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Interference with reelin signaling in the lateral entorhinal cortex impairs spatial memory

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Abstract

Entorhinal neurons receive extensive intracortical projections, and form the primary input to the hippocampus via the perforant pathway. The glutamatergic cells of origin for the perforant pathway are distinguished by their expression of reelin, a glycoprotein involved in learning and synaptic plasticity. The functional significance of reelin signaling within the entorhinal cortex, however, remains unexplored. To determine whether interrupting entorhinal reelin signaling might have consequences for learning and memory, we administered recombinant receptor-associated protein (RAP) into the lateral entorhinal cortex (LEC) of young Long-Evans rats. RAP prevents reelin from binding to its receptors, and we verified the knockdown of reelin signaling by quantifying the phosphorylation state of reelin's intracellular signaling target, disabled-1 (DAB1). Effective knockdown of reelin signaling was associated with impaired performance in the hippocampus-dependent version of the water maze. Moreover, inhibition of reelin signaling induced a localized loss of synaptic marker expression in the LEC. These observations support a role for entorhinal reelin signaling in spatial learning, and suggest that an intact reelin signaling pathway is essential for synaptic integrity in the adult entorhinal cortex.

Keywords

receptor-associated protein; learning; disabled-1

Connectivity between the entorhinal cortex and hippocampal formation is essential for certain forms of learning and memory. Within the entorhinal cortex, the lateral and medial subdivisions differ with respect to their environmentally evoked firing properties and their anatomical connectivity. The medial entorhinal cortex (MEC) shows location-specific firing, while the lateral entorhinal cortex (LEC) does not (Hargreaves, Rao, Lee, & Knierim, 2005). Although previous studies indicate that LEC neurons fire in response to olfactory stimuli (Young, Otto, Fox, & Eichenbaum, 1997), including olfactory cues that distinguish between socially relevant conspecifics (Petrulis, Alvarez, & Eichenbaum, 2004), a consensus regarding the functional role of LEC neurons has yet to be reached. In a manner consistent with regional differences in spatial selectivity, the LEC and MEC are further distinguished by hodological criteria. The LEC receives greater input from the perirhinal cortex, and the MEC receives the bulk of its input from the postrhinal cortex (van Strien, Cappaert, &

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Witter, 2009). Differences in afferent input properties between these two regions could potentially support distinct contributions to behavior, but this distinction has not yet been conclusively demonstrated in the literature.

Notable differences between the LEC and MEC have also been reported in the context of aging. The ‘transentorhinal region,’ which encompasses the LEC, shows earlier susceptibility to neurofibrillary tangle formation in humans (Braak & Braak, 1996). Likewise, in a rodent model of naturally occurring variability in cognitive aging, the LEC emerges as a focal region for molecular alterations in aged rats that are cognitively impaired (Stranahan, Haberman, & Gallagher, 2011). Entorhinal neurons are not lost with age-related cognitive impairment (Rapp, Deroche, Mao, & Burwell, 2002; Merrill, Chiba, & Tuszynski, 2001), but the number of LEC neurons expressing reelin, a glycoprotein involved in synaptic plasticity (Herz & Chen, 2006), is reduced in aged rats that are cognitively impaired (Stranahan et al., 2011) relative to both young adults and aged cohorts with preserved cognitive function. Because reelin is involved in synapse formation in the adult brain (Niu, Yabut, & D’Arcangelo, 2008; Pujadas, Gruart, Bosch, Delgado, Teixeira, Rossi, de Lecea, Martinez, Delgado-Garcia, & Soriano, 2010), reduced reelin expression could alter the connectivity and function of entorhinal circuits.

In the current report, we have suppressed reelin signaling locally in the LEC of young rats to assess the behavioral effects of mimicking a condition associated with age-related cognitive deficits. We used recombinant receptor-associated protein (RAP), which prevents reelin from binding to its receptors (Hiesberger, Trommsdorf, Howell, Goffinet, Mumby, Cooper, & Herz, 1999). With in vivo administration via cannulae targeted to the LEC, we succeeded in reducing the phosphorylation of reelin’s intracellular signaling target, disabled-1 (DAB1). We then employed this method to suppress reelin signaling before assessing hippocampus-dependent memory using the Morris water maze. Inhibition of reelin signaling in the LEC impaired spatial memory and reduced synaptophysin expression in LEC homogenates. These results represent the first behavioral characterization of a regionally specific, inducible knockdown of reelin signaling in the adult brain.

