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Highlighting anatomical sub-structures in rat brain tissue using lipid imaging

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Abstract

Cell membranes are made up of a mixture of glycerolipids, sphingolipids, gangliosides and cholesterol. Lipids play important roles in a cell's life. However many of their functions have still to be discovered. In the present work, we describe an efficient, easy and rapid methodology to accurately localize phosphatidylcholines and sphingomyelins from a single coronal rat brain section in the cerebrum area. Matrix assisted laser desorption/ionization (MALDI) mass spectrometry was used to profile and image lipids. The best resolved structure was 25–50 μm in the hippocampus.

Introduction

Lipids make up half of the brain's dry weight. They are a challenging class of molecules to study due to their diverse structures and functions.^{1–3} Their distribution differs in various cell membranes depending on the cell's function. Thus elucidation of their structure could shed light on the role they play in the physiology and biochemistry of the membrane and on pathological aspects of brain related diseases. Cell membranes are made up of a mixture of glycerolipids, sphingolipids, gangliosides and cholesterol. Proteins and glycoproteins are embedded in this sea of lipids. Lipid rafts which float in the lipid bilayer are particularly rich in sphingomyelin (SM, Fig. 1). They are the assembly centers for signaling biomolecules and protein trafficking.⁴ SM are the major source of lipid signaling molecules. Their degradation is a major source of ceramides. They also play a crucial role in motor and cognitive behavior.⁵ Both SM and Phosphatidylcholine (PC) are zwitterionic lipids, however SM are built on a scaffold of ceramide while PC have a glycerol backbone (Fig. 1). PC are most abundant in the outer layer of the cell's membrane, accounting for more than 50% of all Glycerophospholipids.⁶ PC are also involved in cell proliferation, differentiation and death.⁷ SM and PC are important in normal brain development^{8,9} as well as in some diseases. Indeed, alterations in the level of SM are common in lysosomal diseases such as Niemann-Pick,^{10,11} in ammonia intoxication¹² and brain ischemia,¹³ while changes in PC metabolism have been observed in pathological conditions such as Alzheimer disease¹⁴ and ataxia.^{15,16}

A multidisciplinary approach to lipid analysis is filling holes in our knowledge of their role in cell membranes.^{2,6} A variety of analytical techniques are available to achieve this goal, as shown by the number of reviews dedicated to the subject.^{1,17–20} More specifically, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has proven to be an efficient tool in detecting, characterizing, profiling and mapping these biomolecules while

keeping their spatial distribution, by directly probing tissue sections.^{1,18,19,21} MALDI imaging of proteins²² were first demonstrated by Caprioli's group, while lipid profiling and imaging was extensively documented by the Woods group.^{23–30} Nowadays, biomolecular profiles and images are acquired from various tissue as well as whole bodies.³¹

SM and PC's choline moiety makes their ionization and detection particularly easy in positive mode as their choline moiety is already positively charged. These species were previously profiled, localized and imaged by MALDI MS from rodent brain tissue sections.^{9,23–30,32–34} The present study offers a refining of SM and PC mapping from healthy coronal rat brain tissue sections, providing a more accurate localization with a spatial resolution of 25–50 μm .

Material and methods

Chemicals

All reagents used were HPLC grade: isopentane, ethanol, methanol, chloroform, hexane, diethylether and acetone; diisopropylether was ACS grade, heptafluorobutyric acid (HFBA) from Sigma-Aldrich (Saint-Louis, Missouri). Saline and ammonium sulfate from J.T. Baker (Phillipsburg, New Jersey). Acetic acid and 2,6-dihydroxy-acetophenone (DHA) from Fluka (Steinheim, Germany).

Animals

All animal use and handling in this work abide by the NIH Guidelines for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighting 270 to 350 g (Harlan Industries, Indianapolis, IN) were used for this work. Brains were harvested, frozen in isopentane on dry ice and stored at -80°C before analysis.

