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Interactions Between Repetitive Mild Traumatic Brain Injury and Methylphenidate Administration on Catecholamine Transporter Protein Levels Within the Rodent Prefrontal Cortex

Anna Abrimian
Rowan University

Eleni Papadopoulos
Rowan University

Christopher P. Knapp
Rowan University

J. Loweth
Rowan University

Barry Waterhouse
Rowan University

See next page for additional authors

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ROWAN-VIRTUA School of Translational Biomedical Engineering & Sciences

Anna Abrimian, Eleni Papadopoulos, Christopher P. Knapp, Jessica A. Loweth, Barry D. Waterhouse, Rachel L. Navarra

Department of Cell Biology and Neuroscience, Rowan-Virtua School of Translational Biomedical Engineering & Sciences, Stratford, NJ 08084

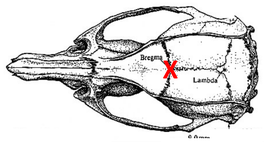
ROWAN-VIRTUA School of Osteopathic Medicine

Introduction

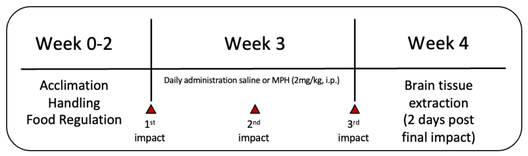
It is theorized that a hypo-catecholaminergic state, i.e., low concentrations of dopamine (DA) and norepinephrine (NE), within in the prefrontal cortex (PFC) following traumatic brain injury (TBI) leads to impairments in cognitive processes that drive increased risk-taking behavior in clinical populations.[1-5] Our lab has demonstrated that repeated mild TBI (rmTBI) sex-differentially increases risky choice behavior using the closed head-controlled cortical impact (CH-CCI) model and a risk/reward decision making task in rodents. Methylphenidate (MPH) is a psychostimulant drug used to treat symptoms of Attention-Deficit Hyperactivity Disorder (ADHD), which are also driven by a hypo-catecholaminergic state within the PFC.[6,7] MPH elevates catecholamine levels by blocking DA and NE transporters, DAT and NET, respectively.[8,9] Catecholamine transporters can be dynamically regulated by various states of pathology and drug treatment. While psychostimulants have been explored to treat post-TBI symptoms, the ability of sub-chronic, low-dose MPH to affect levels of these proteins in young adult rats following rmTBI has not been evaluated.[10,11] To investigate this gap in our knowledge, we used the CH-CCI model to induce 3 mild injuries within one week in age-matched adult male and female Long Evans rats. Rats received either saline or MPH (2 mg/kg, i.p.) daily from the first day of surgery until 48 hours post-final surgical preparation. Rats were sacrificed, brain tissue from the medial (mPFC) and orbitofrontal (OFC) regions of the PFC were collected, and standard western blotting protocols were used to measure protein levels of the vesicular monoamine transporter 2 (VMAT2) and NET within each region.

Methods

- ***Animals:*** Male & female Long Evans rats (Envigo; n = 32) were housed in a 12:12 hour inverted light cycle facility and placed on a food regulated diet (5 grams/100 grams body weight) with ad libitum access to water.
- ***Surgical Procedures:*** All rats (9-10 weeks at the beginning of surgeries) were anesthetized and received either sham surgeries or a series of 3 mild CH-CCI injuries (rmTBI) over the course of 1 week. A 5mm-diameter metal impactor tip was electronically driven into the skull along the sagittal suture line with the edge of the tip aligned with bregma at a velocity of 5.5m/s to a depth of 3.5mm below the zero point.[12]
- ***Location of Impact:***



- ***Treatments:*** Rats received either saline or MPH (2 mg/kg, i.p.) daily from the first day of surgery to 48 hours post-final surgical preparation
- ***Experimental Groups:*** sham/saline, sham/MPH, rmTBI/saline, rmTBI/MPH
- ***Western Blot:*** 48 hours following the final surgical preparation, tissue was collected from the mPFC and OFC, electrophoresed on Criterion XT Bis-Tris Protein Gels, and transferred to Immuno-Blot PVDF membranes. Membranes were probed for: VMAT2 (1:1000, Abcam) and NET (1:1000, Abcam).



