



Should I Stay or Should I Go?

Impaired Reelin Processing and Secretion by Cajal–Retzius Cells Contributes to Granule Cell Dispersion in a Mouse Model of Temporal Lobe Epilepsy.

Duveau V, Madhusudan A, Caleo M, Knuesel I, Fritschy J-M. *Hippocampus*. doi:10.1002/hipo.20793. [Published online ahead of print May 17, 2010].

Cajal–Retzius cells play a crucial role during ontogeny in regulating cortical lamination via release of reelin. In adult brain, they comprise small calretinin-positive interneurons located in the marginal zone of the cerebral cortex and in the hippocampal fissure. Alterations of reelin signaling or expression have been involved in major neurological disorders, and they underlie granule cell dispersion (GCD) in mesial temporal lobe epilepsy (TLE). Here, we investigated in a mouse model of TLE the contribution of Cajal–Retzius cells to reelin production in epileptic hippocampus and the molecular mechanisms underlying GCD. Following unilateral intrahippocampal Kainic acid injection in adult mice to induce an epileptic focus, we observed that Cajal–Retzius cells gradually became strongly immunopositive for reelin, due to intracellular accumulation. This phenotype resembled the morphology of Cajal–Retzius cells in *reeler* Orleans (*reln^{ori/ori}*) mice, which express a secretion-deficient 310-kDa reelin fragment. The possibility that GCD might result from abnormal reelin processing in Cajal–Retzius cells, leading to a lack of reelin secretion, was confirmed by KA injection in *reln^{ori/+}* mice, which induced severe GCD. Furthermore, Western blot analysis in KA-treated wildtype mice revealed increased production of ~300-kDa reelin fragments, confirming abnormal proteolytic processing. This effect was not seen upon treatment with Botulinum neurotoxin E (BoNT/E), which prevents GCD in KA-lesioned hippocampus by chronic blockade of synaptic transmission. Furthermore, BoNT/E blocked upregulation of TrkB in Cajal–Retzius cells, suggesting that production of truncated reelin in KA-treated hippocampus is activity-dependent and regulated by BDNF. Altogether, these data reveal that GCD results from abnormal reelin processing in Cajal–Retzius cells under the control of BDNF. Our findings highlight the critical role played by Cajal–Retzius cells for hippocampal neuronal reorganization in TLE.

Commentary

Young cortical pyramidal neurons migrate away from proliferative zones of the neuroepithelium by translocating along the shafts of radial glial cells. While mostly accomplished before birth in humans, neurogenesis and migration continue in the dentate gyrus of the hippocampus throughout life. Faulty neuronal migration during fetal development is associated with mental retardation and severe drug-resistant forms of pediatric epilepsies, such as lissencephaly, double cortex, and focal cortical dysplasia (1). Granule cell dispersion is a less severe migration defect occurring in adult patients with mesial temporal lobe epilepsy (MTLE) (2).

The search for causes of granule cell dispersion in MTLE is narrowing down on a molecule called Reelin, a secreted signaling protein that provides essential instructions telling young neurons when and where to stop migrating. New studies are aimed at understanding how this molecule becomes cleaved and secreted into its active form. Most of the brain's Reelin is synthesized and secreted by Cajal–Retzius cells, located in the

cortical marginal zone and the hippocampal fissure. In the extracellular matrix, Reelin binds to very-low-density lipoprotein receptors and the apolipoprotein E receptor 2 expressed on neuronal plasma membranes and activates signaling events that stabilize microtubules and regulate cell motility (3). Recent studies also implicate Reelin signaling in dendrite spine formation, synaptic plasticity, and long-term potentiation, so its roles in the brain extend well beyond neuronal migration.

Several studies provide hints that Reelin is required for maintaining the proper lamination of the adult dentate gyrus. When Reelin is depleted experimentally, the tight lamination of the granule cell layer is lost, even in the absence of seizures. Reelin decreases in patients and rodents with MTLE, and this is correlated with granule cell dispersion, suggesting that Reelin normally helps to maintain a well-structured granule cell layer in humans and rodents. Reduced Reelin signaling in MTLE could be caused by interneuron death, since severe seizures contribute to the loss of some hilar GABAergic interneurons that express Reelin. However, Cajal–Retzius interneurons do not appear to die out in MTLE, so further work has focused on understanding why the residual Reelin secreted by these cells fails to maintain the granule cell layer.