Extraction and purification

A total lipid extract was obtained from a rat brain coronal section (100 μm thickness; cut at bregma -5.76 mm) using a modified Folch extraction.³⁵ Briefly, 6.8 mg brain tissue was homogenized by sonication in 136 μL chloroform/methanol 2/1 (v/v) followed by shaking for 2 h, and addition of 27 μL distilled water. The mixture was vortexed for 1 min then centrifuged for 10 min at 3000 rpm. Methanol was added to the lipid extract to restore its initial volume. The lower organic phase containing all lipids except gangliosides was evaporated to dryness using nitrogen and re-suspended in chloroform for lipid separation using aminopropyl-bonded silica gel cartridges (100 mg, 1 mL, LC-NH2 SPE tubes, Supelco Park Bellefonte, PA). A 1 mL LC-NH2 column was conditioned with 2 mL hexane, and the extract loaded on the column. Four fractions containing different lipid species were obtained using the following eluants:³⁶ 2 mL diethylether, 1.6 mL chloroform/methanol 23/1 (v/v), 1.8 mL diisopropylether/acetic acid and 2 mL acetone/methanol 9/1.2 (v/v). Fraction 5 containing neutral phospholipids (sphingomyelin SM and glycerophosphatidylcholine PC) was eluted with 2 mL of chloroform/methanol 2/1 (v/v), dried under nitrogen, resuspended in 10 μL chloroform + 200 μL methanol and analyzed.

Mass spectrometry

18 μm thick rat brain sections were deposited onto MALDI plates. An artistic airbrush was used to deposit our recently described matrix solution, 2,6-dihydroxyacetophenone/ammonium sulfate/heptafluorobutyric acid (DHA/ammonium sulfate/HFBA). Saturated DHA/3mM ammonium sulfate was used for lipids profiling, and saturated DHA/125mM ammonium sulfate/0.05% HFBA for lipids imaging.^{29,30}

A MALDI LTQ mass spectrometer (Thermo Scientific LTQ XL) was used in positive ion mode. The matrix used was saturated DHA with 6 mM ammonium sulfate in 50% ethanol. Optimal settings for lipid detection were 2 microscans and 3 laser shots at 14 mJ laser energy. Pulsed-Q Dissociation (PQD) spectra were performed on each peak in fraction 5 to confirm structures. 100 mM LiCl was added to the DHA matrix to maximize fragmentation.

Data was acquired in the mass range 700–900 amu. The laser spot was a 100 μm $\times$ 80 μm rectangle. The raster step size was 75 μm . The ImageQuest software (Thermo Scientific) was used to generate 2D ion density maps (MALDI images). Each image was normalized as a function of the total ion current.

Results and discussion

Lipids detection from rat brain tissue

The MALDI spectrum of a total lipid extract in positive ion mode with and without 6 mM ammonium sulfate added is seen in Fig. 2. In the absence of ammonium sulfate the spectrum is dominated by sodiated and potassiated peaks while molecular ions are partially suppressed and have the lowest relative abundance (Fig. 2a). PC 34 : 1 was present in its protonated $[\text{PC } 34\text{a}:1 + \text{H}]^+$ (m/z 760.6), sodiated $[\text{PC } 34\text{a}:1 + \text{Na}]^+$ (m/z 782.6) and potassiated $[\text{PC } 34\text{a}:1 + \text{K}]^+$ (m/z 798.6) forms, which made spectrum interpretation difficult. In addition, these ions were sometimes overlaid with isobaric protonated molecular ions as $[\text{PC } 34\text{a}:1 + \text{Na}]^+$ with $[\text{PC } 36\text{a}:4 + \text{H}]^+$ at m/z 782.6, increasing the complexity of peak assignment. Ammonium sulfate addition to DHA (Fig. 2b) significantly decreased the intensity of salt adducts as seen by $[\text{PC } 34\text{a}:1 + \text{K}]^+$ (m/z 798.6) disappearance. Table 1 summarizes detected species from Fig. 2b. Ammonium sulfate clarified peak assignment by minimizing salt adducts formation.³⁰ Indeed, its addition has already been used since 1994 to improve peptides detection.³⁷ The H donor moiety of the salt, NH_4^+ , optimizes lipids' ionization. Its counter ion, SO_4^{2-} , traps cations such as Na^+ and K^+ thus competing with lipids for salt adduct formation. Both actions resulted in what we previously demonstrated,^{29–31,38} as lipid/matrix co-crystallization became more homogeneous, resulting in higher relative abundance of $[\text{M} + \text{H}]^+$ and increase of signal-to-noise ratio, thus improving resolution and decreasing salt adducts.

