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Matrix-assisted laser desorption/ionization analysis of the brain proteome of microgravity-exposed mice from the International Space Station

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Manned spaceflight exposes humans to extreme environmental conditions, including microgravity exposures. The effects of microgravity during spaceflight could lead to changes in brain structure, gene expression, and vascular physiology. Given the known physiological effects, it is highly likely that there are microgravity-initiated proteomic differentials in the brain, possibly domain specific. MALDI-TOF (matrix-assisted laser desorption/ionization time of flight) Imaging Mass Spectrometry allows the visualization of the spatial distribution of highly abundant intact proteins in tissue specimens. This study utilized this technique to visualize global proteomic changes induced by microgravity exposure in brain tissue received from the Rodent Research-1 Center for the Advancement of Science in Space (CASIS)/National Aeronautics and Space Administration (NASA). Proteome profiles were obtained from isolated whole brain tissue from microgravity exposed, Habitat control, and baseline. While a total of 135 mass peaks equating to individual proteins were identified, statistical analysis determined that there were no significant differences in the spectra profiles from the three test groups utilizing this methodology, possibly due to sample collection logistics rather than lack of cellular response.

KEYWORDS

microgravity, MALDI, brain, proteomics, International Space Station, *in vivo*, mice, proteome

1 Introduction

The age of space travel is in an exciting new era where the commercialization of space is fast becoming a reality. For decades, humans have been launching into space while subjected to extreme environmental conditions such as microgravity and radiation. Comprehensive studies to determine the impacts of spaceflight are being performed

to understand the effects on the entirety of the human system. (Demontis et al., 2017; Van Ombergen et al., 2017) NASA's Twin study was pivotal in developing an integrated systems description of the effects of 1 year of spaceflight on the human body. (Garrett-Bakelman et al., 2019) This study collected physiological, telomeric, transcriptomic, epigenetic, proteomic, metabolomic, immune, microbiome, cardiovascular, vision-related, and cognitive data to investigate long term molecular pathway alterations due to lengthy exposures. (Demontis et al., 2017) Despite the tremendous data output from this collaborative research, there were limitations in examining potentially subtle molecular changes induced in the brain by microgravity.

The brain is arguably the most complex organ in the human body. The enormous network of cells, containing billions of neurons and interconnections is responsible for receiving, processing, and executing cognition and perception, controlling motor activities and the storage of memories. (Kandel, 2001) The absence of gravity can affect the brain, possibly inhibiting cognitive function. (Grabherr and Mast, 2010; Mammarella, 2020) Neuroasthenia, also known as "space fog", has been reported by astronauts for some time and prompted extensive cognitive testing. (Kanas et al., 2001; Basner et al., 2015) However, these cognitive evaluation data have not shown statistical linkage to self-reported cognitive decrement, possibly due to individual responsivity as well as certain stressors not influenced by microgravity (Vaughan et al., 2013; Strangman et al., 2014).

Despite discrepancies in cognitive evaluation, it has long been observed that long term spaceflight can change brain physiology and affect the sensory-motor system. (Blaber et al., 2010; Roberts et al., 2017) Visualizing the changes in brain physiology on human astronauts and pilots has been performed *via* diffusion magnetic resonance imaging (dMRI). This instrumentation allows for the observation of Free Water shifts, grey matter, and white matter distribution. Disrupted white matter structural connectivity in various brain regions have been linked with several previous spaceflight missions, mission duration and preflight to postflight balance declines. (Basner et al., 2015) Equivalent changes in brain physiology have also been observed within U2 pilots in the United States Air Force (Nelson et al., 2013). Invasive analytical imaging cannot be performed to examine the human brain proteome, therefore MALDI TOF imaging mass spectrometry (IMS) instrumentation was utilized to search for proteomic shifts due to the effects of spaceflight in a well-characterized mouse model. MALDI-TOF-IMS is a label-free technique that can be used for global screening of proteomic, metabolomic or lipidomic changes on the surface of tissue samples, providing a bridge between histology and deep molecular mass spectrometric analysis in tissue-based research (Aichler and Walch, 2015).

