Electronic Supplementary Material (ESI) for Analyst.
This journal is © The Royal Society of Chemistry 2020

Supporting Information

Label-free Lipidome Study of Paraventricular Thalamic Nucleus (PVT) of Rat Brain with Post-Traumatic Stress Injury by Raman Imaging

Ardalan Chaichi[1], Syed Mohammad Abid Hasan[1], Nishir Mehta[1], Fabrizio Donnarumma[2], Philip Ebenezer[3], Kermit Murray[2], Joseph Francis[3], Manas Ranjan Gartia[1, *]

[1]Department of Mechanical and Industrial Engineering, Louisiana State University, Baton Rouge LA 70803

[2]Department of Chemistry, Louisiana State University, Baton Rouge LA 70803

[3]Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA 70803

*Corresponding author: mgartia@lsu.edu

Supporting Information includes Figures S1-S19 and Tables S1 and S2:

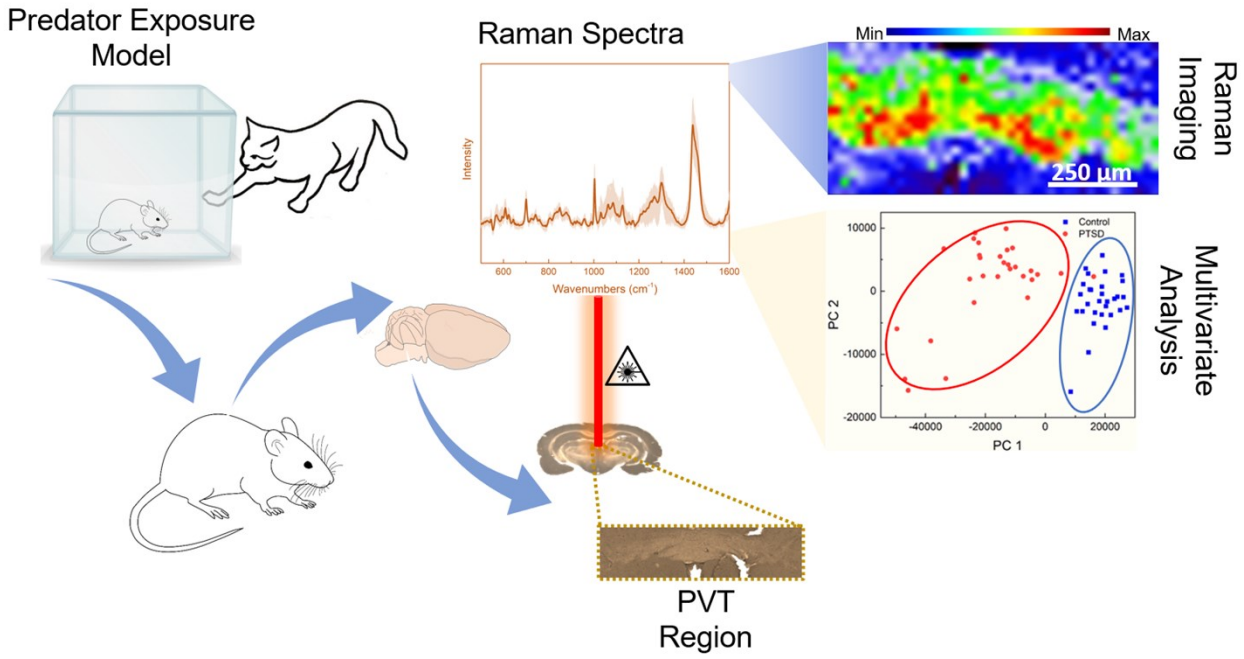


Figure S1. Schematic showing the design of experiment. Brain tissue from control and PTSD rats was obtained. Multivariate statistical analyses were performed on the Raman spectra and Raman images to identify the biochemical changes in the paraventricular nucleus of the thalamus (PVT) region of the brain.

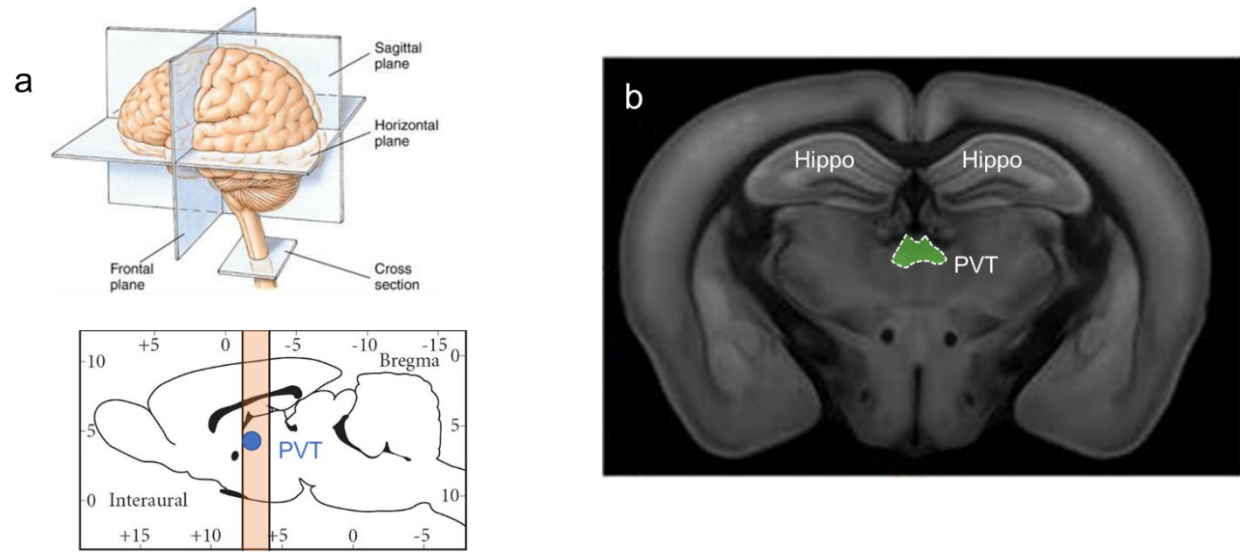
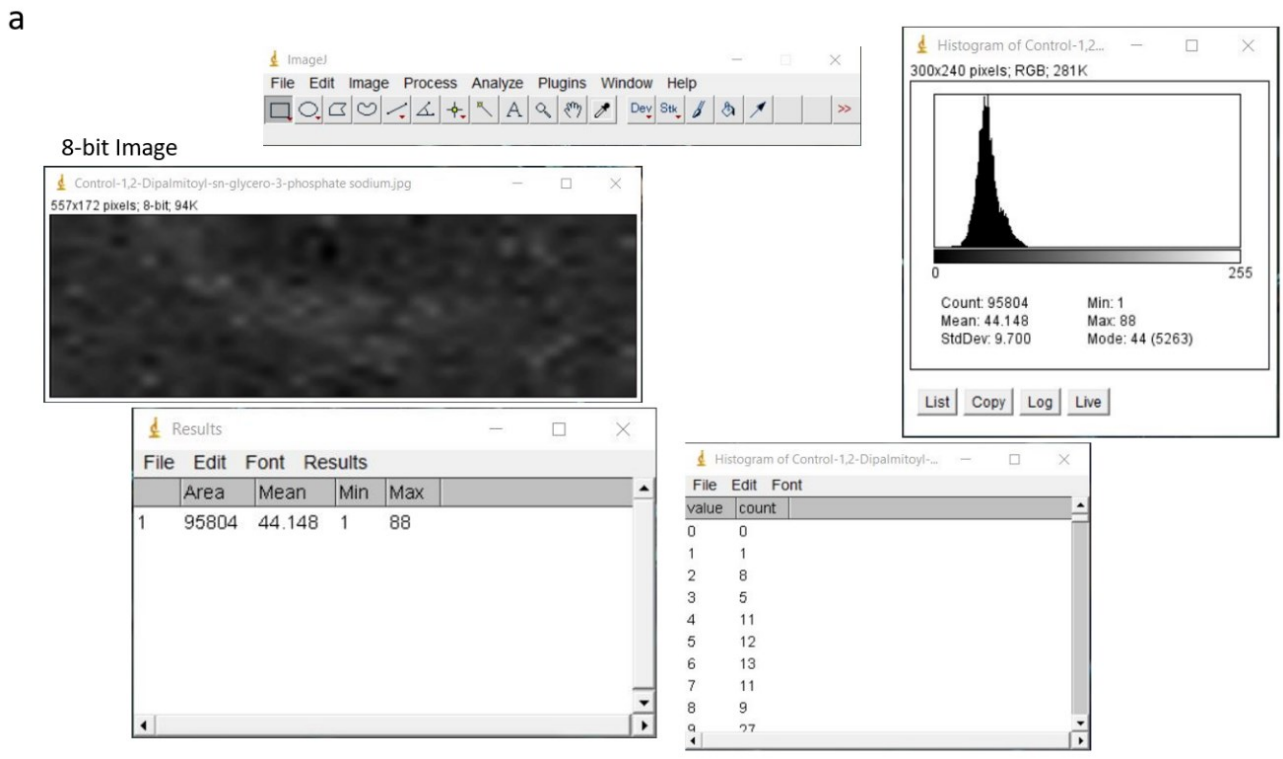
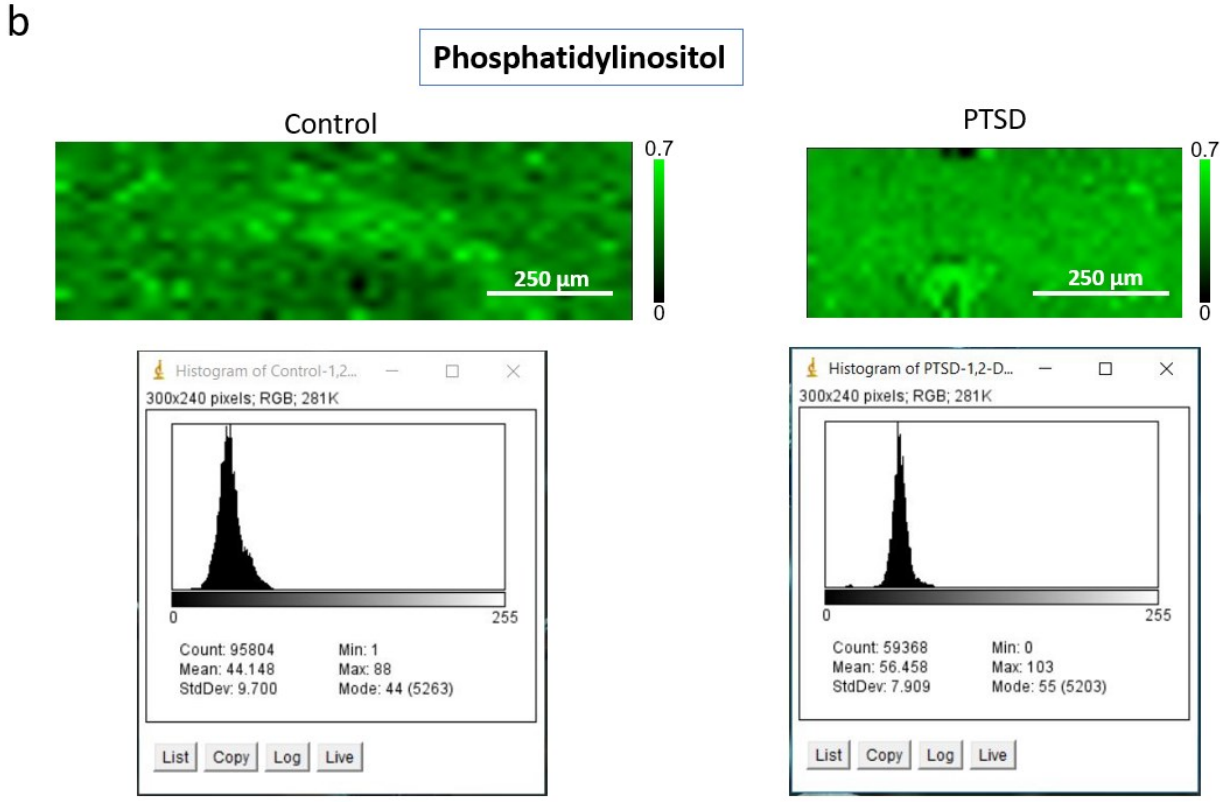


Figure S2. Paraventricular nucleus of the thalamus (PVT) region inside rat brain. The tissue section containing the PVT region using the rat brain stereotaxic coordinates in Bregma number region of -1.20 mm to -3.6 mm.



