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

Alexis M. Stranahan, Sebastian Salas-Vega, Nicole T. Jiam, Michela Gallagher *

Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA

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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.

1. Introduction

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, 2005), 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, & 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, 1995). 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 (Merrill, Chiba, & Tuszynski, 2001; Rapp, Deroche, Mao, & Burwell, 2002), 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 et al., 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 et al., 1999). With in vivo administration via cannulae targeted to

* Corresponding author. Address: Department of Psychological and Brain Sciences, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, USA. Fax: +1 410 516 0494.
E-mail address: michela@jhu.edu (M. Gallagher).

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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.

2. Materials and methods

2.1. Animal treatments

Male Long-Evans rats were purchased from Charles River Laboratories at 3 months of age. All rats were maintained on a 12 h 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 h, followed by dehydration and cryoprotection for 48 h 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.

2.2. Histology

Frozen brains were sectioned on the coronal plane at 40 μm 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).

2.3. Stereotaxic surgery and drug infusion

Rats were anesthetized with Isoflurane and placed in a stereotaxic apparatus. Guide cannulae were mounted bilaterally at bregma -6.8 mm on the anteroposterior axis, and ±7.0 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 μl injection volume) or sterile Dulbecco's phosphate-buffered saline (DPBS) into the lateral entorhinal cortex. Injections began 3 days before water maze training, with the final injection occurring on the first day of training.

2.4. 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, Burwell, & Burchinal, 1993). Behavioral testing took place during the light phase, with training over 8 days, in sessions of three trials per day, as shown in Supple-

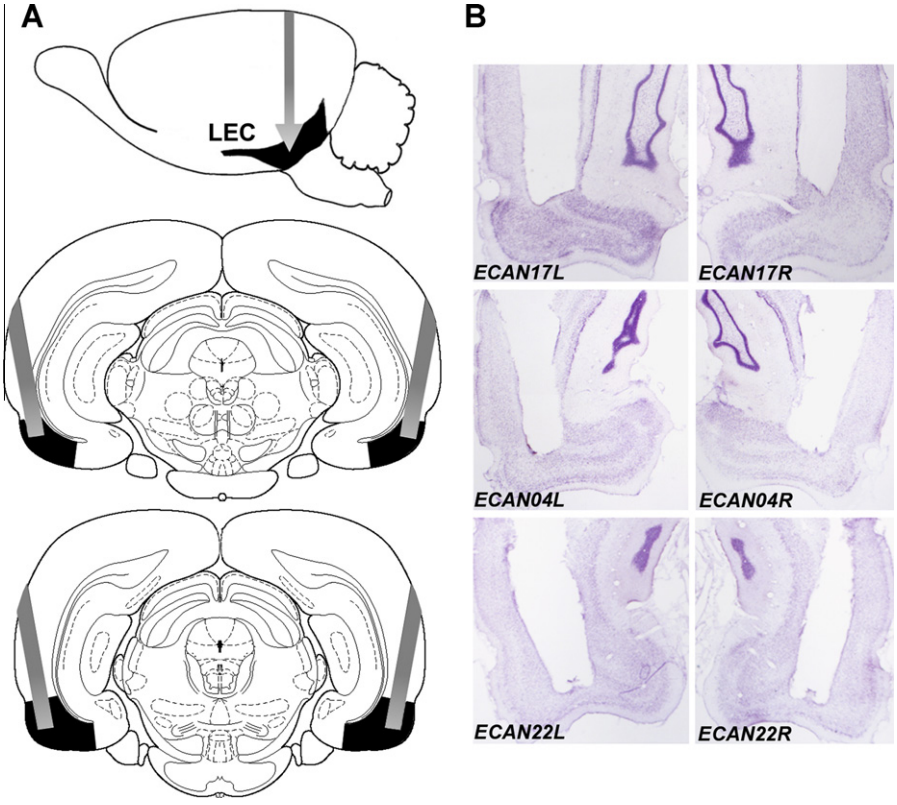

Fig. 1. Cannula placement in the lateral entorhinal cortex. (A) Schematic showing cannula placement in the lateral entorhinal cortex (LEC), using coordinates described in Section 2. Figures used with permission from Paxinos and Watson (1998). (B) Cresyl violet staining of coronal sections from three different rats showing the rostrocaudal extent of LEC cannula tracks.