Gaining insight on protein-associated changes in rodent brains following microgravity exposure provides insight on which regions may be impacted. Mice are an excellent candidate to study any potential effects because of the shared genetic homology (Uhl and Warner, 2015). In this study, nine mice brains were evaluated using MALDI-TOF-IMS. There were three separate groupings: Baseline, Habitat Control and Flight. The highest abundance protein ions were visualized and spatially resolved by brain region. The hippocampus and cerebellum are regions of interest because it is well known that activity between these regions contribute to human spatial navigation and motor control functions that are critical functions for astronauts (Iglói et al., 2015).

This study used MALDI-TOF-IMS to characterize proteomic shifts within different regions. Insight into any shifting behavior can provide a target for in depth proteomic analysis to fully characterize the observed effect. Understanding how the body is affected by space exploration will help shape space travel development and influence the proper measures that must be taken to safeguard against the effects of microgravity.

2 Materials and methods

2.1 Animal study

This study was conducted under an IACUC approved protocol in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011) and approved by the NASA Institutional Animal Care and Use Committee.

The animal transport was conducted on the as part of the resupply launch mission CRS-16. Mature (30–52 weeks old) female BALB/cAnNTac mice (Taconic Biosciences, Rensselaer, NY) were used in this study. The Habitat Control (HC) group was kept at standard Earth gravity in the specialized animal containment (called the Rodent Habitat), a part of the ISS Rodent Habitat Hardware System. The Baseline group (BL) was prepared just prior to launch of the microgravity-exposed group and consists of animals at Earth gravity in normal animal cages. The microgravity-exposed group (FL) were placed in the Rodent Transporter containment cages, launched aboard a Falcon 9 rocket, docked at the International Space Station (ISS), then transferred to a Rodent Habitat using the Animal Access Unit. On the ISS, the animals were fed *ad libitum* using NASA Type 12 Nutrient-upgraded Rodent Food Bars diet plus unrestricted water. The animals were on board the ISS at microgravity a total of 36 days. At Day 37, the animals were placed in a Rodent Transporter cage for return transport to Earth. Within 3 days of FL mice returning to standard Earth gravity (9.80665 m/s^2), all group animals were euthanized *via* ketamine/xylazine overdose, the whole brain removed and quickly frozen in liquid nitrogen. The tissue samples were kept at −80°C prior to MALDI analyses.

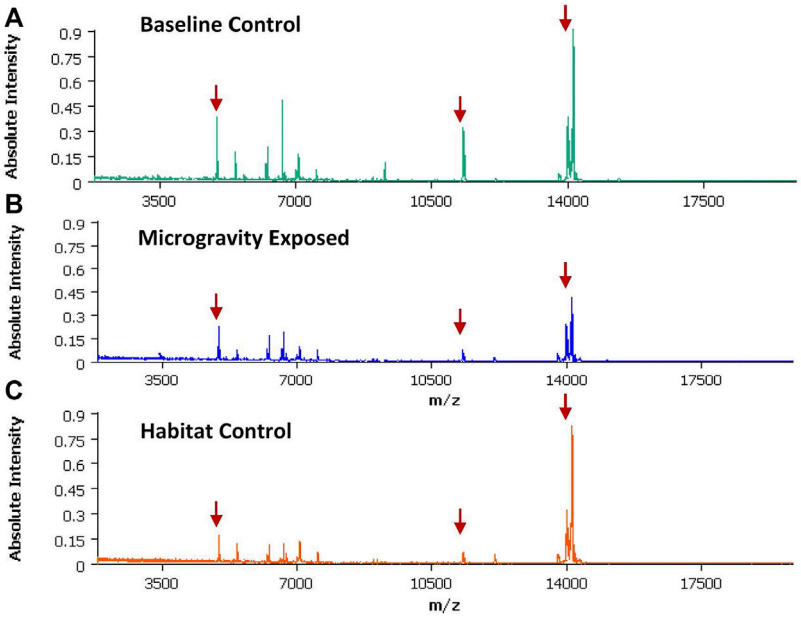

FIGURE 1
An averaged spectra generated SCiLs Lab software utilizing spectra data generated from a total of three brain sections (3 different animals) within the same exposure group. Red arrows indicate the *m/z* used to create the spectral images discussed below **(A)** Averaged spectra from Baseline (BL) group; **(B)** Averaged spectra from the microgravity group (FL); **(C)** Average spectra from the Habitat (HC) group.

