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Brain Tissue Lipidomics: Direct Probing Using Matrix-assisted Laser Desorption/Ionization Mass Spectrometry

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ABSTRACT

Lipidomics is the new frontier in biomolecular structural studies. Not only are lipids the main components in membranes that define the contours of the cell and its organelles, but they are also used for storage. Lipids form stable noncovalent complexes with proteins as well as with many drugs. Lipids are a storage depot for drugs and certain types of organic molecules. To study lipid composition and distribution, complex and time-consuming techniques are used. However, recent advances in mass spectrometry, mainly matrix-assisted laser desorption/ionization (MALDI) have made it possible to directly probe tissues to study structural components, as well as for the localization of drugs. Direct tissue imaging is a powerful tool as it gives a more complete and accurate structural picture and can trace and follow where drugs localize in tissue with minimal anatomical disruption and a minimum of manipulations. Hence, we believe that in addition to its accuracy and efficiency, this new approach will lead to a better understanding of physiological processes as well as the pathophysiology of disease.

KEYWORDS: lipids, MALDI, in situ analysis, tissue

INTRODUCTION

After water, lipids are the most common biomolecules found in the brain (12%) and make up 50% of its dry weight. They perform numerous tasks in the body such as storage of energy, are the major building blocks of cell membranes, and aid in signal transduction across biological membranes. A brief perusal of the physiology, anatomy, and pathophysiology of the brain illustrates the importance of lipids. Altered levels of phospholipids in tissue are associated with Farber disease, Gaucher disease, Niemann-Pick disease, Alzheimer disease, and Down syndrome.[1-4] Furthermore, sphingomyelin and other glycolipids have been shown to form noncovalent complexes with chlorisondamine, a nicotinic antagonist,[5,6] as well as with acetylcholine and peptides containing 2 or more adjacent Arg residues.[7] Brain lipids consist mainly of 3 major categories: cholesterol, sphingolipids (sphingomyelin, cerebrosides, sulfatides, gangliosides), and glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositols).[8]

The interest in genomics started to wane to some extent in the nineties with the completion of the genome projects, followed by the meteoric rise of Proteomics. In the melee that ensued, the scientific community at large, which has always looked on lipids as those compounds that fill fat cells and make up the least interesting part of the plasma membrane, further ignored lipids. However lipids researchers in their usual quiet way proved that lipids are important in immunology,[9] and in interactions with proteins[10] and drugs.[11]

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MALDI MS

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), a modification of laser desorption/ionization mass spectrometry (LDI-MS), has emerged as a powerful method that allows for the analysis and detection of a wide range of biomolecules.[12] In MALDI, the analyte is mixed with an excess of chemical matrix, usually a small organic acid, prior to analysis (molar matrix to analyte ratio: 10^3-10^5:1). In this technique, the matrix absorbs the laser energy and when it vaporizes, it carries off the analyte molecules. The matrix also aids the ionization of analyte molecules in the gas phase.[12] MALDI has low attomole sensitivity, a mass range up to hundreds of thousands of Daltons and is routinely used for the analysis of peptides and proteins. However, several good studies have been conducted for lipid analysis.[13-18]

Caprioli et al[19] were the first to demonstrate that biomolecules, mainly proteins, could be directly detected in tissue using MALDI, followed by Rubakhin et al[20] who probed peptides in neurons and ganglia. In this technique, matrix solution is directly deposited onto a tissue section, which is then mass analyzed with no additional sample preparation. Direct tissue analysis using MALDI produce mass spectral profiles, which can map the location and distribution of biomolecules in tissue. MALDI is well suited for biomolecular

analysis in tissue because of its high sensitivity, large tolerance for salts and other contaminants, and a wide mass range with little fragmentation. Despite the success of MALDI for the direct analysis of peptides and proteins in tissue, very little work has been undertaken for the direct tissue analysis of lipids, probably because most of the lipids in tissue have a molecular weight below 1000 d. In this mass range, identification of analytes can be difficult because of matrix ions or background interference ions from the preparation of tissue sections (ie, stains, optimal cutting temperature compound [OCT], etc). However, recently MALDI-MS has been employed for the direct analysis of lipids in different tissue types.[21-25] An example of the application of MALDI-MS for the direct probing of the distribution of glycerophospholipids and sphingolipids in rat brain tissue is discussed below.

Brain tissue lipidomics is needed because it allows precise anatomic localization of lipid species in various areas of the brain. Lipids are important as many therapeutic and drugs of abuse localize in certain areas of the brain, where they interact noncovalently with lipids in the cell lipid membrane. Furthermore, these interactions control the rate of release of the drugs, thus playing a very important role in drug delivery and drugs effects.