It was previously demonstrated that using DHA gave better lipids detection and resolution than DHB.^{25,28–30} However, its fast sublimation in vacuum (1×10^{-6} Torr or lower) limited its use for imaging to 30–60 min of acquisition.^{26,39,40} We remedied the problem by adding 0.05% of HFBA to the matrix, resulting in increased matrix stability which increased our imaging window from 1 to 6 h.³⁰ HFBA is an ion pairing agent used in HPLC to increase species separation and resolution.⁴¹ In fact, HFBA stabilizes DHA through hydrogen bonding interactions between the carboxylic acid moiety and the matrix ketone and hydroxy moieties. Nonetheless, the hydrogen bonding between the carboxylic acid and the ketone is stabilized by the delocalized π system present in the aromatic ring, thus extending the aromatic resonance to the ketone and the matrix alcohol moieties consequently providing the needed stability.⁴² Effects of the 6 mM ammonium sulfate addition also improved SM detection as highlighted in Fig. 2 for $[\text{SM } 34 : 1 + \text{H}]^+$ at m/z 703.6, $[\text{SM } 36 : 1 + \text{H}]^+$ at m/z 731.6 and $[\text{SM } 42 : 2 + \text{H}]^+$ at m/z 813.7. Using this same matrix solution directly on a brain tissue section, we observed the same species (Fig. 2c).

Lipids characterization

Representative fragmentation mass spectra of two lithiated SM (m/z 737.7) and PC (m/z 766.6) are shown Fig. 3.

The phosphocholine neutral loss from SM (Fig. 3a) yielded the ion $[\text{N}(\text{CH}_3)_3\text{C}_2\text{H}_5\text{O}_4\text{P} + \text{Li}]^+$ at m/z 554.6. The protonated phosphocholine was observed at m/z 184.0, and the singly charged N $(\text{CH}_3)_3\text{C}_2\text{H}_3^+$ at m/z 86.1. The loss of $\text{N}(\text{CH}_3)_3$ gave rise to $[\text{M}-\text{N}(\text{CH}_3)_3 + \text{H}]^+$ at m/z 678.5. SM head group was identified by the presence of these 4 ions. The peak at m/z 264.3 resulted from the loss from m/z 554.6 of Li (Fw = 7.0), H_2O (Fw = 18.0) and $\text{C}_{17}\text{H}_{34}\text{CO}$ (Fw = 266.4). The resulting ion $[\text{M}-\text{N}(\text{CH}_3)_3\text{C}_2\text{H}_4\text{O}_4\text{PLi}-\text{C}_{17}\text{H}_{34}\text{CO} + \text{H}]^+$ was attributed to a form of the sphingoid base, sphingenine d18:1. $\text{C}_{17}\text{H}_{34}\text{CO}$ corresponded to a part of the C18:0 fatty acid. The structure of m/z 737.7 was identified as SM d18:1/C18:0. These observations and interpretations are in agreement with the mechanisms highlighted by Hsu and Turk⁴³ as well as with previous studies.⁴⁴ Nine other SMs were identified (Table 1).

As for SM, PC (m/z 766.6) head group was determined by the loss of the neutral phosphocholine yielding $[\text{M}-\text{N}(\text{CH}_3)_3\text{C}_2\text{H}_5\text{O}_4\text{P} + \text{Li}]^+$ at m/z 583.5. A neutral trimethylamine $\text{N}(\text{CH}_3)_3$ loss yielded $[\text{M}-\text{N}(\text{CH}_3)_3 + \text{Li}]^+$ at m/z 707.4. Its singly charged form $\text{N}(\text{CH}_3)_3\text{C}_2\text{H}_3^+$ was observed at m/z 86.1.