Dissection and initial tissue collection was handled by CASIS and shipped to the Air Force Research Laboratory (AFRL) overnight on dry ice. Upon receipt, the samples were weighed, measured, and prepared for analysis.

2.2 Sample preparation for MALDI-TOF analysis

Frozen whole brain tissues were cyrosectioned along the sagittal axis in 12 µm sections (landmark p56, Allen Brain Atlas) using an Advantic Cryostat in preparation for MALDI-TOF-IMS. Sagittal orientation was selected in order to capture maximal brain region coverage in a 2D image (Fernandes et al., 2016). Brain slices were mounted on Indium-Tin-Oxide (ITO) coated slides using a gelatin/CMC solution (Gemperline et al., 2014) and adhered under vacuum. Once mounted, the slides were stored in a −80°C freezer until ready for imaging. All brain tissues were randomized for processing as well as for imaging.

2.3 MALDI-TOF IMS slide preparation for protein analysis

ITO slides with mounted tissue sections were acclimated to room temperature in a vacuum desiccator for 1 h and washed using the Carnoy method to target proteins. (Puchtler et al., 1970) Prior to MALDI-TOF imaging, an optical image of the tissue ITO slide was generated using a high resolution scanner at a dpi 4,800. This image was utilized to program the MALDI-TOF instrument. A total of 10 mg/ml Sinapinic Acid matrix was applied to the ITO slide using a Bruker ImagePrep sprayer and imaged using a Bruker Ultraflextreme MALDI-TOF (Bruker Corp.). All samples were imaged in linear positive mode, utilizing a 1.8 0 in the 29 kDa mass range with continuous 50 µm raster.

2.4 Sample analysis

Mass spectra and images were obtained from intact protein ions using one brain slice section from each animal from the BL, HC and FL groups (3 animals per group). Individual spectra were obtained for each sample (Figure 1). To compare the microgravity proteome to both the HC and BL groups, the spectra were averaged for each exposure group to take in account biological variability. The developed averaged spectra for each group was then utilized to generate ROC curves to evaluate if there were significant changes between exposure groups. Receiver operating characteristic (ROC) curves were generated using SCiLs Lab software (Bruker Corp.) to compare the averaged spectra between the BL, HC, and FL samples. A two-way analysis of variance (ANOVA)

10.3389/frspt.2022.971229

TABLE 1 AUC values generated from ROC curves for each *m/z*. Data from Figures 3–5 are highlighted in yellow. BL = Baseline; HC = Habitat; FL = Microgravity exposed.

