescence, maturity, and senescence. Although the precise functions of reelin during development remain an active area of investigation, studies of reelin signaling in adulthood and ageing have benefited from the relatively more extensive literature surrounding the role of reelin in appropriate lamination of cortical and subcortical structures. Because both neuronal migration during development and ongoing synaptogenesis in adulthood require some degree of cellular motility, it is possible that common mechanisms for neuronal positioning and dynamic regulation of dendritic architecture may be identified through examination of the evolving literature on reelin signaling during cortical morphogenesis.

During nuclear translocation, the leading process needs to be stabilized and under tension by either attaching to the guiding radial glial fiber, or to the marginal zone during translocation of the soma (Miyata and Ogawa, 2007). These two models of neuronal migration are distinguished by close apposition to a radial glial fiber, with stability along the length of the leading process in the case of glia-assisted migration, versus somal translocation, which involves shortening of the leading process and the movement of the soma along its length (Nadarajah et al., 2001; Nadarajah and Parnavelas, 2002). Somal translocation is employed by early generated neurons when the distance from the ventricular zone is short enough that it can be bridged by the leading process of the migrating cell, whereas glia-assisted locomotion is used at later stages when migrating neurons require a guiding scaffold (Nadarajah and Parnavelas, 2002). Once these neurons are in the terminal phase of their migratory process, where their leading processes have reached the marginal zone, they detach from the radial glial scaffold and switch back to somal translocation (Cooper, 2008). During both events the leading processes needs to be stabilized and under tension, either by attaching to the guiding radial glial fiber in glia-assisted locomotion, or to the marginal zone during somal translocation (Miyata and Ogawa, 2007).

Continuous remodeling of the actin cytoskeleton is required for the changes in cell shape that occur during radial migration and somal translocation, and reelin signaling stabilizes the actin cytoskeleton (Frotscher et al., 2007; Frotscher et al., 2010) and neuronal processes (Chai et al., 2009). The intracellular signaling target for reelin, DAB1, is required for somal translocation and contributes to stabilization of the leading processes (Franco et al., 2011).

Consistent with this role, reelin maintains the uniform ascension of apical pyramidal cell dendrites (Frotscher, 2010), and reeler mice lack the vertical orientation of these dendrites (Rakic and Caviness, 1995). Reelin therefore controls neuronal morphogenesis by regulating actin dynamics. When not phosphorylated, the protein ADF/cofilin depolymerizes actin. Actin depolymerization is constrained by LIM kinase 1 (LIMK1) phosphorylation of cofilin at serine residue 3 (Arber et al., 1998). Reelin stabilizes the cytoskeleton by activating LIMK1, which leads to serine3 phosphorylation of cofilin, leaving cofilin incapable of disassembling F-actin. Different processes of the same neuron exhibit compartmentalized motility depending on the presence or absence of reelin in the local microenvironment. Specifically, processes that encounter a reelin-enriched substrate show decreased motility and increased immunoreactivity for phosphorylated cofilin. Reelin-induced cofilin phosphorylation occurs in the leading processes of radially migrating neurons growing towards the marginal zone, underscoring the conservation of this functional role in development and adulthood. Because cofilin phosphorylation is significantly reduced in ApoER2 knockout mice but not in VLDLR single knockout mice, reelin binding to ApoER2 receptors on the surface of migrating neurons is the likely signal for phosphorylation of cofilin and stabilization of neuronal processes (Chai et al., 2009).

Late in cortical development, reelin promotes extension of dendritic processes and maturation of dendritic spines (Jossin and Goffinet, 2007). In cultured neurons and brain slices, reelin exposure leads to phosphorylation of DAB1, activating phosphatidylinositol 3 kinase (PI3K) and regulating the phosphorylation of protein kinase B (Akt) and glycogen synthase kinase 3 β (GSK3 β) (Beffert et al., 2002; Bock et al., 2003). Normal activity of PI3K and Akt is necessary for pre-plate splitting and cortical layering, but GSK3 β is dispensable in this process (Jossin and Goffinet, 2007). Inhibition of PI3K, Akt and the mammalian target of rapamycin (mTOR), but not GSK3 β , prevents reelin-induced growth and branching of dendrites (Niu et al., 2004; Jossin and Goffinet, 2007). There are several ways in which reelin could activate one or more of the mTOR complexes through Akt, which then stimulates mTOR through the tuberous sclerosis complex 1/2 (TSC1/2) and Ras homolog enriched in brain (Rheb), or through Rho and Rac (for review, see Jossin and Goffinet, 2007). Taken together, these data emphasize interactions between reelin signaling and numerous pathways known to influence dendritic and synaptic plasticity.

