May 6th, 12:00 AM

# Physiological Response and Tissue Damage Following Different Depths of Impact in a Rodent Model of Mild Traumatic Brain Injury

Haven K. Predale
*Rowan University*

Christopher P. Knapp
*Rowan University*

Barry D. Waterhouse
*Rowan University*

Rachel L. Navarra
*Rowan University*

# Physiological Response and Tissue Damage Following Different Depths of Impact in a Rodent Model of Mild Traumatic Brain Injury

Rowan University
Graduate School of Biomedical Sciences

Haven K. Predale, Christopher P. Knapp, Barry D. Waterhouse, Rachel L. Navarra

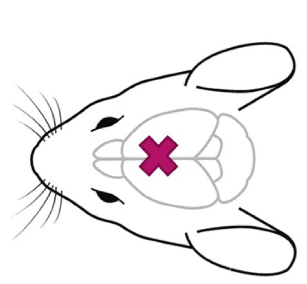
*Department of Cell Biology and Neuroscience, Rowan University GSBS, Stratford NJ*

## Introduction

Mild traumatic brain injury (mTBI) is a serious public health concern that can result in significant neurological and behavioral deficit. mTBI results from impact to the head and can be repetitive in nature, especially in sports and domestic violence cases. Our laboratory studies the effects of repetitive mTBI on risky choice behavior in rodents using a closed-head controlled cortical impact (CH-CCI) model of injury and a well-established probabilistic discounting task that assesses risk-based decision-making behavior. We have recently found that females, but not males, display transient increases in risky choice behavior following three CH-CCI's delivered at 5.5m/s velocity and 2.5mm impact depth. These findings suggest that our injury parameters may produce marginally threshold influences on behavioral outcomes that do not allow observation of the extent of repetitive mTBI-induced effects and have prompted us to explore expansion of our model to include greater depths of injury. In the present work we subjected rats to a series of three fixed velocity impacts at depths of 2.5mm, 3.0mm, or 3.5mm. The goal was to compare physical manifestations of injury in male and female rats following different depths of injury. The survival rate, righting reflex time, skull injury observations, animal weights, and histological markers of tissue damage were evaluated post-injury. Our hypothesis was that these indices of injury would be more prominent as injury depth increased.