Fatty acid losses were deduced by subtraction from m/z 510.4 the loss of C16:0 (Fw $\text{C}_{16}\text{H}_{32}\text{O}_2 = 256.4$) and assigned as $[\text{M}-\text{C}_{16}\text{H}_{32}\text{O}_2 + \text{Li}]^+$, and m/z 484.4 resulting from the loss of C18:1 (Fw $\text{C}_{18}\text{H}_{34}\text{O}_2 = 282.4$) and assigned as $[\text{M}-\text{C}_{18}\text{H}_{34}\text{O}_2 + \text{Li}]^+$. Consecutive losses of trimethylamine were observed at m/z 451.3 $[\text{M}-\text{C}_{16}\text{H}_{32}\text{O}_2-\text{N}(\text{CH}_3)_3 + \text{Li}]^+$ and m/z 425.3 $[\text{M}-\text{C}_{18}\text{H}_{34}\text{O}_2-\text{N}(\text{CH}_3)_3 + \text{Li}]^+$. The peak at m/z 265.3 was assigned as $\text{C}_{18}\text{H}_{33}\text{O}^+$ and is a C18:1 fatty acid. The structure of m/z 766.6 was identified as PC C16:0/C18:1. Hsu *et al.* suggested and confirmed PC fragmentation mechanisms which explained our observations.^{45,46} These results were also in agreement with previous studies.^{44,23,47} Thirteen other PCs were identified however six had one or more isobaric isomers (Table 1).

Lipids localization

Tissue sections used for imaging, extraction and microscopy were cut from the same bregma (−5.76 mm); this is easily accomplished as sections were 18 microns in thickness. The body cell layer was localized in a Cresyl violet stained section (Fig. 4). SM and PC MALDI images are displayed in Fig. 5. The spectrum range was m/z 700 to 900. Species images were normalized as a function of the total ion count. SM and PC localization were carefully detailed by comparing the MALDI images with the stained section (Table 2). A total of nine SMs and thirteen PCs were imaged. Three of each are displayed and show distinctive mapping.

SMs mapping—Three complementary locations of SMs images are displayed in Fig. 5, **1st row** and summarized in Table 2. Overlays of SMs and PCs highlight locations of the species of interest. SM containing the short chain fatty acid d18:1/C18:0 (m/z 731; **3rd col.**), predominates in grey matter, while SM species d18:1/C24:1 (m/z 813; **2nd col.**), containing long chain fatty acid, is mainly located in white matter, in agreement with previous results.⁴⁹ In addition to this bimodal distribution, both SMs were observed communally in the cerebral cortex (Cx). This localization confirmed our earlier profiling work on rat brain where SM d18:1/C18:0 was even more concentrated in Cx than SM d18:1/C24:1.²³ These images demonstrate the good spatial resolution achieved in our study as two thin hippocampal layers were indeed resolved, the pyramidal cell layer (Py; ~ 25 μm at its upper end, Fig. 4) and the granular cell layer of the dentate gyrus (GrDG; ~ 25 μm at its lower end, Fig. 4). Note the high spatial resolution achieved, considering that our laser's raster step was 75 μm . This result is easily explained by the methodology used in this work and was described by Sweedler in a study dedicated to overcoming the technical limit imposed by the coverage of the laser beam.⁵⁰ Basically, three parameters were controlled by experience or automation:

the wet matrix layer thickness, its rapid drying, and the correct number of laser shots. The obtained spatial resolution of 25 has been outreached by using SIMS.^{51,52} However, this technique unfortunately leaves an ambiguity between PC and SM characterization as detection relies on their head group (m/z 184).⁵³

A third SM, d18:1/C16:0 (m/z 703.6; **4th col.**), was exclusively localized in the dorsal 3rd ventricle (D3V), both lateral ventricles (LV) of the midbrain and both lateral subnucleus of the interpeduncular nucleus (IPL). This last example emphasizes the various locations of SMs.