Duveau and colleagues investigated whether, in experimental MTLE, losing Reelin signaling from Cajal–Retzius cells



would cause granule cell dispersion. They induced seizures unilaterally in the dorsal hippocampus of adult mice with the excitotoxin kainic acid, and then compared Reelin expression in Cajal-Retzius cells during the process of granule cell dispersion. While multiple types of interneurons express Reelin, Cajal-Retzius cells have small ovoid or fusiform cell bodies and thin dendrites that distinguish them from other Reelin-expressing GABAergic interneurons in the hippocampus. Moreover, they also co-express the calcium-binding protein calretinin and reside chiefly within the hippocampal fissure. While many of the Reelin-expressing dentate gyrus interneurons were destroyed by the excitotoxin, the Cajal-Retzius cells survived. However, they seemed to have a defect either in Reelin cleavage or secretion because these cells exhibited abnormally elevated levels of Reelin after seizures.

This finding suggested that defective Reelin cleavage might account for granule cell dispersion in MTLE, leading the authors to examine mutant mice heterozygous for the Orleans mutation ($reln^{ori/ori}$ mice). The homozygous mutation causes severe disruption of granule cell layer lamination, but without a seizure phenotype. In contrast, the dentate gyrus of heterozygous $reln^{ori/+}$ littermates shows normal granule cell layer lamination and only slight increases in Reelin staining of Cajal-Retzius cells near the hippocampal fissure. Following unilateral injection of kainic acid into the hippocampus of mice heterozygous for the Orleans mutation, the authors found even more pronounced granule cell dispersion than observed after seizures in wild-type mice, suggesting that either seizures or a mutation causing Reelin accumulation can cause granule cell dispersion.

Duveau and colleagues wondered whether the accumulation resulted from defective proteolysis of Reelin. The possibility that Cajal-Retzius cells might abnormally accumulate an inactive form of Reelin was tested in biochemical experiments. Reelin exists in three isoforms in the brain, corresponding to a full-length isoform of ~400,000 Da and two smaller proteolytic isoforms corresponding to 310,000 and 180,000 Da. The mice with kainate-induced MTLE showed increases in the higher molecular weight inactive isoforms, and concomitant reductions in the secreted, active 180,000 molecular weight fragment that signals to other neurons.

To determine whether epileptic activity was connected to a buildup of the larger inactive isoforms of Reelin, Duveau and colleagues asked whether blocking neural activity with botulinum neurotoxin E (Botox) after they induced seizures would prevent the abnormal accumulation of Reelin. Botox acts to block synaptic neurotransmission and was previously shown to prevent granule cell dispersion in the kainic acid model of MTLE. Duveau and colleagues found that the Botox treatment prevented Reelin from accumulating in Cajal-Retzius cells in the hippocampus.

These findings suggest a chain of events leading from hyperactive neural firing in the dentate gyrus to granule cell dispersion. In this scenario, epileptic hippocampal networks result in abnormal proteolysis and buildup of the high molecular weight, inactive forms of Reelin. Without an active gradient of Reelin in the dentate gyrus, granule neurons would lack their normal positional cues to stop migrating and disperse.

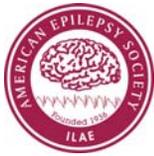
This study linked epileptiform activity with abnormal Reelin processing. Reelin appears to build up in Cajal-Retzius cells in the hippocampal fissure, but the exact mechanism causing defective proteolysis in epilepsy is not yet clear. As with many other secreted molecules, Reelin is synthesized in the endoplasmic reticulum, raising the possibility that endoplasmic reticulum stress is responsible for defective proteolysis of Reelin in MTLE. Possibly so, but additional new evidence suggests that Reelin secretion is constitutive and activity independent. If so, then the locus for abnormal cleavage of Reelin might be in the extracellular matrix surrounding the Cajal-Retzius cells (4). In fact, recent work suggests that epileptic activity might impair the activity of matrix metalloproteinases (MMPs), a family of endopeptidases that proteolyze extracellular proteins. MMPs have been linked to homeostatic plasticity and synaptic scaling. Their expression is altered by seizures, and it was shown that epileptic discharges reduce MMP activity in hippocampal slices (4). These observations led to the speculation that reduced MMP activity in the dentate gyrus, rather than endoplasmic reticulum stress in Cajal-Retzius cells, causes accumulation of the high molecular weight, inactive forms of Reelin in the extracellular matrix surrounding the hippocampal fissure.

This new work suggests that drugs that increase MMP activity could protect against granule cell dispersion in epilepsy by restoring proteolysis of Reelin. Working out whether impairments in Reelin signaling are caused by endoplasmic reticulum stress, impaired secretion, loss of extracellular MMP activity, or other mechanisms could impact future efforts to identify effective pharmacologic treatments to prevent granule cell dispersion and hippocampal sclerosis.

by Janice R. Naegele, PhD

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American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

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Dr Coulter, one of the co-authors on one of the papers that I reviewed in my commentary, serves on an NIH study section that recently reviewed one of my grant applications (it was not funded). I wrote the commentary subsequent to the grant panel recommendations to not fund my grant application.