Lipidome Alterations in Mild Traumatic Brain Injury Mapped via FTICR Mass Spectrometry Imaging

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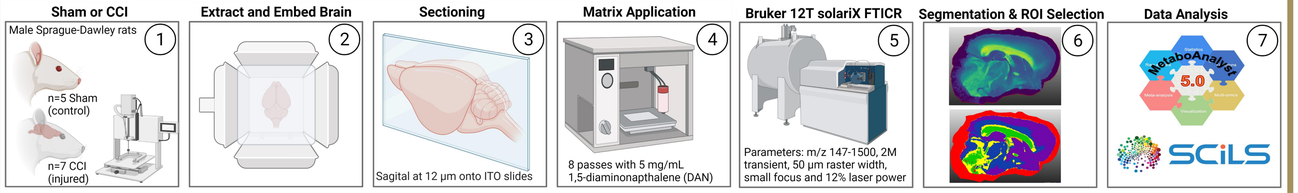
BACKGROUND

- Traumatic Brain Injury (TBI) is caused by excessive external force to the brain that alters normal brain function, affecting quality of life. [1]
- TBI is a significant cause of death and disability worldwide for all ages. [2]
- There are more than 50 million TBI incidents worldwide annually, of which 70-90% are estimated to be mild TBI, and this costs the economy $400 billion annually. [2]
- Despite its widespread occurrence, there are no FDA approved neuroprotective treatments for TBI and diagnosis is challenging due to the heterogeneity of the disease and lack of objective measurements. [3]
- TBI is biphasic; the secondary injury occurs after the tissue damage from the initial impact and involves damage from biochemical and physiological processes including neuroinflammation and oxidative stress. This secondary phase remains poorly understood.
- Mass spectrometry imaging (MSI) will be used to study the biochemical changes of lipids involved in the secondary phase in order to gain more insight on the pathology of mild TBI in the brain.
- **Objective: Study spatial lipidome alterations in rat brains following closed head injury using an ultrahigh resolution MSI approach.**

METHODS

- Mild repetitive TBI was modeled by subjecting rats to three closed head impacts (2 min interval; 5 m/s; 5mm, 2mm, and 2mm head displacement) to the dorsal head surface using a modified controlled cortical impact device.
- 72h after injury rats were perfused with cold phosphate buffer, the brains were extracted, flash frozen and embedded in a gelatin mixture.



RESULTS

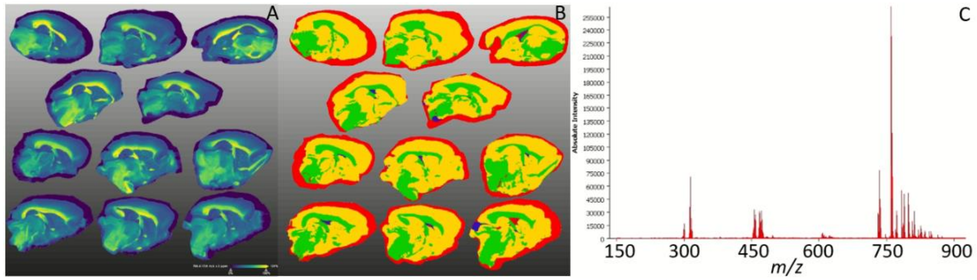


Figure 1. Overview of the Dataset. (A) Images of brains at *m/z* 788.61738 +/- 2 ppm (PC 36:1), localized to the white matter (light yellow). Top 5 brains are control and bottom 6 are injured. RMS normalization and medium denoising were used. **(B)** Segmentations of all brains, demonstrating good reproducibility and that brains are similar in anatomy. **(C)** Average mass spectrum of all 11 brains from *m/z* 147-900 (no visible peaks beyond 900).