Highly resolved PCs images—For PCs, images of the three most common species found in the brain^{54,55} are shown Fig. 5, **2nd row** and Table 2. PC 18:0a/18 : 1 localization (m/z 788.6; **3rd col.**) is highlighted by overlaying (**1st col.**) it with PC 16:0a/16 : 0 image (m/z 734.6, **2nd col.**). The heterogeneous presence of PC 16:0a/16 : 0 in the hippocampus confirmed results obtained with its potassiated form showing the species inside the hippocampal dendritic region and outside Py and GrDG.⁹ PC 16:0a/16 : 0 and PC 18:0a/18 : 1 mapping was mostly complementary; PC 18:0a/18 : 1 being more concentrated in the deep cerebral white matter (dcw) of the corpus callosum (Cc) and midbrain areas such as the cerebral peduncle (cp) and red nuclei (R). In addition, comparison between PC 16:0a/18 : 1 (**4th col.**) and PC 18:0a/18 : 1 showed the specific presence of the last one only in the cortex laminar layers close to the hippocampus.⁵⁶ Its absence was noticeable in the oculomotor nerve (3n) and the hippocampus area except around Py and GrDG. With high spatial resolution, it was also possible to highlight the quasi-omnipresence of PC 16:0a/18 : 1 (m/z 760.6; **4th col.**) supporting previous observations.⁹ The overlay of PC 16:0a/18 : 1 and SM d18:1/C16:0 (**4th col., 3rd row**) clearly indicated PC absence from D3V, LVs of each Cc, and cp.

These results were in agreement with our previous observations obtained for PCs potassiated forms by profiling the global cortex using a MALDI-TOF and a MALDI-Ion mobility TOF.²⁴ Other MALDI IMS studies provided similar localizations for both protonated or potassiated PCs in coronal^{28,32} as well as sagittal^{9,33} sections. For sagittal sections, results had to be subtly compared by observing their PCs location along the vertical line going through D3V (*i.e.*, close to the bregma of interest in our work).

Previous studies show similar species with little differences from our results.³⁴ For example, PC 16:0a/18 : 1 was imaged in the cited work using its adducted forms, *i.e.* $[M + Na]^+$ (m/z 782.7) and $[M + K]^+$ (m/z 798.6). Both of their images display PC locations overall similar to ours. However, we also detected PC 16:0a/18 : 1 in the entire midbrain whereas they only observed it partially. Their study highlights the presence of isobaric species due to salt adducts. Additional images show the localization of lipid fragment ions formed after the trimethylamine neutral loss ($Fw = 59 \text{ g mol}^{-1}$). In this case, the new localizations differ from the original $[PC + Na]^+$ and $[PC + K]^+$ and corroborate our $[PC + H]^+$ images. Same observations were made in their study for PC 18:0a/18 : 1 which was finally located through its m/z 751 fragment ion $[M-59 + Na]^+$ image, yielding similar results as ours.

The precision of SMs and PCs localization was confirmed by a second imaging experiment showing similar results. In addition, observed localization of SMs and PCs in brain tissue structures indicate either no or negligible molecular delocalization otherwise molecules would be more randomly distributed.

Conclusion

To our knowledge no other combination of matrix (DHA/ammonium sulfate/HFBA) and MALDI mass spectrometer has given such precision and high localization of molecular ions in SMs and PCs imaging as shown in this study. With this simple and efficient methodology, images description is not anymore a matter of localization in white *versus* grey matter, as we are now able to distinguish layers as narrow as 25 to 50 μm . In addition, we highlighted the heterogeneous locations of SMs and PCs, which are of high interest in the field of neuroscience. These lipids are good potential biomarkers of diseased tissues. In brain development, SMs presence and quantity vary as a function of axons myelination.⁸ Thus; MALDI MS can play an important role in identifying, characterizing and mapping specific SMs involved in such a process. Another application would be the mapping of PCs from brain sections of an animal model of ataxia because of the reduction of GrDG where certain PCs are located.¹⁶