Materials and Methods

Animal treatments

Male Long-Evans rats were purchased from Charles River Laboratories at 3 months of age. All rats were maintained on a 12hr light/dark schedule with food and water available ad libitum. Rats were handled daily for one week prior to starting experiments. For euthanasia, rats were transcardially perfused under Isoflurane anesthesia with phosphate-buffered saline followed by 4% paraformaldehyde in phosphate buffer. Brains were postfixed for 24 hours, followed by dehydration and cryoprotection for 48 hours in paraformaldehyde with 20% sucrose. A subset of rats were anesthetized with Isoflurane and decapitated for entorhinal cortex microdissections and western blotting. All animal procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines.

Histology

Frozen brains were sectioned on the coronal plane at 40µm thickness using a freezing microtome. Tissue sections were mounted onto coated slides, dried, and stained with cresyl violet (Sigma-Aldrich) according to standard protocols. After cresyl violet staining, slides were dehydrated in progressively increasing concentrations of ethanol, cleared in Histoclear, and coverslipped under Permount. Cannula placements were verified with reference to the atlas of Paxinos and Watson (1998).

Stereotaxic surgery and drug infusion

Rats were anesthetized with Isoflurane and placed in a stereotaxic apparatus. Guide cannulae were mounted bilaterally at bregma -6.8mm on the anteroposterior axis, and $\pm 7.0\text{ mm}$ from bregma mediolaterally. The temporal muscle was gently pulled away from the skull in order to access the lateral surface. Cannulae were purchased from Plastics One. One side of the cannula base was trimmed at a 45° angle to accommodate the curvature of the skull. All animals were fitted with dummy cannulae to keep the guide cannula free from obstructions and implants were held in place with four support screws and dental cement. After recovering from surgery for a minimum of one week, rats received four daily injections of RAP (BioMol International, $1.0\mu\text{l}$ injection volume) or sterile Dulbecco's phosphate-buffered saline (DPBS) into the lateral entorhinal cortex. Injections began three days before water maze training, with the final injection occurring on the first day of training.

Water maze training

The water maze protocol used in the current experiment followed previously published methods used to detect cognitive impairment in aged rats (Gallagher et al., 1993). Behavioral testing took place during the light phase, with training over 8 days, in sessions of 3 trials per day, as shown in Supplementary Figure 1. During the trials, rats were placed in the water at the perimeter of the pool, with starting locations varied across trials. Each trial lasted for 90 seconds or until the rat successfully located the platform, with a 60-second intertrial interval. Every sixth trial was a probe trial to assess the rat's spatial bias during its search. Rats were permitted to escape on probe trials when a retracted platform was made available after 30 seconds for completion of those trials. An index score, derived from the proximity of the rat to the escape platform location during the 30-second free swim on probe trials, was used to characterize performance of the rats in the maze for the purpose of neurobiological analyses. This index is the sum of the weighted proximity scores measured during the probe trials, with lower scores reflecting better spatial memory as indicated by shorter average distances from the platform location (Gallagher et al., 1993). Rats were tested with a visible platform on the ninth day. Rats that were unable to perform visible platform training, indicated by latencies > 25 seconds during a thirty-second trial, were excluded from the study.

Entorhinal cortex microdissections

To dissect the entorhinal cortex, we removed the cortex from one hemisphere and spread it flat. In this configuration, the cortex is approximately 16.0mm long. According to the atlas of Paxinos and Watson (1998), the lateral entorhinal cortex encompasses the temporal cortex from bregma -4.8 mm to bregma -8.0 mm . The medial entorhinal cortex occupies the caudal temporal cortex from bregma -8.3 mm to bregma -9.3 , proximal to the posterior edge of the cortex. Using the rhinal sulcus as an anatomical landmark, we excised the temporal cortex starting 12.0mm from the anterior pole, with the aid of a dissecting microscope. The anterior 3.0mm of the excised temporal region was collected for the lateral entorhinal cortex, and the posterior 1.0mm was taken to sample the medial entorhinal cortex as shown in Figure 2A. Samples were frozen on dry ice and stored at -80°C .

