The synaptic scaffolding proteins Homer1b/c are necessary for contextual fear conditioning and for group I metabotropic glutamate receptor mediated long-term depression

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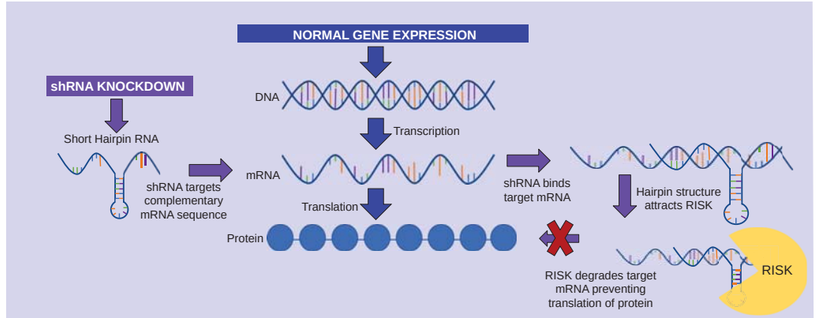
ABSTRACT

The effect of aging on cognitive ability is considerably diverse. Age-associated cognitive decline can range from mild cognitive impairment to severe dementia, yet many individuals experience no cognitive deficits even while surpassing ages of one hundred years. Advanced age is the number one risk factor for the development of Alzheimer's disease and individuals experiencing mild cognitive impairment are at an increased risk. Currently, little is known regarding the changes within the brain which underlie differing cognitive fates. However, it is likely that changes in gene expression occur with aging which alter the expression of specific proteins within neurons. Previous research in our lab has indicated that, changes in expression of the protein Homer1b/c within the hippocampus may be involved in age-associated cognitive decline. This protein is reduced in hippocampi of aged learning impaired (AI) rats, compared to superior learners (SL) after training in a hippocampal dependent learning task. Previously, we have developed a Homer1c expressing viral vector; that when injected into rat hippocampi overexpresses this protein. Overexpression of Homer1c in the hippocampi of AI rats rescues their learning and memory deficits, improving performance on behavioral memory tasks to a level similar to SL animals. Since we have shown that Homer1c is sufficient to rescue memory in AI animals, we are now investigating whether maintenance of this protein's expression, as well as a nearly identical variant: Homer1b, is necessary for cognitive success. To answer this question, we are using a viral vector to express a short hairpin RNA (shRNA) targeting *Homer1b/c* messenger RNA (mRNA). The process by which proteins are synthesized in cells begins with the transcription of a DNA sequence into mRNA which is then translated into protein. This *Homer1b/c* targeting shRNA binds to *Homer1b/c* mRNA and triggers its degradation, thereby significantly reducing, or 'knocking down' the amount of Homer1b/c protein expressed. After Homer1b/c knock down in adult rats, behavioral analyses were conducted to test learning and memory abilities. Additionally, we investigated the molecular function of Homer1b/c in learning and memory. On a cellular level, learning and memory are manifest as long-lasting changes in the strength of certain synapses. One form of this synaptic plasticity, long-term depression (LTD), can be triggered by activation of specific glutamate receptors in hippocampal synapses: group 1 metabotropic glutamate receptors (mGluR1/5). We used electrophysiological techniques to determine the impact Homer1b/c knock down on mGluR1/5 mediated LTD (mGluR-LTD). The results of this study indicate that Homer1b/c is necessary for contextual fear conditioning and mGluR-LTD, indicating that Homer1b/c knock down leads to deficits in learning and memory, and synaptic plasticity. Future studies will focus on the role of Homer1b/c in cognitive aging. We will assess the impact of knockdown on learning and synaptic plasticity in aged SL rats, comparing memory task performance pre- and post- knockdown, and characterizing the effect of knockdown on LTD. This research has the potential to elucidate unknown mechanisms of learning and memory in the hippocampus as well as how these mechanisms change with age, potentially uncovering novel therapeutic targets for the treatment and prevention of age-associated neuropathies.

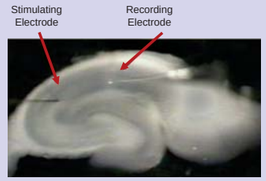
METHODS

shRNA Knockdown

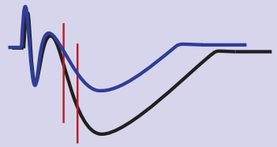


Synaptic Plasticity

Hippocampal slices are incubated at 32ºC in artificial cerebral spinal fluid bubbled with oxygen and carbon dioxide