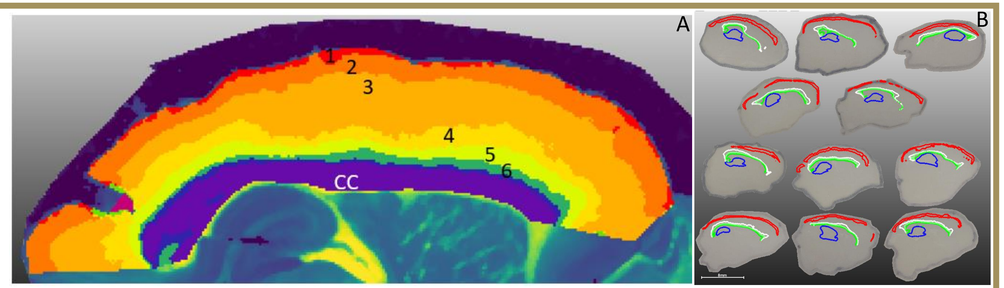


Figure 2. Segmentation of Rat 3 Cortex and Region of Interest (ROI) Selection in All Brains. (A) Example of how the brain layers and corpus callosum were chosen using segmentation in rat 3. Layers are numbered and roughly correspond to cortical brain layers. Layer 6 is the two layers directly above the corpus callosum. **(B)** Selection of ROIs for all brains: hippocampus in blue, layer 2 in red, layers 4 and 5 in white, layer 6 in green.

Tentative ID	Error (ppm)	Experimental	Exact
LPC (O-16:1)	-3.46	480.34319	480.34485
ROIs	**AUC**	**FC**	**P-value**
HC	0.67	1.45	0.20
CC	0.70	1.85*	0.48
Layer 2	0.73	1.30	0.19
Layers 4&5	0.77	1.56	0.20
Layer 6	0.80*	1.82*	0.20
EB	0.80*	1.52	0.13

Tentative ID	Error (ppm)	Experimental	Exact
LPC (16:0)	-1.45	496.33905	496.33976
ROIs	**AUC**	**FC**	**P-value**
HC	0.93*	1.23	0.01*
CC	0.70	1.18	0.32
Layer 2	0.80*	1.20	0.09*
Layers 4&5	0.83*	1.19	0.08*
Layer 6	0.77	1.18	0.19
EB	0.90*	1.22	0.02*

Tentative ID	Error (ppm)	Experimental	Exact
SM (32:6;O5)	1.99	713.45147	713.45005
ROIs	**AUC**	**FC**	**P-value**
HC	0.80*	1.25	0.19
CC	0.60	2.17*	0.30
Layer 2	0.70	1.43	0.16
Layers 4&5	0.67	1.28	0.28
Layer 6	0.63	1.72*	0.26
EB	0.72	1.22	0.32

Tentative ID	Error (ppm)	Experimental	Exact
PC (36:2)	-1.20	786.59979	786.60073
ROIs	**AUC**	**FC**	**P-value**
HC	0.83*	0.94	0.11
CC	0.60	0.93	0.27
Layer 2	0.63	0.98	0.31
Layers 4&5	0.80*	0.94	0.22
Layer 6	0.70	0.93	0.23
EB	0.67	0.97	0.34

Tentative ID	Error (ppm)	Experimental	Exact
PC (36:1)	0.54	788.61681	788.61638
ROIs	**AUC**	**FC**	**P-value**
HC	0.83*	0.93	0.11
CC	0.73	0.94	0.14
Layer 2	0.70	0.95	0.18
Layers 4&5	0.67	0.93	0.17
Layer 6	0.80*	0.93	0.08*
EB	0.57	0.98	0.71

Tentative ID	Error (ppm)	Experimental	Exact
CL (74:0)	0.16	1494.13624	1494.13601
ROIs	**AUC**	**FC**	**P-value**
HC	0.80*	1.79*	0.07*
CC	0.80*	1.45	0.10*
Layer 2	0.70	1.52	0.19
Layers 4&5	0.83*	1.67	0.07*
Layer 6	0.80*	1.52	0.09*
EB	0.77	1.56	0.09*

Table 1. Ions of Interest from ROC Analysis Found in Multiple ROIs. The AUC , FC and p-values from ROC analysis for select ions in ROIs (HC = hippocampus; CC = corpus callosum; EB = entire brain).