Western blotting

Protein was extracted and processed for western blot as described, with minor modifications (Stranahan, Norman, Lee, Cutler, Telljohann, Egan, & Mattson, 2008). Briefly, lateral and medial entorhinal cortices were homogenized in lysis buffer containing protease (Roche) and phosphatase inhibitors (Sigma-Aldrich). Samples were separated by centrifugation and protein content was determined in the supernatant using the Bradford assay. Samples ($25\mu\text{g}$ protein for synaptophysin, $75\mu\text{g}$ protein for DAB1) were separated by SDS-PAGE (10%

acrylamide) and transferred to a nitrocellulose membrane (Invitrogen). Membranes were incubated in blocking buffer containing 5% nonfat milk before being reacted overnight in the presence of a 1:1,000 dilution of a rabbit polyclonal antibody raised against a chemically synthesized phosphopeptide derived from the region of the DAB1 protein containing tyrosine 220 (Invitrogen), rabbit polyclonal anti-synaptophysin (Santa Cruz Biotechnologies), and β -actin (Sigma-Aldrich). After incubation with alkaline phosphatase-conjugated secondary antibody, the signal was visualized with a chemiluminescence detection kit (Amersham Pharmacia), and optical density was measured with ImageJ (NIH). For analysis of total DAB1 protein levels, blots used to detect phosphorylated DAB1 were stripped using a commercially available stripping buffer (Thermo Scientific) and re-probed with a 1:1,000 dilution of a rabbit polyclonal antibody raised against a peptide containing the 12 C-terminal amino acids of the DAB1 protein (a kind gift from A. Goffinet).

Statistics

Behavioral data were compared across rats that received RAP or vehicle using repeated measures ANOVA with Tukey's post hoc. Western blot data were analyzed using 2×2 ANOVA to compare band intensities across RAP- or vehicle-treated rats in the LEC and MEC. For all analyses, statistical significance was set at $p < 0.05$.

Results

RAP infusion effectively reduces phosphorylation of reelin signaling targets in vivo

Recombinant receptor associated protein (RAP) has previously been used in vitro to prevent reelin from binding to its receptors, the apolipoprotein-E receptor 2 (APOER2) and low-density lipoprotein receptor 2 (LDLR2) (Hiesberger et al., 1999). However, no studies had previously used this agent to block reelin signaling in vivo. To test the efficacy of RAP as a means to inhibit reelin signaling in vivo, we implanted cannulae targeting the lateral entorhinal cortex (LEC; Figure 1A–B). The LEC was selected because this region shows prominent age-related loss of reelin-immunoreactive cells (Stranahan et al., 2011), without loss of neurons (Rapp et al., 2002; Merrill et al., 2001), specifically in aged rats that are cognitively impaired. In this regard, we chose to interrupt reelin signaling in young rats within an anatomical region with known sensitivity to age-related cognitive decline.

After recovering from surgery, rats received four daily injections of $10\mu\text{g}/\mu\text{l}$ RAP bilaterally to the LEC. This dose was selected because it has previously been shown to inhibit reelin signaling in culture (Hiesberger et al., 1999). Immediately after the last injection, the lateral and medial entorhinal cortices were microdissected on ice as shown (Figure 2A). Protein was extracted and quantified, and phosphorylation of reelin's intracellular signaling target, disabled-1 (DAB1), was assessed. RAP infusions effectively reduce DAB1 phosphorylation in tissue samples obtained from the LEC (Figure 2B; $F_{1,12}=15.99$, $p=0.008$), while DAB1 phosphorylation in the MEC was unaffected. No changes in total DAB1 expression were detected following treatment with RAP (data not shown).

