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

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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, 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

Introduction

It is theorized that a hypo-catecholaminergic state, i.e., low concentrations of dopamine (DA) and norepinephrine (NE), within the prefrontal cortex (PFC) following traumatic brain injury (TBI) leads to impairments in cognitive processes that drive increased risk-taking behavior in clinical populations. Our lab has demonstrated that repetitive mild TBI (rmTBI) sex-differentially increases risky choice behavior using the closed-head-controlled cortical impact model (CH-CCI) 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. MPH elevates catecholamine levels by blocking DA and NE transporters, DAT and NET, respectively. 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. 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-surgical procedures. 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.

Medial Prefrontal Cortex

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<th>Effect</th>
<th>Male</th>
<th>Female</th>
<th>Combined</th>
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<tbody>
<tr>
<td>MPH</td>
<td>+</td>
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Methods

- **Animals:** Male & female Long Evans rats (Envip; 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 (5-10 weeks at the beginning of surgeries) were anesthetized and received either sham surgeries or a series of 3 mild CH-CCI injuries (mTBI) over the course of 1 week. A 5mm-diameter metal impactor tip was electronically driven into the skull along the sagittal suture line, and the edge of the tip aligned with bregma at a velocity of 5.5ms to a depth of 3.5mm below the zero point.
- **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-surgical procedure
- **Experimental Groups:** sham/saline, sham/MPH, rmTBI/saline, rmTBI/MPH
- **Western Blot:** 48 hours following the final surgical procedure, 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).

![Figure 1: Protein density analysis of the mPFC](image1)

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) at 48 hours post-surgical surgery. *denotes p<0.05 and **denotes p<0.01 from sham.

![Figure 2: Western blot images of the mPFC proteins](image2)

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

Orbitofrontal Cortex

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![Figure 3: Protein density analysis of the OFC](image3)

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) at 48 hours post-surgical surgery. *denotes p<0.05 and **denotes p<0.01 from sham.

![Figure 4: Western blot images of the OFC proteins](image4)

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

Conclusion

Decreased levels of VMAT2 suggest less packaging, storage, and release, while decreased levels of NET suggest less uptake and clearance of catecholamines transporters 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 the greatest therapeutic effects, and further elucidate mechanisms for sex-specific differences in MPH treatment.

Acknowledgments

References:

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Figure 5: Diagram of a non-competitive synapse depicting the observed alterations of catecholaminergic transporter proteins within the mPFC and OFC following rmTBI and MPH administration. Decreased net VMAT2 and NET seem to be driven by injury while CPA restores levels in females only. Decreased OFC NET seem to be driven by MPH while injury potentiates the effect to significance in males.

mPFC

- In males, VMAT2 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 VMAT2 levels were both decreased in male rmTBI/MPH groups only.

Significant levels of VMAT2 and NET are shown to be decreased in both the mPFC and OFC in both male and female rats. However, only in males did the protein levels approach significance in the OFC.