b


Phosphatidylinositol
Control
PTSD
0.7
0
250 µm
Histogram of Control-1,2...
300x240 pixels; RGB; 281K
0
255
Count: 95804
Mean: 44.148
StdDev: 9.700
Min: 1
Max: 88
Mode: 44 (5263)
List
Copy
Log
Live
Histogram of PTSD-1,2-D...
300x240 pixels; RGB; 281K
Count: 59368
Mean: 56.458
StdDev: 7.909
Min: 0
Max: 103
Mode: 55 (5203)

c

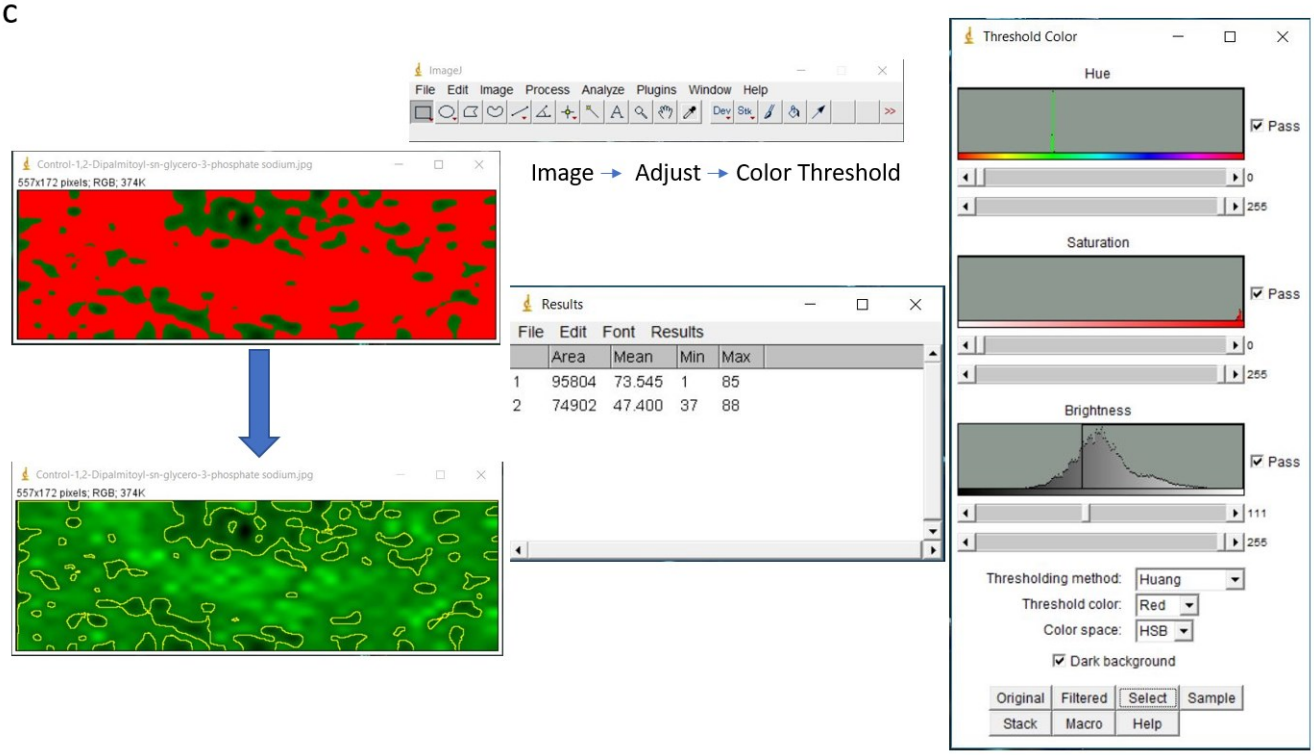

ImageJ
File Edit Image Process Analyze Plugins Window Help
Control-1,2-Dipalmitoyl-sn-glycero-3-phosphate sodium.jpg
557x172 pixels; RGB; 374K
Image → Adjust → Color Threshold
Results
File Edit Font Results
Area Mean Min Max
1 95804 73.545 1 85
2 74902 47.400 37 88
Threshold Color
Hue
Saturation
Brightness
Pass
Thresholding method: Huang
Threshold color: Red
Color space: HSB
Dark background
Original Filtered Select Sample
Stack Macro Help

Figure S3. The method used for quantification of Raman maps by means of brightness intensity values. (a) 8-bit image should be used for calculation of weighted mean value. (b) Weighted mean value can be obtained from pixel values of the 8-bit image. (c) Selected area (red) shows the region with higher pixel value than a certain threshold.

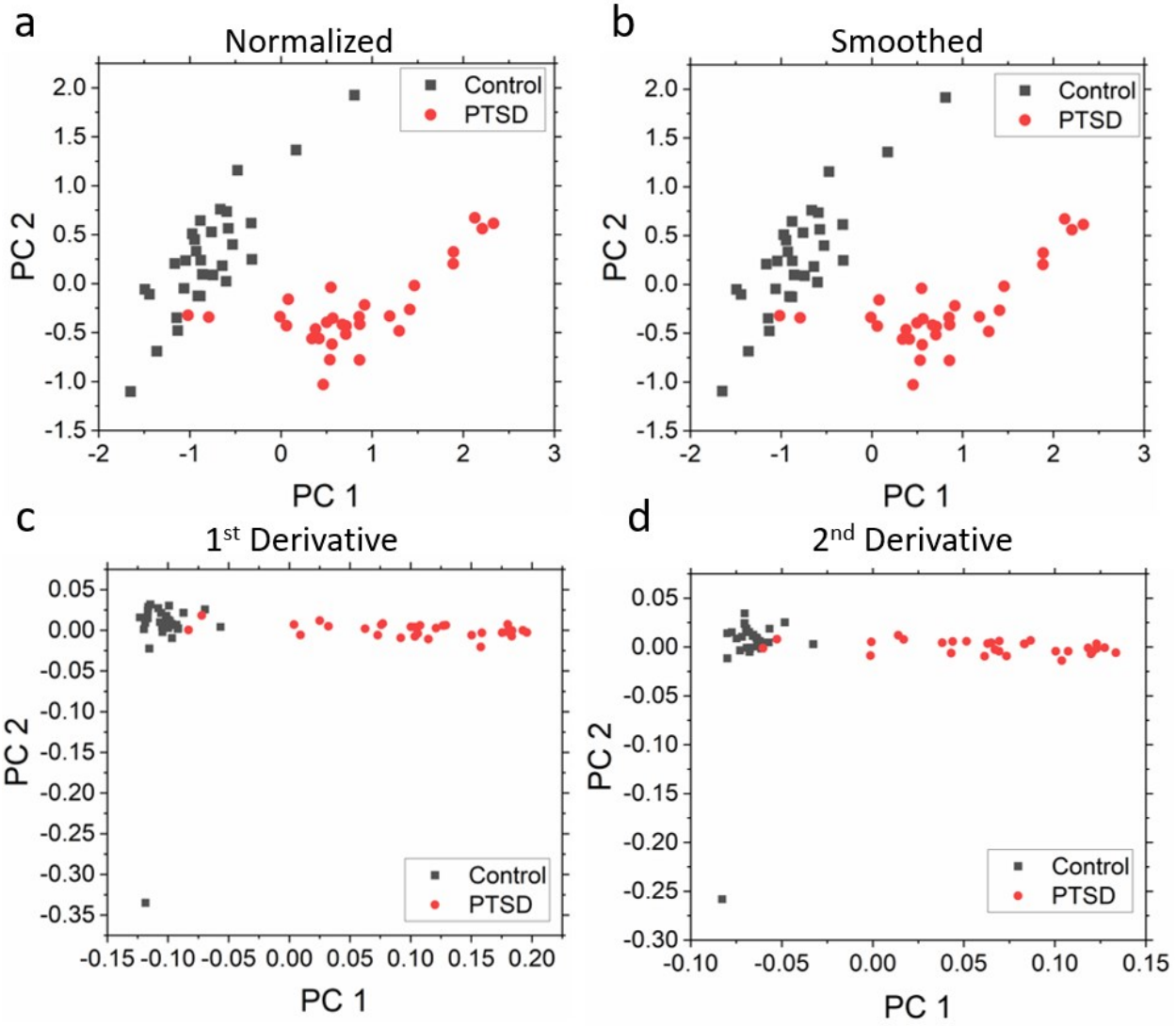


Figure S4. Different preprocessing methods performed on Control and PTSD datasets. (a) normalized, (b) smoothed, (c) 1st derivative and (d) 2nd derivative. In all the figures, there is an obvious separation between Control and PTSD.

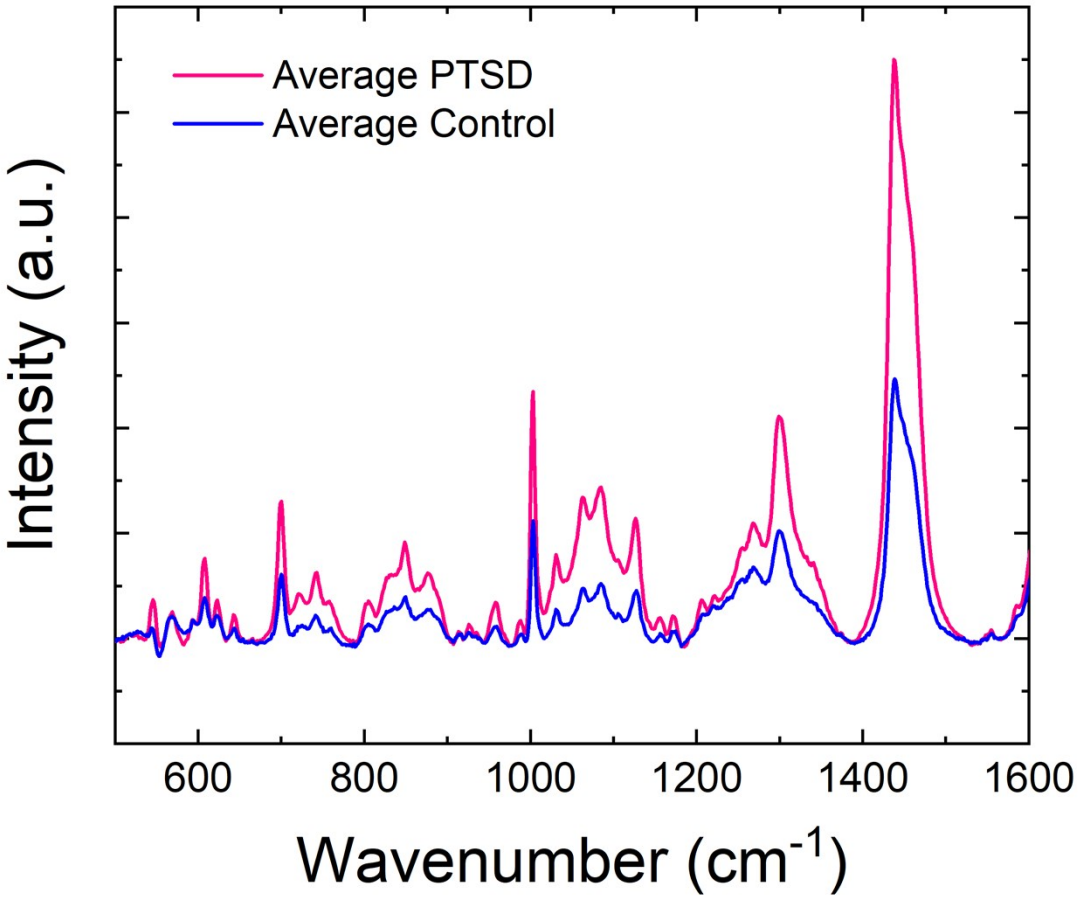


Figure S5. Average spectra of Control and PTSD samples.