Reelin signaling knockdown impairs spatial memory

Reelin is synthesized in entorhinal cortical layer II neurons, and transported into the hippocampus along perforant path axons (Martinez-Cerdeno, Galazo, & Clasca, 2003). In an earlier study (Stranahan et al., 2011), we observed that naturally occurring cognitive decline during aging is associated with loss of reelin immunoreactivity in the LEC. To determine whether suppression of reelin in the LEC could interfere with hippocampus-dependent learning in young rats, we used the same RAP infusion protocol, with four daily injections terminating on the first day of water maze training. Rats received three, ninety-second training trials per day, over eight days, with interpolated probe trials occurring every sixth

trial (Supplementary Figure 1). It is important to note that after each probe trial, the submerged platform was available for the rat to successfully escape on that trial. In this regard, the behavioral training and testing protocol used in the current study is identical to the one in which we observed a correlation between age-related cognitive impairment and reelin-immunoreactive cell numbers in the LEC (Stranahan et al., 2011).

During training trials, rats treated with RAP traveled longer distances to locate the platform (Figure 3A, $F_{1,19}=6.66$, $p=0.04$). Likewise, during probe trials, RAP treated rats were significantly less accurate in searching for the location of the goal platform (Gallagher, Burwell, & Burchinal, 1993; Figure 3B, $t_{19}=5.79$, $p < 0.001$). There was no effect of RAP on swim speed during training trials (data not shown), and when we assessed hippocampus-independent learning using the visible platform version of the water maze, there was no impairment following RAP infusion ($t_{19}=1.41$, $p=0.17$). This indicates that selective knockdown of reelin signaling in the LEC of young rats impairs spatial performance in the water maze.

Selective loss of synaptic marker expression following RAP infusion

In age-related cognitive impairment, reductions in the number of reelin-immunoreactive neurons are accompanied by reduced expression of the synaptic marker synaptophysin in the LEC (Stranahan et al., 2011). Reelin both localizes to synapses (Rodriguez, Pesold, Liu, Kriho, Guidotti, Pappas, & Costa, 2000) and promotes synaptogenesis (Pujadas et al., 2010, Niu et al., 2008). To determine whether suppression of reelin signaling in the entorhinal cortex might influence synaptic marker expression in young rats, we quantified synaptophysin expression in extracts from the LEC and MEC of rats treated with RAP or vehicle. In this analysis, four daily injections of RAP in the LEC reduced synaptophysin expression (Figure 4, $F_{1,12}=10.58$, $p=0.011$). Reductions in synaptophysin band intensity occurred only in LEC extracts, but not in MEC extracts. The results of these assays support the possibility that suppression of reelin signaling compromises synaptic integrity in vivo.

Discussion

We have assessed the behavioral consequences of interfering with reelin signaling in the lateral entorhinal cortex (LEC). Reduced reelin signaling was associated with impaired spatial performance, both during acquisition training trials and retention-based probe trials. Suppression of reelin signaling compromised the expression of synaptophysin locally within the LEC. Overall, these data support a role for entorhinal cortical reelin signaling in both the maintenance of synaptic integrity and the behavioral expression of learning and memory in a spatial task.

The observation that interfering with reelin signaling impairs synaptophysin expression is consistent with previous reports in heterozygous *reeler* mice. *reeler* heterozygotes have a fifty percent reduction in reelin expression, without the migratory abnormalities present following complete ablation of reelin (Liu, Pesold, Rodriguez, Carboni, Auta, Lacor, Larson, Condi, Guidotti, & Costa, 2001). Neurons in *reeler* heterozygotes have a number of structural deficits, including reduced dendritic spine density (Niu et al., 2008) and simplification of the dendritic arbor (Niu, Renfro, Quatrocci, Sheldon, & D'Arcangelo, 2004). In culture, treatment of normal wildtype neurons with reelin-blocking antibodies reduces dendritic outgrowth and complexity (Niu et al., 2004). Conversely, reelin overexpression enhances hippocampal synaptic density (Pujadas et al., 2010). Because interfering with reelin signaling reduced synaptic marker expression, behavioral deficits might be secondary to synaptic loss in the LEC, or might arise from a direct requirement for intact reelin signaling in the entorhinal cortex during learning. Although the precise mechanistic relationship between LEC reelin signaling, synaptic integrity, and learning

remains obscure, the outcome of these experiments suggests that an intact reelin pathway is necessary for spatial memory.