M/z	HC	FL	*M/z*	HC	FL	*M/z*	HC	FL
3459	0.568	0.483	7199	0.562	0.475	10373	0.542	0.42
3479	0.567	0.483	7374	0.552	0.459	10394	0.541	0.428
4940	0.569	0.478	7541	0.56	0.477	10618	0.524	0.418
4967	0.581	0.527	7668	0.571	0.478	10648	0.556	0.456
4986	0.575	0.491	7755	0.557	0.464	10954	0.562	0.444
5174	0.566	0.474	7799	0.565	0.465	11016	0.542	0.415
5190	0.561	0.464	7936	0.553	0.457	11090	0.546	0.442
5349	0.561	0.469	8033	0.543	0.442	11131	0.535	0.413
5375	0.558	0.472	8078	0.551	0.445	11192	0.54	0.43
5410	0.559	0.468	8110	0.547	0.444	11228	0.55	0.432
5430	0.559	0.467	8145	0.546	0.442	11271	0.581	0.451
5463	0.565	0.477	8277	0.546	0.441	**11308**	**0.662**	**0.598**
5448	0.569	0.492	8300	0.54	0.431	11330	0.612	0.531
5556	0.557	0.466	8334	0.564	0.459	11356	0.641	0.566
5654	0.576	0.494	8370	0.544	0.432	11397	0.579	0.469
5679	0.576	0.491	8389	0.544	0.437	11409	0.561	0.451
5710	0.569	0.483	8449	0.546	0.441	11437	0.553	0.429
5728	0.562	0.468	8494	0.56	0.461	11454	0.55	0.43
5755	0.569	0.478	8550	0.557	0.459	11478	0.546	0.43
5771	0.566	0.473	8571	0.558	0.458	11527	0.557	0.438
5973	0.56	0.468	8667	0.555	0.46	11562	0.554	0.435
6050	0.567	0.475	8702	0.556	0.457	12138	0.531	0.444
6069	0.564	0.474	8798	0.551	0.452	12183	0.542	0.46
6088	0.566	0.485	8837	0.538	0.434	12328	0.524	0.418
6226	0.573	0.491	8883	0.541	0.437	12376	0.526	0.429
6279	0.575	0.491	8952	0.541	0.445	12445	0.52	0.418
6294	0.572	0.476	8993	0.556	0.47	13389	0.528	0.413
6373	0.554	0.457	9069	0.536	0.427	13432	0.526	0.422
6484	0.559	0.463	9086	0.553	0.462	13646	0.525	0.415
6546	0.568	0.479	9101	0.54	0.438	13781	0.545	0.497
6576	0.566	0.475	9172	0.547	0.448	13801	0.539	0.501
6634	0.565	0.455	9213	0.559	0.465	13816	0.541	0.494
6652	**0.599**	**0.509**	9250	0.549	0.442	13836	0.529	0.521
6704	0.569	0.479	9293	0.59	0.495	13872	0.535	0.457
6722	0.566	0.49	9332	0.558	0.457	13909	0.547	0.47
6736	0.553	0.455	9375	0.545	0.44	13968	0.564	0.503
6860	0.566	0.467	9410	0.539	0.429	14012	0.571	0.578
6902	0.57	0.489	9466	0.541	0.432	14040	0.563	0.582
6955	0.556	0.456	9538	0.531	0.417	14063	0.544	0.615
6984	0.569	0.478	9625	0.545	0.44	14096	0.572	0.524
7006	0.571	0.491	9645	0.55	0.448	**14144**	**0.536**	**0.558**
7026	0.568	0.49	9922	0.533	0.426	14222	0.543	0.526
7048	0.57	0.489	9985	0.528	0.429	15066	0.534	0.446
7075	0.566	0.505	10020	0.541	0.421	15325	0.589	0.509
7110	0.569	0.494	10262	0.534	0.42	18412	0.511	0.451

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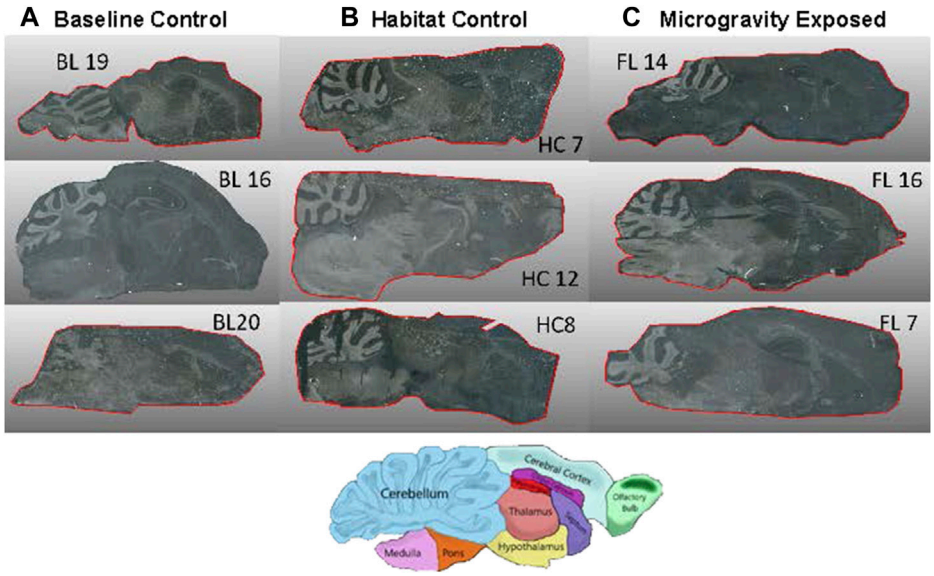

FIGURE 2
High Resolution images of 12 μm tissue samples from three animals per exposure group. Diagram indicates brain domains in orientation as spectral images. **(A)** BL = Baseline control, **(B)** HC = Habitat Control, **(C)** FL = Microgravity exposed.

comparison was performed utilizing GraphPad Prism V7.0 to confirm the data.