Tentative ID	Error (ppm)	*m/z* (exper)	AUC	FC	P-value
Hippocampus					
SHexCer (33:0;O2)	2.2	768.53068	0.83	0.48*	0.12
PC (O-36:2)	-1.5	772.62033	0.83	0.60	0.06*
PC (O-36:1)	0.4	774.63742	0.83	0.56	0.06*
PC (36:2) [^{13}C]	1.0	787.60492	0.80	0.94	0.26
PC (O-38:6)	2.6	792.59223	0.80	4.76*	0.05*
PC (40:6)	-0.6	834.60021	1.00*	0.91	0.00*
PC (42:1)	1.2	872.71133	0.80	0.38*	0.15
Corpus Callosum					
SM (40:2;O2) [^{13}C]	-0.9	786.65571	0.83*	5.26*	0.04*
SHexCer (35:3;O2)	-0.8	790.51271	0.73	1.41	0.27
PC (36:2) [M+K]$^+$	-0.3	824.55638	0.77	0.87	0.25
PC (36:1) [M+K]$^+$	-0.2	826.57211	0.73	0.90	0.20
Layer 2					
LPC (18:1)	-2.2	522.35426	0.80*	1.39	0.18
PC (34:1)	0.2	760.58521	0.77	0.98	0.19
PC (38:4)	-2.1	810.59903	0.77	0.93	0.11
SM (42:1;O2)	-2.7	815.69789	0.60	0.18*	0.18
TG (64:17;O3)	1.8	1045.71456	0.72	0.50*	0.17

Table 2. Ions of Interest from ROC Analysis Unique to Specific ROIs. The AUC, FC and t-test values from ROC analysis for select ions in specific ROIs. No ions from Table 1 are included here.

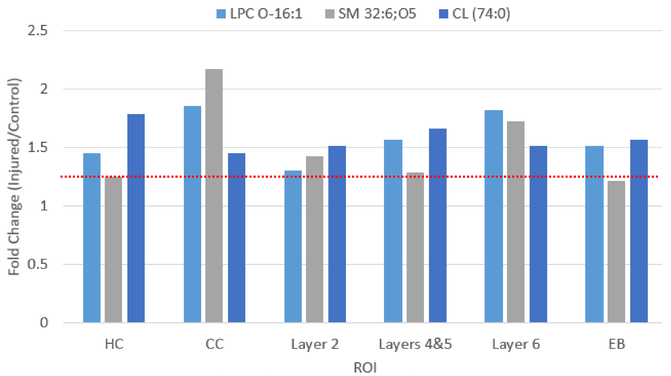


Figure 3. Fold Change Plot of Select Ions for All ROIs. Average intensity values for an ROI were RMS normalized. The plot demonstrates increases of the select ions in the injured ROIs. Red line is 1.25 FC.

CONCLUSIONS

- Utilized SCiLS Lab's segmentation tool to reproducibly select ROIs including cortical brain layers despite the biological variability of brains (Figure 2).
- ROC analysis identified several ions that showed discriminatory potential between control and injured brains (Tables 1 and 2).
- LPC (16:0) and CL (74:0) had significant AUC and p-values for almost all ROIs (Table 1) → LPC (16:0) is a demyelinating agent that has been shown to increase after TBI and is associated with memory performance.
- PC (40:6) had an AUC value of 1.00 and a p-value of 0.00085 in the hippocampus (Table 2).
- LPC (O-16:1), SM (32:6;O5) and CL (74:0) had fold changes around 1.5, ranging between 1.22 and 2.17, in the injured ROIs relative to control (Table 1 and Figure 3) → LPC, SM and CL have all previously been associated with TBI.
- Future work
  - Recalibration of m/z scale to improve accuracy
  - MS/MS for more definitive annotation
  - Multivariate analysis

AKNOWLEDGEMENTS & REFERENCES

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