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Examining Levels of Catecholamine Neurotransmitter Regulatory Proteins Within the Prefrontal Cortex of Rodents Following Traumatic Brain Injury

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Introduction

Traumatic brain injury (TBI) resulting from impact to the head can cause long lasting impairments of cognitive processes that lead to increased risk-taking behavior in clinical populations.[1,2] Our laboratory has recently shown that female, but not age-matched male, rats increase preference for risky choices after multiple experimentally-induced mild TBI's. Our overarching goal is to understand the neural mechanisms underlying TBI-induced increases in risk-taking behavior. The prefrontal cortex (PFC) plays a prominent role in risk-based decision making. Sub-regions of the PFC include the medial PFC (mPFC), the orbitofrontal cortex (OFC), and the anterior cingulate cortex (ACC), and these sub-regions play specific roles in decision-making processes. Catecholamine neurotransmitter circuits, such as the dopamine (DA) and norepinephrine (NE) systems, project to the PFC and modulate the PFC's control over executive functions.[3] Previous studies have demonstrated that both dopamine (DA) and norepinephrine (NE) transmitter levels are increased in the PFC immediately following TBI, which is then followed by a persistent hypo-catecholaminergic state.[4-14] These results suggest that an imbalance of catecholamine levels within the PFC may underlie aberrant decision-making behavior following TBI; however, it is not presently known what processes contribute to TBI-induced catecholamine imbalance. Here we examined how levels of catecholamine neurotransmitter regulatory proteins responsible for packaging (VMAT2) and degrading (COMT and MAO) are altered to explain chronic decreases in DA and NE levels observed in the PFC following TBI. Age-matched adult male and female Long Evans rats (n=6-8) were exposed to either a single or a series of three closed head controlled cortical impact (CH-CCI) injuries over the course of one week. Rats were sacrificed and brain tissue (mPFC, OFC, and ACC) were collected and standard western blotting protocols were used to measure the levels of VMAT2, COMT, and MAO in each sub-region.

Anterior Cingulate Cortex

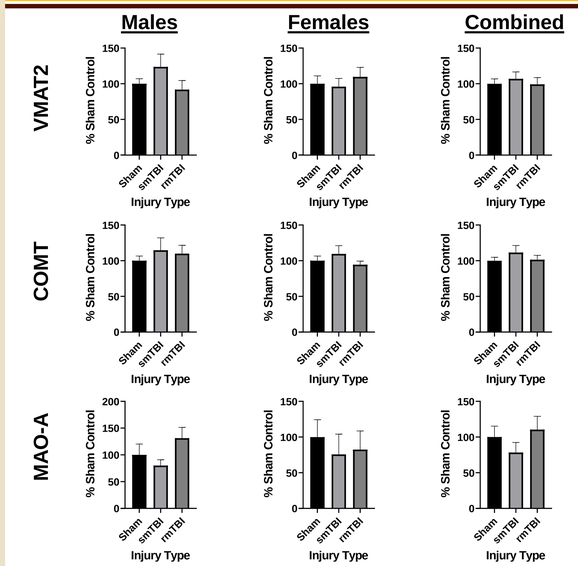


Figure 1: Protein density in ACC. Graphs represent mean percent change in total protein levels ± SEM as compared to sham controls (n=6-8 per group) 2 days post-final injury.

Medial Prefrontal Cortex

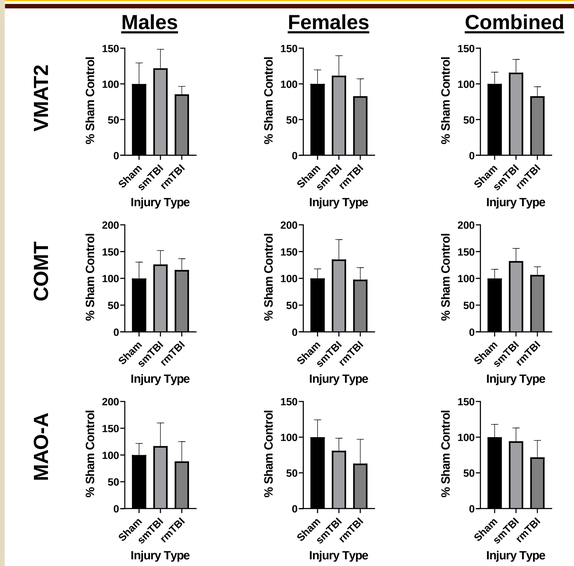


Figure 2: Protein density in mPFC. Graphs represent mean percent change in total protein levels ± SEM as compared to sham controls (n=6-8 per group) 2 days post-final injury.

Summary

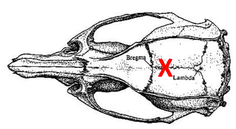
- No overall differences were found in protein levels across all three sub-regions of the PFC using one-way ANOVAs ($p > 0.05$).
- Multiple comparisons revealed several alterations of protein levels in the OFC, but not in the mPFC or ACC.
- In the OFC, rmTBI decreased VMAT2 and COMT of males and females combined. rmTBI tended to differentially increase MAO-A in males, but decrease MAO-A in females.