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Glossary

3n	oculomotor nerve
Aq	Aqueduct
CA1	Field CA1 of the hippocampus
Cc	Corpus callosum (dcw + LV)
cp	cerebral peduncle
Cx	Cerebral cortex
dcw	deep cerebral white matter
DG	Dentate Gyrus
DS	Dorsal Subiculum
D3V	Dorsal 3rd Ventricle
GrDG	Granular layer of the Dentate Gyrus
IP	InterPeduncular nucleus
IPL	InterPeduncular nucleus, Lateral subnucleus
Lmol	Lacunosum Molecular layer of the hippocampus
LV	Lateral ventricle
MG	Medial Geniculate nucleus
ml	medial lemniscus
MODG	Molecular Layer of the Dentate Gyrus
Or	Oriens layer of the hippocampus

PAG	PeriAqueductal Gray
PoDG	Polymorph layer of the Dentate Gyrus
Py	Pyramidal cell layer of the hippocampus
R	Red nucleus
Rad	Radiatum layer of the hippocampus
Slu	Stratum Lucidum of the hippocampus
SN	Substantia Nigra
SuG/Zo	Superficial gray and Zona layers of the superior colliculus

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Fig. 1.
Chemical structures of a) sphingomyelin SM d18:1/C18:0, b) phosphatidylcholine PC C16:0/C18:1.

Fig. 2. Positive ion mode MALDI LTQ mass spectra obtained for total lipid extract from a 100µm thick section of a rat brain using a) saturated DHA and b) saturated DHA with 6 mM ammonium sulfate. In these latter conditions, an average mass spectrum was obtained from a coronal rat brain section c).

Fig. 3. MALDI-LTQ Pulsed-Q Dissociation (PQD) spectra from a) m/z 737.7 [SM d18:1/C18:0 + Li]⁺ and m/z 766.6 [PC C16:0/C18:1 + Li]⁺.

Fig. 4. Representation of a rat brain section left side at bregma -5.76 mm with anatomical structure nomenclature (left panel adapted from Paxinos and Watson, 1998) and its cresyl violet staining (right panel). Arrows indicate points of thickness measurement.

Fig. 5. Differential localization by MALDI IMS for SM or PC horizontally and for both together perpendicularly. MALDI images were obtained from a single coronal section of a rat brain at bregma -5.76 mm.

SM and PC $[M + H]^+$ detected by MALDI. i) Linkages between fatty acid and the glycerol backbone of PC is noted as **a** for an acyl, **e** for an alkyl ether and **p** for a plasmalogen

