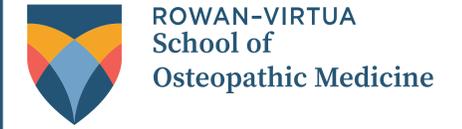# Low-Dose Methylphenidate Counteracts Increased Risky Decision Making Following Repetitive Mild Traumatic Brain Injury in Female Rodents

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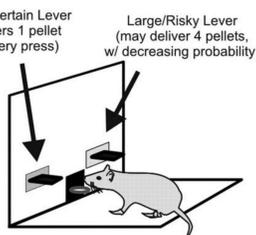ROWAN–VIRTUA School of Osteopathic Medicine

Introduction

Mild traumatic brain injury (mTBI) is the most common type of non-penetrating wound often characterized as concussion, which accounts for 80% of all reported TBI cases. Many individuals including athletes and military personnel experience repeated mild traumatic brain injuries (rmTBIs).[1,2] Sustaining head trauma impairs high-order decision making processes mediated within the prefrontal cortex (PFC) of the brain and leads to increased risk-taking behavior.[3-5] The catecholamine neurotransmitters, dopamine (DA) and norepinephrine (NE), modulate the PFC's actions. Imbalances in catecholamine function have been associated with TBI and are theorized to underlie aberrant decision making following TBI.[6-18] Currently, there are no FDA-approved treatments for rmTBI. The psychostimulant, methylphenidate (MPH), elevates catecholamine levels by blocking DA and NE reuptake transporters and because of its efficacy in reducing impulsive and risky behavior in patients with attention deficit hyperactivity disorder (ADHD), it has been considered as a potential therapy for alleviating similar neurocognitive symptoms following TBI.[19-22] At low doses, MPH increases levels of these catecholamines with regional specificity for the prefrontal cortex.[23] However, no studies have comprehensively measured how low dose MPH can influence risk/reward decision making following rmTBIs. Here we used a closed head-controlled cortical impact model to induce up to 3 mTBIs and the probabilistic discounting task of risk/reward decision making to determine the effects of chronic administration of low-dose MPH (0.5 and 2 mg/kg, i.p.) on risky behavior in male and female rats.

Methods

- **Animals:** Male (n=54) & Female (n=54) Long Evans rats (3-4 weeks upon arrival) 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.

- **Closed Head-Control Cortical Impact (CH-CCI) Model:** All rats (9-10 weeks, 150-200g, at the beginning of surgeries) were anesthetized and subjected to either sham, a single mild injury (smTBI), or a series of three mild CH-CCI injuries (rmTBI) over the course of one week (Tuesday, Friday, Monday). The edge of a 5mm-diameter rounded metal impactor tip was aligned with bregma at the skull surface along the sagittal suture and then electronically driven at a velocity of 5.5m/s to a depth of 3.5mm below the zero point.
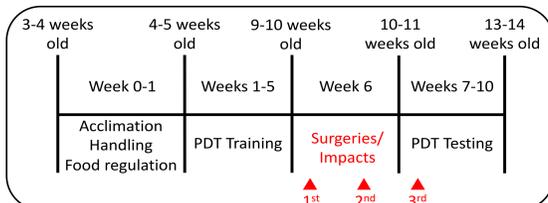
- **Probabilistic Discounting Task (PDT):** The PDT requires rats to choose between small/certain rewards delivered with 100% certainty and large/risky rewards delivered with decreasing probabilities across a series of trial blocks: 100%, 50%, 25%, 12.5%, 6.25%.[9] Rats were trained on the PDT for ~21-26 days until stable baseline was achieved. Rats then received their assigned surgery and returned to the PDT 48 hours post-final surgery for 4 weeks.

  Small/Certain Lever (delivers 1 pellet on every press)

  Large/Risky Lever (may deliver 4 pellets, w/ decreasing probability)

- **Treatments:** MPH (Sigma-Aldrich) was prepared fresh daily, dissolved in sterile saline, and administered intraperitoneally daily on weekdays beginning 48 hours post-final injury (15 min prior to task) with a dosing volume of 1mL/kg.

