ROBO4 and its relationship to Alzheimer's Disease

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ABSTRACT

Aging is associated with the impairment of the neurovascular unit, and this potentially leads to increased Alzheimer's disease pathology and cognitive impairment. A specific axon guidance receptor, Robo4, is important in maintaining the structure and restrictive barrier of the blood-brain barrier (BBB). Therefore, Robo4 signaling pathways may potentially be a valuable target for therapeutic treatments of AD. In the present study, we studied Robo4 knockout (Robo4 -/-) and wild type (Robo4 +/+) mice crossed with mice containing mutations in amyloid precursor protein (APP), leading to greater aberrant amyloid-beta production. We predicted that the knockout of Robo4 will increase BBB permeability, leading to increased Alzheimer's disease-related neuropathology and cognitive dysfunction. To examine the effect of aging, we studied young and old wildtype C57BL6 mice. We assessed cognitive function by conducting Nest Building tests and Morris Water Maze. We found that old C57BL6 mice had impaired cognitive function compared to young C57BL6 mice. However, when Robo4 x APP groups were compared, we found no differences in cognitive function. These preliminary results suggest that aging has a stronger effect on cognitive function than Robo4 knockout. Additional studies are needed to determine the effect of Robo4 knockout on blood-brain barrier permeability and amyloid-beta accumulation.

INTRODUCTION

The physiological mechanisms of Alzheimer's Disease (AD) include the formation of amyloid-beta (Aβ) plaques and neurofibrillary tangles from tau protein (Yeo, 2019). In the neurovascular unit, the blood-brain barrier (BBB) is a highly selective permeable membrane (Suzuki, Nagai, & Umemura, 2016). Loss of these restrictive barrier characteristics in the BBB may be a possible mechanism for AD as it can lead to neurotoxicity and precedes Aβ plaque formation. Robo4 is an axon guidance receptor that increases vascular stability, prevents hyperpermeability, and angiogenesis. Thus, Robo4 signaling may help maintain BBB permeability and stability, therefore reducing cognitive impairment and neuropathology related to AD.

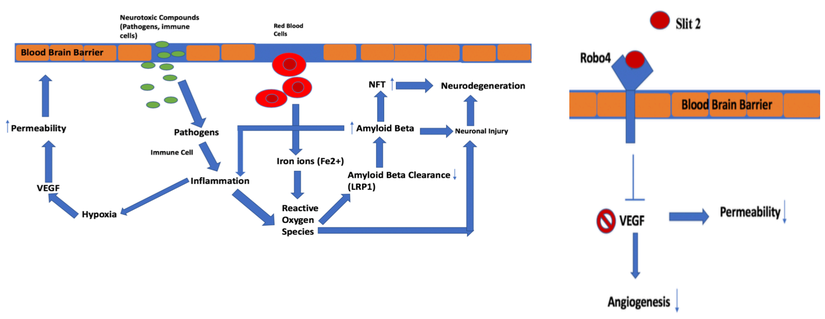


Figure 1: **Robo4 signaling mechanism.** It is believed that a leaky BBB can lead to neurodegeneration through multiple pathways. The binding of Slit2 to Robo4 may potentially suppress the permeability of the BBB.

In this set of studies, I sought to establish a set of tests of cognitive function in mice. I then used these tests to compare young and old wildtype mice (C57BL6), and to compare mice with a knockout for Robo4 (Robo4-/-) combined with a mutation leading to the overproduction of aberrant Aβ (APP).

HYPOTHESIS

1) Old mice will exhibit greater cognitive dysfunction than young mice.
2) Robo4 knockout mice crossed with APP mutation will exhibit greater cognitive dysfunction.

METHODS

C57BL6 Mice

Gene Expression

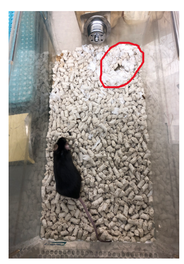
Robo4 +/+
Robo4 -/-
Robo4 +/+ APP
Robo4 -/- APP

Nest Building (Nest Scores 0-5)

Objective: Creating a nest using paper nestlet

- Each mice was left overnight in a cage containing paper nestlet.
- Nesting scores were rated according to Accessing Nest Construction, and Hoarding in Mice by Robert Deacon.