Reelin orchestrates hippocampal development

The developmental functions of reelin as a migratory signal in the cortex differ slightly from those in the hippocampus, where reelin initially attracts newly generated neurons towards their terminal destination (Zhao et al., 2004; Cooper, 2008; for review see Förster et al., 2006). The developing hippocampus has a marginal zone containing Cajal-Retzius cells that corresponds to the future stratum lacunosum-moleculare, a termination zone for afferent fibers from the entorhinal cortex (Tamamaki, 1997). In normal hippocampal development, commissural fibers gives rise to compact, sharply delineated projections to the inner molecular layer that arrive during early postnatal development, once the majority of granule cells have been born (Supér and Soriano, 1994). Unlike the more precocious entorhinal afferents, commissural afferents do not need a transient target, but instead establish their synapses directly with the granule cells. The granule cells of homozygous reeler mice do not

form the normal densely packed granule layer but instead are loosely dispersed throughout the hilus (Supér and Soriano, 1994). The developmental defects in the reeler hippocampus can be partially rescued in slice culture by exogenous reelin administration, which lengthens neuronal processes but does not rescue radial glial fiber orientation or granule cell lamination (Zhao et al., 2004). Reelin therefore serves as a positional cue for hippocampal granule cells during development.

Neuronal precursor cells are guided from the ventricular zone to a secondary neurogenic zone in the hilar region by a primary radial glial scaffold. The cells are then guided by a secondary glial scaffolding to the emerging dentate gyrus, and the presence of this secondary scaffold is dependent on the presence of reelin, ApoER2, VLDLR, and DAB1 (Förster et al., 2002; Zhao et al., 2004, Zhao et al., 2006). The homozygous reeler mouse does not have the characteristic radial glial scaffold in the dentate gyrus (Weiss et al., 2003) which leads to persistent deficits in dentate morphogenesis. Reelin's contributions are not limited to the developmental period, as the process of adult neurogenesis in the dentate gyrus is also responsive to reelin signaling. Overexpression of reelin accelerates dendritic growth and maturation of adult-generated dentate gyrus granule neurons, and inactivation of the reelin signaling pathway impairs adult neurogenesis and contributes to a decrease in dendritic development among newly generated dentate granule neurons (Teixeira et al., 2012), implicating the reelin pathway as a conserved regulator of adult neurogenesis and dendritic morphology among newly generated neurons.

There is also evidence for a role of the signaling protein Notch1 in the control of radial glia-guided migration in the dentate gyrus (Sibbe et al., 2009). Notch receptors mediate cell-cell communication through binding to Delta-like and Jagged proteins on proximal cells in the local microenvironment, and emerging data support direct interactions between the reelin and notch signaling pathways. These observations revealed that DAB1 co-localizes with Notch1 in the dentate gyrus and that this interaction is essential for the development and maintenances of radial glial cells located there (Sibbe et al., 2009). A rodent model where Notch1 was conditionally inactivated, specifically in radial glial cells, would help to characterize the specific crosstalk of reelin and notch in radial glia, but such a model remains to be developed.

ApoER2 and VLDLR make distinct contributions to the migration of dentate granule cells during early postnatal development. This distinction may also be recapitulated by a unique contribution of each receptor to the regulation of structural plasticity in the adult hippocampus (Figure 1). While VLDLR acts as a stop signal for migrating neurons reaching the marginal zone, ApoER2 promotes granule cell migration (Förster et al., 2010). A possible mechanism suggested for this distinction involves differential sorting of the receptors to the domains of the plasma membrane which control endocytosis. Lipid rafts are specialized membrane microdomains that compartmentalize cell signaling based on receptor localization, and there is some indication that ApoER2 and VLDLR may be differentially sorted into raft (ApoER2) and non-raft (VLDLR) domains (Duit et al., 2010). The possibility of targeting either the ApoER2 or the VLDLR to specific areas of membrane specialization has yet to be assessed, therefore the functional consequences of such a manipulation remain obscure.