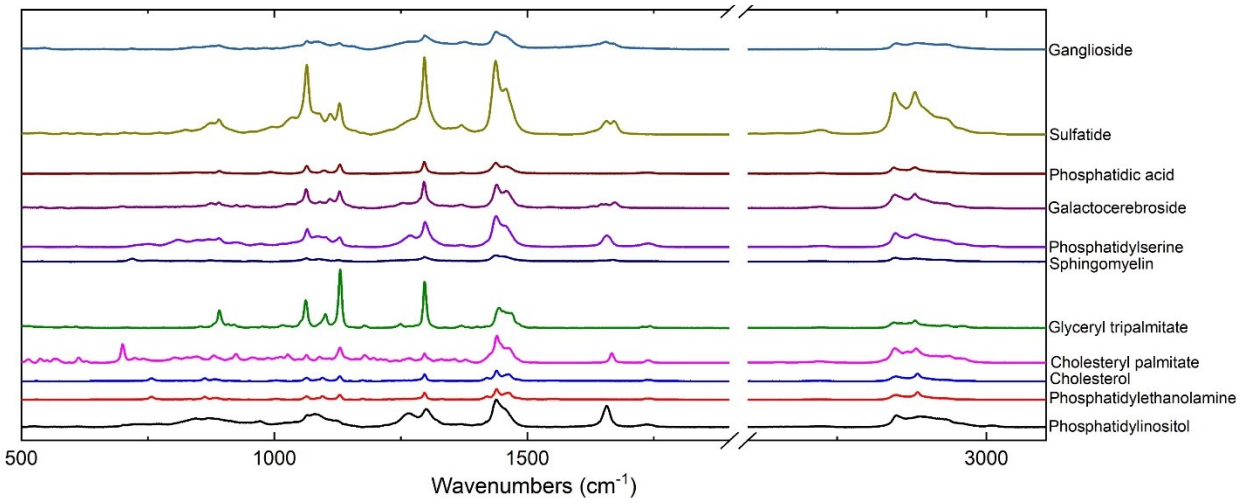


Figure S6. Full range Raman spectra of all the lipids used in this study.

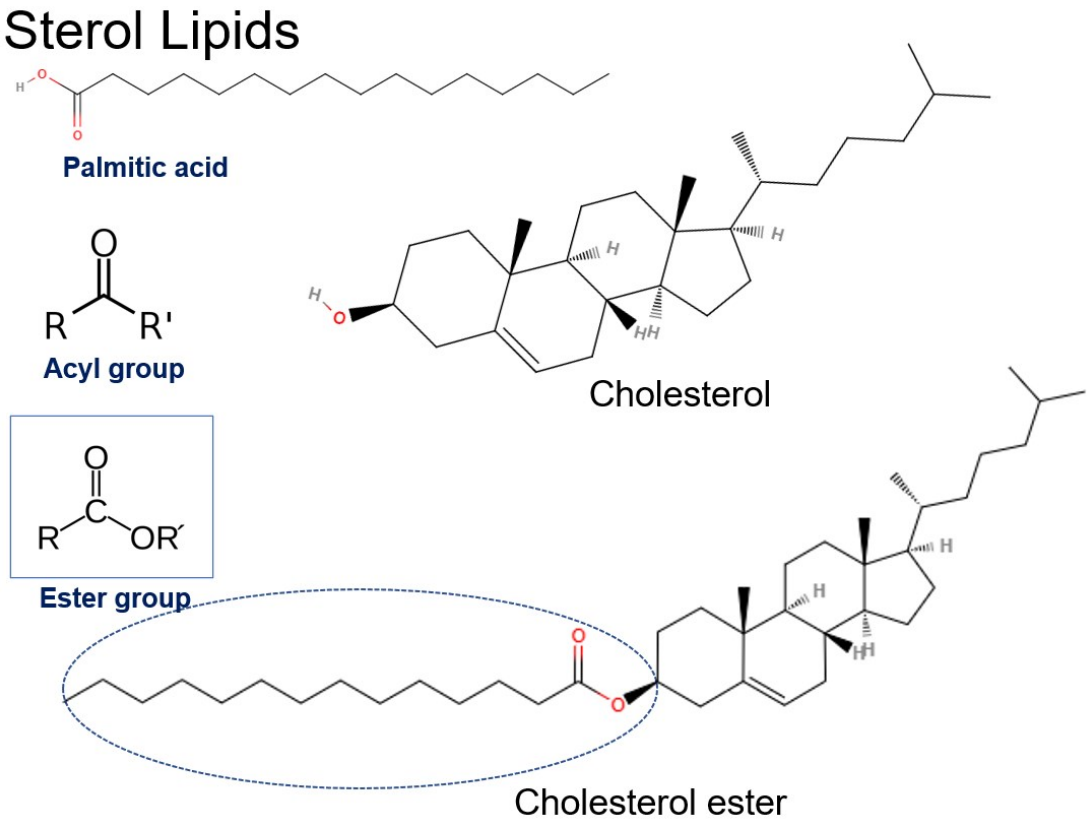


Figure S7. Comparison of cholesterol (FC) and cholesteryl palmitate (or cholesterol ester, CE) molecular structures. The Raman band due to ester group at 1737 cm^{-1} is absent in

cholesterol. Spectral band at 1065, 1130, and 1297 cm^{-1} are due to palmitic acid group in the cholesterol ester.

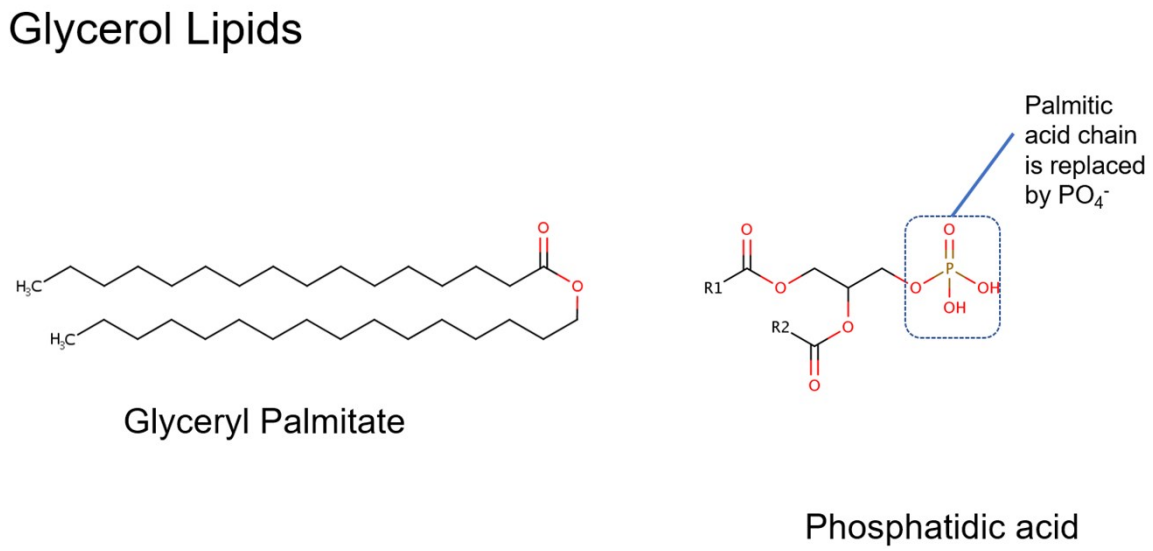


Figure S8. Comparison of glyceryl palmitate (or triacylglyceride, TAG) and phosphatidic acid (PA) molecular structures. They share quite similar structure with one palmitic acid chain in TAG is replaced by a phosphate group for PA.

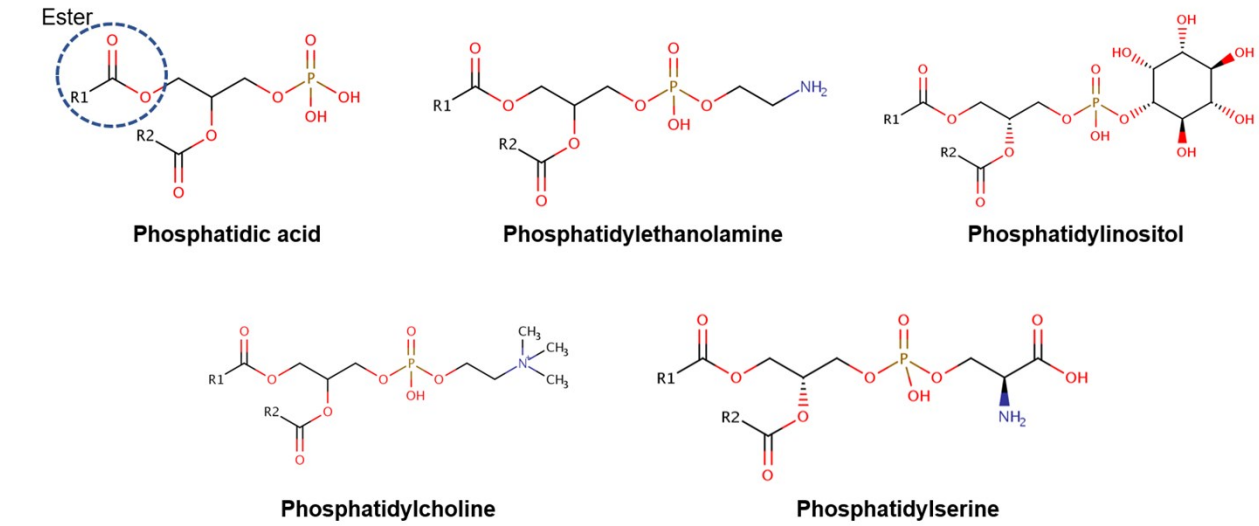


Figure S9. Molecular structure of different phospholipids (PE, PI, PC, PS). Raman bands of phospholipids share features similar to that of phosphatidic acid.

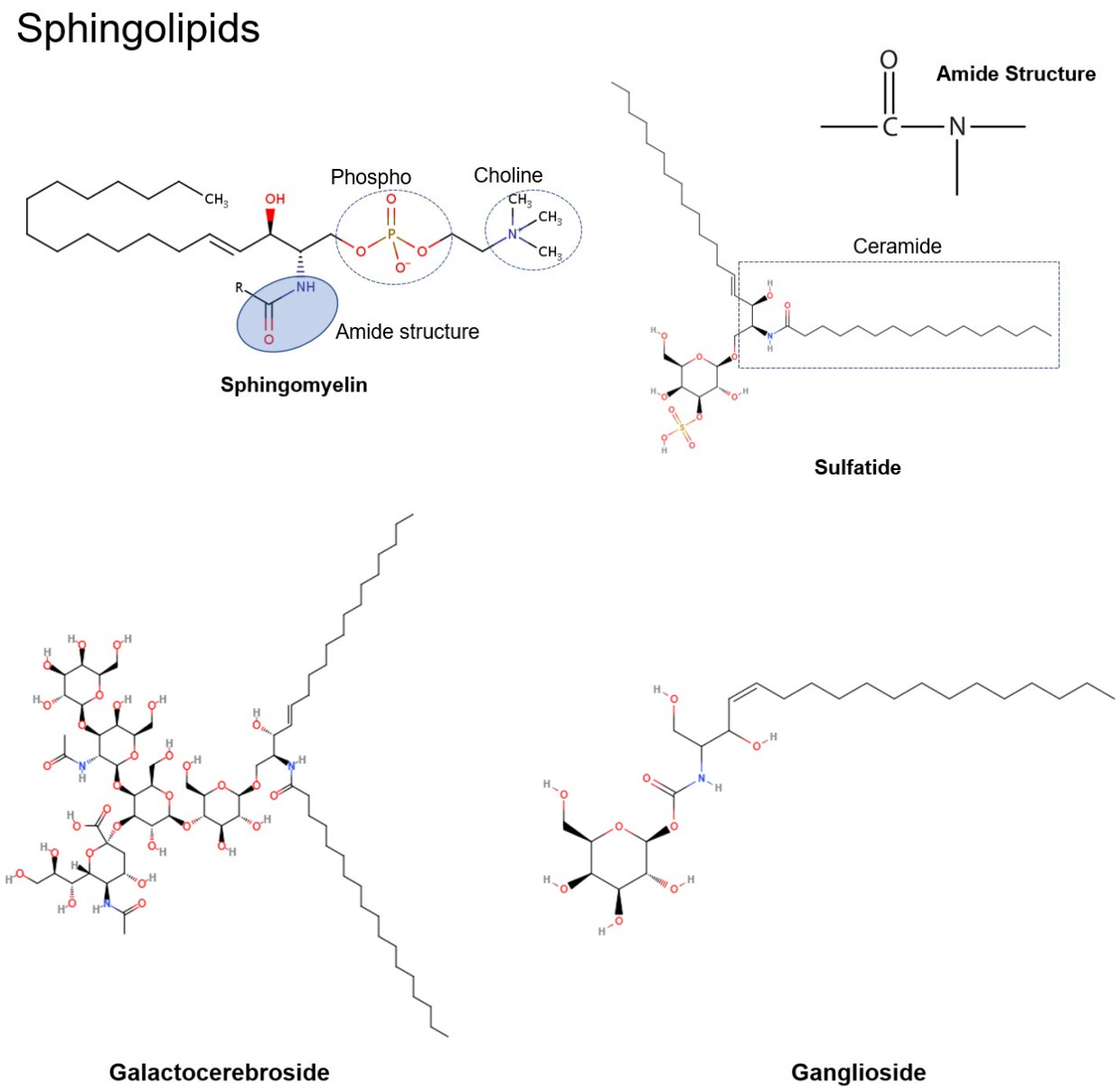


Figure S10. Comparison of the molecular structure of sphingolipids. Sphingolipids are composed of lipids, a ceramide backbone, and glucose rings with or without linker groups; the linker group for galactocerebroside (GalCer) is galactose, sulfate containing monosaccharide for sulfatide (ST), and oligosaccharides for ganglioside (GM).

