INVESTIGATING SPACE BRAIN: HOW DO BRAIN CELLS RESPOND TO THE EFFECTS OF INCREASED INTRACRANIAL PRESSURE?

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Abstract

The human brain adapts to space conditions such as changes in acceleration due to take-off and landing, and exposure to microgravity and radiation, leading to increased intracranial pressure (ICP). The objective of this work was to understand how changes in ICP affect neurochemical adaptation. Methods were performed by first characterizing real-time changes in ICP for a range of applied overpressure magnitudes in a preclinical model. ICP was found to be greater than the applied overpressure magnitude by a factor of 1.6 on average. Metabolic profiles of amino acids extracted from the cortex and hippocampus were then determined at 4 and 24 hours after the applied overpressure using high performance liquid chromatography where neurochemical concentrations were quantified. Glutamate, glutamine, and GABA were identified as promising therapeutic targets to mitigate potential long-term neurological deficits, all of which are quantifiable metabolites using magnetic resonance spectroscopy (MRS) in the clinic. This work has shown metabolic adaptation to a defined ICP increase leads to quantifiable changes in metabolites. To ensure the neurological safety of our astronauts, future studies should consider investigation of glutamate, glutamine, and GABA concentrations in the brain after space flight using MRS as well as the development of neuroprotective therapeutic interventions.

Introduction

Recent evidence suggests that human space travel poses a threat to the central nervous system (CNS). Space brain, a phenomenon where degeneration of neurological function occurs due to prolonged exposure to microgravity and radiation, remains an untreatable side effect of space exploration[1, 2]. While the mechanisms of this degeneration are unknown, it has been documented that intracranial pressure (ICP) increases, which could be due to a number of combined events such as take-off and landing, microgravity, radiation, or changes in vacuum pressure[1, 3, 4]. The magnitude of ICP increase depends on the specific event conditions, however, neurological adaptation to this change is required for all cases. As part of the Exploration Systems Development Mission Directorate (ESDMD) and Space Operations Mission Directorate (SOMD), we aim to propel humans to the moon, Mars, and beyond. Before we can get there, it is critical that we further explore the mechanisms that contribute to space brain. This work explores how brain cells respond to changes in ICP.

One approach to assess CNS health can be best performed through the quantification of free amino acids. Amino acids are directly and indirectly involved in neurotransmission and cellular metabolism through complex molecular pathways[5]. Both clinically and in preclinical models, amino acids can be quantified within various brain regions where shifts in metabolite concentrations can be detected using magnetic resonance spectroscopy (MRS) methods in clinical models and high-performance liquid chromatography (HPLC) methods in preclinical models[5-9]. Thus, the objective of this experiment was to detect and characterize the neurochemical shifts in amino acid concentrations that result

following a defined increase in ICP where we hypothesize that increased ICP will lead to distinct neurochemical shifts consistent across brain regions and ICP magnitudes.

Methods

Preclinical Model

Male Sprague Dawley rats (10 weeks old) were purchased from Envigo (Dublin, VA) and all procedures were performed using an established animal model following approval from the Virginia Tech Institutional Animal Care and Use Committee. The animals were acclimated for at least three days and followed a 12 hour light-dark cycle with food and water administered ad libitum. Two main experiments were performed to: 1) measure real-time ICP response to a range of applied overpressure magnitudes and 2) develop metabolic profiles in the cortex and hippocampus brain regions at 4- and 24-hours following increased ICP.

Applied Overpressure Magnitudes

The Virginia Tech ABS (**Fig. 1**), located in the Center for Injury Biomechanics, was used to direct overpressures of defined magnitudes along the length of the rectangular test section and over the test specimen to elicit a change in ICP. The wave was driven by compressed helium, and peak static overpressure was controlled by the thickness of an acetate membrane between the driver and the transition section. The end wave eliminator absorbed the energy by sliding along the rails to prevent wave reflections on the subject. The test subjects were anesthetized and secured in a taut mesh sling. Static overpressure was measured in three locations along the wall of the test section using piezoelectric sensors. These methods are described in more detail in Cho et al. (2013)[10].