The current evidence that interference with reelin signaling impairs spatial memory is consistent with the prior observation that reductions in the number of reelin-expressing neurons in the LEC occur in aged rats with a similar behavioral impairment (Stranahan et al., 2011). While the LEC is not required for spatial learning in the water maze as assessed after restricted brain lesions (Burwell, Saddoris, Bucci, & Wiig, 2004; Ferbinteanu, Holsinger, & McDonald, 1999), neurons in the LEC do express the immediate early gene *Arc/Arg3.1* at high levels following water maze training (Gusev, Cui, Alkon, & Gubin, 2005). Reelin enhances the posttranscriptional processing of *Arc/Arg3.1* mRNA in synaptosomes (Dong, Caruncho, Liu, Smalheiser, Grayson, Costa, & Guidotti, 2003), so it is possible that there may be a role for LEC reelin signaling in the modulation of proteins associated with neuronal activation. Taken together with the absence of spatial learning deficits previously reported following LEC lesions (Burwell et al., 2004; Ferbinteanu et al., 1999), the current report suggests that altered neuronal function following interruption of LEC reelin signaling may actually be more harmful than the complete absence of LEC neurons. Whether deficient reelin signaling directly among LEC neurons exerts deleterious effects on behavioral performance or whether that outcome is due to downstream effects in the hippocampus after this entorhinal manipulation remains to be determined. Future studies would be needed in order to understand the consequences of reduced reelin signaling in the entorhinal cortex for hippocampal processing and plasticity.

Reelin expression is reduced in aged rats that are impaired across a battery of memory tasks that recruit entorhinohippocampal networks (Gallagher et al., 1993; Robitsek, Fortin, Koh, Gallagher, & Eichenbaum, 2008), opening the possibility that reelin signaling in the entorhinal cortex may contribute to other forms of memory in addition to spatial learning. Heterozygous *reeler* mice also show deficits in contextual fear conditioning (Qiu, Korwek, Pratt-Davis, Peters, Bergman, & Weeber, 2006), and mice deficient in a particular splice variant of the *APOER2* exhibit spatial learning impairments (Beffert, Weeber, Durudas, Qiu, Masiulis, Sweatt, Li, Adelman, Frotscher, Hammer, & Herz, 2005), lending further support to the idea that reelin expression in the medial temporal lobe is associated with memory across a number of different domains. In humans, a particular allelic variant in the reelin gene is associated with cognitive dysfunction (Wedenoja, Tuulio-Henriksson, Suvisaari, Loukola, Paunio, Partonen, Varilo, Lonnqvist, & Peltonen, 2010), suggesting that across species, reelin expression covaries with and may contribute to learning and memory.

Several questions remain regarding the behavioral outcome that we observed following selective ablation of reelin signaling in the LEC. First, how do reelin-positive neurons in the LEC contribute to spatial memory? Reelin-positive neurons in the MEC are more likely than reelin-negative neurons to contribute axons to the perforant path (Varga, Lee, & Soltesz, 2010), but it remains to be determined whether this relationship holds true for the LEC. Second, do local changes in LEC reelin signaling have downstream effects within the hippocampus? Lastly, because we have employed a method for inhibiting reelin signaling that prevents binding to both the apolipoprotein-E receptor 2 (*APOER2*) and low-density lipoprotein receptor 2 (*LDLR2*), the question of whether either receptor specifically mediates the role of LEC reelin signaling in learning and memory remains unanswered. There is some agreement that reelin exerts its effects on synaptic plasticity within the hippocampus primarily through *APOER2* (Beffert, Durudas, Weeber, Stolt, Giehl, Sweatt, Hammer, & Herz, 2006), but there are no data to suggest that this relationship holds true specifically for the LEC, although the *APOER2* is expressed among principal neurons in this region (Lein et al., 2007, <http://mouse.brain-map.org>). While much work remains to be done to fully elucidate the role of entorhinal cortical reelin signaling in learning, the significance