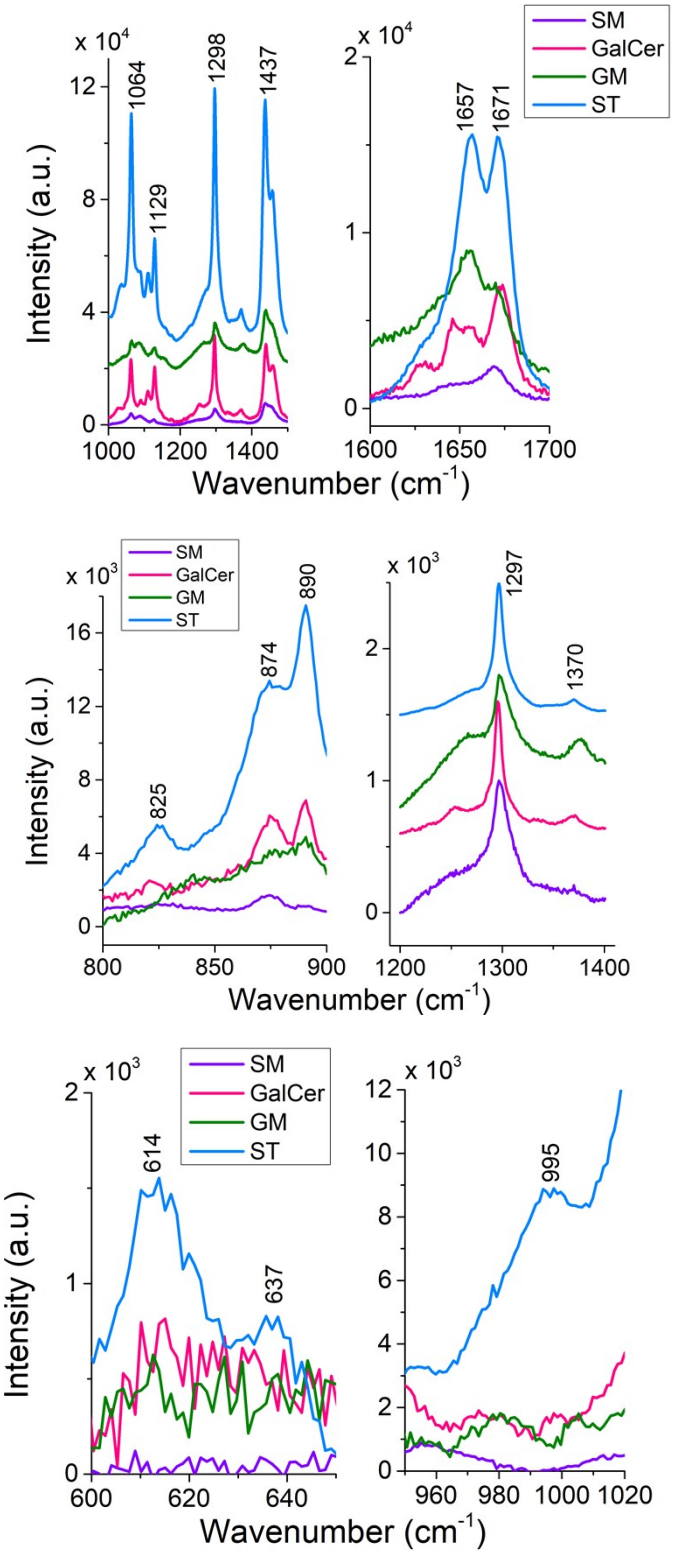


Figure S11. Raman spectra of sphingolipids at fingerprint region. Raman signature of lipids (at 1064, 1129, 1298, 1437 cm^{-1}), ceramide backbone (1657, 1671 cm^{-1}; C=C stretching), and sugar

chain (1370 cm^{-1}). The Raman peak at 890 cm^{-1} (C-O-O skeletal) mode is present in all the sphingolipids except SM as SM does not have any C-O-O group. The sulfate band in ST is characterized by 614 and 995 cm^{-1}.

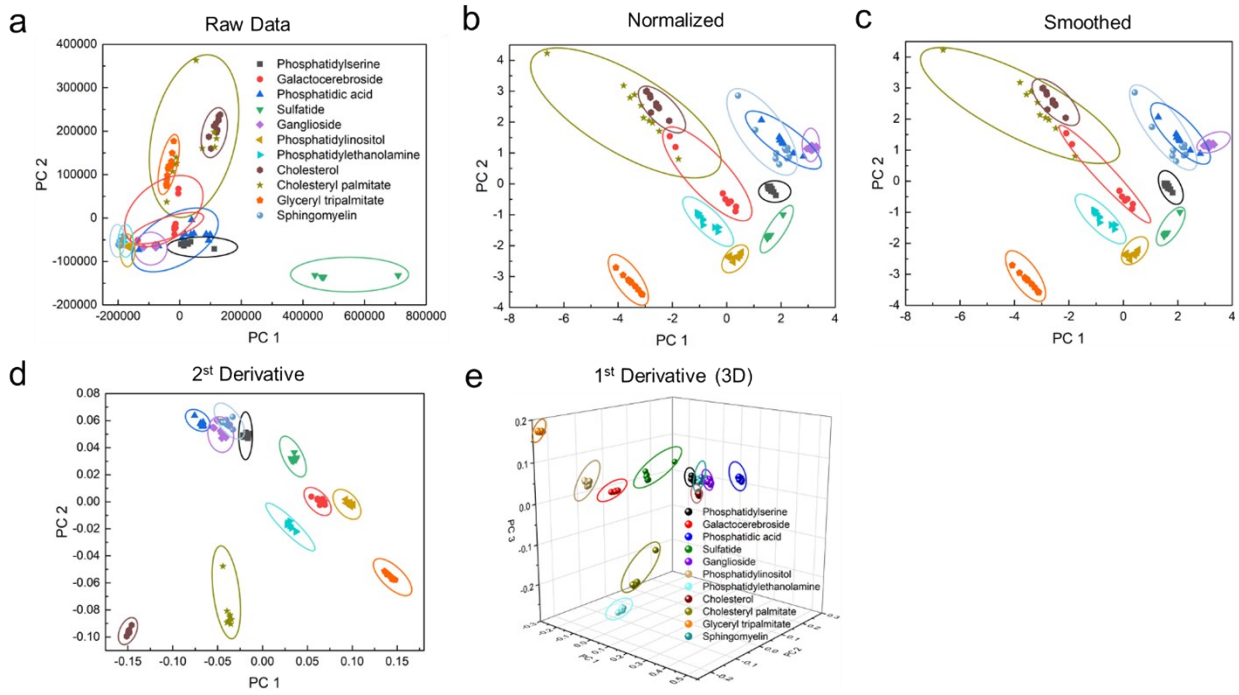


Figure S12. PCA analysis of the most common lipids in brain tissue for (a) raw data, (b) normalized data, (c) smoothed data and (d, e) derivatives of data. PCA analysis on raw data does not distinguish the lipids very well. Meanwhile, after normalization, smoothing and taking the derivatives of the data, a well distinguishability is observed in the PCA results (details are in the methods section).

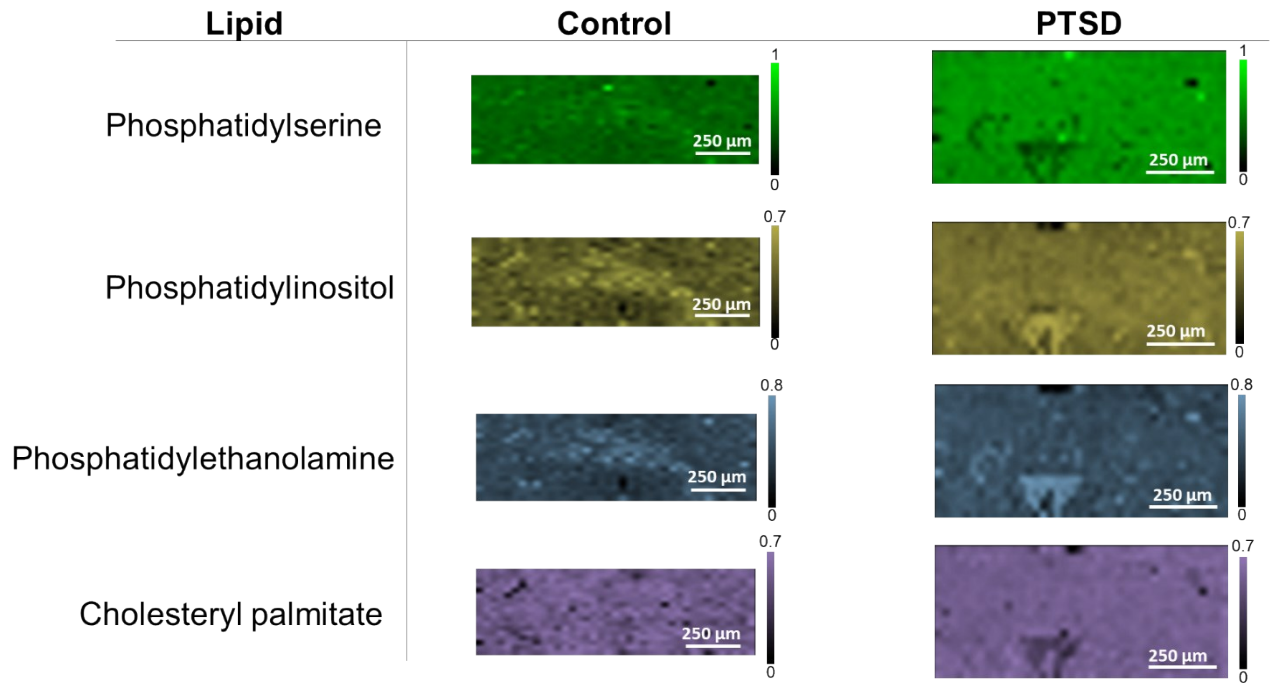


Figure S13. Raman maps of phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine and cholesteryl palmitate in PVT region of the rat brain. The bars beside each image indicate the value of correlation coefficient between the corresponding lipid and brain spectra.