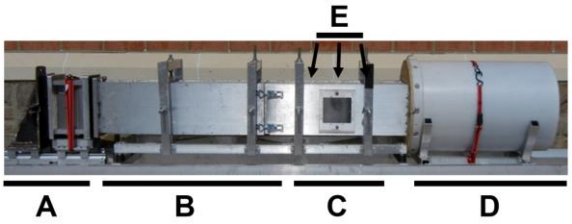

Figure 1. Virginia Tech ABS. **A)** Driver section where compressed gas builds. **B)** Sloped transition section followed by rectangular test section. **C)** Location of test specimen. **D)** End wave eliminator. **E)** Placement of wall sensors.

Experiment 1: Measuring Intracranial Pressure

ICP was measured in five subjects where each was exposed to three overpressures at magnitudes of 5, 10, 15, and 20 psi. To collect real-time changes in subject ICP, a single SPR-524 Millar Mikro-Tip pressure gauge (ADInstruments, Colorado Springs, CO) was implanted in the brain through the back of the skull at a depth of approximately 10 mm, parallel to the top of the skull. The sensor was inserted through the occiput so as to minimize disruption of wave profile or skull dynamics during the head-on wave exposure. The SPR-524 was particularly beneficial due to its small size (tip diameter = 1.2 mm) and sensitivity (1.296 mV/psi). Surgical methods were adapted from Leonardi et al. (2012)[11] where subjects were briefly anesthetized with 4% isoflurane prior to administration of ketamine/xylazine (80/10 mg/kg IP), a stereotaxic high-speed drill was used to drill a 1.2 mm diameter hole taking great care not to damage the vasculature at the back of the brain, approximately 3 mm to the right of the mid-line and 3.5 mm from the top of the skull, where the sensor was inserted. The gaps around the sensor exiting the back of the skull were sealed using a viscous acrylate resin (InstaCure+). This sealing around the sensor was a critical step in maintaining a closed environment inside of the skull. If not sealed completely, pressures

will leak. The scalp was then sutured shut and reinforced with Vetbond. To prevent snagging and removal of the sensor during the overpressure event, the sensor was sutured to the skin on the upper neck and the torso. Quarter doses of ketamine/xylazine were administered every 30 minutes, for a total of approximately 2-3 hours until all testing was completed. Subjects were oriented facing the driver compartment and extra padding was inserted to reduce relative motion inside the sling. Euthanasia immediately followed the final wave exposure and sensor placement was confirmed using biplane X-ray and Hematoxylin and Eosin (H&E) stain (Abcam, Waltham, MA). Data was processed in MATLAB R2018b (MathWorks, Natick, MA). Offsets from baseline were corrected for all pressure traces. Filtering was not performed so as not to corrupt any frequency responses seen in the tissue. Peak static overpressure and ICP magnitudes were then measured for analysis.

Experiment 2: Creating Metabolic Profiles

The objectives of this experiment were to see if metabolic adaptation was consistent for a small change in ICP (ΔICP) compared to a large ΔICP, to determine which metabolites, if any, were sensitive to ΔICP, and to determine if outcomes were specific to brain region. Based on results from Experiment 1, we chose to compare metabolic profiles for ΔICP = 10 psi and ΔICP = 30 psi. For each case, amino acid concentrations were quantified in the hippocampus and cortex at 4 and 24 hours after exposure to a single overpressure. Group sizes and average overpressure characteristics are provided in **Table 1**. Control subjects received the same treatment as the ΔICP group, but were placed adjacent to the ABS during the overpressure event, thus not experiencing ΔICP.

Table 1. Experimental design including time points, group sizes, average overpressure peak magnitude and duration $\pm$ standard deviation, and approximated ΔICP based on the model created in Experiment 1.

