The SAGE Rebreather: Point-of-Injury CO2 Neuroprotection for Blast-Induced TBI in Contested Combat Environments

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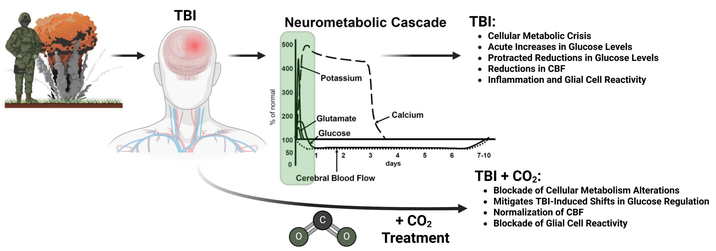
Background

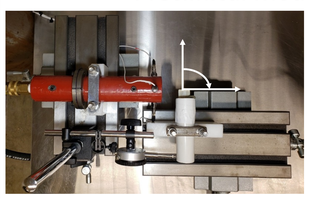
- Traumatic Brain Injury (TBI) is a major public health burden with no FDA-approved pharmacotherapies available specifically for either acute injury or it's enduring comorbidities.
- Mild Traumatic Brain Injury (mTBI) is the most common form of closed head injury in both civilian and military personnel.
- Given available prevalence numbers, our current preventative and/or treatment methods are clearly inadequate.
- TBI can result in dizziness, balance issues, vision loss, diffuse axonal injury, the generation of neuropsychiatric disorders and a greater risk of developing neurodegenerative disease.
- Within US military populations, blunt impact and blast-induced TBI are the most common modalities of service-related neurotrauma.
- These forms of military service-related TBI can stem from several environmental factors, including repeated exposure to high and/or low-level overpressure events and acceleration/deceleration forces encountered in various training and battlefield exposures.
- We have previously discovered that pretreatment with carbon dioxide (CO_2) attenuates specific detrimental effects of mild blast- and acceleration-deceleration-elicited TBI.
- The single most powerful determinant of cerebral blood flow (CBF) is the concentration of respired CO_2.

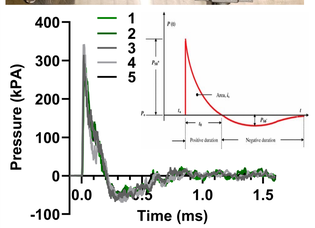
- **We hypothesize that exposure to CO_2 immediately *post-injury* will act to provide *protective effects against blast- and acceleration-deceleration injury* by altering aspects of secondary injury cascades driving the detrimental effects of mTBI.**

- ***Proposed Mechanism:***



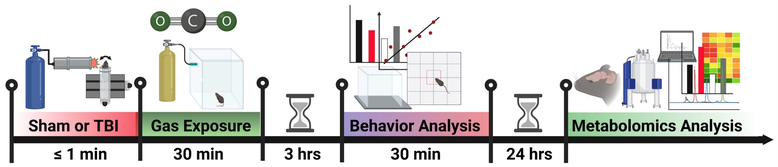
Preclinical Model For Blast-Induced mTBI





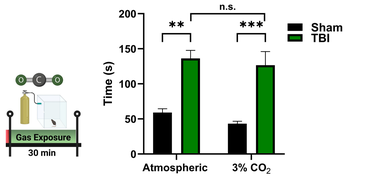
- Blast shock tube model for mTBI generates blast waves mimicking a classic Friedlander waveform reminiscent of improvised explosive devices.
- Blast waves are scaled in intensity and duration for murine subjects.
- Helium is used as a driver gas. Dividing the driver section and driven section is separated by a Mylar™ membrane (0.0762 mm).
- Model results in concomitant blast wave exposure and a rapid head acceleration event (HAE) in murine subjects.

Experimental Methods and Timeline



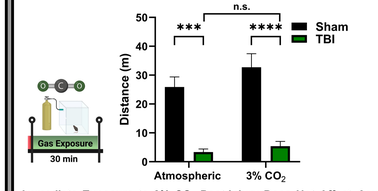
- Subjects underwent mTBI or Sham procedures under isoflurane anesthesia.
- Immediately following injury paradigm, subjects were exposures to medical grade compressed ambient air or a 3% CO_2 mixture containing atmospheric levels of O_2 (20%) for a period of 30 minutes.
- Righting reflex time (RRT) and functional locomotor activity was measured during the 30 min CO_2 post-injury exposure period.
- Functional locomotor activity was measured 3 hours post-injury.
- 24 hours post-injury bilateral cortex and hippocampal samples were collected and subjected to metabolomics analysis using the Cincinnati Children's Hospital Medical Center (CCHMC) Metabolomics Core Facility.