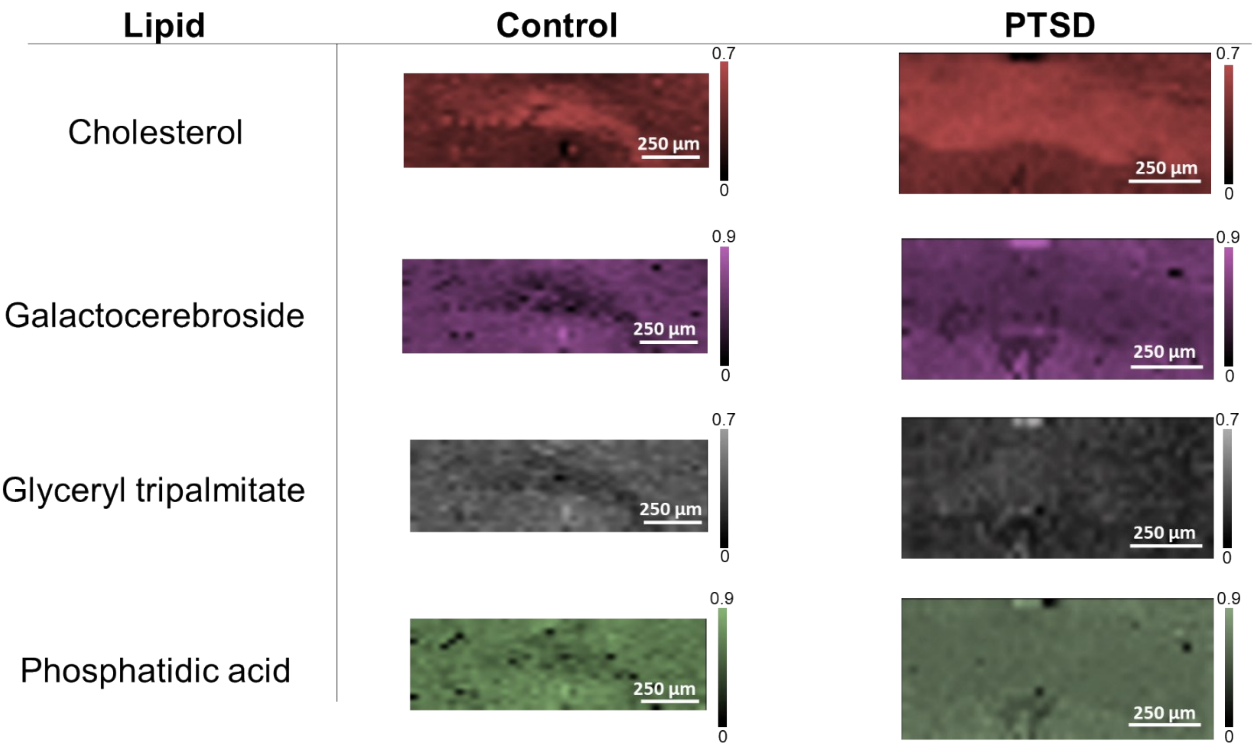


Figure S14. Raman maps of cholesterol, galactocerebroside, glyceryl tripalmitate and phosphatidic acid in PVT region of the rat brain. The bars beside each image indicate the value of correlation coefficient between the corresponding lipid and brain spectra.

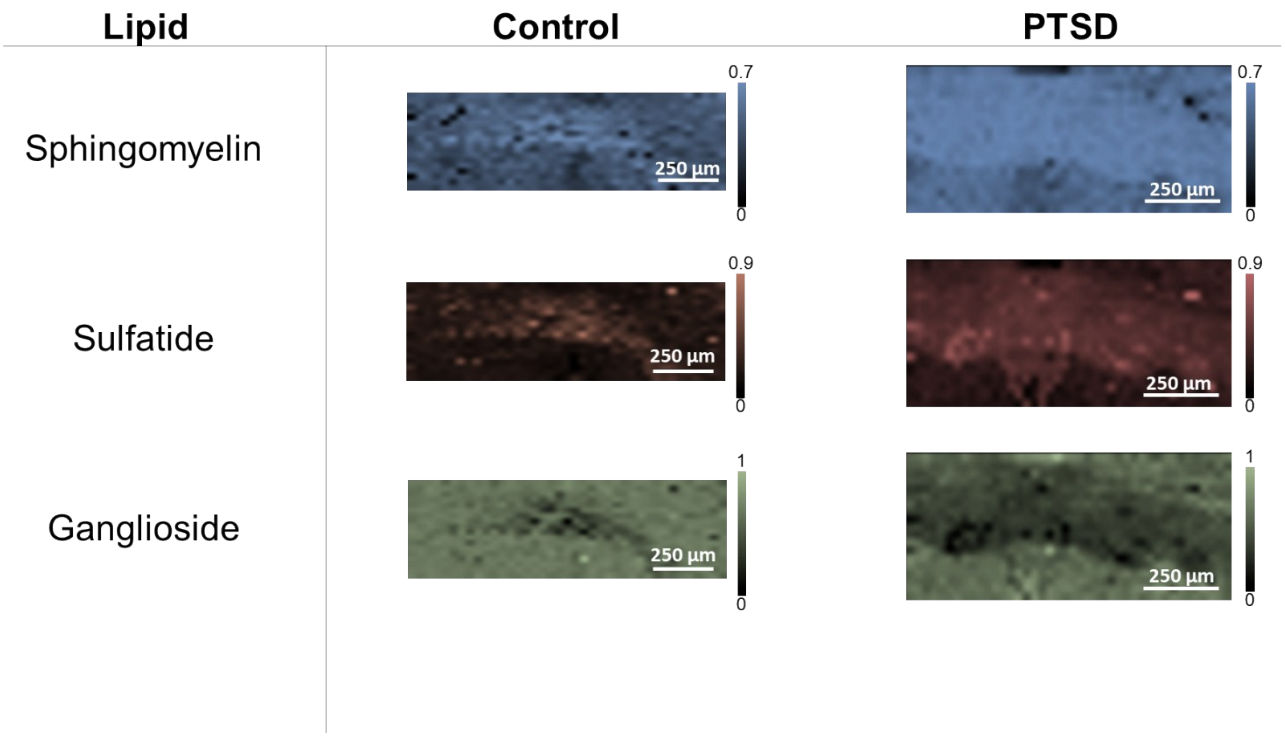


Figure S15. Raman maps of sphingomyelin, sulfatide and ganglioside in PVT region of the rat brain. The bars beside each image indicate the value of correlation coefficient between the corresponding lipid and brain spectra.

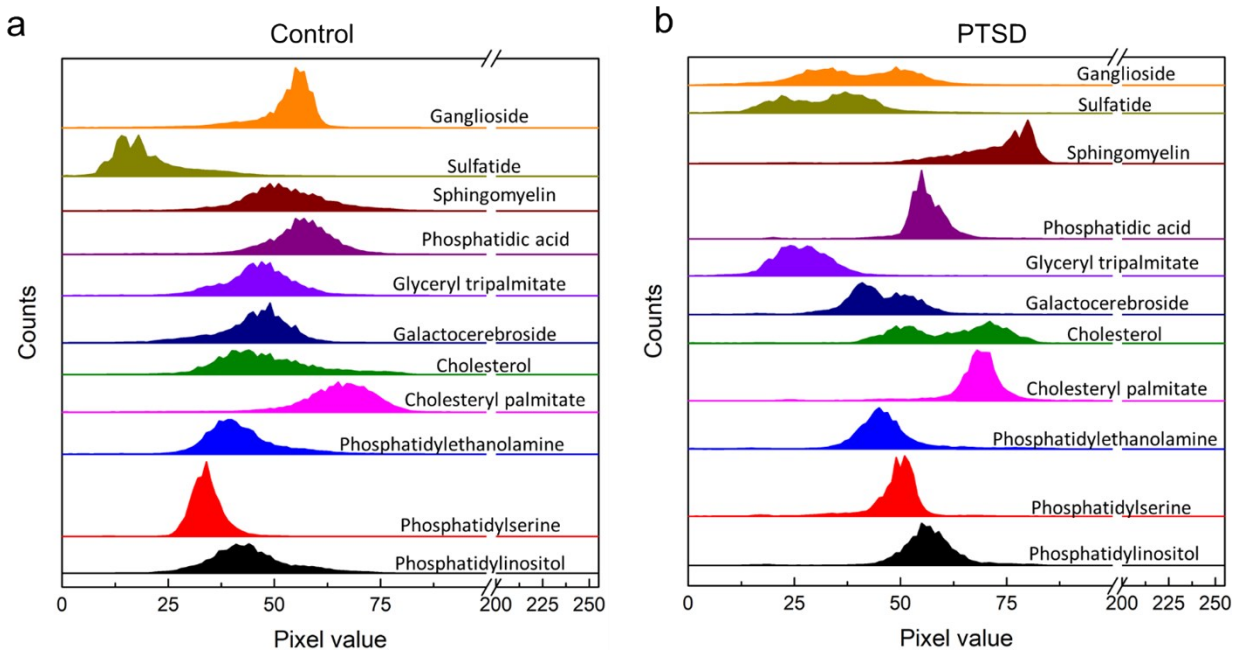


Figure S16. Pixel value distributions of different lipids in control and PTSD samples. Relative shifts of the peaks to the right (higher pixel values), indicates an increase in the relative concentration of lipids. Weighted mean value is obtained for each lipid from this diagram. This value directly indicates the concentration changes of different lipids.