Time Point (hr)	Group Sizes C=Control P=ΔICP	Peak Pressure (psi)	Positive Duration (ms)	ΔICP (psi)
4	C=4, P =4	18.3 $\pm$ 1.0	2.3 $\pm$ 0.1	29.3
24	C=4, P =5	18.9 $\pm$ 0.8	2.5 $\pm$ 0.1	30.2
4	C=4, P =4	6.3 $\pm$ 0.8	1.6 $\pm$ 0.02	10.1
24	C=4, P =4	6.3 $\pm$ 0.8	1.6 $\pm$ 0.01	10.1

Subjects were euthanized at the time points of interest, the brain was quickly extracted (<10 min), rinsed with 0.9% saline solution, and placed on ice for dissection. The hippocampus and cortex were stored in centrifuge vials on dry ice and transported to long term storage at -80°C. Tissue mass in each vial was recorded. Amino acids were extracted from the tissue by first adding 500 µl of 1% methanol to remove any enzymatic activity, adding a known concentration of tyrosine as the internal standard, mechanically homogenizing the tissue, and centrifuging the supernatant through a hydrophilic PVDF 0.2 µm filter. HPLC methods using electrochemical detection were performed using the parameters outlined in **Table 2**. The amino acids of interest included: alanine, arginine, aspartate, serine, threonine, glutamine, glutamate, GABA, and glycine. Known concentrations of each were used to make calibration curves before each run. All calibration curves exceeded regression coefficients of 0.98 prior to loading samples for testing. The concentration of each amino acid was then quantified in µg per g of tissue based on these calibration curves. Data was collected and processed using Chromeleon

Chromatography Data System Software (Chromeleon 7). Concentrations were then normalized to the control group. Statistical analysis on the changes in amino acid concentration between the control group and the ΔICP group was then performed using an unpaired two-sided t-test where $p < 0.05$ was significant. All p-values were reported to discuss evidence of amino acid sensitivity to changes in concentration.

Table 2. Optimized HPLC methods for amino acid analysis.

Mobile phase	76.5% 75 mM Phosphate buffer, pH 6.3, 20% Methanol, 3.5% Acetonitrile
Analytical column	C18 Hypersil™ 3 µm BDS 3 mm × 150 mm
Guard cartridge	Universal Uniguard Holder 3 mm i.d.
Flow rate	0.4 mL/min
Column temperature	45 °C
Voltage	550 mV
Derivatizing agent	27 mg of OPA; 1 ml of methanol; 9 mL of 0.2 M H_3BO_3 and 5 µL of C_2H_6OS (βME)
Calibration method	External Standard
Type of detection	Electrochemical detection
Run time	28 min
Analytes quantified	Ala, Arg, Asp, Ser, Thr, Glu, Gln, Gly, GABA
Sample	Amino acids extracted from rat brain

Results

Experiment 1: Increased ICP

The applied external overpressures followed a Friedlander waveform and in all cases, ICP magnitude was greater than the corresponding overpressure magnitude (**Fig. 2**). Additionally, ICP magnitude increased linearly by a factor of 1.6 times the static overpressure with a regression coefficient of 0.91 (**Fig. 3**). The exponential fit coefficient was 0.92, however, regression coefficients showed a linear dependence on a subject-level ranging from 0.94-0.99. Sensor placement was confirmed using biplane X-ray and H&E staining (**Fig. 4**). Any variability within the ICP linear model predictions is most likely due to sensor placement as the vertical depth from the top of the skull varied by approximately 4 mm.