- **Experimental Groups:** sex (2; male, female) x injury condition (3; sham, smTBI, rmTBI) x treatment (3; saline, 0.5mg/kg MPH, 2mg/kg MPH) = 18 total groups

- **Experimental Timeline:**

3-4 weeks old	4-5 weeks old	9-10 weeks old	10-11 weeks old	13-14 weeks old
Week 0-1	Weeks 1-5	Week 6		Weeks 7-10
Acclimation Handling Food regulation	PDT Training	Surgeries/ Impacts		PDT Testing

1st 2nd 3rd

Week 1 Post Injury PDT Performance
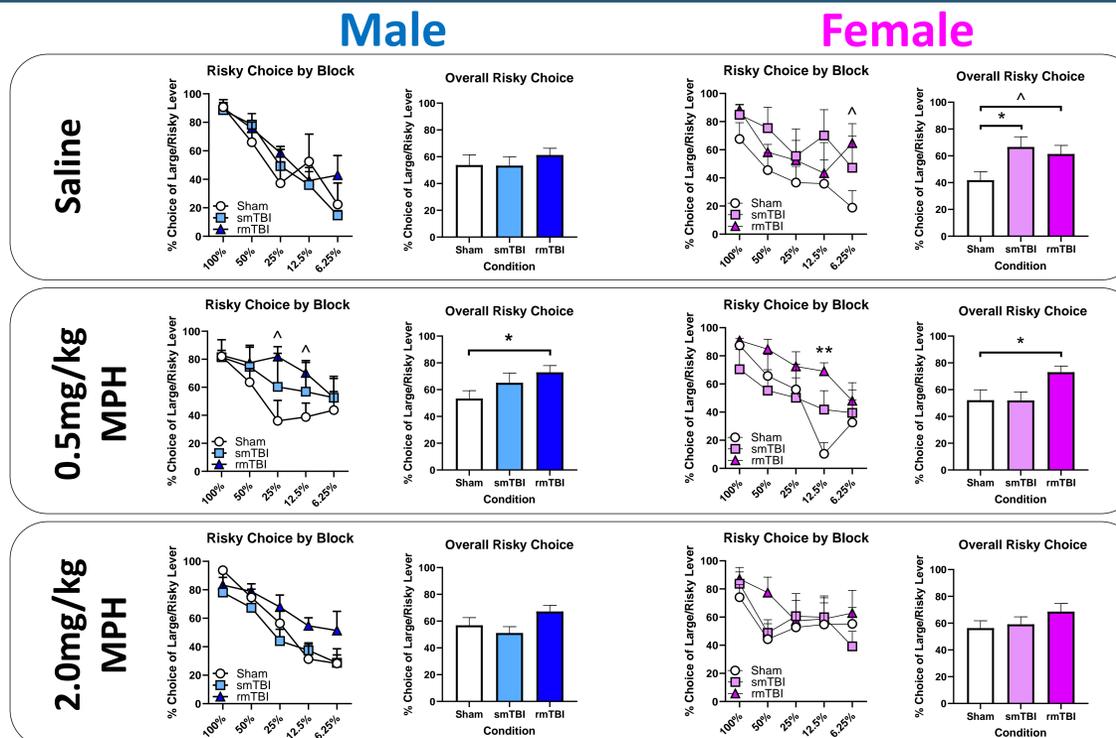
Male   Female

Figure 1. Male (blue) and Female (pink) performance on the probabilistic discounting task in the first week post-final surgery. Line graphs represent percent choice of the large/risky option across five trial blocks of decreasing reward probability between each group and treatment (n=4-6 per treatment, sex and injury type). Bar graphs represent average total percentage of large/risky choice made across all trial blocks. * on graph denotes p < 0.05, ** on graph denotes p < 0.01, ^ on graph denotes p < 0.1 when smTBI and rmTBI were compared to sham using Dunnett's multiple comparisons test. Symbols and bars represent mean ± SEM.