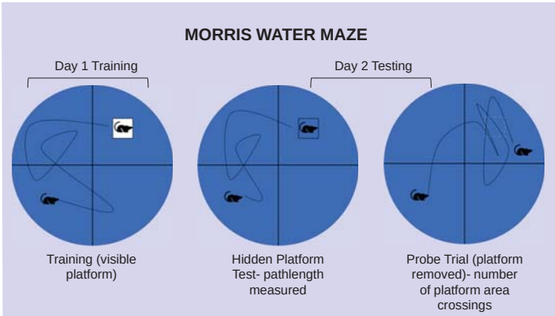


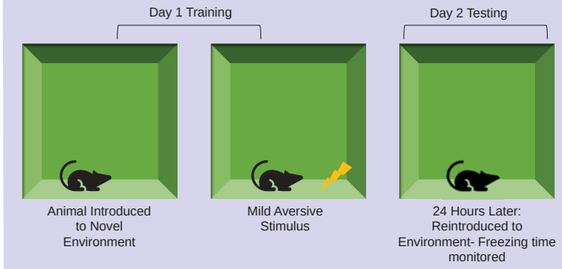
Neurons in each slice at one end of a synaptic pathway are stimulated with an electric stimulus, and the response from downstream neurons is recorded



In black: a typical recorded response. The slope of the response between the two red lines is indicative of its magnitude. The blue waveform shows reduced magnitude in a subsequent response, known as synaptic depression

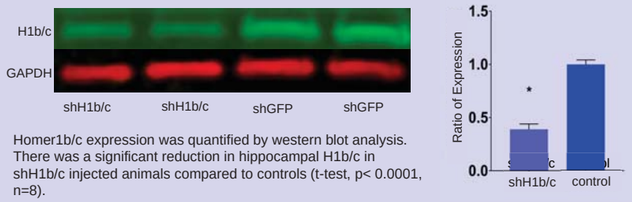
Spatial Learning and Memory





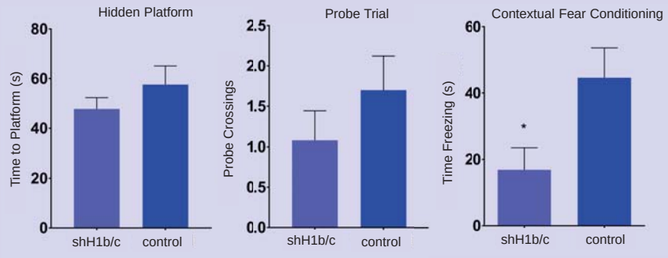
RESULTS

shH1b/c Yields a 61% Reduction in Hippocampal H1b/c Expression



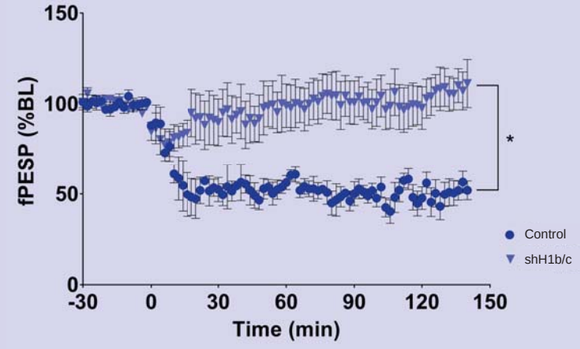
Homer1b/c expression was quantified by western blot analysis. There was a significant reduction in hippocampal H1b/c in shH1b/c injected animals compared to controls (t-test, p< 0.0001, n=8).

Knockdown of Hippocampal H1b/c Causes Significant Deficits in Contextual Fear Conditioning, but Does Not Cause Deficits in the MWM Hidden Platform or Probe Tasks



Behavioral analysis of adult shH1b/c injected rats and control animals. H1b/c knockdown did not cause significant deficits in MWM Hidden Platform trial (n=23, p=.255), or MWM Probe trial (n=23, p=.277). However, significant learning deficits were observed in Contextual Fear Conditioning (n=18, p=.025).

H1b/c is Necessary for mGluR1/5 Mediated LTD in the Hippocampus



Hippocampal slices from animals treated with shH1b/c (n= 7 slices, 4 animals) showed significant deficits in LTD expression compared to those harvested from control animals (n= 4 slices 3 animals); repeated measures ANOVA $F_{(1,9)} = 10.19$, p= 0.011).

SUMMARY

CONCLUSIONS

- Hippocampal Homer1b/c is necessary for induction of mGluR mediated LTD in adult animals
- Reduced expression of hippocampal Homer1b/c results in contextual fear conditioning memory deficits, but not in MWM tasks

FUTURE DIRECTIONS

- H1b/c knockdown in aged superior learners
- CRISPR/Cas9 for complete knockout of hippocampal H1b/c expression
- H1b/c mechanism of action in mGluR-LTD

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