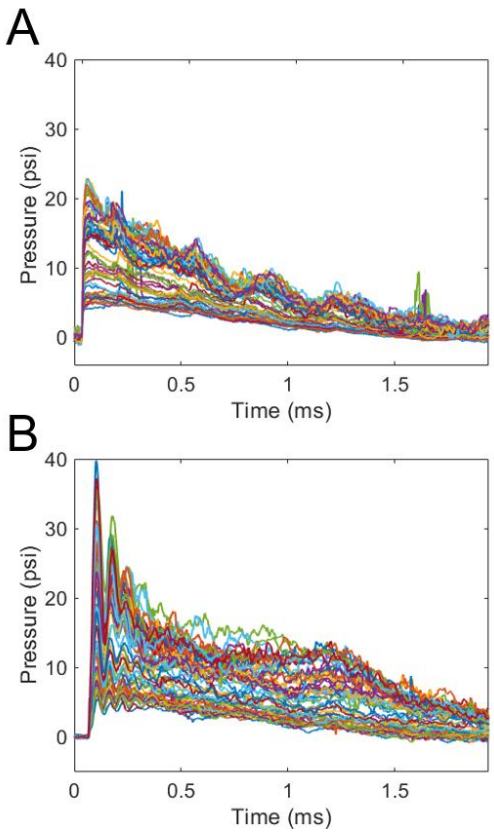

Figure 2. Comparison of all **A)** Overpressure traces to all **B)** ICP traces. All ICP magnitudes were greater than the corresponding overpressure magnitudes.

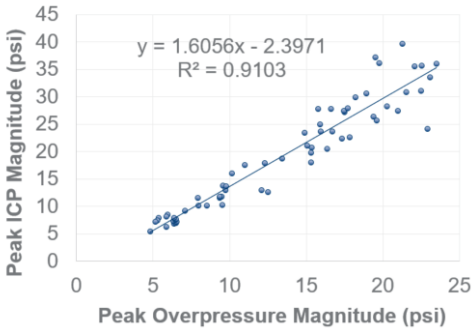

Figure 3. Peak ICP magnitude increases linearly relative to the peak static overpressure by a factor of 1.6056.

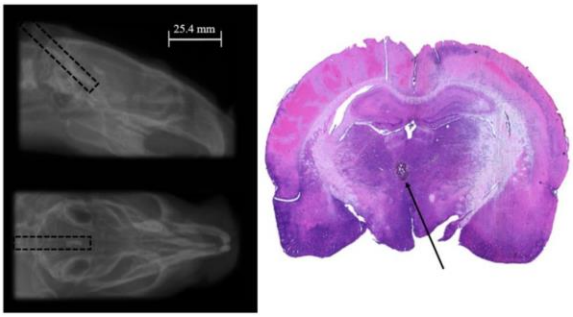

Figure 4. Confirmation of sensor location using biplane X-ray (left) and H&E staining (right). The dashed box on the left and arrow on the right indicate sensor location.

Experiment 2: Metabolic Profiles

Profiles for glutamate, glutamine, GABA, threonine, serine, aspartate, arginine, alanine, and glycine were created. Metabolite profiles followed similar trends in the hippocampus versus the cortex and most changes in concentration occurred at 4 hours after increased ICP compared to 24 hours. These findings indicate that metabolic adaptation may be consistent across brain regions and are more likely to occur within the first 24 hours following ICP changes.

In most cases, the 30 psi group had more significant changes in metabolites compared to the 10 psi group, as anticipated. A few exceptions include glutamine, GABA, and threonine, which were more sensitive to a change in 10 psi than 30 psi at 4 hours in the hippocampus. These metabolites may have a pressure dependence or the feedback loops may be keeping up with metabolic demand at 10 psi, but are not able to meet demands at 30 psi, leading to prolonged stress in this region at 24 hours.

Glutamate, glutamine, GABA, and their relationships were most sensitive to changes in ICP and are compared in the cortex (**Fig. 5**) and hippocampus (**Fig. 6**). These amino acids all relate to each other in the glutamate-glutamine/GABA cycle where glutamate is directly converted to glutamine, and vice versa, and GABA is converted to glutamate[12]. Glutamate decreases at 4 hours after a change in 10 psi, but increases after a change in 30 psi. However, at 24 hours, glutamate remains relatively unchanged at both ICP magnitudes. Glutamine decreased in the cortex, except at 4 hours at 30 psi, and increased in the hippocampus. GABA showed trending increases at 4 hours and then a slight decrease in average concentration at 24 hours.