Week 2 Post Injury PDT Performance
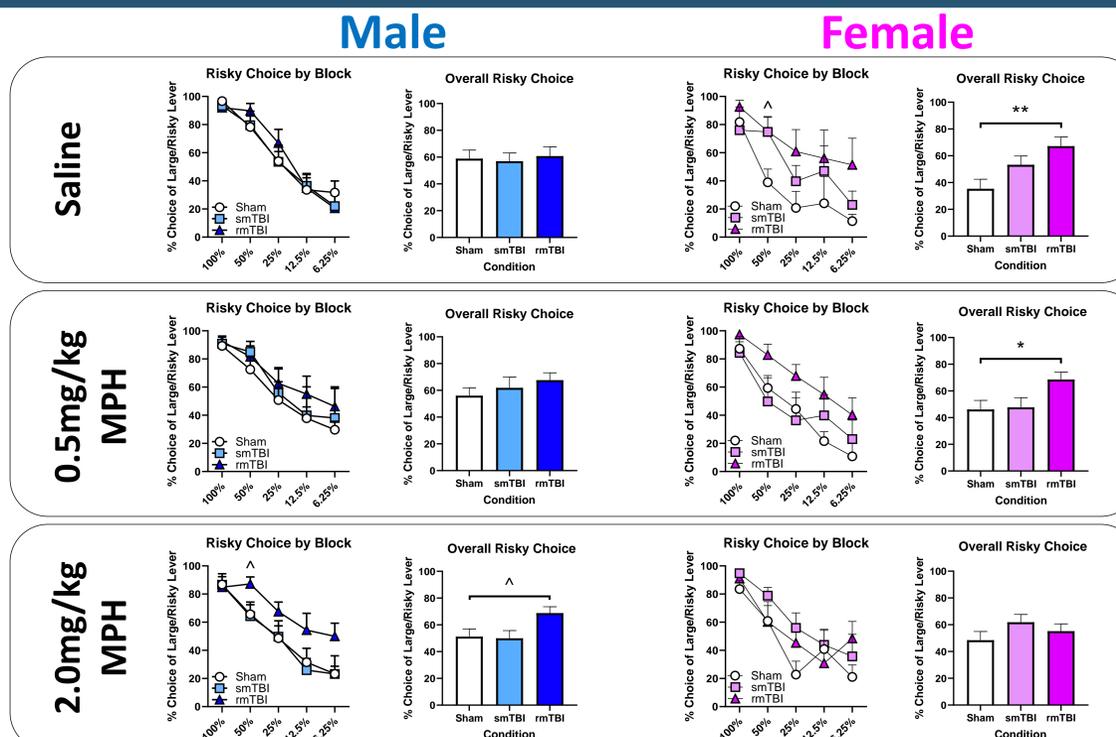
Male   Female

Figure 2. Male (blue) and Female (pink) performance on the probabilistic discounting task in the second week post-final surgery. Line graphs represent percent choice of the large/risky option across five trial blocks of decreasing reward probability between each group and treatment (n=4-6 per treatment, sex and injury type). Bar graphs represent average total percentage of large/risky choice made across all trial blocks. * on graph denotes p < 0.05, ** on graph denotes p < 0.01, ^ on graph denotes p < 0.1 when smTBI and rmTBI were compared to sham using Dunnett's multiple comparisons test. Symbols and bars represent mean ± SEM.

Summary

Independent injury-induced effects on performance

- Injury alone increased overall risky choice preference in saline-treated female, but not male rats as compared to sham controls up to 2 weeks post-injury.

Treatment by injury-induced effects on performance

- MPH (0.5 mg/kg) increased overall risky choice preference males and seems to potentiate risky choice preference in females following rmTBI as compared to treated sham controls, but attenuated the smTBI-induced risky choice preference observed in saline-treated females.

- MPH (2 mg/kg) increased overall risky choice preference in the 2nd week post-injury rmTBI males, but attenuated the injury-induced risky choice preference in females.

Conclusions

- Overall, these results suggest that daily administration of low-doses of MPH have the potential to produce detrimental or additive effects towards increasing risky choice behavior in injured males, but may resolve injury-induced increases in risky choice behavior in females.

- These sex-differential interactions between injury and MPH treatment are both dose and injury dependent.

Significance

Our results show rmTBI alone increased risky choice behavior in females, but not males, indicating females are more susceptible to changes in risk/reward decision making following injury. MPH decreased risky choice behavior in females for up to 2 weeks post-rmTBI. Conversely, MPH increased risky choice behavior in injured males. These results indicate that low doses of MPH have sex-specific modulating effects on risky choice behavior following rmTBI, where effects are beneficial for females, but detrimental for males. This study provides insight regarding the potential efficacy of psychostimulants for treating neurocognitive symptoms following rmTBI and reveals a potential sex-specific therapeutic strategy for treating rmTBI-induced increases in risky behavior. Our ongoing work continues to expand the present results, while also investigating the roles of injury and drug interactions on catecholamine regulatory proteins within the PFC as plausible mechanisms of injury- and drug-induced effects on risk/reward decision making behavior.

Acknowledgments