Reelin also segregates neuronal subpopulations in the dentate gyrus, specifically the granule cells and the hilar mossy cells, by a temporally specific mechanism. In reeler homozygotes, the dentate stratum granulosum consists of a mosaic of both granule cells and hilar mossy cells (Drakew et al., 2002). Both populations express ApoER2, VLDLR, and DAB1, but mossy cells are generated before the radial glial scaffold has been fully established, so that only the later generated granule cells can use the scaffold as a guide to migrate to the granule cell layer (Weiss et al., 2003; Zhao et al., 2004). In this regard, the timing of reelin stimulation is a critical determinant for appropriate morphogenesis of the hippocampal dentate gyrus.

Reelin maintains neural networks in the adult brain

Recent work has underscored the importance of reelin signaling in postnatal synaptic function. Following termination of neuronal migration, the majority of reelin-expressing Cajal-Retzius cells disappear, and, instead, a subset of GABAergic interneurons originating from the medial ganglionic eminence express reelin throughout adulthood (Martinez-Cerdeno et al., 2002; Abraham et al., 2003; Pesold et al., 1998; Ramos-Moreno et al., 2006; Roberts et al., 2005). In the adult brain, reelin expression is highest in the hippocampus, cerebral cortex, and olfactory bulb (Martinez-Cerdeno et al., 2002; Abraham et al., 2003; Pesold et al., 1998; Ramos-Moreno et al., 2006; Roberts et al., 2005), regions associated with ongoing plasticity throughout adulthood and ageing. In this regard, reelin is anatomically poised to modulate neural networks involved in multiple aspects of cognitive function.

Studies have implicated postnatal reelin signaling in hippocampal synaptic plasticity, memory formation, and cognitive function, and mounting evidence suggests that the role of reelin signaling in network maintenance and function continues beyond development. In the adult hippocampus, reelin expression is localized to interneurons residing in the hilus of the dentate gyrus (DG) and the stratum lacunosum-moleculare layer of the hippocampus (Figure 1; Pesold et al., 1998; Alcantara et al., 1998). Reelin-expressing interneurons are also located in the stratum radiatum and stratum oriens of CA1 and CA3, where they form inhibitory synaptic contacts with pyramidal cells (Förster et al., 2002; Frotscher et al., 2003). Reelin appears to be expressed predominantly in interneurons, with the exception of glutamatergic cerebellar granule cells and layer II stellate neurons of the entorhinal cortex, which also express reelin (Chin et al., 2007). Reelin produced by entorhinal neurons is axonally transported and released into the DG, although it is unclear whether this release is constitutive or activity-dependent. Given that reelin is present at the predominant point of entry for synaptic inputs to the hippocampal trisynaptic circuit, it is likely that reelin released from medial perforant path terminals contributes to plasticity, learning, and memory, although the precise mechanisms for this role are still being elucidated.

Reelin participates in synaptic transmission, learning, and memory across multiple subfields of the adult hippocampus. Long-term potentiation (LTP) is a form of synaptic plasticity that results in lasting increases in synaptic efficacy. Induction of LTP in hippocampal area CA1 is dependent on N-methyl-D-aspartate receptor (NMDAR) activation and consequent increases in α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor

insertion at excitatory synapses (Harris et al., 1984; Lu et al., 2001; Pickard et al., 2001; Grosshans et al., 2002). In the adult brain, reelin binding to post-synaptic ApoER2 and VLDLR modulates NMDAR and AMPAR activity (Chen et al., 2005; Herz and Chen et al., 2006; Qiu et al., 2006c) via control of NMDA Ca^{2+} entry (Sala and Sheng et al., 1999; Chen et al., 2005). ApoER2 and VLDLR receptors form a postsynaptic functional unit with NMDARs, where the response to reelin depends on the presence of both receptors (Weeber et al., 2002). The alternatively spliced domain of ApoER2 encoded by exon 19 is attached to postsynaptic scaffolding protein postsynaptic density-95 (PSD-95) and also forms a signaling complex with NMDARs (Beffert et al., 2005). In this regard, reelin interacts with known master regulators of synaptic plasticity.