Medial Prefrontal Cortex

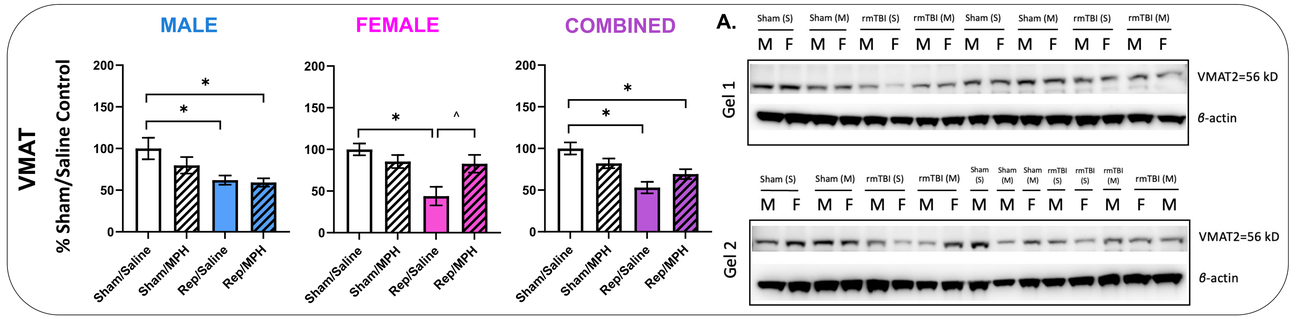


Figure 1: ***Protein density analysis of the mPFC.*** Graphs represent mean percent change in total protein levels ± SEM as compared to sham controls (n=3-5 per group) 48 hours post-final surgery. *denotes p<0.05 and ^denotes p<0.1 from sham.

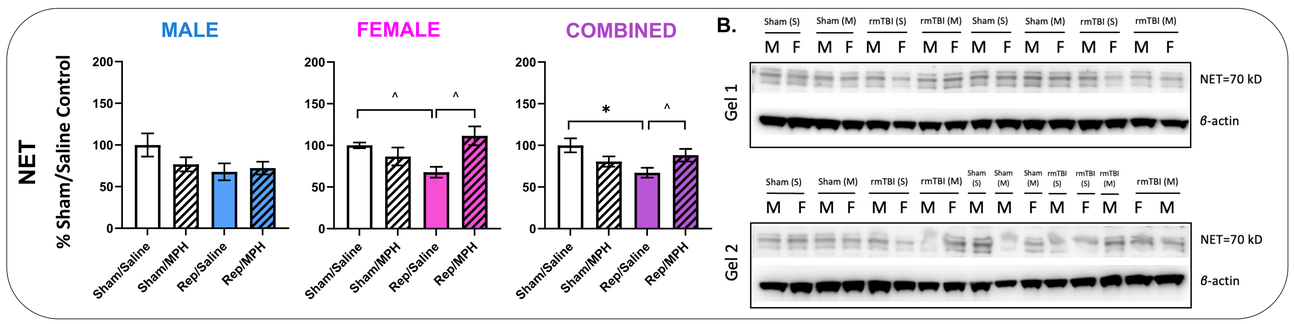


Figure 2: ***Western blot images of the mPFC proteins.*** A. Membrane probed with rabbit anti-VMAT2. B. Membrane probed with rabbit anti-NET. Beta-actin was used as the loading control.

Orbitofrontal Cortex

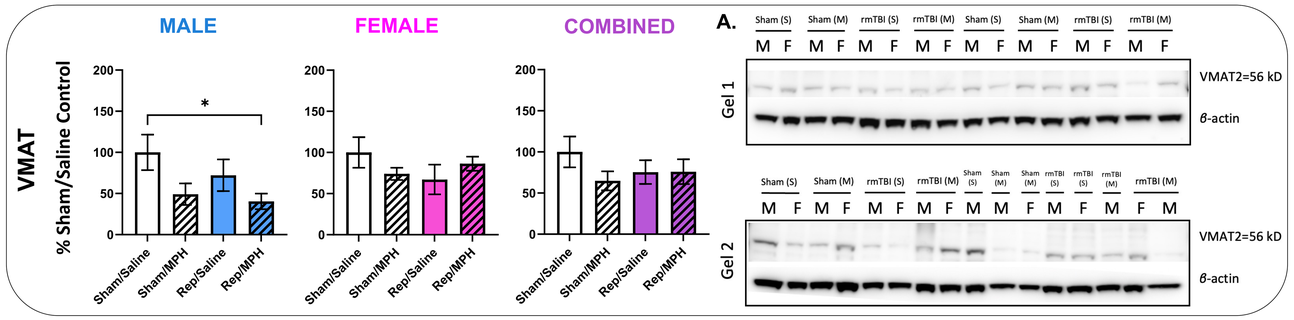


Figure 3: ***Protein density analysis of the OFC.*** Graphs represent mean percent change in total protein levels ± SEM as compared to sham controls (n=3-5 per group) 48 hours post-final surgery. *denotes p<0.05 and ^denotes p<0.1 from sham.

