Northwestern Medicine®
Feinberg School of Medicine

CONSTITUTIVE LOSS OF SHANK3 IN MICE PRESERVES ASSOCIATIVE LEARNING AND MEMORY AFTER TRAUMATIC BRAIN INJURY

Mecca B.A.R. Islam, MS, Booker T Davis IV, PhD, MS , Kacie P Ford, BS, Madeline E. Timken, BS, Zhangying Chen , BS and Steven J. Schwulst, MD, FACS, FCCM

Department of Surgery, Division Of Trauma and Critical Care

ABSTRACT

The CDC estimates that nearly 3 million Americans sustain a traumatic brain injury each year with a high degree of long-term neurocognitive morbidity. TBI results in a variety of neuropathological hallmarks including the generation of amyloid beta oligomers (AβOs). AβOs cause memory dysfunction by inhibiting long term potentiation during synaptic remodeling. AβOs insert into neuronal membranes via interaction with specific membrane protein receptors. Shank3 (SH3 and ankryin repeat domains 3) is a scaffolding protein located in the postsynaptic density of glutamatergic synapses and has recently been shown to bind to AβOs. RNA sequencing data from our laboratory has shown that Shank3 is serially upregulated within the brains of TBI mice long-term. These data suggest that Shank3 may play a role in TBI-associated neurocognitive decline. We hypothesized that mice with the constitutive loss of Shank3 would have attenuated deficits in learning and memory after traumatic brain injury. 14-17 week-old male Shank 3 knockout (Shank3 KO; n=19) and wild type C57BL/6 (n=18) mice underwent severe TBI via controlled cortical impact **(Figure 1)**. At 14 days and 60 days post injury or sham injury, mice underwent neurocognitive testing with both contextual and cued fear conditioning to assess for deficits in associative learning and memory. Data were analyzed using one-way ANOVA and Tukey's multiple comparison post-test. Shank3 knockout resulted in marked preservation of associative learning and memory at both acute and chronic time points after TBI. At 14 days post TBI, Shank At 14 days post TBI, Shank3 KO mice displayed significant preservation of hippocampus based learning and memory compared to wild type TBI mice as tested with contextual fear conditioning (**Figure 4A,**70.0± 11.0% freezing vs. 35.0± 13.0% freezing, **** p<0.01). This preservation persisted at 60 days post injury (**Figure 4B ,**43.0 ± 16.0% freezing vs. 21.0 ± 5.0% freezing, *** p<0.02). In fact, there was no statistical difference between the Shank3 KO TBI group and any of the sham injury groups. Lastly, Shank3 KO TBI mice also demonstrated preservation of amygdala-hippocampus-prefrontal cortex pathways at 14D (**Figure 5A ,**80.0 ± 26.0% freezing vs. 22.0 ± 1.0% freezing,**** p<0.01) and at 60D (**Figure 5B ,** 64.0 ± 13.0% freezing vs. 23.0 ± 11.0% freezing, ***p<0.02)as tested by cued fear conditioning.

INTRODUCTION

Trauma is the leading cause of death and disability in patients between the ages of 1-44 with TBIs contributing to a nearly a third of them. Presently, approximately 2% of the U.S. population are afflicted with disabilities and behavior deficits as a result of a TBI. Despite promising preclinical data, clinical trials have failed to produce effective therapies for this highly morbid injury process.

The lack of effective therapies has led to an increased interest in understanding the genetic differences involved in cognition and neurodegeneration. This has led to the study of Shank3, which plays a key role in protein scaffolding and synaptic functioning.