Reelin treatment enhances CA1 pyramidal neuronal NMDAR-mediated whole-cell current (Chen et al., 2005) and the addition of recombinant reelin to acute hippocampal slices enhances LTP (Weeber et al., 2002). Akin to reelin signaling during development, this effect is dependent upon SRC family kinase (SFK) activity (Beffert et al., 2005; Chen et al., 2005; Qiu et al., 2006a; Durakoglugil et al., 2009) and results in increased tyrosine phosphorylation of the NMDAR NR2B subunit, thus controlling Ca^{2+} entry (Sala and Sheng et al., 1999). Apolipoprotein E may interfere with this pathway by competing with reelin for ApoER2, and thereby inhibiting ApoER2 interaction with NMDARs (D'Arcangelo et al., 1999; Brandes et al., 2001). Exon 19 of ApoER2 is necessary for coupling ApoER2 and the activated DAB1-SFK complex to the NMDAR and for the consequent postsynaptic tyrosine phosphorylation of this complex (Beffert et al., 2005; Chen et al., 2005). ApoER2 coupling with NMDARs can arise from both extracellular and intracellular interactions (Beffert et al., 2005; Hoe et al., 2006a; Hoe et al., 2006b). While acute reelin exposure at intervals less than twenty minutes predominantly affects NMDAR activity, increased reelin exposure at intervals above this temporal threshold modifies AMPAR-mediated synaptic responses through an increase in AMPARs at synaptic sites (Qiu et al., 2006c). Reelin has also been shown to influence synaptic function through modulation of presynaptic release mechanisms (Hellwig et al., 2011). Altered neurotransmitter release and vesicle fusion at Schaffer collateral synapses of adult reeler mice were attributable to a decrease in SNAP25, a protein necessary for vesicular fusion (Hellwig et al., 2011). Vesicle release-dependent presynaptic plasticity was therefore impaired in the CA1 of the striatum radiatum in reeler mice, implicating reelin in pre- and post-synaptic forms of functional plasticity.

Reelin signaling also makes a direct contribution to the behavioral expression of learning and memory. Intracerebroventricular injections of recombinant reelin increase DAB1 and cAMP-response element binding protein (CREB) activation, and enhance dendritic spine density in hippocampal area CA1. These cellular and structural responses occurred in the context of improved CA1 LTP, and enhanced associative and spatial learning and memory performance, suggesting that transient increases in reelin lead to long term effects on synaptic function, dendritic morphology, and cognition (Rogers et al., 2011). Deletion of ApoER2 blocks the reelin-mediated increase in DAB1 and CREB activation, emphasizing the importance of this receptor for induction of signals that support plasticity (Rogers et al., 2011). These observations suggest that enhancement of reelin signaling in the adult brain might be one strategy to improve cognition and reduce or delay memory impairment with ageing.

Layer II entorhinal cortex neurons form the primary input to the hippocampal dentate gyrus and exhibit selective vulnerability in the context of ageing (Stranahan and Mattson, 2010). In addition to interneurons, certain populations of excitatory neurons also express reelin, with entorhinal cortical neurons prominent among this group (Ramos-Moreno et al., 2006). Natural variability in cognitive ageing is a useful model to identify molecular signatures that distinguish between aged rats with impaired learning and aged rats that perform within the range of young rats on hippocampus-dependent learning tasks. Aged, cognitively impaired rats exhibit reduced entorhinal cortical reelin expression (Stranahan et al., 2011a), without any loss of entorhinal neurons (Rapp et al., 2002). This pattern suggests that reductions in entorhinal cortical reelin expression may contribute to synaptic dysfunction during age-related memory loss. Entorhinal infusions of the reelin scavenger receptor-associated protein (RAP) in young rats revealed that interruptions in reelin signaling impair hippocampal-dependent spatial learning and memory (Stranahan et al., 2011b). Additionally, suppression of reelin signaling leads to localized reductions in synaptic marker expression in the entorhinal cortex, suggesting that reelin depletion compromises adult synaptic integrity in vivo (Stranahan et al., 2011b). Taken together, these observations support an essential role for reelin in synaptic plasticity, learning, and memory, although the relative contributions of reelin released from excitatory entorhinal projections and local release of reelin from hippocampal interneurons remain to be determined in relationship to their neurocognitive consequences.

