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Sex Matters in Traumatic Brain Injury

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ABSTRACT

Approximately 176 Americans died from TBI related injuries every day in 2020 and more than 233,000 hospitalizations in 2019. Although studies have shown markedly different outcomes in TBI between males and females, there is a paucity of data in examining sex as an independent variable. We hypothesized that female mice would have greater neuropsychiatric deficits after TBI as compared to male mice. Age-matched C57Bl/6 female mice (N=21) and male mice (N=20) were grouped into TBI and sham-injury groups. To account for sex hormonal differences, female mice were monitored for three estrous cycles and injured during the pro-estrous phase. Open head controlled cortical impact was used to induce a severe TBI vs. sham injury. Neuropsychiatric outcomes were examined using open field testing for general activity, anxiety, and willingness to explore while the zero maze was used to examine anxiety-like behaviors at 45 days post TBI. Data was analyzed using one way ANOVA with Tukey's Multiple Comparison Post Test. Female mice demonstrated significantly increased levels of post-traumatic anxiety as compared to male mice after TBI. Female TBI mice spent notably less time in the open space of the zero maze as compared to their male counterparts indicating increased anxiety (49.0 ± 11% time spent in open vs. 46.0 ± 8% time spent in open, $p<0.01$). Likewise, female TBI mice spent less time in the center of the open field as compared to male mice demonstrating increased anxiety and less exploratory behavior (12.0 ± 4% time spent in center vs. 24.0 ± 5.0% time spent in center, $p<0.0001$). Additionally, over the course of the open field testing, more distance was traveled by female TBI mice as compared to male TBI mice indicating an increase in generalized activity (7941.0 ± 932.0m vs. 6078.0 ± 523.0m, $p<0.0001$). Increased post-traumatic anxiety-like behavior, increased exploratory behavior, and disinhibition of generalized activity were all noted in female TBI mice when compared to male TBI mice. These data suggest marked sex-linked differences in neuropsychiatric outcome after TBI. This supports the need for sex to be evaluated as an independent variable in future clinical trial design.

INTRODUCTION

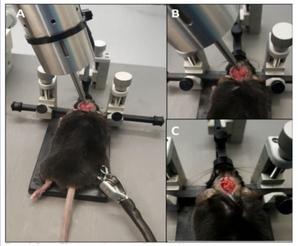
The Centers for Disease Control and Injury Prevention report that 611 Traumatic Brain Injury (TBI) related hospitalizations and 176 TBI-related deaths occur every day in the United States. Significant long-term complications can occur with resultant motor, cognitive, and behavioral deficits. Outcomes differ markedly between men and women after TBI with women suffering from greater neuropsychiatric sequelae. However, there is a paucity of data examining sex as an independent variable in TBI outcome.

RESEARCH OBJECTIVES

Our research objective was to assess sex as an independent variable in neurocognitive tests of learning, memory, and anxiety after TBI .**Therefore, we hypothesized that female mice would have greater neuropsychiatric deficits after TBI as compared to male mice.**

METHODS

Figure 1. Severe TBI via Murine Model

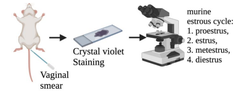


(Islam et al, JoVE 2019)

(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.



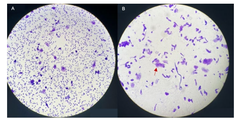
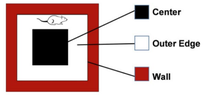


Figure 3. Open Field Behavioral Test

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.

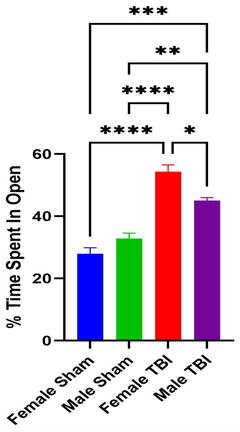


(Islam et al, Experimental Neurology 2021)

RESULTS

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

A.



B.

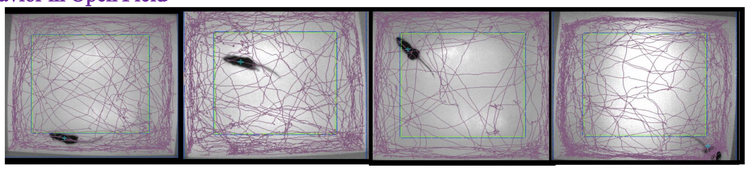
(A) At 45 days post injury, Female TBI mice displayed significant loss of hippocampus based learning compared to male TBI mice as tested in Zero Maze (49.0 ± 11% time spent in open vs. 46.0 ± 8% time spent in open *$p<0.01$). Male TBI mice showed a marked difference from both the male sham (46.0 ± 8% time spent in open vs. 36.0 ± 5% time spent in open ***$p<0.004$) female sham group (46.0 ± 8% time spent in open vs. 33.0 ± 8% time spent in open ***$p<0.005$). The largest difference seen is the significant increase in %time spent in open for Female TBI mice as compared to both male sham (49.0 ± 11% time spent in open vs. 36.0 ± 5% time spent in open ****$p<0.0001$) and female sham ((49.0 ± 11% time spent in open vs. 33 ± 8% time spent in open ****$p<0.0001$).

(B) Qualitative data showing that Female TBI mice spent an increased amount of time in the open regions as compared to any other group indicating an inhibition of anxiety like behaviors after TBI

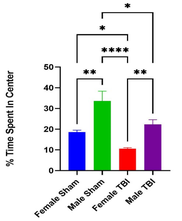
RESULTS

Figure 5. Female Mice Show Disinhibition of Normal Anxiety-Like and increase Exploratory Behavior in Open Field

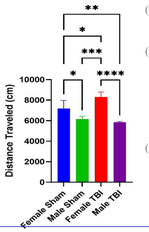
A.



B.



C.



(A) Qualitative data showing that Female TBI mice spent less time in the center of open field as compared to any other group indicating a disinhibition of anxiety like behaviors after TBI

(B) Female TBI mice show disinhibition of generalized anxiety-like behavior and spend less time in the center of open field as compared to male TBI mice (12.0 ± 4.0% vs. 24.0 ± 5.0%,** $p<0.0034$), female sham (12.0 ± 4.0% vs. 16.0 ± 6.0%,**** $p<0.0001$), and male sham (12.0 ± 4.0% vs. 34.0 ± 8.0%,** $p<0.04$). Male TBI mice showed marked difference between male sham and (24.0 ± 5.0% vs, 13.0 ± 4.0 * $p<0.04$). An interaction between male sham and female sham (13.0 ± 4.0 vs 16.0 ± 6.0%, ** $p<0.0034$)

(C) Female TBI showed increased in exploratory behavior in compared to male TBI (7941.0 ± 932.0m vs. 6078.0 ± 523.0m,**** $p<0.0001$), male sham (7941.0 ± 932m vs. 8130.0 ± 4208.0m,*** $p<0.0002$), and female sham (7941.0 ± 932.0m vs. 5644.0 ± 959m,*$p<0.04$). Female sham explored more than both male TBI (5644.0 ± 959m vs. 6078.0 ± 523.0m,**$p<0.008$), and male sham (5644.0 ± 959m vs. 8130.0 ± 4208.0m ,*$p<0.03$) . No interaction was seen between male groups.

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 :

- Increased post-traumatic anxiety-like behavior, increased exploratory behavior, and a disinhibition of generalized activity were all noted in female TBI mice when compared to male TBI mice.
- These data suggest marked sex-linked differences in neuropsychiatric outcome after TBI.
- This supports the need for sex to be evaluated as an independent variable in future clinical trial design.

ACKNOWLEDGMENTS

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