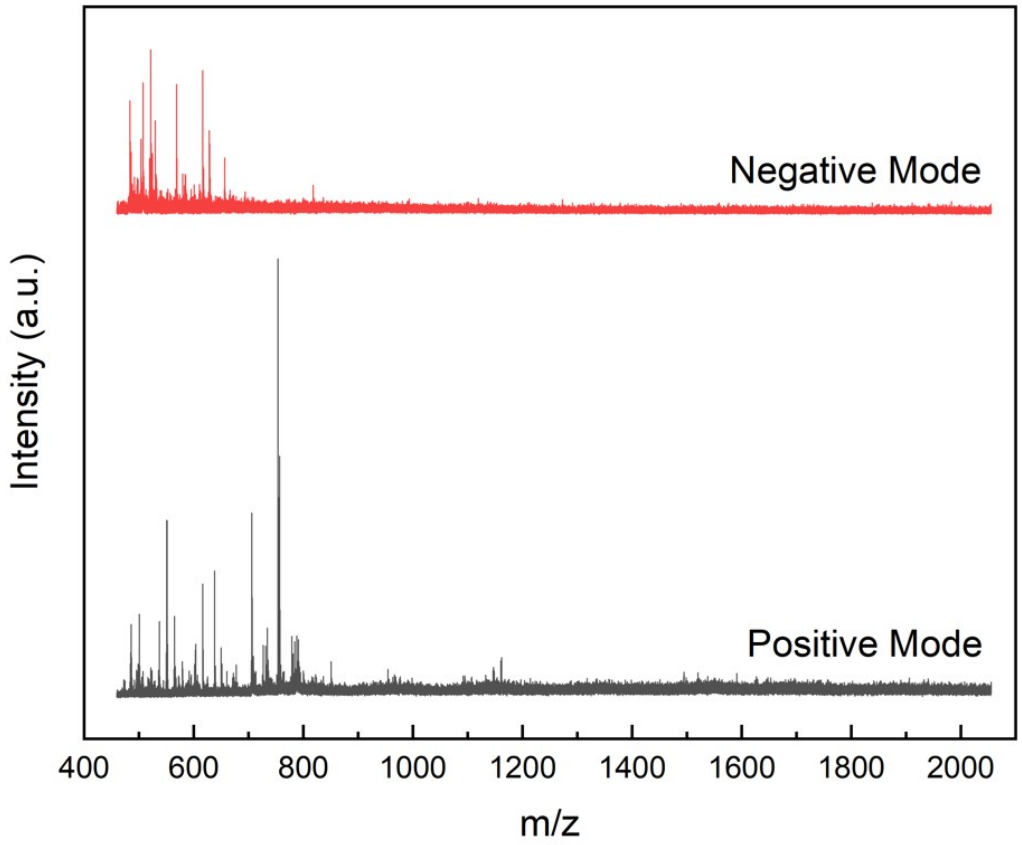
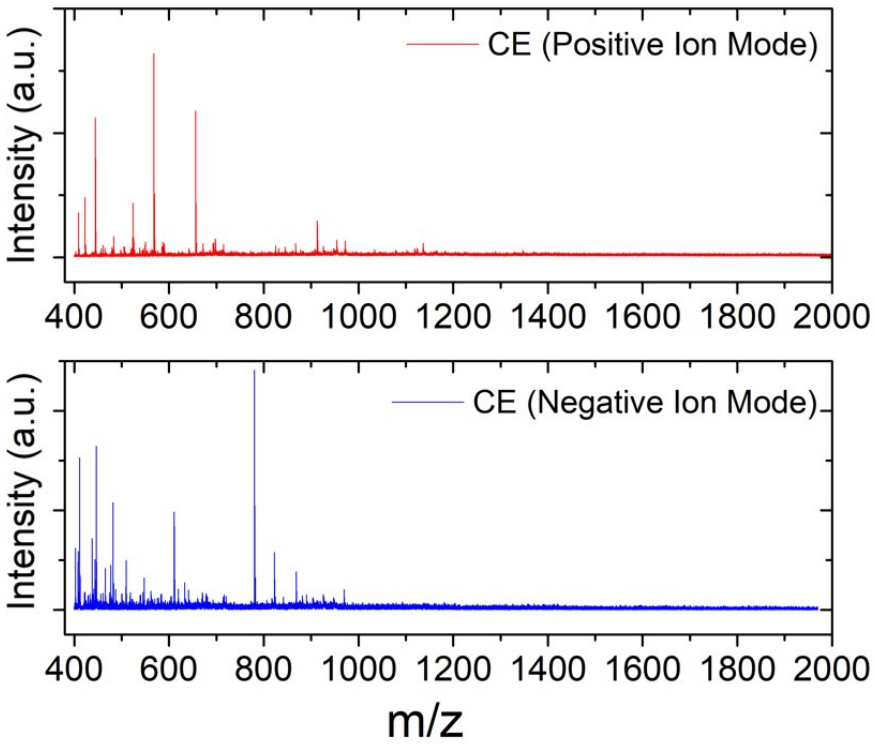
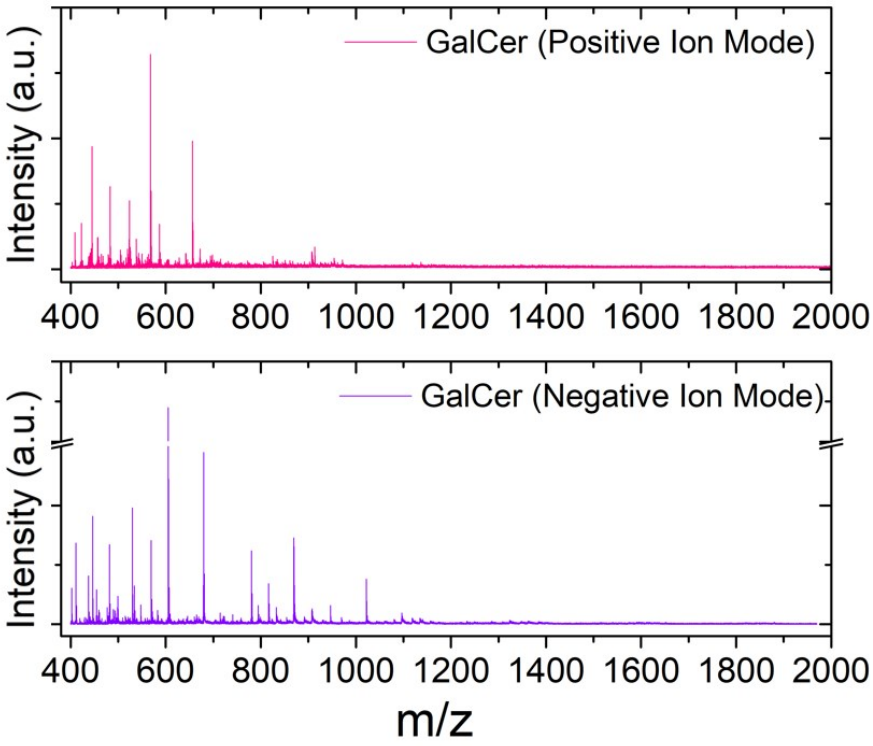
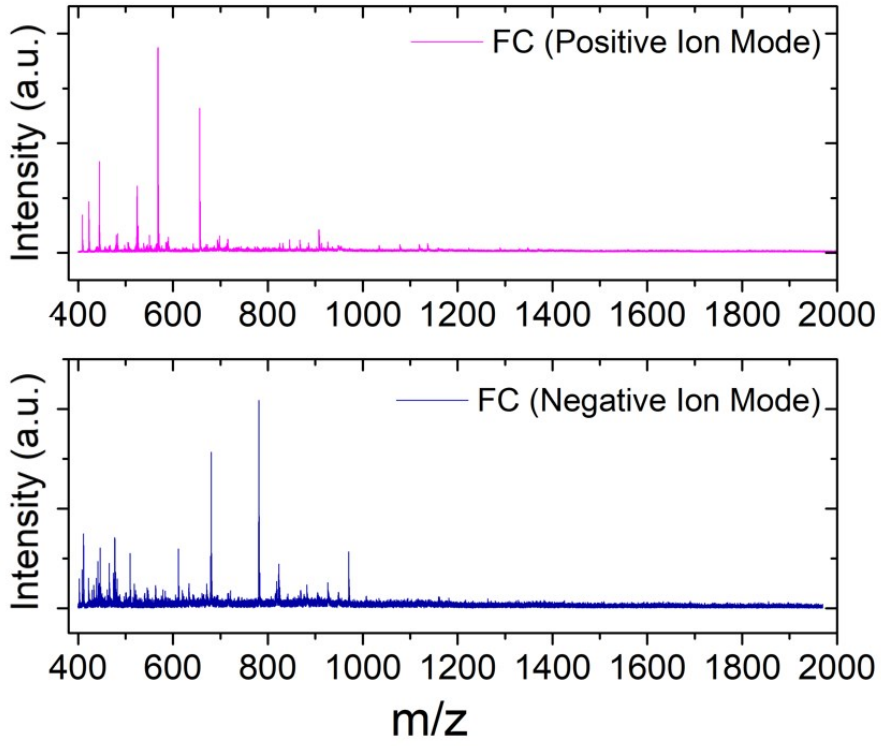
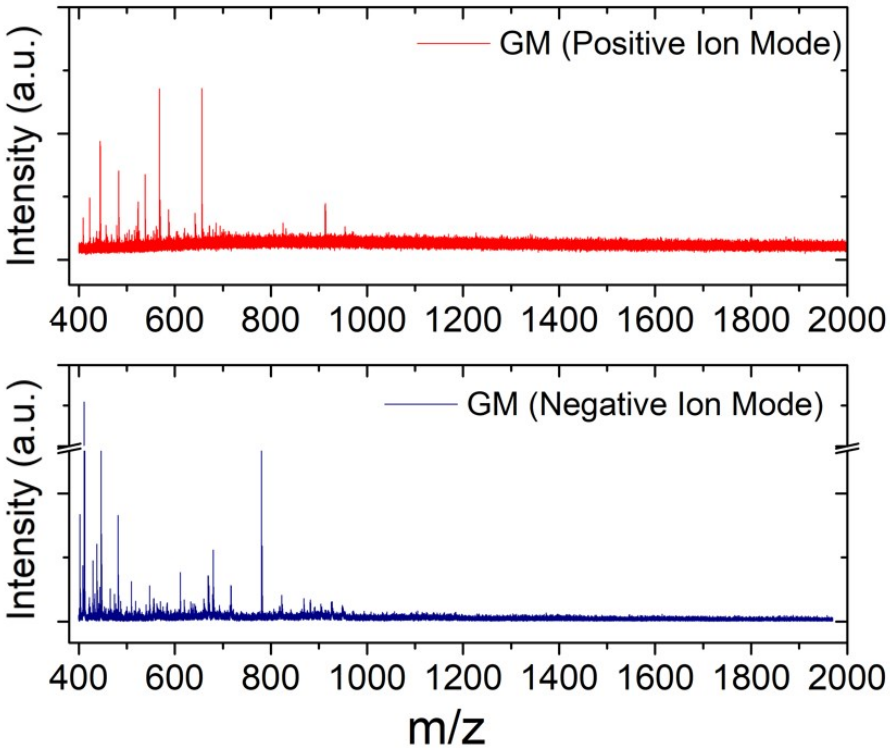


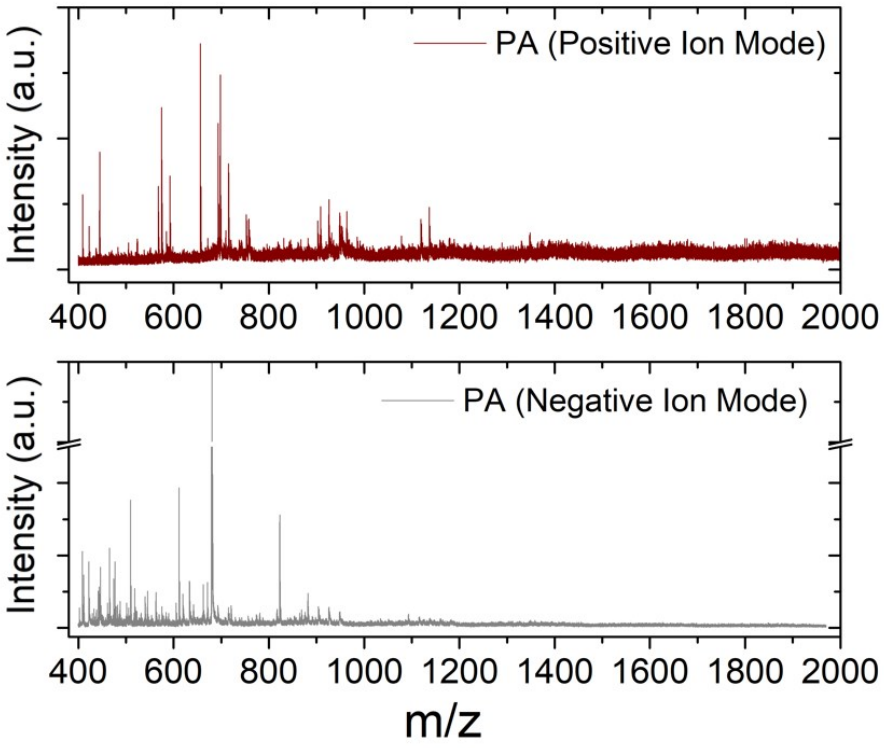
Figure S17. MALDI signal obtained from rat brain tissue in both positive and negative modes.

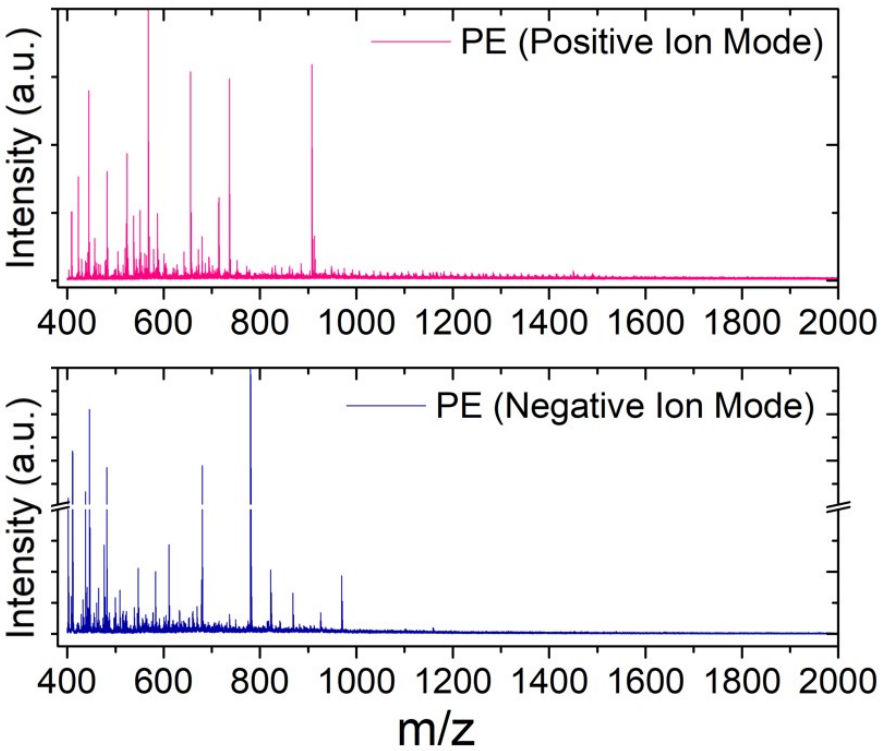

CE (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
CE (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z

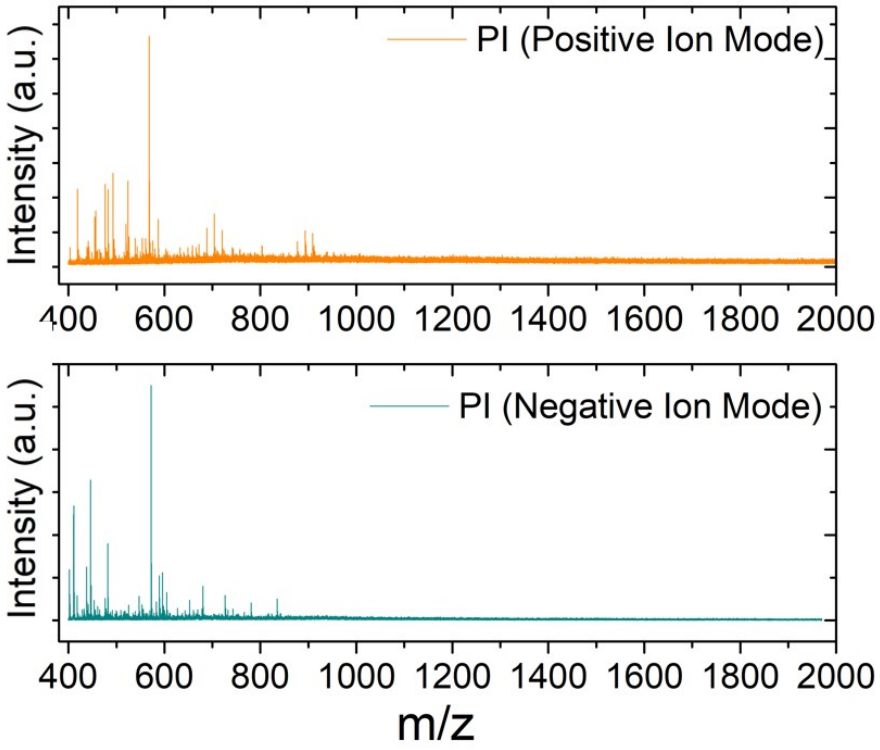

GalCer (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
GalCer (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z