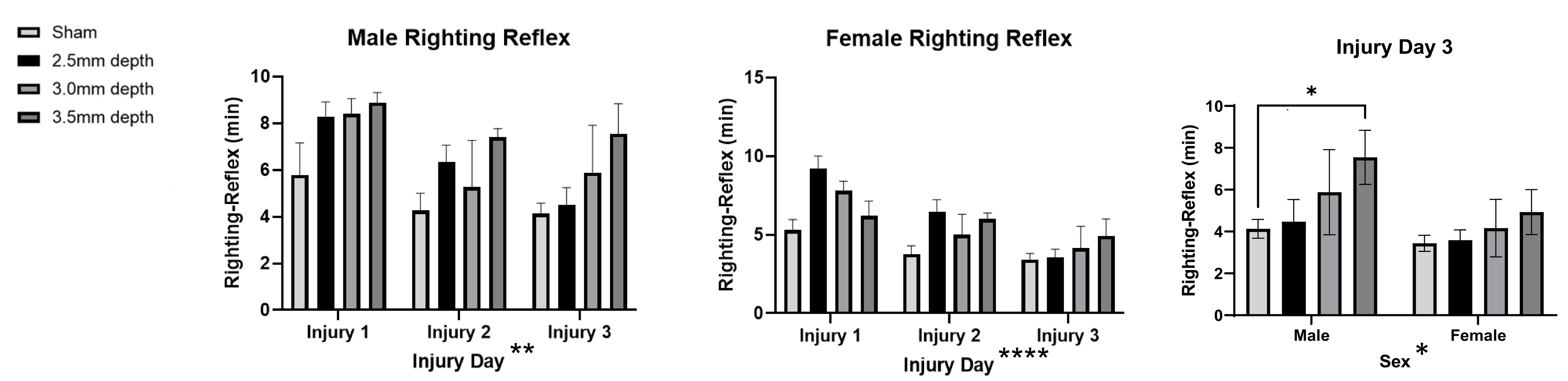
## Methods

- Male and female Long Evans rats (n = 63, 75-100g upon arrival) were housed in a 12 : 12 hour inverted light cycle facility and placed on a food restricted diet (5 grams/100 grams body weight) with *ad libitum* access to water.
- Injury model: All rats (150-200g at the beginning of surgeries) were anesthetized and subjected to either sham surgery or mild closed head-controlled cortical impact CH-CCI) injuries occurring three times over the course of one week. Briefly, a 5mm-diameter metal impactor tip was zeroed at the skull surface along the sagittal suture line so that the edge of the tip was aligned with bregma. The tip was then electronically driven into the skull at a velocity of 5.5m/s to a depth of 2.5mm, 3.0mm, or 3.5mm below the zero line.



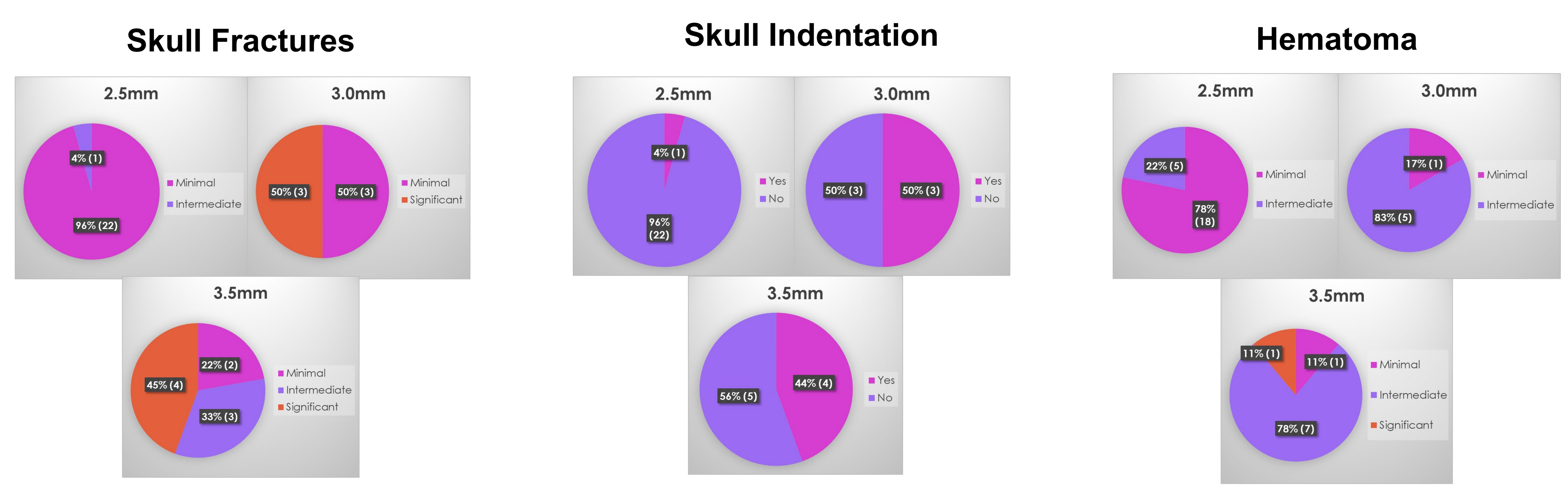
- The righting reflex was recorded by measuring the time it took for the animal to regain normal posture after being placed in the supine position.
- The site of impact was examined for indentation or fracture of the skull and sub-dural hematoma.
- 5 days following final injury, rats were perfused, and brains were extracted. Brains were coronally sectioned at 40 microns thick from bregma to -7.0 and Nissl stained to visualize structural damage under the impact site.

