Does Reelin Affect Recovery from a Stroke?

Charisma DeSai[1], Joachim Herz[1,2,3,4], Ann M. Stowe[3] , Courtney Lane-Donovan[1,2]

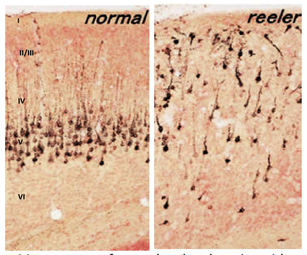
Departments of [1]Molecular Genetics, [2]Neuroscience, [3]Neurology and Neurotherapeutics, [4]Center for Translational Neurodegeneration Research

at the University of Texas Southwestern Medical Center, Dallas, Texas, USA

WHAT IS REELIN?

Reelin is a large extracellular glycoprotein that plays important roles in brain development and synaptic plasticity.

In the embryonic brain, Reelin directs neuronal migration and the ordered arrangement of neuron layers in the cortex. Mice born without Reelin are called *reeler* mice. These mice exhibit abnormal cortical layering and ataxia.



Motor cortex of normal and reeler mice with cortical layers numbered in normal. [1]

In the mature brain, the binding of Reelin to the ApoE receptor (ApoER2, also known as LRP8) activates a conserved signaling pathway which results in activation of neuronal enhancers. ApoER2 is essential for the induction of long term potentiation in the adult brain, and Reelin has been experimentally shown to enhance long-term potentiation.[2]

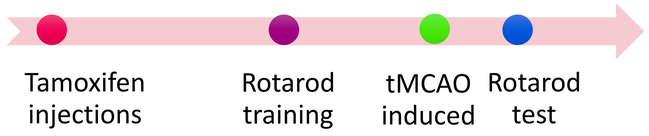
BACKGROUND

We have previously shown that Reelin has neuroprotective effects in models of Alzhimer's disease[3] and were interested in seeing if it plays a role in recovery after a stroke since both events cause significant neurological damage.

Earlier papers describing experiments with *reeler* mice showed that mice lacking Reelin have increased susceptibility to stroke and suffer more damage post-stroke.[4, 5] Reelin levels are modestly reduced for some time after a stroke due to increased expression of miR200-c shortly after ischemia. Antagonism of miR200-c protects against tMCAO.[4] The impact of Reelin regulation in this effect is unclear since there are alternative implicated targets for miR200-c. Since Reelin is important in brain development, it was also possible that the observed results were due to brain defects.

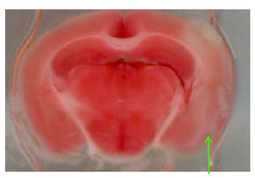
The experiments described here were designed to overcome the limitation of using *reeler* mice by instead using Reelin conditional knockout (cKO) mice. This inducible Reelin knockout mouse model ($Reln^{fl/fl}$, CAG-Cre driver) allows us to see the effects of Reelin loss while permitting normal brain development. Mouse behavior and indirect infarct volume were measured to assess the impact of Reelin loss on strokes and stroke recovery.

PROCEDURE



First, CAG-Cre mice were given intraperitoneal **injections of Tamoxifen** for five days. Sixteen days after the fifth injection, the mice were **trained on the Rotarod** machine until their learning ability peaked. Ten days after training was completed, **tMCAO**s were induced in the mice. They were allowed to heal for two days, after which they were **tested on the Rotarod.** The mice were subsequently sacrificed, and tissues were harvested for study.

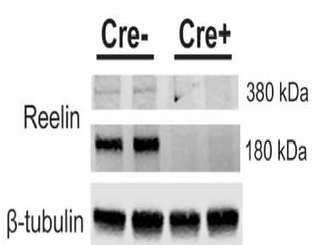
- Tamoxifen injections
  - Tamoxifen was used in the CAG-Cre conditional knockout mouse system[3] to activate the flox gene system and turn off the production of Reelin.
- Rotarod training
  - The Rotamex rotarod is a device used to test a mouse's motor function. The rotarod has a wheel which spins progressively faster in a controlled manner. The mice are placed on this wheel, once a day for multiple days, until their run time on the wheel reaches a plateau. Their motor ability is measured by the amount of time they run before falling off the wheel (latency of fall).
- tMCAO
  - In this procedure, mice are anesthetized and a small suture is inserted into the middle cerebral artery of the mouse. It remains in the mouse for 45 minutes, after which it is removed. The occlusion or the artery is confirmed using Doppler flowmetry.



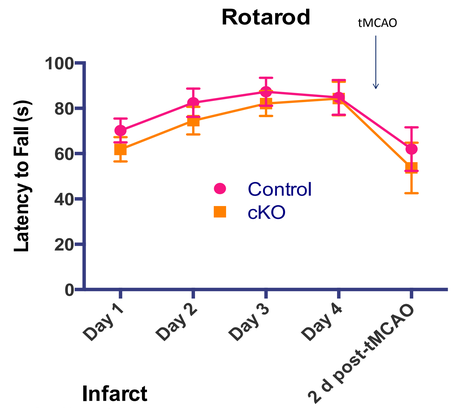
This is a brain slice from a mouse which had a tMCAO. The brains were stained with TTC, which selectively stains active mitochondria (darker colored areas). The area of the infarct can be seen as the lighter area on the right side of the image.

Photo credit: Courtney Lane-Donovan

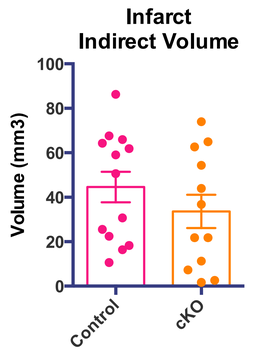
- Rotarod test
  - The same device is used to train and test the mice. The Rotarod test is conducted post-stroke. The results are compared with the plateau of training in order to see the difference in the mouse's balance and coordination pre- and post-stroke.
- One additional step taken was to confirm the absence of Reelin from CAG-Cre+ mice. This was done by doing a Western blot of brain tissue with antibody markers for Reelin. As can be seen in the two examples below, Reelin expression was lost in the CAG-Cre+ mice.



RESULTS



Both sets of mice learned at similar rates and demonstrated similarly reduced ability on the rotarod two days after the tMCAO (unpaired t-test $p = 0.5756$).



The indirect infarct volume (a measure of the swelling of the infarct) was calculated using this formula: [contralateral hemisphere volume – (ipsilateral hemisphere volume – infarct volume)].[6] The infarct area was visualized using TTC staining.

tMCAO induced infarct volumes were variable for both groups, but there was no significant effect of Reelin loss on average indirect infarct size ($p=0.2909$).

CONCLUSIONS

No significant difference was seen between wild type and Reelin cKO mice in infarct size or post-stroke motor coordination , suggesting that while Reelin plays an important function in the brain, it does not play a significant role in short-term post-stroke recovery. It is possible that the effects seen previously were due to improper brain development rather than Reelin deficiency. Although Reelin does not appear to have an endogenous effect on post-stroke recovery, it may still have potential as a therapy.

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