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Sex Matters in Traumatic Brain Injury

Madeline E. Timken[1], Zhangying Chen[1,2], Mecca B.A.R. Islam[1], Booker T Davis IV[1], Steven J. Schwulst[1]
1 Department of Surgery, Division of Trauma and Critical Care, Northwestern University Feinberg School of Medicine, Chicago IL
2 Driskill Graduate Program in Life Science, Northwestern University Feinberg School of Medicine, Chicago IL

ABSTRACT

Approximately 3 million Americans sustain a traumatic brain injury (TBI) each year with a high rate of subsequent neuropsychiatric morbidity. TBI outcomes differ markedly between males and females in both clinical and preclinical studies. Nonetheless, there are limited studies examining sex as an independent variable in neuropsychiatric outcomes after TBI. We hypothesized that female mice would have attenuated post-traumatic anxiety after TBI as compared to male mice. Age-matched C57Bl/6 male mice (N=21) and female mice (N=20) were grouped into TBI and sham-injury groups. An open-head controlled cortical impact was used to induce a severe TBI vs. sham-injury. At 45 days post-TBI, neuropsychiatric outcomes were assessed with the zero maze for anxiety-like behavior and the open field test for generalized activity levels, anxiety, and willingness to explore. Data was analyzed using one-way ANOVA and Tukey's multiple comparison test. Contrary to our hypothesis, female mice demonstrated markedly increased levels of post-traumatic anxiety after TBI as compared to male mice. Female TBI mice spent significantly less time in the open space of the zero maze as compared to male TBI mice indicating increased levels of anxiety (22.86 ± 4.98% vs. 31.62 ± 9.7%, p=0.0171). Similarly, female TBI mice spent less time in the center of the open field demonstrating increased anxiety and less exploratory behavior than male TBI mice (12 ± 4% vs. 24.3 ± 5.4%, p<0.0001). This corresponded to more distance traveled over the course of open field testing in female TBI mice as compared to male TBI mice indicating a marked increase in generalized activity (7515.3 ± 1335.3cm vs. 6742.2 ± 1229.2cm, p=0.0047). Female mice had increased levels of post-traumatic anxiety-like behavior, less exploratory behavior, and increased generalized activity as compared to male mice after TBI. These data suggest marked sex-linked differences in neuropsychiatric outcome after TBI. Future clinical trials should make sex an a priori consideration in future trial design.

INTRODUCTION

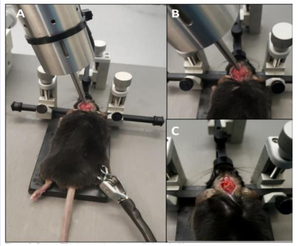
Trauma is the leading cause of death and disability in patients between the ages of 1-44 with TBIs contributing to a nearly a third of them. Presently, approximately 25% of the TBI population are females afflicted with disabilities and behavior deficits as a result of a TBI. Despite high prevalence rate, there is a severe lack of understanding on pathophysiology and mechanisms that may be sex-specific. Some recent clinical studies have also suggested possible worse long-term outcomes for females.

RESEARCH OBJECTIVES

Our research objective was to assess sex as an independent variable in neurocognitive tests of learning, memory, and anxiety after TBI. **We hypothesized that female mice would have attenuated post-traumatic anxiety after TBI as compared to male mice.**

METHODS

Figure 1. Severe TBI via Murine Model



(Islam 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. Vaginal Smear for Estrous Cycle Staging Identification

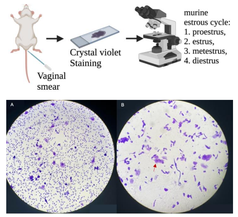
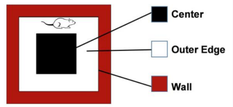


Fig 2 **Top** showing the schematic for vaginal smear and murine estrous assessment. **Bottom A** showing metestrus featured by high number of leukocytes and **B** proestrus featured by the presence of large nucleated cells (indicated by a red arrow). Magnification: 20X.

Figure 3. Open Field Behavioral Test

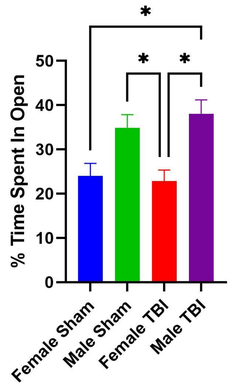
Mice were placed in the center of an enclosed box for the open field (OF) test. Each trial lasted 5 minutes and was tracked using LimeLight 4 software. OF is used to measure anxiety, exploratory behavior, and locomotive activity.



(Islam et al, Experimental Neurology 2021)

RESULTS

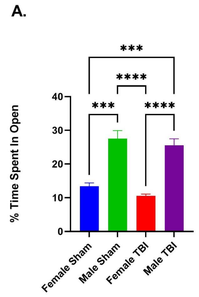
Figure 4. Females Show Increased Levels of Anxiety-Like Behavior in Zero Maze

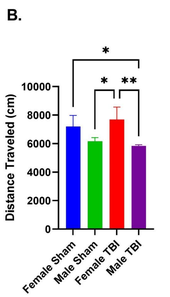


Female mice spent less time in the open regions as compared to male mice (22.86 ± 4.98% vs. 31.62 ± 9.7%, p=0.0171) indicating increased levels of anxiety-like behavior.

RESULTS

Figure 5. Female Mice Show Increased Anxiety-Like and Exploratory Behavior in Open Field





(A) Female mice show increased generalized anxiety-like behavior and spend less time in the center of open field as compared to male mice (12 ± 4% vs. 24.3 ± 5.4%, p<0.0001)

(B) Female mice showed more exploratory behavior and traveled more in OF as compared to male mice (7515.3 ± 1335.3cm vs. 6742.2 ± 1229.2cm, p=0.0047).

CONCLUSION

Contrary to the hypothesis, female mice had increased levels of post-traumatic anxiety-like behavior, less exploratory behavior, and increased generalized activity as compared to male mice after TBI :

- These data demonstrate marked sex-linked differences in neuropsychiatric outcome after TBI suggesting that clinical studies and possible treatment options should consider grouping by sex
- These data also show female sex-linked differences should be further researched as an independent variable in TBI outcomes

ACKNOWLEDGMENTS

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