In these studies,[21-23] male Spray-Dawley rats (300 to 420 g) were used. The rats were killed with intraperitoneal injection (>65 mg/kg) of sodium pentobarbital and were decapitated upon cessation of respiration. The brains were quickly removed from the skull and frozen in dry-ice-chilled isopentane for 15 seconds, prior to storage at –80°C. Frozen brain tissue was cut into thin sections (14 μm thickness) in a cryostat. The tissue samples were attached to the cryostat sample stages using ice slush made from distilled water. The ice slush only came in contact with the tissue blocks at the surface opposing the sample stages and was frozen into a thin layer of ice within 5 seconds. OCT was not used because of past studies showing that OCT interference reduced the quality of mass spectra.[26] Serial brain sections were alternately collected onto a MALDI sample target and poly-L-lysine-coated microscopic slide. After collection, tissue sections were stored at 4°C until MALDI analysis. The following MALDI matrices were used in this study: 6-aza-2-thiothymine (ATT); 2,6-dihydroxyacetophenone (DHA); α-cyano-4-hydroxy cinnamic acid (CHCA); 2,5-dihydroxybenzoic acid (DHB); and sinapic acid (SA). All matrices were initially prepared as a saturated solution in 50% ethanol. Upon further testing, DHA at a concentration of 30 mg/mL in 50% ethanol was shown to yield optimal mass spectral results for lipid analysis.

Figure 1 illustrates the basic structure of common classes of glycerophospholipids (phosphatidylcholine [PC], phosphatidylethanolamine [PE], phosphatidylinositol [PI], phosphatidylserine [PS]) and sphingolipids (sphingomyelin [SM] and sulfatides [ST]). Note in Figure 1 the different head groups for each lipid class, which greatly affect the ionization efficiency of the lipids by MALDI-MS. PCs and SMs are easily ionized in positive ion mode, while PIs, PSs, and STs are easily ionized in negative ion mode. PEs can be analyzed in both positive ion and negative ion mode. The lipid nomenclature used below is as follows: PC, PE, PI, and PS species numbers (x:y) denote the total length and number of double bonds of both acyl chains, respectively, except for PE plasmalogen species (denoted with p) in which the acyl chain at the sn-1 position is replaced with an alkenyl. SM and ST species numbers correspond to the length and number of double bonds of the acyl chain attached to the sphingosine base. Hydroxylated ST species are denoted with OH.

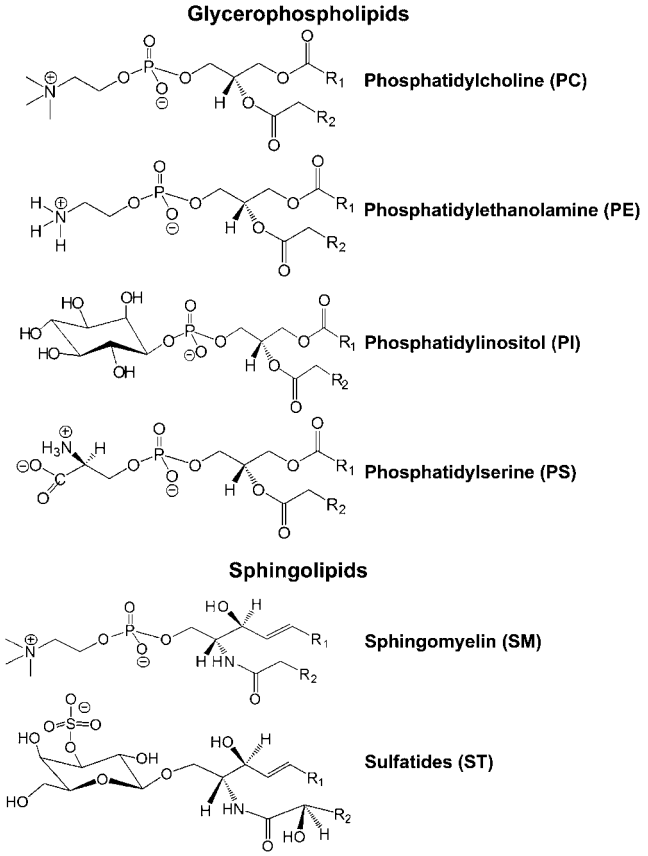

Figure 1. Major classes of brain glycerophospholipids and sphingolipids.