of this molecular pathway in aging and age-related dementias such as Alzheimer's disease makes reelin an attractive therapeutic target for the prevention and treatment of cognitive decline.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Beffert U, Durudas A, Weeber EJ, Stolt PC, Giehl KM, Sweatt JD, Hammer RE, Herz J. Functional dissection of Reelin signaling by site-directed disruption of Disabled-1 adaptor binding to apolipoprotein E receptor 2: distinct roles in development and synaptic plasticity. *J Neurosci*. 2006; 26:2041–52. [PubMed: 16481437]
- Beffert U, Weeber EJ, Durudas A, Qiu S, Masiulis I, Sweatt JD, Li WP, Adelmann G, Frotscher M, Hammer RE, Herz J. Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. *Neuron*. 2005; 47:567–79. [PubMed: 16102539]
- Braak H, Braak E. Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol Aging*. 1995; 16:271–8. [PubMed: 7566337]
- Burwell RD, Saddoris MP, Bucci DJ, Wiig KA. Corticohippocampal contributions to spatial and contextual learning. *J Neurosci*. 2004; 24:3826–3. [PubMed: 15084664]
- Dong E, Caruncho H, Liu WS, Smalheiser NR, Grayson DR, Costa E, Guidotti A. A reelin-integrin receptor interaction regulates Arc mRNA translation in synaptoneurosome. *Proc Natl Acad Sci U S A*. 2003; 100:5479–84. [PubMed: 12707415]
- Ferbinteanu J, Holsinger RM, McDonald RJ. Lesions of the medial or lateral perforant path have different effects on hippocampal contributions to place learning and on fear conditioning to context. *Behav Brain Res*. 1999; 101:65–84. [PubMed: 10342401]
- Gallagher M, Burwell R, Burchinal M. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci*. 1993; 107:618–26. [PubMed: 8397866]
- Gusev PA, Cui C, Alkon DL, Gubin AN. Topography of Arc/Arg3.1 mRNA expression in the dorsal and ventral hippocampus induced by recent and remote spatial memory recall: dissociation of CA3 and CA1 activation. *J Neurosci*. 2005; 25:9384–97. [PubMed: 16221847]
- Hargreaves EL, Rao G, Lee I, Knierim JJ. Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. *Science*. 2005; 307:1792–4. [PubMed: 15961670]
- Herz J, Chen Y. Reelin, lipoprotein receptors and synaptic plasticity. *Nat Rev Neurosci*. 2006; 7:850–9. [PubMed: 17053810]
- Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz J. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron*. 1999; 24:481–9. [PubMed: 10571241]
- Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, et al. Genome-wide atlas of gene expression in the adult mouse brain. *Nature*. 2007; 445:168–76. [PubMed: 17151600]
- Liu WS, Pesold C, Rodriguez MA, Carboni G, Auta J, Lacor P, Larson J, Condie BG, Guidotti A, Costa E. Down-regulation of dendritic spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous reeler mouse. *Proc Natl Acad Sci U S A*. 2001; 98:3477–82. [PubMed: 11248103]