While the trends and changes in glutamate, glutamine, and GABA are interesting, the relationship between glutamate and GABA (Glu/GABA) is critical to understanding metabolic stress. A significant change in Glu/GABA indicates there is an imbalance between excitatory and inhibitory neurotransmitters[13-15] where Glu/GABA significantly increased at 24 hours in the hippocampus, but only after a change in 30 psi, indicating that a change in 10 psi may result in effective adaptation of amino acids rather than adaptation leading to a stressed state.

The ratio between glutamate and glutamine (Glu/Gln) can provide more information on where metabolic cycles may be altered. Glu/Gln decreased at 4 hours in both the cortex and hippocampus following a change in 10 psi and increased following a change in 30 psi. Similar to Glu/GABA, this could be due to appropriate adaptation at 10 psi and adaptation leading to a stressed state at 30 psi.

Discussion

The experiments outlined in this report demonstrated consistent, controlled ICP increases across subjects and metabolic profiles were successfully created. Brain adaptation following a change in 10 psi was found to occur similarly to the response at 30 psi with a few exceptions. At 30 psi in the hippocampus, the tissue region showed signs of metabolic stress.

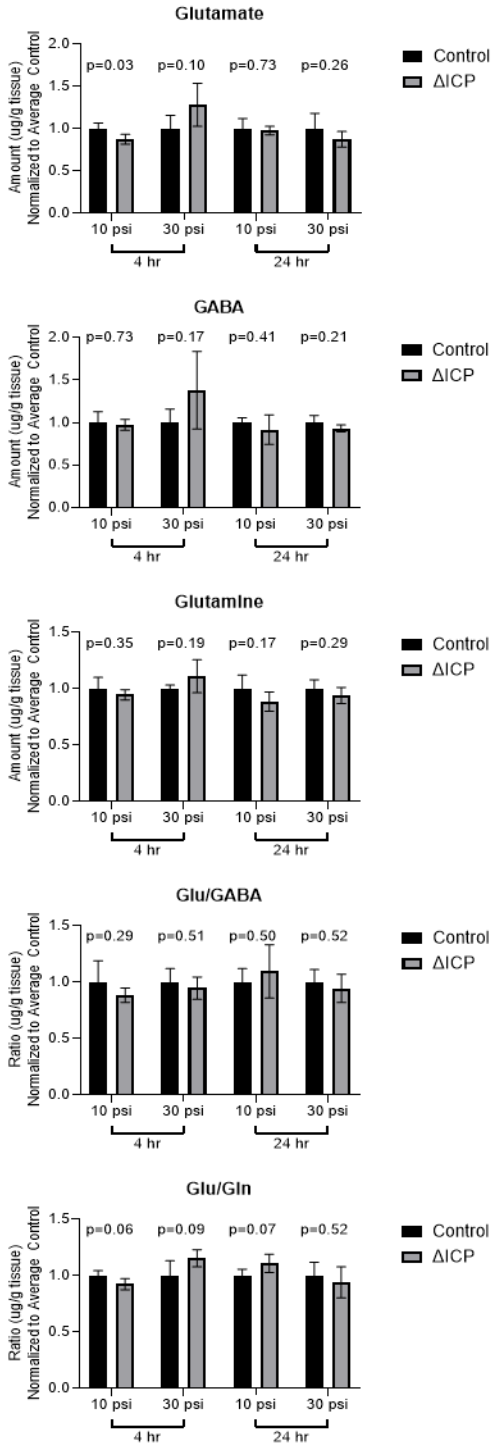

Figure 4. Glutamate, Glutamine, GABA, Glu/GABA, and Glu/Gln concentrations normalized to the control in the **cortex**. P-values are from two-tailed t-tests comparing control and ΔICP groups.