3 Results

MALDI-TOF imaging captures the highest abundance proteins on the surface of the tissue sample. A total of 135 intact protein ions were acquired *via* mass spectrum across tissue slices generated from each of the 9 brains (3 per exposure group) analyzed. All images were normalized by Total Ion Current (TIC). An average mass spectra (Table 1) was generated for each group as described above to account for biological variability between animals (Figure 1). For each *m/z* peak on the spectra acquired, an image was generated with the corresponding ion. Select sample images reflecting *m/z* 6,652 (Figure 3), *m/z* 11,308 (Figure 4), and *m/z* 14,144 (Figure 5) from each exposure group indicate some areas of high level proteomic alterations (approximate hypothalamic region, Figure 5C, FL), but no uniformity within groups or across groups were noted. Landmark notation of specific brain regions was difficult due to brain tissue distortion after collection during freezing. The cerebellum was noted (Figure 2) but other more subtle domains could not be discerned.

Receiver operating characteristic (ROC) curves were generated in SCiLs Lab software to determine significant change that occurred during exposure to the microgravity environment found onboard the ISS. Area under the curve (AUC) values between 0.3 and 0.7 convey that there were no significant shifts seen between FL and HC compared against the Baseline. To confirm this data, Prism was utilized to perform statistical analysis (Table 2). A 2-way ANOVA comparison was utilized to verify that there were no significant changes determined between the protein ions detected. There were no adjusted *p*-values that indicated statistically significant changes in the microgravity brain proteome compared to baseline or habitat control values. There are several potential study design variables, discussed below, which may have impacted our ability to parse out the proteomic signature differences between the exposure groups.

4 Discussion

MALDI-TOF-IMS can be utilized to include differential expression of lipids and metabolites, in addition to proteins, to create a time-specific map of spatially-connected molecular alterations. (Murphy et al., 2009; Aichler and Walch, 2015) Such multi-omic molecular maps are especially important in the multi-domain brain, where targeted Omic analyses are problematic if one does not have *a priori* knowledge on which or now many brain regions are modulated in response to a given external exposure or condition. Identification of the specific locales of differential expression allow for a secondary targeted Omics approach, more likely to produce statistically stronger data as the tissue analyzed has first been identified as relevant to the response rather than generated *via* a "tissue-averaged" signal. Precise isolation of very small tissue regions can then be accomplished using targeted laser dissection for in-depth

TABLE 2 *p* values resulting from a two-way ANOVA Statistical Analysis of spectra values, as compared to Baseline. HC = Habitat; FL = Microgravity exposed. Specific *m/z* comparisons from spectral images (Figures 3–5) are highlighted in yellow.