- Martínez-Cerdeño V, Galazo MJ, Clascá F. Reelin-immunoreactive neurons, axons, and neuropil in the adult ferret brain: evidence for axonal secretion of reelin in long axonal pathways. *J Comp Neurol*. 2003; 463:92–116. [PubMed: 12811805]
- Merrill DA, Chiba AA, Tuszyński MH. Conservation of neuronal number and size in the entorhinal cortex of behaviorally characterized aged rats. *J Comp Neurol*. 2001; 438:445–56. [PubMed: 11559900]
- Niu S, Renfro A, Quattrocchi CC, Sheldon M, D'Arcangelo G. Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway. *Neuron*. 2004; 41:71–84. [PubMed: 14715136]
- Niu S, Yabut O, D'Arcangelo G. The Reelin signaling pathway promotes dendritic spine development in hippocampal neurons. *J Neurosci*. 2008; 28:10339–48. [PubMed: 18842893]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1998.
- Petrulis A, Alvarez P, Eichenbaum H. Neural correlates of social odor recognition and the representation of individual distinctive social odors within entorhinal cortex and ventral subiculum. *Neuroscience*. 2005; 1:259–74. [PubMed: 15561442]
- Pujadas L, Gruart A, Bosch C, Delgado L, Teixeira CM, Rossi D, de Lecea L, Martínez A, Delgado-García JM, Soriano E. Reelin regulates postnatal neurogenesis and enhances spine hypertrophy and long-term potentiation. *J Neurosci*. 2010; 30:4636–49. [PubMed: 20357114]
- Qiu S, Korwek KM, Pratt-Davis AR, Peters M, Bergman MY, Weeber EJ. Cognitive disruption and altered hippocampus synaptic function in Reelin haploinsufficient mice. *Neurobiol Learn Mem*. 2006; 85:228–42. [PubMed: 16376115]
- Rapp PR, Deroche PS, Mao Y, Burwell RD. Neuron number in the parahippocampal region is preserved in aged rats with spatial learning deficits. *Cereb Cortex*. 2002; 12:1171–9. [PubMed: 12379605]
- Robitsek RJ, Fortin NJ, Koh MT, Gallagher M, Eichenbaum H. Cognitive aging: a common decline of episodic recollection and spatial memory in rats. *J Neurosci*. 2008; 28:8945–54. [PubMed: 18768688]
- Rodriguez MA, Pesold C, Liu WS, Kriho V, Guidotti A, Pappas GD, Costa E. Colocalization of integrin receptors and reelin in dendritic spine postsynaptic densities of adult nonhuman primate cortex. *Proc Natl Acad Sci U S A*. 2000; 97:3550–5. [PubMed: 10725376]
- Stranahan AM, Haberman RP, Gallagher M. Cognitive decline is associated with reduced reelin expression in the entorhinal cortex of aged rats. *Cerebral Cortex*. 2011; 21:392–400. [PubMed: 20538740]
- Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus*. 2008; 18:1085–1088. [PubMed: 18651634]
- van Strien NM, Cappaert NL, Witter MP. The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. *Nat Rev Neurosci*. 2009; 10:272–82. [PubMed: 19300446]
- Varga C, Lee SY, Soltesz I. Target-selective GABAergic control of entorhinal cortex output. *Nat Neurosci*. 2010; 13:822–4. [PubMed: 20512133]
- Wedenoja J, Tuulio-Henriksson A, Suvisaari J, Loukola A, Paunio T, Partonen T, Varilo T, Lönnqvist J, Peltonen L. Replication of association between working memory and Reelin, a potential modifier gene in schizophrenia. *Biol Psychiatry*. 2010; 67:983–91. [PubMed: 19922905]
- Young BJ, Otto T, Fox GD, Eichenbaum H. Memory representation within the parahippocampal region. *J Neurosci*. 1997; 13:5183–95. [PubMed: 9185556]

Figure 1. Cannula placement in the lateral entorhinal cortex

(A), Schematic showing cannula placement in the lateral entorhinal cortex (LEC), using coordinates described in Materials and Methods. Figures used with permission from Paxinos and Watson (1998). (B), Cresyl violet staining of coronal sections from three different rats showing the rostrocaudal extent of LEC cannula tracks.

Figure 2. Infusion of recombinant receptor-associated protein (RAP) effectively reduces disabled-1 phosphorylation

(A), Methods for microdissection of the LEC and medial entorhinal cortex (MEC). (B), disabled-1 (DAB1) phosphorylation at tyrosine 220 is selectively reduced in the LEC following four daily infusions of 10 μ g/ml RAP. No changes in total DAB1 were detected. Asterisk (*) indicates significance at $p < 0.05$ following 2×2 ANOVA.

Figure 3. Suppression of reelin signaling in the lateral entorhinal cortex impairs spatial memory in the water maze

(A), Rats treated with receptor-associated protein (RAP) navigate less efficiently to the goal platform, relative to vehicle-infused rats. Asterisk (*) indicates significance at $p < 0.05$ following one-way repeated measures ANOVA with Tukey's post hoc. (B), Administration of RAP to the LEC impairs performance during probe trials. The learning index is derived from the average distance from the goal platform, with higher scores indicating poorer performance.

Figure 4. Suppression of reelin signaling is associated with reduced synaptophysin expression (A), Synaptophysin expression is reduced in the lateral entorhinal cortex (LEC) following receptor-associated protein (RAP) treatment. Impairment of synaptic marker expression was localized to the target region, as no corresponding decrease was detectable in the medial entorhinal cortex (MEC). Asterisk (*) indicates significance at $p < 0.05$ following 2×2 ANOVA.