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The Impact of Traumatic Brain Injury on Noradrenergic Innervation of the Prefrontal Cortex

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References

Introduction

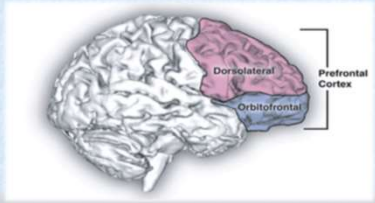
Traumatic brain injury (TBI) often causes cognitive problems that can lead to increased risk-taking behaviors. To better understand this in humans, my laboratory developed a preclinical model to evaluate risk-based decision-making behavior after experimental TBIs. These studies have shown that repetitive mild TBI (rmTBI) leads to increased preference for risky choices in rodents. We now aim to understand the neural mechanisms driving these injury-induced increases in risk-taking behaviors.

The specific areas of the brain we are interested in looking at are the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), and/or anterior cingulate cortex (ACC) of the prefrontal cortex (PFC). Higher order executive functions that guide complex decision-making are regulated by the prefrontal cortex (PFC) and are most often impacted by TBIs.[1,2] The circuits of catecholamine neurotransmitters, dopamine (DA) and norepinephrine (NE), project to the PFC and modulate control of executive functions. Previous research has found that DA and NE levels in the PFC are increased immediately following a TBI, but then persistently decreased over time.[2-6] These findings suggest that catecholamine dysregulation within the PFC may have an impact on the aberrant decision-making behavior after a TBI.

Our lab has demonstrated decreased levels of norepinephrine transporters (NET) within the orbitofrontal (OFC) subregion of the PFC 2 days after repetitive rmTBI in rats. However, it is not known whether these decreases are due to downregulation of NET protein levels within intact NE fibers terminating in the OFC or a loss of OFC NE innervation. My research analyzed NE fiber density within the OFC following either rmTBI or repeated control sham surgeries.

Objective

- During my Summer Medical Research Fellowship, I studied the impact of rmTBI on noradrenergic innervation of the OFC.
- My goal was to investigate acute and chronic changes in NE innervation within OFC following rmTBI. I compared the density of noradrenergic fibers by measuring the presence dopamine beta-hydroxylase (DBH, the enzyme responsible for NE biosynthesis) within the OFC between animals that received rmTBI or repeated control sham surgeries at 1 and 4 weeks post-final surgery.



Methods

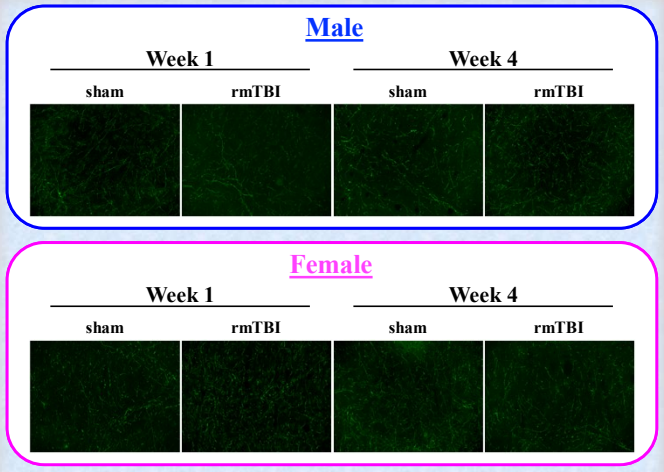
Animals: Male and female Long-Evans rats (5-6 weeks old upon arrival) were housed in a 12:12hr inverted light cycle facility and placed on a food regulated diet (5 grams/100 grams body weight/day) with ad *libitum* access to water prior to injuries.

Closed Head Control Cortical Impact (CH-CCI) Model: Rats (9-10 weeks old, 150-200g, at the beginning of surgeries) were anesthetized and exposed to either a series of 3 sham (prepared for impact surgery, but received no impact) or mild TBI surgeries over the course of 1 week. Briefly, a 5mm-diameter metal impactor tip was placed along the sagittal suture line with the edge of the tip aligned with bregma. The tip was then driven into the skull at a velocity of 5.5m/s to a depth of 3.5mm below the surface point.

Surgery Groups: sham and rmTBI

Immunohistochemistry: Tissue containing the OFC (5-6 sections per rat) were double-stained with DBH (1:2000) primary antibody followed by Alexa donkey anti-mouse wavelength 488 (1:250) secondary antibody. Images of the OFC were taken at 20X using a Keyence BZ-X710. Image-J was then used to threshold and visualize DBH-positive axons to quantify the fibers of interest.

Results



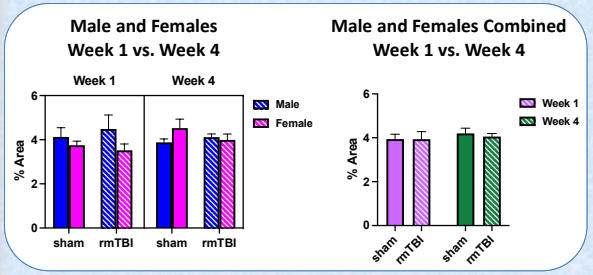
Immunohistochemical staining of dopamine beta-hydroxylase (green) expressing fibers in the orbitofrontal cortex (OFC) of sham and repetitive mild traumatic brain injury (rmTBI) male (Top) and female (Bottom) rats at weeks 1 and 4 post-final surgery.

Results Continued

Males: There were no differences in DBH fiber density between sham or rmTBI conditions at 1 or 4 weeks post-final surgery in male animals.

Females: There were no differences in DBH fiber density between sham or rmTBI conditions at 1 or 4 weeks post-final surgery in female animals.

Males and Females Combined: There were no differences in DBH fiber density between sham or rmTBI conditions at 1 or 4 weeks post-final surgery when male and female animals were combined.



Quantification of post-surgery dopamine beta-hydroxylase fiber density. Left: Bar graphs represent quantification of DBH expressing fibers within the OFC of male and female rats, separately. Right: Bar graphs represent a combined male and female analysis of DBH expressing fibers within the OFC. Bars represent mean ± SEM.

Conclusion

- Previous results have demonstrated that rmTBI reduces the level of NET within the OFC of rodents within 1 week post-final injury. My research aimed to examine whether rmTBI-induced NET reductions were due to downregulation of the NET protein or loss of NE innervation within the OFC.
- My results showing no significant differences in DBH-positive fiber density between rmTBI and sham OFC tissue indicate that our model of rmTBI did not alter the presence of noradrenergic fibers at either 1 or 4 weeks post-final surgery in this region.

These findings suggest that rmTBI-induced reductions of NET proteins levels within the OFC are due to less expression of NET proteins and not a loss of noradrenergic innervation within this region.