	Adjusted *p* -Value			Adjusted *p* -Value			Adjusted *p* -Value			Adjusted *p* -Value	
M/z	**HC**	**FL**	***M/z***	**HC**	**FL**	***M/z***	**HC**	**FL**	***M/z***	**HC**	**FL**
3459	0.9997	0.9996	6860	0.9999	0.9999	9069	0.9999	0.9999	11478	0.9999	0.9999
3479	0.9996	0.9998	6902	0.9999	0.9999	9086	0.9999	0.9999	11527	0.9999	0.9999
4940	0.9996	0.9917	6955	0.9999	0.9999	9101	0.9999	0.9999	11562	0.9999	0.9999
4967	0.8757	0.9330	6984	0.9999	0.9999	9172	0.9999	0.9999	12138	0.9965	0.9999
4986	0.9871	0.9935	7006	0.9974	0.9984	9213	0.9999	0.9999	12183	0.9997	0.9999
5174	0.9999	0.9999	7026	0.9999	0.9999	9250	0.9999	0.9999	12328	0.9999	0.9999
5190	0.9999	0.9999	7048	0.9999	0.9999	9293	0.9999	0.9999	12376	0.9999	0.9999
5349	0.9999	0.9999	7075	0.9973	0.9875	9332	0.9729	0.9733	12445	0.9999	0.9999
5375	0.9998	0.9999	7110	0.9999	0.9999	9375	0.9999	0.9999	11478	0.9999	0.9999
5410	0.9999	0.9999	7199	0.9998	0.9999	9410	0.9999	0.9999	11527	0.9999	0.9999
5430	0.9999	0.9999	7374	0.9999	0.9999	9466	0.9999	0.9999	11562	0.9999	0.9999
5463	0.9897	0.9676	**7541**	**0.9999**	**0.9999**	9538	0.9999	0.9999	12138	0.9999	0.9999
5448	0.9999	0.9999	7668	0.9999	0.9999	9625	0.9999	0.9999	13389	0.9965	0.9999
5556	0.9999	0.9999	7755	0.9999	0.9999	9645	0.9999	0.9999	13432	0.9999	0.9999
5654	0.9989	0.9990	7799	0.9999	0.9999	9922	0.9999	0.9999	13646	0.9999	0.9999
5679	0.9991	0.9999	7936	0.9999	0.9999	9985	0.9999	0.9999	13781	0.9999	0.9999
5710	0.9999	0.9999	8033	0.9999	0.9999	10020	0.9999	0.9999	13801	0.9999	0.9999
5728	0.9999	0.9999	8078	0.9999	0.9999	10262	0.9999	0.9999	13816	0.9997	0.9999
5755	0.9999	0.9999	8110	0.9999	0.9999	10373	0.9999	0.9999	13836	0.9999	0.9999
5771	0.9999	0.9999	8145	0.9999	0.9999	10394	0.9999	0.9999	13872	0.9999	0.9999
5973	0.9999	0.9999	8277	0.9999	0.9999	10618	0.9999	0.9999	13909	0.9999	0.9999
6050	0.9997	0.9999	8300	0.9999	0.9999	10648	0.9999	0.9999	13968	0.9999	0.9999
6069	0.9999	0.9999	8334	0.9999	0.9999	10954	0.9999	0.9999	14012	0.9891	0.9441
6088	0.9999	0.9999	8370	0.9999	0.9999	11016	0.9999	0.9999	14040	0.9991	0.9947
6226	0.9918	0.9980	8389	0.9999	0.9999	11090	0.9999	0.9999	14063	0.9988	0.9919
6279	0.9769	0.9961	8449	0.9999	0.9999	11131	0.9999	0.9999	14096	0.9999	0.9998
6294	0.9997	0.9998	8494	0.9999	0.9999	11192	0.9999	0.9999	**14144**	**0.9750**	**0.5072**
6373	0.9999	0.9999	8550	0.9999	0.9999	11228	0.9999	0.9999	14222	0.9993	0.9999
6484	0.9999	0.9999	8571	0.9999	0.9999	11271	0.9999	0.9999	15066	0.9999	0.9999
6546	0.9999	0.9999	8667	0.9999	0.9999	**11308**	**0.8288**	**0.8427**	15325	0.9997	0.9997
6576	0.9999	0.9999	8702	0.9999	0.9999	11330	0.9857	0.9813	18412	0.9999	0.9999
6634	0.9999	0.9903	8798	0.9999	0.9999	11356	0.9597	0.9662	13389	0.9999	0.9999
6652	**0.6854**	**0.7819**	8837	0.9999	0.9999	11397	0.9999	0.9999			
6704	0.9993	0.9996	8883	0.9999	0.9999	11409	0.9999	0.9999			
6722	0.9999	0.9999	8952	0.9999	0.9999	11437	0.9999	0.9999			
6736	0.9999	0.9999	8993	0.9999	0.9997	11454	0.9999	0.9999			

discovery of mechanistic pathways. Thus, imaging is the critical initial approach to use an integrated top down and bottom up Omics approach for the identification of molecules of interest in a targeted brain region. (Ntai et al., 2016)

Technology and software have made it possible to construct three dimensional images of the organ being analyzed using MALDI-IMS capabilities. (Seeley and Caprioli, 2012; Vos et al., 2020) This would provide an ultimate mapping of spatial shifts in protein, lipids and metabolites. (Seeley and Caprioli, 2011) The employment of the full Omic suite could be employed on multiple organs in *vivo* studies allowing for an understanding of how all the biological functions are impacted by spaceflight. Bridging the medicinal and biochemical studies together can lead to steps to develop countermeasures to combat detrimental effects caused by microgravity.