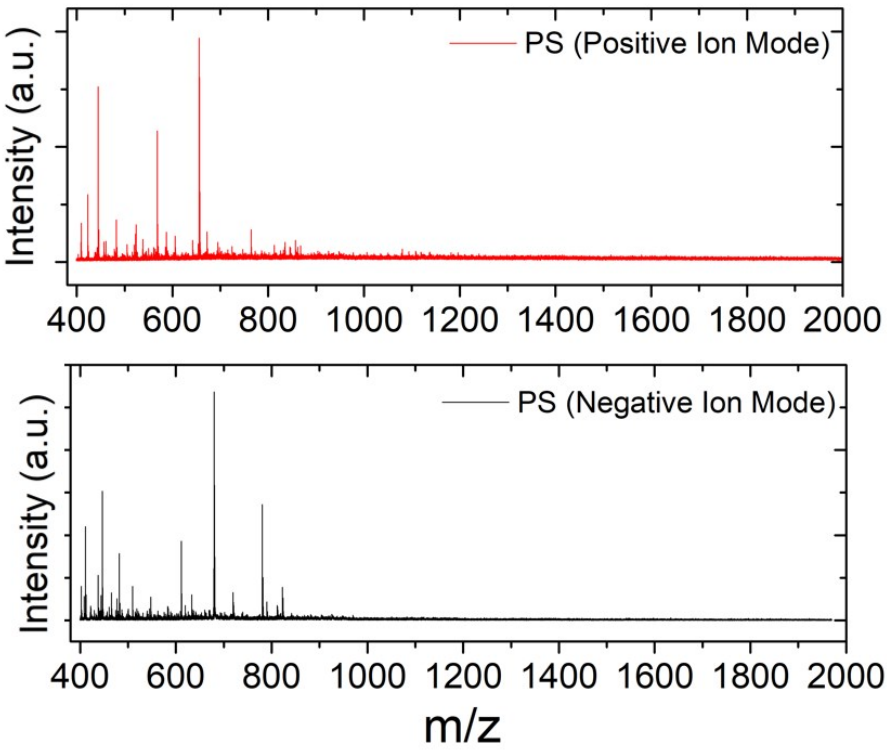

FC (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
FC (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z

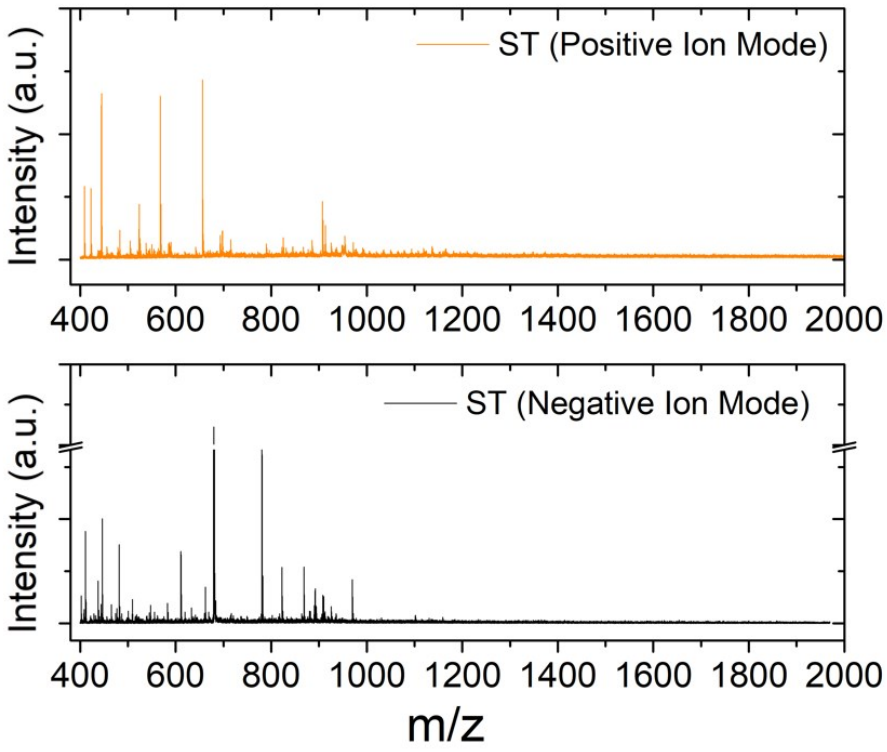

GM (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
GM (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z


PA (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
PA (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z


PE (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
PE (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z


PI (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
PI (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z


PS (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
PS (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z


ST (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
ST (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z

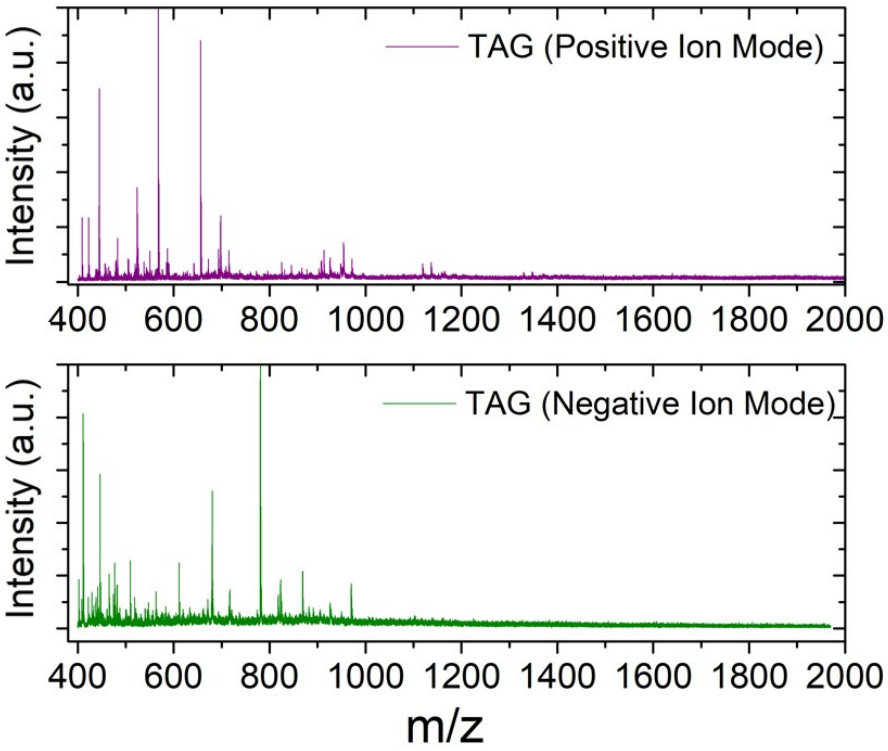

TAG (Positive Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
TAG (Negative Ion Mode)
Intensity (a.u.)
400 600 800 1000 1200 1400 1600 1800 2000
m/z

Figure S18. Comparison of positive and negative ion mode spectra. The MS spectra indicate both low molecular weight (400 – 650 Da) and high molecular weight (700 – 1200 Da) lipid species.

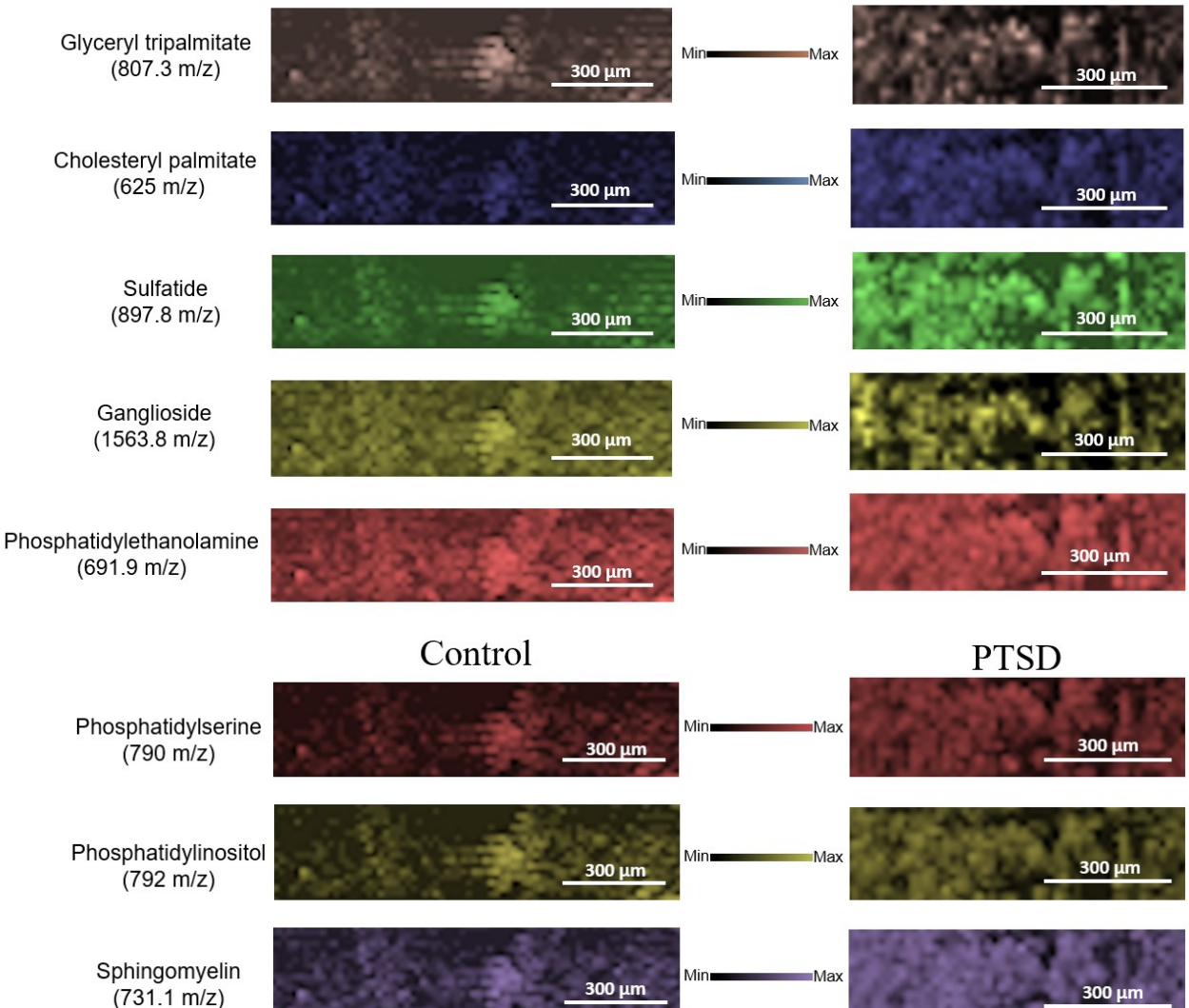

Glyceryl tripalmitate
(807.3 m/z)
Cholesteryl palmitate
(625 m/z)
Sulfatide
(897.8 m/z)
Ganglioside
(1563.8 m/z)
Phosphatidylethanolamine
(691.9 m/z)
Control
PTSD
Phosphatidylserine
(790 m/z)
Phosphatidylinositol
(792 m/z)
Sphingomyelin
(731.1 m/z)
Min
Max
300 µm

Figure S19. MALDI images acquired from PVT region of rat brain for control and PTSD. The corresponding mass value used for plotting the images is showed beside each lipid name. In all the Images, brightness of the pixels shows the relative concentration of the lipid (brighter: higher concentration; darker: lower concentration).

Table S1. Peak positions of Raman spectrum for control and PTSD samples. Peak positions are mostly remained unchanged or negligibly changed.