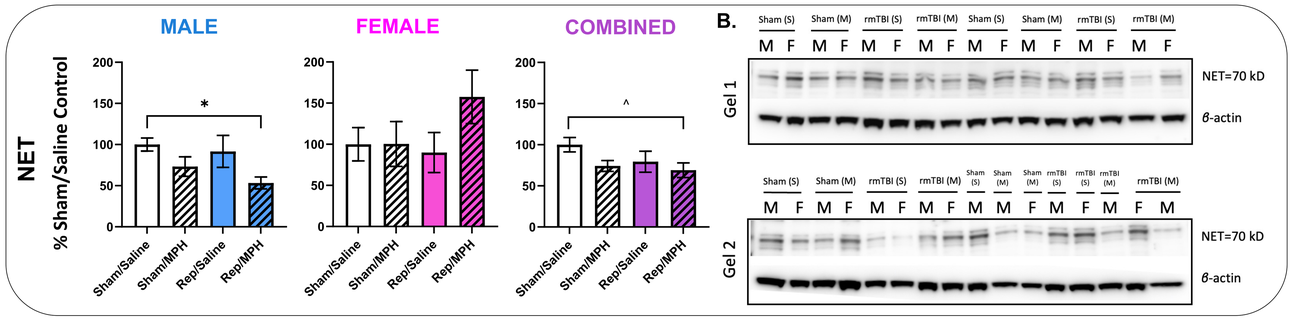


Figure 4: ***Western blot images of the OFC proteins.*** A. Membrane probed with rabbit anti-VMAT2. B. Membrane probed with rabbit anti-NET. Beta-actin was used as the loading control.

Summary

mPFC

- In males, VMAT levels were decreased in the rmTBI/saline and rmTBI/MPH groups.
- In females, VMAT and NET levels were decreased in the rmTBI/saline group, while the rmTBI/MPH group's protein levels did not differ from sham/saline controls.

OFC

- NET and VMAT levels were both decreased in male rmTBI/MPH groups only.

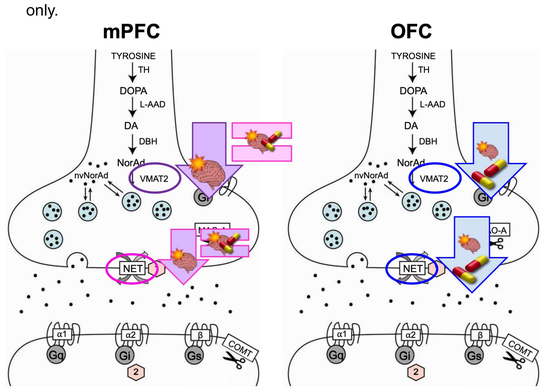


Figure 5: Diagram of a noradrenergic synapse depicting the observed alterations of catecholamine transporter protein levels within the mPFC and OFC following rmTBI and MPH administration. Decreased mPFC VMAT and NET seem to be driven by injury while MPH restores levels in females only. Decreased OFC VMAT and NET seem to be driven by MPH while injury potentiates this effect to significance in males.

Conclusion

Decreased levels of VMAT2 suggest less packaging, storage, and release, while decreased levels of NET suggest less uptake and clearance of catecholamine transmitters in these regions. We believe these transporters are downregulated in a compensatory manner in response to a hypo-catecholaminergic state following rmTBI, and therefore, reduced need for transport. Sub-chronic MPH administration appears to restore rmTBI-induced decreased transporter levels in females but may have an additive effect in males.

Significance

Based upon these findings, we conclude that the effects of rmTBI, MPH, and the interactions between rmTBI/MPH on levels of catecholamine regulatory proteins may begin to elucidate sex differential changes in risk-taking behavior following injury and subsequent treatment. Further studies will use varying dosages and timeframes for MPH administration to pinpoint when its introduction into rmTBI recovery can have greatest therapeutic effects, and further elucidate mechanisms for sex-specific differences in MPH treatment.

Acknowledgments

References:

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