Reelin as an obligatory signaling component in memory circuits of the medial temporal lobe

Given that reelin figures prominently in synaptic plasticity and learning, modulation of reelin transcription likely leads to changes in learning and memory. Loss of function at any point in the reelin signaling cascade has major consequences for cognition and neural development. Likewise, gain-of-function within this pathway could substantially improve plasticity and cognitive function. In this way, reelin is poised to modify and affect neuronal function, and regulation of reelin expression via epigenetic modifications of the reelin promoter region represents a sensitive and robust system for regulation of synaptic plasticity (for review, see Levenson et al., 2008).

Reelin expression is classically regulated via transcriptional activation, in which transcription factors bind to cis-acting regulatory elements within the reelin gene to directly influence RNA polymerase II binding and initiation of reelin transcription. Characterization and cloning of the reelin promoter exposes multiple potential cis-elements for a variety of distinct transcription factors (Royaux et al., 1997; Grayson et al., 2006). Many of these potential transcription factor binding sites are functional in vivo. Genetically altered mice lacking either transcription factor T-box brain 1 (Tbr1) or paired box protein 6 (Pax6) exhibit decreased reelin expression and phenotypes similar to those of homozygous reeler mutant mice (Bulfone et al., 1995; Hevner et al., 2001; Rice et al., 2001; Tarabykin et al., 2001; Swanson et al., 2005). Neural-associated cell line studies co-transfecting the NT2 neural progenitor cell line with a reelin promoter-reporter construct and either Tbr1 or Pax6, suggest that either transcription factor is sufficient to promote reelin expression (Chen et al.,

2002). Neuronal PAS-domain proteins 1 and 3 are key transcription factors modulating reelin expression in cortical interneurons. Mice lacking these transcription factors exhibit significantly reduced reelin expression and share many behavioral characteristics of the reeler phenotype (Erbel-Sieler et al., 2004). Each of these transcription factors represents a potential therapeutic target to increase reelin expression and promote plasticity in select neuronal populations.

Epigenetic mechanisms within the nervous system regulate gene expression through covalent modification of DNA and its associated proteins. Histone acetylation and cytosine methylation of the reelin promoter represent two independent modes for epigenetic regulation of reelin expression (for review, see Levenson et al., 2008). Methylation of cytosine residues by DNA methyltransferases (DNMTs) ultimately results in decreased gene expression (Royaux et al., 1997; Chen et al., 2002). Acute treatment with DNMT inhibitors leads to a significant decrease in reelin promoter methylation in hippocampal slices (Levenson et al., 2006). In vivo, direct infusion of DNMT inhibitors into the CNS causes a significant decrease in methylation of the reelin promoter, suggesting that the promoter is actively methylated in the adult CNS (Miller and Sweatt, 2007). Following fear conditioning, rats exhibited rapid demethylation and transcriptional activation of reelin, indicative of epigenetic regulation of reelin expression during memory consolidation (Miller and Sweatt, 2007). Histone deacetylase (HDAC) inhibitors also increase reelin expression in a dose and time dependent manner, similar to DNMT inhibitors (Kundakovic et al., 2009). These studies point to multiple mechanisms, including transcription factors and pharmacological modifiers of reelin promoter methylation, that could be targeted to promote reelin signaling for therapeutic purposes in the adult and aging brain.