This study aimed to examine proteome shifts initiated by microgravity by spatially resolving surface protein ions on brain

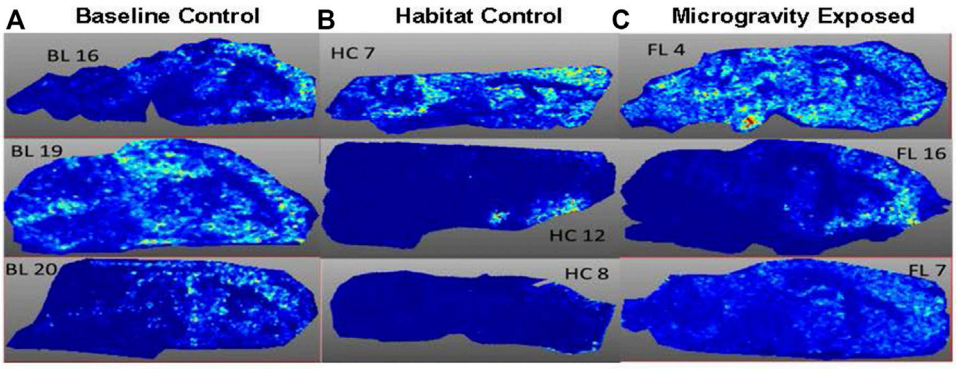

FIGURE 3
Spatial Image obtained at ***m/z* 6,652** from three animals per exposure group. **(A)** BL = Baseline group; **(B)** HC = Habitat control group; **(C)** FL = Microgravity exposed group.

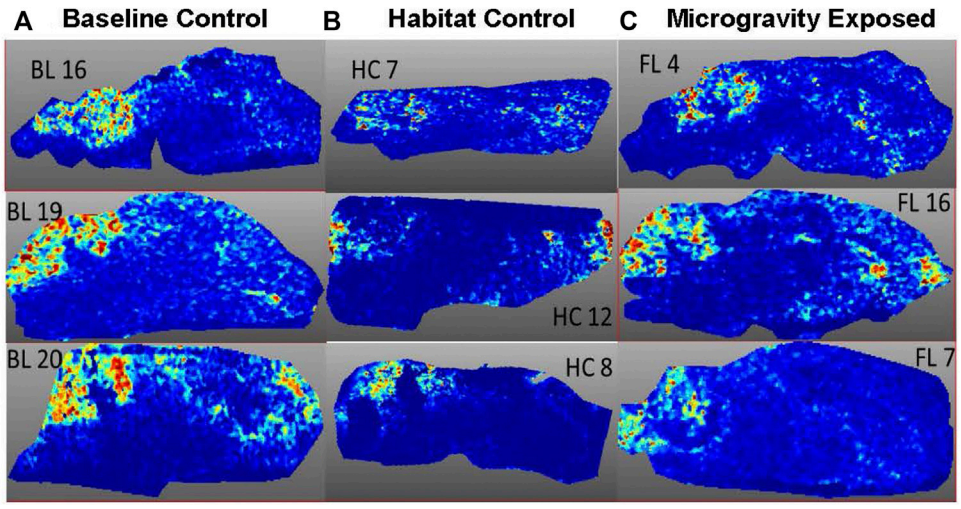

FIGURE 4
Spatial Image obtained at ***m/z* 11,308** from three animals per exposure group. **(A)** BL = Baseline control, **(B)** HC = Habitat control, **(C)** FL = Microgravity exposed.

tissue samples in order to visualize proteomic shifts between different exposure groups. The results also served to observe biological variation between each animal in different brain regions. After 36 days in a microgravity environment, no significant changes in protein expression were observed using MALDI-TOF-IMS within the highest abundant intact protein ions that were detected. Other research has indicated that microgravity does have an effect on protein expression, although these studies only utilized simulated microgravity. (Sarkar et al., 2006; Ishikawa et al., 2017) These published experiments had the distinct advantage of performing dissection and tissue preparation quickly after exposure. In this study, the logistics of retrieval of the animals from ISS and tissue dissection post-spaceflight posed an unavoidable delay from exposure time to tissue collection, a delay of over 3 days. Therefore, it is likely that the failure to identify statistically significant proteome alterations was due to this time lapse, allowing the mice in this study reacclimatized in the period before dissection, and that any unique proteome signatures induced by the microgravity exposure were lost. A previous proteomic study by Mao et al. utilizing LC-MS analysis confirmed that there were 26 significant protein changes in the mouse brain after spaceflight. (Mao et al., 2018) The samples were processed for analysis within 3–5 h after Space