Table 1

[M + H] ⁺ assignment (major species)							
obs. <i>m/z</i>	error ppm	theo. <i>m/z</i>	precise structure ⁱ	global structure	Formula	ref.	
703.58	14	703.57	SM	d18:1/C16:0	34 : 1	C ₃₉ H ₈₀ N ₂ O ₈ P	43,48
731.67	82	731.61		d18:1/C18:0	36 : 1	C ₄₁ H ₈₄ N ₂ O ₈ P	43,48
759.62	26	759.64		d18:1/C20:0	38 : 1	C ₄₃ H ₈₈ N ₂ O ₈ P	43
785.64	13	785.65		d18:1/C22:1	40 : 2	C ₄₅ H ₉₀ N ₂ O ₈ P	43
787.66	13	787.67		d18:1/C22:0	40 : 1	C ₄₅ H ₉₂ N ₂ O ₈ P	43
799.66	13	799.67		d18:1/C23:1	41 : 2	C ₄₆ H ₉₂ N ₂ O ₈ P	43
801.67	12	801.68		d18:1/C23:0	41 : 1	C ₄₆ H ₉₄ N ₂ O ₈ P	43
813.67	12	813.68		d18:1/C24:1	42 : 2	C ₄₇ H ₉₄ N ₂ O ₈ P	43
815.68	24	815.70		d18:1/C24:0	42 : 1	C ₄₇ H ₉₆ N ₂ O ₈ P	43
732.67	164	732.55	PC	16:0a/16 : 1	32a:1	C ₄₀ H ₇₈ NO ₈ P	48
734.67	136	734.57		16:0a/16 : 0	32a:0	C ₄₀ H ₈₀ NO ₈ P	45,48
746.67	161	746.55		15:0a/18 : 1	33a:1	C ₄₁ H ₈₀ NO ₈ P	48
				16:0a/17 : 1	33a:1	C ₄₁ H ₈₀ NO ₈ P	48
				18:1e/16 : 0	34e:1	C ₄₁ H ₈₂ NO ₇ P	48
				16:0e/18 : 1	34e:1	C ₄₁ H ₈₂ NO ₇ P	48
756.67	159	756.55		16:1a/18 : 2	34a:3	C ₄₂ H ₇₈ NO ₈ P	48
				16:0a/18 : 3	34a:3	C ₄₂ H ₇₈ NO ₈ P	48
758.58	132	758.57		16:0a/18 : 2	34a:2	C ₄₂ H ₈₀ NO ₈ P	45,48
760.67	105	760.59		16:0a/18 : 1	34a:1	C ₄₂ H ₈₂ NO ₈ P	45,48
762.67	92	762.60	16:0a/18 : 0	34a:0	C ₄₂ H ₈₄ NO ₈ P	45,48	
782.67	128	782.57	16:0a/20 : 4	36a:4	C ₄₄ H ₈₀ NO ₈ P	45,48	
784.67	102	784.59	18:1a/18 : 2	36a:3	C ₄₄ H ₈₂ NO ₈ P	45,48	
			16:0a/20 : 3	36a:3	C ₄₄ H ₈₂ NO ₈ P	48	
786.67	89	786.60	18:1a/18 : 1	36a:2	C ₄₄ H ₈₄ NO ₈ P	48	
788.67	63	788.62	18:0a/18 : 1	36a:1	C ₄₄ H ₈₆ NO ₈ P	45,48	

obs. <i>m/z</i>	error ppm	theo. <i>m/z</i>	[M + H] ⁺ assignment (major species)			ref.
			precise structure ⁱ	global structure	Formula	
790.63	76	790.57	18:0p/18:0	38p:6	C ₄₆ H ₈₀ NO ₇ P	48
			20:0a/16:0	36a:0	C ₄₄ H ₈₈ NO ₈ P	48
			14:0a/22:0	36a:0	C ₄₄ H ₈₈ NO ₈ P	48
804.67	99	804.55	16:1a/22:6	38a:7	C ₄₆ H ₇₈ NO ₈ P	48
806.58	12	806.57	16:0a/22:6	38a:6	C ₄₆ H ₈₀ NO ₈ P	45,48
810.67	86	810.60	18:0a/20:4	38a:4	C ₄₆ H ₈₄ NO ₈ P	45,48
			16:0a/22:4	38a:4	C ₄₆ H ₈₄ NO ₈ P	45,48
814.67	74	814.63	18:0a/20:2	38a:2	C ₄₆ H ₈₈ NO ₈ P	48
816.67	24	816.65	18:0a/20:1	38a:1	C ₄₆ H ₉₀ NO ₈ P	48
			16:0a/22:1	38a:1	C ₄₆ H ₉₀ NO ₈ P	48
			20:0a/18:1	38a:1	C ₄₆ H ₉₀ NO ₈ P	48
832.67	96	832.59	18:1a/22:6	40a:7	C ₄₈ H ₈₂ NO ₈ P	48
834.67	84	834.60	18:0a/22:6	40a:6	C ₄₈ H ₈₄ NO ₈ P	48

Table 2

Description of SM and PC localization obtained from MALDI images of a single rat brain coronal section at bregma -5.76 mm. (See Fig. 4 for structure acronyms)