Conclusions

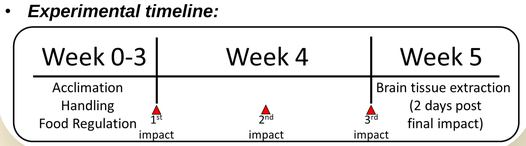
Decreased VMAT2 levels in the OFC indicate less capacity for packaging, storage, and release of catecholamine transmitters in this region, leading to a hypo-catecholaminergic tone as previously reported.[4-14] Decreased levels of COMT in the OFC suggest reduced capacity for catecholamine degradation; however, this downregulation may result as a secondary compensatory response to lowered catecholamine levels due to less necessary degradation. Interestingly, MAO-A increased in males, but decreased in females following injury which may underlie the sex-specific differences observed in previous risk/reward decision making behavioral data.

Methods

- ***Animals:*** Male & Female Long Evans rats (n = 48, 5-6 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.
- ***Surgical Procedures:*** All rats (9-10 weeks, 150-200g, at the beginning of surgeries) were anesthetized and subjected to either a sham surgery, a single mild injury (smTBI) or a series of three mild CH-CCI injuries (rmTBI) 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 below the zero point.[15]
- ***Location of Impact:***



- ***Western Blot:*** Two days following the final impact, brains were removed and the mPFC, OFC, and ACC were dissected. Tissue was homogenized in lysis buffer and protein was electrophoresed on Criterion XT Bis-Tris Protein Gels and transferred to Immuno-Blot PVDF membranes. Membranes were probed for: VMAT2 (1:1000, Abcam), COMT (1:1000, Thermo Fisher Scientific), and MAO-A (1:1000, Abcam).
- ***Experimental timeline:***



Orbitofrontal Cortex

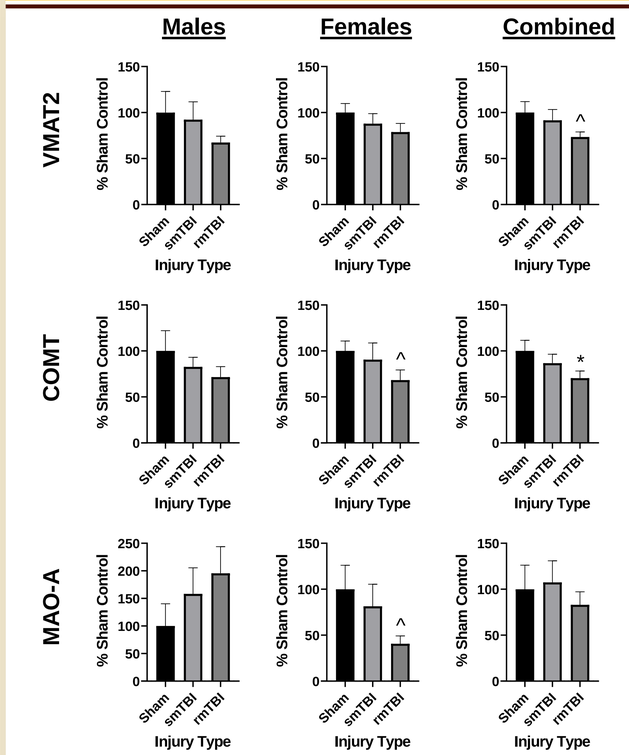


Figure 3: Protein density in OFC. Graphs represent mean percent change in total protein levels ± SEM as compared to sham controls (n=6-8 per group) 2 days post-final injury. *denotes $p<0.05$ and ^denotes $p<0.1$ from sham.

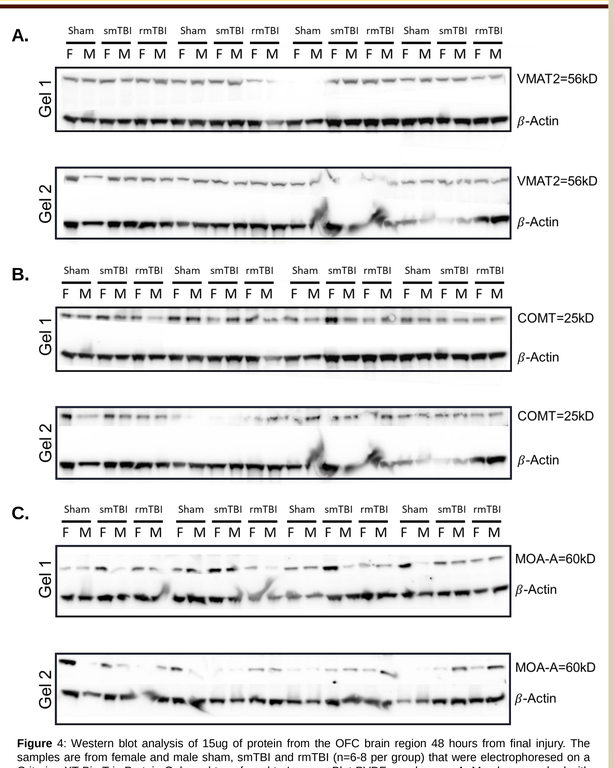


Figure 4: Western blot analysis of 15ug of protein from the OFC brain region 48 hours from final injury. The samples are from female and male sham, smTBI and rmTBI (n=6-8 per group) that were electrophoresed on a Criterion XT Bis-Tris Protein Gels and transferred to Immuno-Blot PVDF membranes. A. Membrane probed with rabbit anti-VMAT2. B. Membrane probed with rabbit anti-COMT. C. Membrane probed with rabbit anti-MAO-A. Beta-actin was used as the loading control.

Significance

Based upon these findings, we conclude that alterations in the levels of catecholamine neurotransmitter regulatory proteins may underlie functional catecholamine imbalances in the PFC. Additional studies will use pharmacological agents to further probe how sex-specific changes in catecholamine regulatory proteins impact sex-specific preference for risky choice following head injury.

Acknowledgements

References:

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