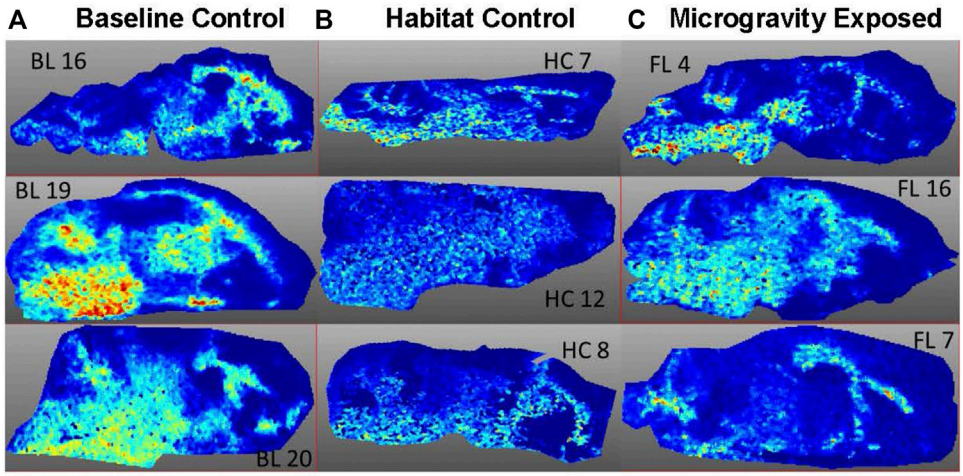

FIGURE 5
Image of ***m/z* 14,144** from three animals per exposure group. **(A)** BL = Baseline, **(B)** HC = Habitat, **(C)** FL = Microgravity exposed.

Shuttle Atlantis returned to Earth after a 13 days space mission. However, the study did not confirm whether the proteome alterations were induced by the microgravity or due to the stress induced from landing. To parse out stress vs. microgravity-induced alternations, future studies should include a secondary control group that could mimic the launch exposure without gravitational changes, possibly by use of NASA astronaut training equipment.

In addition, the ability to perform dissection and sample collection on board the ISS would clarify the lack of proteome responsivity to microgravity between the microgravity exposed (FL) animals and the control sets as analyzed by MALDI-TOF-IMS. MALDI-TOF-IMS is a complex technique and sample preparation is a critical issue. In this study, despite care, the brain morphology were not uniform across the specimens (Figure 2). As such, cryosectioning of the brain tissue using landmarks for spatial sectioning provided its own unique challenges in order to acquire comparable sagittal sections for imaging. However, certain brain regions are clearly observable (note cerebellum in Figure 2 and corresponding proteome activity in Figures 3–5).

Data quality with respect to signal intensity and reproducibility is also impacted by sample handling. (Watrous and Dorrestein, 2013) Matrix application is critical to protonating the proteins on the surface tissue and influences sensitivity. Matrix crystal formation is critical in this application. Improving deposition or sublimation in the matrix application process can possibly lead to an increase in reproducibility of application and better sensitivity of analytes (Hankin et al., 2007; Gemperline et al., 2014).

With future improvements in sample collection as discussed above, MALDI-TOF-IMS analysis of a second set of microgravity-exposed brain tissue could reveal not only the global proteome alterations but brain domain and spatial locale of those shifts. With further analyses to identify the proteins within these proteome alteration 'hot spots', an in-depth understanding of pathway alterations underpinning the subtle cognitive neuro and/or physiological effects of microgravity could be determined. Such effects have been seen using MRI analysis of post-flight astronauts and include increases in brain ventricular volume and spaceflight-associated neuro-ocular syndrome (Roberts et al., 2019).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by This study was conducted under an IACUC approved protocol in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011) and approved by the NASA Institutional Animal Care and Use Committee.

Author contributions

CV, HC, and CM wrote original CASIS proposal. CV, HC, and AD developed methodologies, conducted sample analyses, and completed final statistical evaluations. JS conducted sample preparation and analyses. CM provided project guidance and management. All authors contributed to the development of the manuscript, and CM provided final edits and submissions.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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