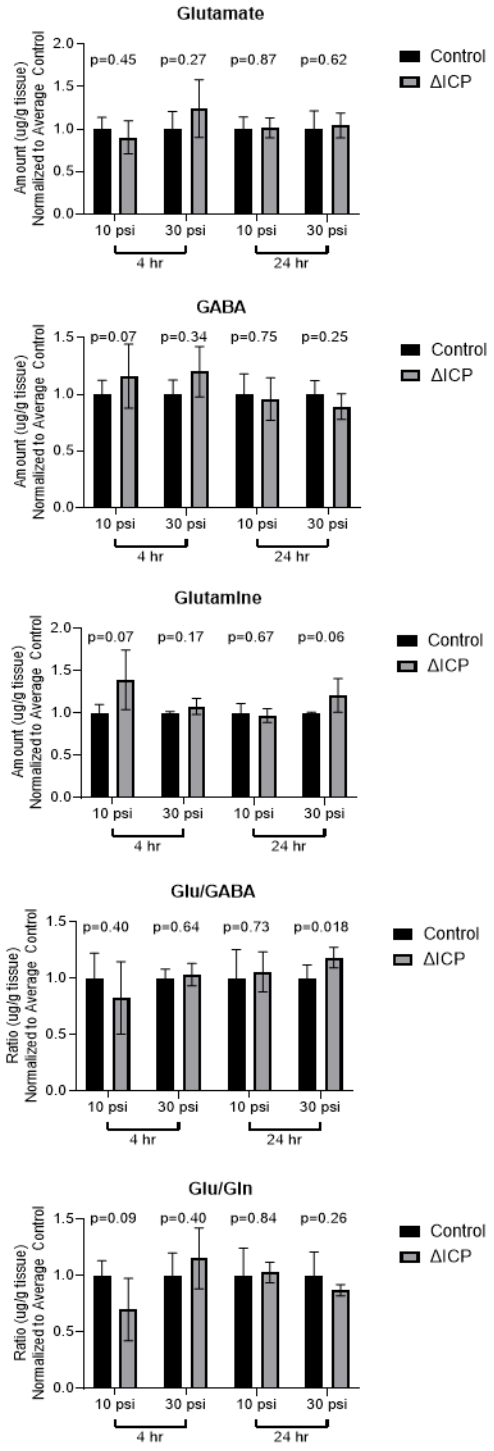

Figure 5. Glutamate, Glutamine, GABA, Glu/GABA, and Glu/Gln concentrations normalized to the control in the **hippocampus**. P-values are from two-tailed t-tests comparing control and ΔICP groups.

Potential causes of metabolic stress at 30 psi could be related to trending increases in the Glu/Gln concentrations at 4 hours, rather than decreases at 10 psi. This reversal may mean that glutamate is not being converted to glutamine at the necessary rate to maintain homeostasis. Astrocytes are the main brain cells responsible for this conversion. Following exposure to an 18 psi overpressure wave, or a 30 psi ICP increase, astrogliosis has been detected at 24 hours and could be a consequence of the disrupted metabolic cycles[16, 17]. Future work should be performed to quantify changes in protein and mRNA expression of glutamine synthetase, the enzyme critical to the conversion of glutamate to glutamine. Additionally, as this is a preliminary study, more subjects will be added to improve the statistical power of the created profiles. Lastly, we aim to translate this work *in vivo* using MRS to validate these metabolic profiles.

Conclusion

ICP measurements were repeatable across subjects and allow for a translational understanding of how ICP changes relative to external changes in pressure. Metabolic profiles showed that glutamate, glutamine, and GABA were sensitive to ICP changes at both 10 and 30 psi and the glutamate-glutamine/GABA cycle should be further investigated for potential therapeutic targets.

Collectively, the proposed studies aim to identify how the CNS adapts to an increase in ICP. These studies may not simulate specific changes in ICP due to microgravity, take-off or landing, or from changes in vacuum pressures, which is what astronauts are likely to experience during spaceflight, as these are not currently known. However, characterization of the neurological response to a range of ICP has the potential to provide a map for stages of neurological recovery upon landing back on Earth. The need for a deeper understanding of neurobiological mechanisms was specifically outlined by Jandial et al. (2018)[1] as an important model of expected risk. With this method of detecting neurological alteration and recovery status, we expect accelerated treatments for men and women affected by space brain, thus accelerating us to Mars where the risk of long-term neurological deficits are mitigated.

Acknowledgements

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