Reelin signaling in the pathophysiology of the adult and ageing brain

The emergence of a prominent role for Reelin and ApoERs as mediators of synaptic function and plasticity stimulated interest in a role for reelin signaling in various neuropathologies that afflict the adult CNS. Reelin localizes to synapses (Rodriguez et al., 2010) and reelin signaling is required for normal dendritic structural development in cultured hippocampal neurons (Niu et al., 2004). In the absence of reelin, or its downstream signaling target DAB1, dendritic structural development is stunted and dendritic complexity is reduced, similar to what is seen in neurons lacking ApoER2 and VLDLR (Niu et al., 2004). Reelin depletion is a common feature in multiple psychiatric and neurological disorders including schizophrenia, autism, major depression, temporal lobe epilepsy, and Alzheimer's disease (Fatemi, 2008). In these conditions, reelin expression is perturbed, although the extent to which these perturbations arise during critical periods in brain development, or later in life, remains uncertain. The timing of reelin depletion likely varies in different disease states; moreover, the regional specificity for loss-of-function in the reelin signaling pathway is also likely to correspond to the functional circuits that are selectively vulnerable in each condition. Disease pathogenesis could be attributable to neurodevelopmental dysfunction of the reelin signaling pathway, or to reelin-mediated mechanistic effects at synapses in the adult brain. It is currently difficult to determine to what degree reelin function during adulthood is impacted by its behavior during development given that loss-of-function mutations make it impossible to assess whether

effects observed in adulthood are a result of the disruption of developmental neuronal positioning, impaired establishment of neuronal architecture and connectivity, or whether reductions in adult reelin signaling selectively impair synaptic function.

Reelin loss-of-function in Alzheimer's disease pathogenesis

Alzheimer's disease (AD) is a form of dementia characterized by accumulation of amyloid-beta (A β) plaques and neurofibrillary tau tangles. Reelin and ApoER2 began to receive attention as potential contributors to the late-onset form of AD when it was reported that the ϵ 4 isoform of apolipoprotein E (ApoE) significantly predisposes its carriers to sporadic AD (Schmechel et al., 1993; Strittmatter et al., 1993). The ApoE ϵ 4 allele has consistently been shown to influence AD risk, age of onset, A β accumulation, and cognition. The effects of ApoE on A β clearance are mediated via endocytosis by lipoprotein receptor family members, including ApoER2 and VLDLR (Herz and Chen, 2006), providing support for a competitive interaction between synaptic reelin and ApoE-mediated signaling in the modulation of NMDA receptor-mediated neurotransmission and synaptic plasticity. This interaction positions reelin-ApoER2 signaling as a potential counterbalancing strategy to oppose or attenuate A β - and tau-related neuropathology.

Reelin decreases amyloid precursor protein (APP) processing in vitro (Hoe et al., 2006a, Hoe et al., 2008) by binding directly to APP (Hoe et al., 2006a) and interacting with DAB1 (Trommsdorff et al., 1999; Howell et al., 1999). This DAB1-APP interaction directly affects the processing and trafficking of APP and ApoER2 (Hoe et al., 2006a). Reelin increases this interaction and enhances APP cleavage while decreasing A β accumulation in the presence of DAB1 (Hoe et al., 2006a). Exposure to A β oligomers impairs the trafficking and endocytosis of NMDARs and AMPARs in hippocampal neurons, thereby decreasing LTP (Kamenetz et al., 2003). Application of recombinant reelin reduces the A β -induced suppression of LTP via mechanisms dependent on Src-family kinase activation (Durakoglugil et al., 2009). In vivo, reelin co-localizes with A β in aged wild-type mice, although the nature and extent of their functional interaction is still being elucidated (Doehner et al., 2010). Collectively, these findings support a model in which reelin, ApoER2, and A β interact to modulate glutamatergic transmission, with ApoER2/VLDLR-reelin binding counterbalancing the deleterious effects of A β accumulation (Förster et al., 2010). Reductions in reelin signaling may therefore underlie cognitive impairment in AD and other neuropathological conditions (Chin et al., 2007).