Raman band (cm^{-1})		**Assignment**			
Control	***PTSD***	***DNA/RNA***	***Proteins***	***Lipids***	***Carbohydrates***
543	545			CE	
568	569			PI	
593	592			PS	
608	608			FC, Glycerol	
622	622	Z-DNA, dG ($\nu(C\text{-}C)_{ring}$) [1]	Phe (t(C-C)) [1]		
643	642		Stretching C-S, tyrosine twisting C-C [1]		
700	699			Cholesterol, cholesterol ester [2]	
725	722	Adenine base/DNA [2]			
741	740				
761	760		Tryptophan, d (ring) [2]		
806	804	OPO backbone stretching [1]			
847	849		Tyrosine, C-C stretching [2]		

879	878			Phosphocholine CCN symmetric stretching [1]	
914	914				Glucose [2]
927	926	OPO backbone stretching [1]			
959	957			Cholesterol [2]	
987	986		C-C [2]		C-O ribose [2]
1002	1002		Phenylalanine, C-C skeletal [2]		
1031	1031		d(C-H), phenylalanine [2]		
1064	1064	C-O stretching [1]	C-C & C-N stretching [1]	C-C & C-O stretching [1]	C-C & C-O stretching, OCH in-plane deformation [1]
1085	1084			Phospholipids [2]	
1127	1126	C-O stretching	C-N stretching	C-C stretching	C-C & C-O stretching
1158	1156		In-plane vibrations of the conjugated C=C-C [2]		
1173	1172		C-H bending, tyrosine [2]		
1208	1206		Amide III [2]		
1220	1219	(PO_2), nucleic acids [2]			
1267	1267	RNA C-C stretching [1]			
1300	1300			CH_2 bending	
1439	1439			CH_2 and CH_3 deformation vibrations [2]	

Table S2. ANOVA with Levene test for Homoscedasticity for the Raman spectra of the lipids.

Comparisons significant at the 0.001 level are indicated by *.**				
Lipid Comparison	**Difference Between Means**	**95% Confidence Limits**		
Glyceryl tripalmit - Cholesterol	0.073252	0.066209	0.080294	***
Glyceryl tripalmit - Sulfatide	0.138848	0.131805	0.145891	***
Glyceryl tripalmit - Phosphatidic acid	0.145274	0.138231	0.152317	***
Glyceryl tripalmit - Ganglioside	0.148326	0.141284	0.155369	***
Glyceryl tripalmit - Sphingomyelin	0.150536	0.143493	0.157579	***
Glyceryl tripalmit - Phosphatidylserine	0.151309	0.144266	0.158352	***
Glyceryl tripalmit - Phosphatidylinosit	0.153514	0.146471	0.160557	***
Glyceryl tripalmit - Galactocerebroside	0.161893	0.154850	0.168936	***
Glyceryl tripalmit - Cholesteryl palmit	0.308696	0.301653	0.315739	***
Glyceryl tripalmit - Phosphatidylethano	0.459798	0.452755	0.466841	***
Cholesterol - Glyceryl tripalmit	-0.073252	-0.080294	-0.066209	***
Cholesterol - Sulfatide	0.065596	0.058554	0.072639	***
Cholesterol - Phosphatidic acid	0.072022	0.064980	0.079065	***
Cholesterol - Ganglioside	0.075075	0.068032	0.082117	***
Cholesterol - Sphingomyelin	0.077284	0.070242	0.084327	***
Cholesterol - Phosphatidylserine	0.078057	0.071015	0.085100	***
Cholesterol - Phosphatidylinosit	0.080262	0.073219	0.087305	***
Cholesterol - Galactocerebroside	0.088641	0.081599	0.095684	***
Cholesterol - Cholesteryl palmit	0.235444	0.228401	0.242487	***
Cholesterol - Phosphatidylethano	0.386546	0.379504	0.393589	***
Sulfatide - Glyceryl tripalmit	-0.138848	-0.145891	-0.131805	***

Comparisons significant at the 0.001 level are indicated by ***.				
Lipid Comparison	**Difference Between Means**	**95% Confidence Limits**		
Sulfatide - Cholesterol	-0.065596	-0.072639	-0.058554	***
Sulfatide - Phosphatidic acid	0.006426	-0.000617	0.013469	
Sulfatide - Ganglioside	0.009478	0.002436	0.016521	***
Sulfatide - Sphingomyelin	0.011688	0.004645	0.018731	***
Sulfatide - Phosphatidylserine	0.012461	0.005418	0.019504	***
Sulfatide - Phosphatidylinosit	0.014666	0.007623	0.021709	***
Sulfatide - Galactocerebroside	0.023045	0.016002	0.030088	***
Sulfatide - Cholesteryl palmit	0.169848	0.162805	0.176891	***
Sulfatide - Phosphatidylethano	0.320950	0.313907	0.327993	***
Phosphatidic acid - Glyceryl tripalmit	-0.145274	-0.152317	-0.138231	***
Phosphatidic acid - Cholesterol	-0.072022	-0.079065	-0.064980	***
Phosphatidic acid - Sulfatide	-0.006426	-0.013469	0.000617	
Phosphatidic acid - Ganglioside	0.003052	-0.003990	0.010095	
Phosphatidic acid - Sphingomyelin	0.005262	-0.001781	0.012305	
Phosphatidic acid - Phosphatidylserine	0.006035	-0.001008	0.013078	
Phosphatidic acid - Phosphatidylinosit	0.008240	0.001197	0.015283	***
Phosphatidic acid - Galactocerebroside	0.016619	0.009576	0.023662	***
Phosphatidic acid - Cholesteryl palmit	0.163422	0.156379	0.170465	***
Phosphatidic acid - Phosphatidylethano	0.314524	0.307481	0.321567	***
Ganglioside - Glyceryl tripalmit	-0.148326	-0.155369	-0.141284	***
Ganglioside - Cholesterol	-0.075075	-0.082117	-0.068032	***
Ganglioside - Sulfatide	-0.009478	-0.016521	-0.002436	***
Ganglioside - Phosphatidic acid	-0.003052	-0.010095	0.003990	
Ganglioside - Sphingomyelin	0.002210	-0.004833	0.009252	
Ganglioside - Phosphatidylserine	0.002983	-0.004060	0.010025	
Ganglioside - Phosphatidylinosit	0.005188	-0.001855	0.012230	

Comparisons significant at the 0.001 level are indicated by ***.				
Lipid Comparison	**Difference Between Means**	**95% Confidence Limits**		
Ganglioside - Galactocerebroside	0.013567	0.006524	0.020610	***
Ganglioside - Cholesteryl palmit	0.160370	0.153327	0.167412	***
Ganglioside - Phosphatidylethano	0.311472	0.304429	0.318515	***
Sphingomyelin - Glyceryl tripalmit	-0.150536	-0.157579	-0.143493	***
Sphingomyelin - Cholesterol	-0.077284	-0.084327	-0.070242	***
Sphingomyelin - Sulfatide	-0.011688	-0.018731	-0.004645	***
Sphingomyelin - Phosphatidic acid	-0.005262	-0.012305	0.001781	
Sphingomyelin - Ganglioside	-0.002210	-0.009252	0.004833	
Sphingomyelin - Phosphatidylserine	0.000773	-0.006270	0.007816	
Sphingomyelin - Phosphatidylinosit	0.002978	-0.004065	0.010021	
Sphingomyelin - Galactocerebroside	0.011357	0.004314	0.018400	***
Sphingomyelin - Cholesteryl palmit	0.158160	0.151117	0.165203	***
Sphingomyelin - Phosphatidylethano	0.309262	0.302219	0.316305	***
Phosphatidylserine - Glyceryl tripalmit	-0.151309	-0.158352	-0.144266	***
Phosphatidylserine - Cholesterol	-0.078057	-0.085100	-0.071015	***
Phosphatidylserine - Sulfatide	-0.012461	-0.019504	-0.005418	***
Phosphatidylserine - Phosphatidic acid	-0.006035	-0.013078	0.001008	
Phosphatidylserine - Ganglioside	-0.002983	-0.010025	0.004060	
Phosphatidylserine - Sphingomyelin	-0.000773	-0.007816	0.006270	
Phosphatidylserine - Phosphatidylinosit	0.002205	-0.004838	0.009248	
Phosphatidylserine - Galactocerebroside	0.010584	0.003541	0.017627	***
Phosphatidylserine - Cholesteryl palmit	0.157387	0.150344	0.164430	***
Phosphatidylserine - Phosphatidylethano	0.308489	0.301446	0.315532	***
Phosphatidylinosit - Glyceryl tripalmit	-0.153514	-0.160557	-0.146471	***
Phosphatidylinosit - Cholesterol	-0.080262	-0.087305	-0.073219	***
Phosphatidylinosit - Sulfatide	-0.014666	-0.021709	-0.007623	***

Comparisons significant at the 0.001 level are indicated by ***.				
Lipid Comparison	**Difference Between Means**	**95% Confidence Limits**		
Phosphatidylinosit - Phosphatidic acid	-0.008240	-0.015283	-0.001197	***
Phosphatidylinosit - Ganglioside	-0.005188	-0.012230	0.001855	
Phosphatidylinosit - Sphingomyelin	-0.002978	-0.010021	0.004065	
Phosphatidylinosit - Phosphatidylserine	-0.002205	-0.009248	0.004838	
Phosphatidylinosit - Galactocerebroside	0.008379	0.001337	0.015422	***
Phosphatidylinosit - Cholesteryl palmit	0.155182	0.148139	0.162225	***
Phosphatidylinosit - Phosphatidylethano	0.306284	0.299242	0.313327	***
Galactocerebroside - Glyceryl tripalmit	-0.161893	-0.168936	-0.154850	***
Galactocerebroside - Cholesterol	-0.088641	-0.095684	-0.081599	***
Galactocerebroside - Sulfatide	-0.023045	-0.030088	-0.016002	***
Galactocerebroside - Phosphatidic acid	-0.016619	-0.023662	-0.009576	***
Galactocerebroside - Ganglioside	-0.013567	-0.020610	-0.006524	***
Galactocerebroside - Sphingomyelin	-0.011357	-0.018400	-0.004314	***
Galactocerebroside - Phosphatidylserine	-0.010584	-0.017627	-0.003541	***
Galactocerebroside - Phosphatidylinosit	-0.008379	-0.015422	-0.001337	***
Galactocerebroside - Cholesteryl palmit	0.146803	0.139760	0.153845	***
Galactocerebroside - Phosphatidylethano	0.297905	0.290862	0.304948	***
Cholesteryl palmit - Glyceryl tripalmit	-0.308696	-0.315739	-0.301653	***
Cholesteryl palmit - Cholesterol	-0.235444	-0.242487	-0.228401	***
Cholesteryl palmit - Sulfatide	-0.169848	-0.176891	-0.162805	***
Cholesteryl palmit - Phosphatidic acid	-0.163422	-0.170465	-0.156379	***
Cholesteryl palmit - Ganglioside	-0.160370	-0.167412	-0.153327	***
Cholesteryl palmit - Sphingomyelin	-0.158160	-0.165203	-0.151117	***
Cholesteryl palmit - Phosphatidylserine	-0.157387	-0.164430	-0.150344	***
Cholesteryl palmit - Phosphatidylinosit	-0.155182	-0.162225	-0.148139	***
Cholesteryl palmit - Galactocerebroside	-0.146803	-0.153845	-0.139760	***

Comparisons significant at the 0.001 level are indicated by ***.				
Lipid Comparison	**Difference Between Means**	**95% Confidence Limits**		
Cholesteryl palmit - Phosphatidylethano	0.151102	0.144060	0.158145	***
Phosphatidylethano - Glyceryl tripalmit	-0.459798	-0.466841	-0.452755	***
Phosphatidylethano - Cholesterol	-0.386546	-0.393589	-0.379504	***
Phosphatidylethano - Sulfatide	-0.320950	-0.327993	-0.313907	***
Phosphatidylethano - Phosphatidic acid	-0.314524	-0.321567	-0.307481	***
Phosphatidylethano - Ganglioside	-0.311472	-0.318515	-0.304429	***
Phosphatidylethano - Sphingomyelin	-0.309262	-0.316305	-0.302219	***
Phosphatidylethano - Phosphatidylserine	-0.308489	-0.315532	-0.301446	***
Phosphatidylethano - Phosphatidylinosit	-0.306284	-0.313327	-0.299242	***
Phosphatidylethano - Galactocerebroside	-0.297905	-0.304948	-0.290862	***
Phosphatidylethano - Cholesteryl palmit	-0.151102	-0.158145	-0.144060	***