Score=0



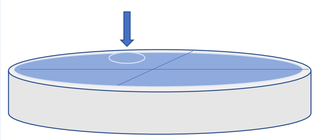
Score=5

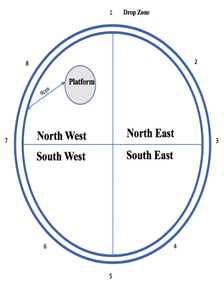
Morris Water Maze

Objective: Allow the mice to determine the location of the hidden platform

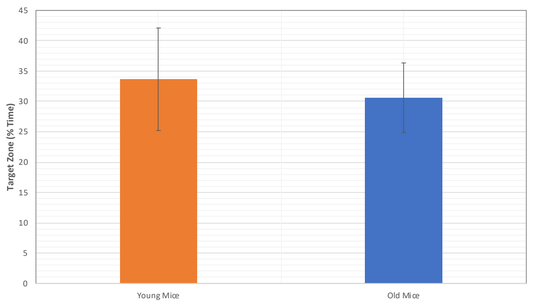
* The water maze experiment was conducted for five days.
* During Day 3 and Day 5, the platform was removed for the probe trial.
* All data analysis was conducted using Ethovision

Hidden Platform





RESULTS



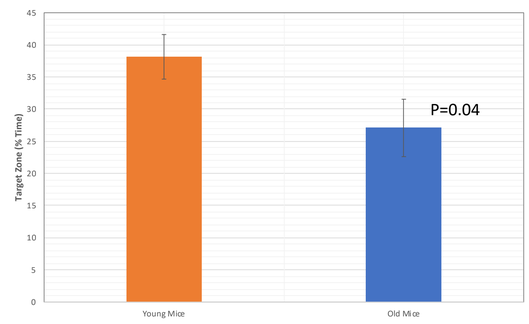


Figure 2. Morris Water Maze. (A) After two days of training, young and old C57BL6 mice perform similarly on the Morris Water Maze test (p=0.39). (B) After four days of training, young mice spend a greater amount of time in the target zone, indicating better memory, compared with old mice (p=0.04).

RESULTS (CONTINUED)

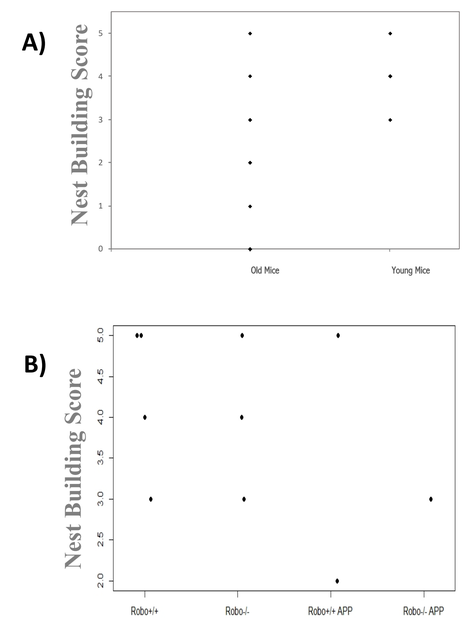


Figure 3. Nesting building test. (A) Nesting score, a measure of cognitive function, is higher in young vs. old wildtype C57BL6 mice ($p<0.001$). (B) No significant difference in nesting score is found between groups with Robo4 deletion (Robo4-/-) or Aβ overproduction (APP) ($P>0.05$).

CONCLUSION

1) Old C57BL6 mice had impaired cognitive function, compared to the young C57BL6 mice.
2) There was no significant difference in cognitive function with Robo4 knockout or APP mutation on the Nest Building Test.

FUTURE STUDIES

1) Test additional Robo4 X APP mice for cognitive function.
2) Measure BBB permeability in Robo4 mice.
3) Examine Aβ and neuroinflammation in brains from Robo4 mice.

REFRENCES

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