Acute Assessment of Righting Reflex Time (RRT)



Immediate Exposure to 3% CO_2 Post-Injury Does Not Affect Righting Reflex Time (RRT) Following mTBI. Experiment serves two purposes; one is to provide evidence that all subjects across groups received consistent injuries (prior to 3% CO_2 exposure) and two is to delineate whether immediate CO_2 exposure would alter times of unconsciousness following mTBI paradigm. All injury subjects, regardless of group were found to have received consistent injuries. Post-injury exposure to CO_2 was found to not alter RRT immediately post-injury paradigm. N = 7-8/group, Two-Way ANOVA, followed by post hoc Tukey's multiple comparison tests. ** = *P ≤ 0.01*, *** = *P ≤ 0.001*.

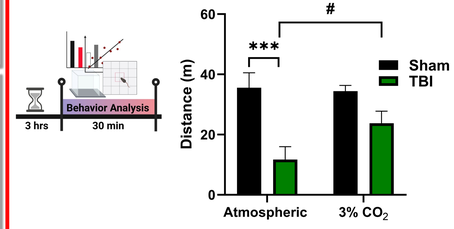
3% CO_2 Exposure Does Not Alter Locomotor Activity



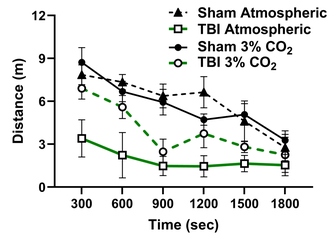
Immediate Exposure to 3% CO_2 Post-Injury Does Not Affect *Acute* Locomotor Activity. Experiment serves three purposes; 1: Provide evidence that all subjects across groups received consistent injuries (prior to CO_2 exposure, mTBI elicits decreases in general locomotor activity); 2: Delineate whether immediate CO_2 exposure alters acute deficits in locomotor activity following mTBI; and 3: Determine whether CO_2 exposure alone alters locomotor activity. Post-injury exposure to CO_2 was found to not alter locomotor activity measurements immediately post-injury paradigm. N = 7-8/group, Two-Way ANOVA, followed by post hoc Tukey's multiple comparison tests. *** = *P ≤ 0.001*, **** = *P ≤ 0.0001*.

Post-Injury Exposure to 3% CO_2 Attenuates mTBI-Induced Reductions in Functional Locomotor Activity

Total Distance Travelled

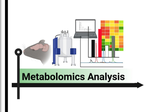


Distance Travelled - Segmented



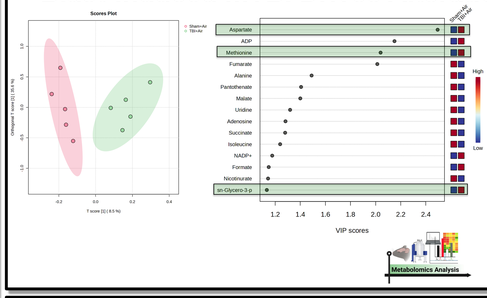
Exposure to 3% CO_2 Immediately Following mTBI Results in the *Latent* Blockade of Injury-Elicited Deficits in Functional Locomotor Activity. Blast-induced mTBI results in significant deficits in functional, general locomotor activity 3 hrs post injury. Exposure to 3% CO_2, as compared to compressed atmospheric air, results in the significant attenuation of functional deficits post-injury. 3 hrs post-injury paradigm, subjects underwent locomotor activity analysis using standard locomotor chambers in combination with ANY-Maze video tracking software. Overall effect is seen at nearly every timepoint over the 30 min locomotor activity session. Two-Way ANOVA, followed by post hoc Fisher's multiple comparison tests. ***= *P ≤ 0.001 vs. Atmospheric-Sham*, #= *P ≤ 0.05 vs. Atmospheric-TBI*

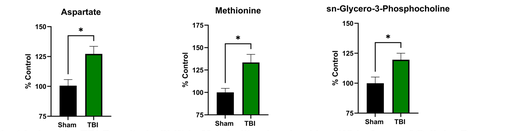
Metabolomics Methods



- The CCHMC Metabolomics Core utilized NMR-based metabolomics analyses using a state-of-the-art fully-automated Bruker 600Mhz NMR system.
- Bilateral cortex and hippocampal samples underwent quantitative metabolic profiling using this NMR-based system.