## Righting Reflex



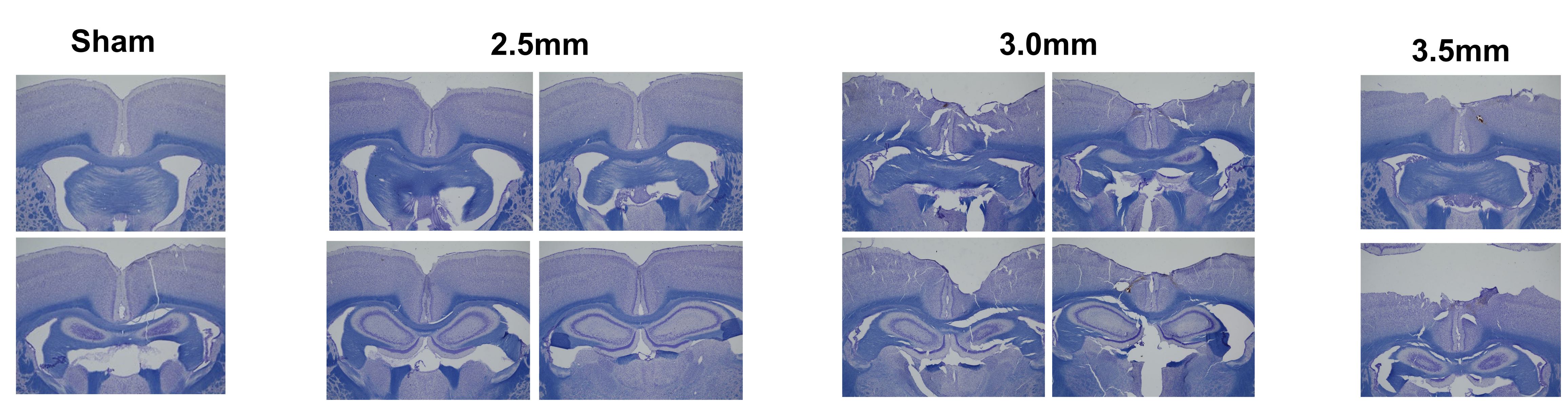

Bar graphs represent righting reflex on each injury day (sham n = 15 females, n = 10 males, 2.5mm n = 14 females, n = 9 males, 3.0mm n = 3 females, n = 3 males, 3.5mm n = 5 females, n = 4 males.) Left: righting reflex for males across all three injury days. ** denotes $p < 0.05$ per 2-way ANOVA. Middle: righting reflex for females across all three injury days. **** denotes $p < 0.0001$ per 2-way ANOVA. Right: analysis of righting reflex on injury day 3. * denotes $p < 0.05$ per Sidak's multiple comparisons test.

## Physical Observations




The pie charts represent the percentage of rats with the noted severity of the specific physical observation. All observations were made subjectively by the individual administering the impacts. Left: incidence of skull fractures. Middle: presence of skull indentations. Right: incidence of hematomas.

## Nissl Staining




Nissl staining of tissue at bregma level -1.0 (top row) and -2.0 (bottom row) across all injury depths. Each column indicates tissue sections from one animal. More significant structural damage to the brain was noted in 3.0mm and 3.5mm injury depths as compared to 2.5mm injury depth. Skull fracture and hematoma were observed in animals receiving injuries at the 3.0mm and 3.5mm depths. Quantitative analysis is pending.

## Conclusions

### Summary

Righting Reflex

- Average righting reflex time increased as the injury depth increased for both males and females
- Males exhibited overall longer righting reflex times as compared to females at the 3.5mm impact depth
- Righting reflex differed significantly between sexes and between sham and 3.5mm (males) on injury day 3

Physical Observations

- In comparison to the 2.5mm group, a greater percentage of animals in the 3.0mm and 3.5mm groups sustained moderate and significant skull fractures and hematomas, as well as a greater incidence of skull indentation
- Weight gain post-injury did not differ significantly amongst injury depths (data not shown)

Nissl Staining

- Structural damage of tissue under the impact site increased as the depth of injury increased

### Significance

The current results show that physiological observational measures of injury are positively correlated with depth of impact in the CH-CCI model of TBI. These data will be used to guide selection of CH-CCI impact parameters in future studies designed to reveal changes in behavior following repetitive mTBI. Based upon the current findings we predict that progressively greater depths of impact will produce greater post-injury deficits in behavioral tests of executive function and that these deficits will be more pronounced in female vs male animals.

Ongoing histological studies are using silver and glial fibrillary acidic protein (GFAP) stains to examine axonal injury and the astrocytic response to injury, respectively.

### Acknowledgements and Support

Supported by the New Jersey Commission on Brain Injury Research, CBIR20PIL004 (Navarra), CBIR19IRG055 (Waterhouse), and the Osteopathic Heritage Foundation for Primary Care Research (Navarra).

We would like to thank Douglas Fox for his technical assistance with this project.