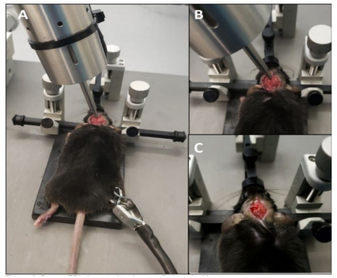
β-Amyloid proteins (Aβ) have been known to play a key role in the synaptic loss associated with Alzheimer's disease (AD). In early stages of AD, AβOs are markedly increased and localized at or within the synapses. The presence of AβOs at the synapses incite dysfunction and degradation of synapses. Recent studies have identified Shank3 as one of the binding target proteins of AβOs. AβOs were found to target shank3 to alter (NMDA) receptor pathways which contributes to synaptic dysfunction in AD.

HYPOTHESIS

We hypothesized that mice with the constitutive loss of Shank3 would attenuate deficits in learning and memory after traumatic brain injury.

METHODS

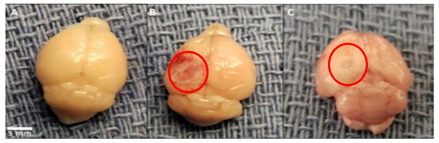
Figure 1. Severe TBI via Murine Model of Controlled Cortical Impact



(Schwulst et al, JoVE 2019)

(A) The grounding cable is clipped to the mouse's hind region and the impacting tip is lowered onto the dura mater. This is the zero point. (B) The impacting tip is retracted, a 2 mm depth of injury is dialed into the stereotaxic frame, and the impact is applied. (C) After the CCI is applied, the impacting tip is rotated out of the field and the mouse is recovered from the stereotaxic frame

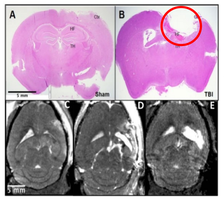
Figure 2. Gross Anatomy of Severe TBI in Mice



(Schwulst et al, JoVE 2019)

(A) Brain from a 12-week-old naïve mouse. (B) Brain from a 12-week-old mouse 24 h after injury (C) Brain from a 12-week-old mouse 7 days after injury

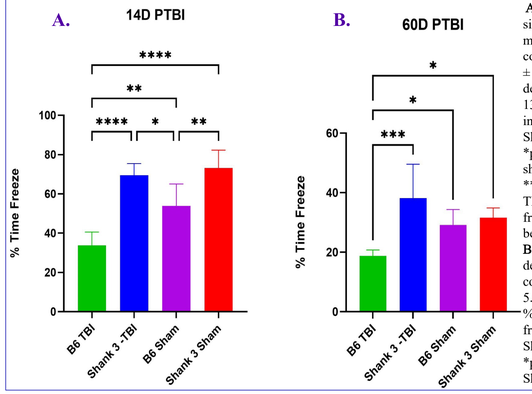
Figure 3. Histology and MRI Confirm Severe TBI



(A) Shows sham injury with craniectomy (B)CCI results in a severe TBI with massive loss of cortex at the site of injury as well as loss and distortion of the underlying hippocampal formation and thalamus . (C) MRI at 1-day post-TBI shows tissue trauma and edema over the left parietotemporal cortex. (D–E) Representative images from post-injury days 7 and 14 showing increased areas of hyper attenuation representing progressive replacement of devitalized tissue with cerebrospinal fluid.

RESULTS

Figure 4. Shank3 KO TBI Mice Show Preservation Of Hippocampus Dependent Learning and Memory In Contextual Fear Conditioning