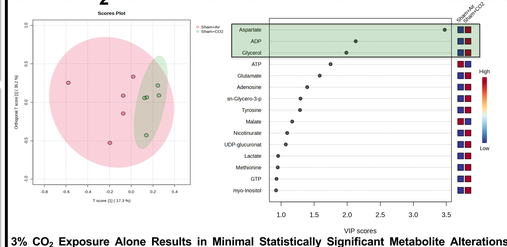
Blast-Induced mTBI Drives Alterations in Metabolites within Brain Tissue

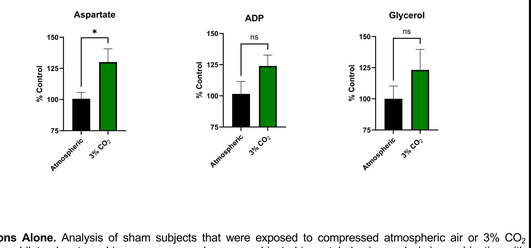




Blast-Induced mTBI Results in Shift in Metabolites Involved in a Wide Array of Cellular Processes within the Brain. Subjects were exposed to compressed atmospheric air immediately following injury or sham paradigms for a period of 30 minutes. 24 hrs post-injury/sham + compressed medical grade atmospheric air exposure paradigms, bilateral cortex + hippocampus samples were subjected to metabolomics analysis in combination with the CCHMC Metabolomics Core facility. Analysis of samples revealed wide-spread changes in metabolites associated with cellular metabolic cascades. Scores plot shows overall divergence between sham and mTBI groups across metabolites analyzed. VIP scores plot shows significantly altered metabolites with corresponding directions of changes when comparing Sham vs. mTBI subjects. Highlighted metabolites (aspartate, methionine & sn-glycerol-3-phosphocholine) are used as examples of metabolites significantly altered by mTBI alone. Two-Way ANOVA, followed by post hoc Fisher's multiple comparison tests, * = *P ≤ 0.05*.

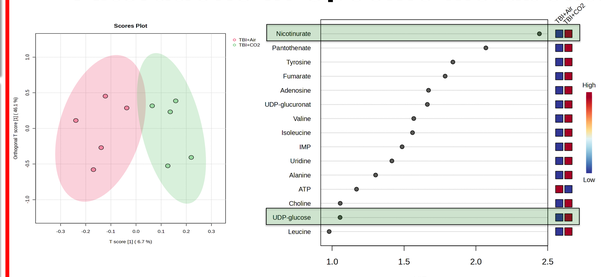
3% CO_2 Administration Has Minimal Effects on Metabolites within Brain Tissue

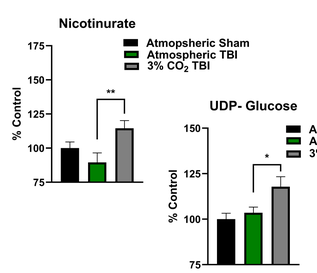




3% CO_2 Exposure Alone Results in Minimal Statistically Significant Metabolite Alterations Alone. Analysis of sham subjects that were exposed to compressed atmospheric air or 3% CO_2 immediately following sham paradigms for a period of 30 minutes. 24 hrs post-exposure paradigms, bilateral cortex + hippocampus samples were subjected to metabolomics analysis in combination with the CCHMC Metabolomics Core facility. Scores plot shows minimal divergence between atmospheric air and 3% CO_2 exposed groups across metabolites analyzed. VIP scores plot shows altered metabolites with corresponding directions of changes when comparing atmospheric air and 3% CO_2 exposed subjects. Of all the metabolites analyzed, 3% CO_2 exposure was found to significantly alter only aspartate levels within brain tissue 24 hrs post-exposure. Two-Way ANOVA, followed by post hoc Fisher's multiple comparison tests, * = *P ≤ 0.05*.

3% CO_2 Exposure Post Injury Attenuates Blast-Induced mTBI Alterations in Specific Metabolites within Brain Tissue





3% CO_2 Exposure Immediately Post-Injury Attenuates or Alters Metabolites within the Brain Following Blast-Induced mTBI. Subjects underwent blast-induced mTBI or sham procedures immediately followed by exposure to 3% CO_2 or compressed atmospheric air for a period of 30 min. 24 hrs post-exposure paradigms, bilateral cortex + hippocampus samples were subjected to metabolomics analysis in combination with the CCHMC Metabolomics Core facility. Scores plot shows divergence between mTBI subjects exposed to atmospheric air as compared to mTBI subjects exposed to 3% CO_2 immediately post-injury providing evidence for differences across the two groups. VIP scores plot shows significantly altered metabolites with corresponding directions of changes when comparing blast-induced mTBI + atmospheric air *or* 3% CO_2 exposed subjects. Post-injury 3% CO_2 exposure significantly increases nicotinurate and UDP-glucose levels as compared to mTBI + atmospheric air exposure. Two-Way ANOVA, followed by post hoc Fisher's multiple comparison tests, ** = *P ≤ 0.01*, * = *P ≤ 0.05*.

Conclusions and Future Directions

- **Post-mTBI exposure to 3% CO_2 attenuates functional locomotor deficits elicited by injury.**
- **Blast-induced mTBI results in defined cellular metabolite changes within the brain 24 hrs post-injury.**
- **Although exhibiting minimal overt effects alone, exposure to 3% CO_2 immediately following mTBI leads to potentially beneficial alterations in cellular metabolites within brain tissue.**

Acknowledgements