Phosphorylation of the microtubule-associated protein tau, another pathological hallmark of AD, is also constrained by reelin signaling. Interruption of reelin signaling by either loss-of-function mutations in the reelin gene or deletion of both ApoER2 and VLDLR leads to tau hyperphosphorylation (Hiesberger et al., 1999). The phosphatidylinositol 3-kinase (PI3K) signaling pathway and its downstream effector Akt/protein kinase B (Akt) constitute the PI3K/Akt pathway. Activation of this pathway leads to down regulation of glycogen synthase kinases 3 α (GSK-3 α) and 3 β (GSK-3 β) activity (Cross et al., 1995; Kaytor and Orr, 2002). GSK-3 α and 3 β have been implicated in A β peptide production and in tau over-phosphorylation, respectively (Phiel et al., 2003; Hanger et al., 1992; Hong et al., 1997; Pei et al., 1999; Lucas et al., 2001). Reelin binding to ApoER2 and VLDLR stimulates PI3K,

leading to inhibition of GSK-3 β (Beffert et al., 2002). This process is dependent on both the ApoER2 and VLDLR, as well as the intracellular signaling target DAB1 (Beffert et al., 2002, Ohkubo et al., 2003). Mutant mice with reelin, ApoER2, or VLDLR defects exhibit increased hyperphosphorylated tau levels in the brain (Hiesberger et al., 1999, Bricht et al., 2003), underscoring the importance of reelin signaling as a negative regulator of AD-associated neuropathology in the ageing brain.

Double transgenic mouse models expressing genes associated with familial AD, as well as loss-of-function mutations in the gene for reelin, exhibit increased amyloidogenic APP processing, significantly increasing plaque burden. Moreover, ageing evoked hippocampal neurofibrillary tangles composed of hyperphosphorylated tau in double transgenic mice (Kocherhans et al., 2010), thereby recapitulating A β plaque and tau pathology in a spatial and temporal configuration closely resembling human AD pathology. Increased plaque burden and tau phosphorylation in double transgenic mice are accompanied by accelerated cognitive impairment, further underscoring the role of reelin as a brake on age-related neuropathology and cognitive decline.

Summary and Future Directions

The glycoprotein reelin represents one example of a developmentally regulated signal recruited by neurons to sequentially guide cells, then neuronal processes, and then other motile elements of the central nervous system, such as dendritic spines. Appropriate guidance is essential for regional specialization and ontogenesis, formation of appropriate connections, and maintenance of excitatory and inhibitory synaptic contacts in the adult and ageing brain. Reelin signals via the ApoER2 and VLDLR but the distinct cellular consequences of activating each receptor are still being elucidated with much of the attention focused on ApoER2, due to its association with synaptic NMDA receptors. The hippocampal dentate gyrus is a region of particular interest with respect to reelin signaling because it receives excitatory projections from excitatory, reelin-expressing entorhinal cortical neurons, as well as the more widespread innervation from reelin-positive interneurons in the local environment. We propose that, in this specific region of the hippocampus where reelin may be co-released with either the excitatory neurotransmitter glutamate, or the inhibitory neurotransmitter GABA, the ApoER2 and VLDLR may partition into excitatory and inhibitory synapses, respectively, and thereby exert distinct effects depending on synapse type (Figure 1). In addition to this speculative proposition, we highlight the role of dysregulated reelin signaling in the pathogenesis of age-related cognitive impairment and Alzheimer's disease, with a focus on strategies to promote reelin expression and signaling in order to prevent or delay cognitive decline.

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Figure 1. Reelin is anatomically poised to modulate neurotransmission at excitatory and inhibitory synapses on dentate granule neurons
 Histological section shows immunofluorescent detection of reelin (green), synaptophysin (red), with nuclei counterstained with DAPI (blue). Schematic shows hippocampal circuitry represented in terms of the synaptic sites for reelin release. Inset on schematic shows a possible model for partitioning of reelin signaling; we speculatively propose that the reelin receptor very-low density lipoprotein receptor (VLDLR) segregates to inhibitory postsynaptic contacts where reelin is released from interneurons residing in the stratum lacunosum-moleculare. This would likely lead to distinct consequences relative to reelin released at excitatory terminals arising from entorhinal neurons, which would signal in concert with glutamate via the postsynaptic complex made up of apolipoprotein E receptor 2 (APOER2) and the N-methyl-D-aspartate receptor (NMDAr).