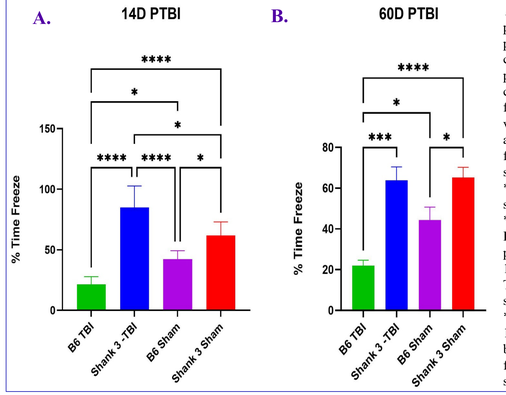


A. At 14 days post injury, Shank3 KO TBI mice displayed significant preservation of hippocampus based learning and memory compared to WT TBI mice as tested with contextual fear conditioning (70.0± 11.0% freezing vs. 35.0 ± 13.0% freezing, ****p<0.01). WT TBI had a significant decrease in %time freeze in compared to it's sham (35.0 ± 13.0% freezing vs. 56.0 ± 15.0% freezing, **p=0.04). An interaction was also seen between Shank3 TBI and WT Sham (70.0± 11.0% freezing vs. 56.0 ± 15.0% freezing, *p=0.04) as well as between both WT sham and Shank3 sham (56.0 ± 15.0% freezing vs. 70.0 ± 18.0% freezing, **p=0.005). The greatest difference was seen between WT TBI and Shank3 sham (35.0± 13.0% vs. 70.0 ± 18.0% freezing, **p<0.01). There were no statistical difference between Shank3 groups

B. At 60 days post-injury, Shank3 KO TBI mice still demonstrated preservation of learning and memory compared to WT TBI (43.0 ± 16.0% freezing vs. 21.0 ± 5.0% freezing, *** p<0.02). WT TBI also had a decrease in %time freeze in compared to WT sham (21.0 ± 5.0% freezing vs. 29.0 ± 5.0% freezing, *p=0.04) and Shank3 Sham (21.0 ± 5.0% freezing vs. 32.0 ± 10.0% freezing, *p=0.03). There were no statistical difference between Shank3 groups and sham.

RESULTS

Figure 5. Shank3 KO Mice Show Preservation Of Connectivity between Amygdala, Hippocampus, and Prefrontal Cortex after TBI



A. At 14 days post injury, Shank3 KO TBI demonstrated preservation of amygdala-hippocampus-prefrontal cortex pathways compared to WT TBI mice as tested with cued fear conditioning (80.0± 26.0% vs. 22.0 ± 1.0% freezing,**** p<0.01). WT TBI had a decrease in %time freeze in compared to WT sham (22.0 ± 1.0% freezing vs. 39.0 ± 9.0% freezing, *p=0.03) and Shank3 sham (22.0 ± 1.0% freezing vs. 62.0± 15.0% freezing, ****p<0.01). An interaction was also seen between Shank3 TBI and WT Sham (80.0 ± 26.0% freezing vs. 39.0± 9.0% freezing, ****p<0.01) and Shank3 sham (80.0 ± 26.4% freezing vs. 62.0± 15.0% freezing, *p=0.04), as well as between both B6 sham and Shank3 sham (39.0 ± 9.0% freezing vs. 62.0 ± 15.0% freezing, *p=0.04).

B. At 60 days post-injury, Shank3 KO TBI mice s preservation continued in compared to wild-type TBI (65.0± 13.0% freezing vs. 23.0 ± 11.0% freezing, ***p<0.02). B6 TBI also had a decrease in %time freeze in compared to B6 sham (23.0 ± 11.0% freezing vs. 44.4 ± 11.0% freezing, *p=0.04) and Shank3 Sham (21.0 ± 5.0% freezing vs. 58.0 ± 18.0% freezing, ****p<0.01). An interaction was seen between WT sham and Shank 3 sham (44.00 ± 11.0% freezing vs. 58.0 ± 18.0% freezing, *p=0.05). There were no statistical difference between Shank3 groups.

CONCLUSION

Consistent with our hypothesis, Shank3 KO in our model of TBI resulted in striking preservation of learning and memory at acute and chronic time points:

- These data suggest Shank3 a significant role in post-injury synaptic remolding preservation.
- Therapeutic targeting of Shank3 may represent a novel treatment strategy to mitigate TBI-associated neurocognitive morbidity.
- Shank3's newly discovered role as an AβO receptor may be one of the putative mechanisms behind the neurocognitive protection seen after the constitutive loss of Shank3

ACKNOWLEDGMENTS

This research was funded by NIH Grant 1R01GM130662 and 1R01GM13066