Detection of Basic Phospholipids and Sphingolipids

Figure 2 shows MALDI mass spectra of rat brain tissue in positive ion reflectron mode with DHA matrix. The mass spectrum illustrated in Figure 2A was obtained from the cerebellar cortex (gray matter region), while the mass spectrum illustrated in Figure 2B was acquired from the cerebellar peduncle (white matter region). Lipid species

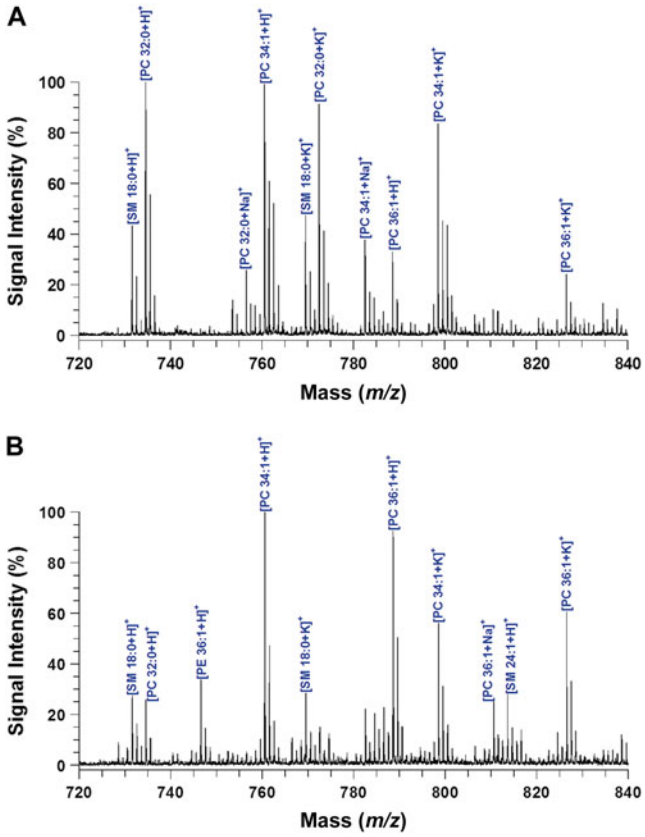

Figure 2. MALDI mass spectra of PC, PE, and SM species in the (A) cerebellar cortex and (B) cerebellar peduncle regions in rat brain with DHA matrix in positive-ion mode.

corresponding to PCs, PEs, and SMs were detected. For each phospholipid specie identified, 3 molecular ions, $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ were seen. The mass spectra in Figure 2 are dominated by phosphatidylcholine species. Although phosphatidylcholines and phosphatidylethanolamines are present in similar amounts in brain tissue, the relative abundance of phosphatidylethanolamine species is weak compared with phosphatidylcholine in the mass spectral profiles, owing to the presence of the positively charged quaternary ammonium group in the choline head of phosphatidylcholines, which immensely facilitates molecular ions formation in positive ion mode.

Comparison of the cerebellar cortex and cerebellar peduncle mass spectra shows similar phospholipid species. However, the relative abundance of the various species varies greatly because of the presence of different fatty acids at the sn-1 and sn-2 positions on the glycerol backbone. In the cerebellar cortex, the major mass peaks detected correspond to PC 32:0 and PC 34:1 species, while in the cerebellar peduncle, the major mass peaks detected correspond to PC 34:1 and PC 36:1 species. The major difference in the lipid profile between the 2 regions is the relative abundance of PC 32:0, in which a strong signal peak was observed in the cerebellar cortex, while only a weak signal peak was observed in the cerebellar peduncle. Previous studies of phosphatidylcholine species distribution in the rat brain have measured PC 34:1 as the most abundant specie followed by PC 32:0.[27,28] These earlier studies involved extraction from brain homogenate, which does not allow the spatial distribution afforded by in situ MALDI analysis. Phosphatidylcholine distribution profiles obtained from direct tissue analysis by MALDI were in relative agreement with these previous studies if the 2 regions probed are grouped together; however these results demonstrate the potential spatial variation of lipid species in the brain.

Detection of Acidic Phospholipids and Sphingolipids

In order to overcome the innate advantage that lipid classes containing quaternary amines have, negative ion mode was used to detect the other lipid classes. Figure 3 shows MALDI mass spectra of rat brain tissue in negative ion reflectron mode with DHA matrix. The mass spectrum shown in Figure 3A was recorded from the cerebellar cortex, while the mass spectrum shown in Figure 3B was acquired from the cerebellar peduncle (white matter region). Lipid species corresponding to PEs, PIs, PSs, and STs were detected.

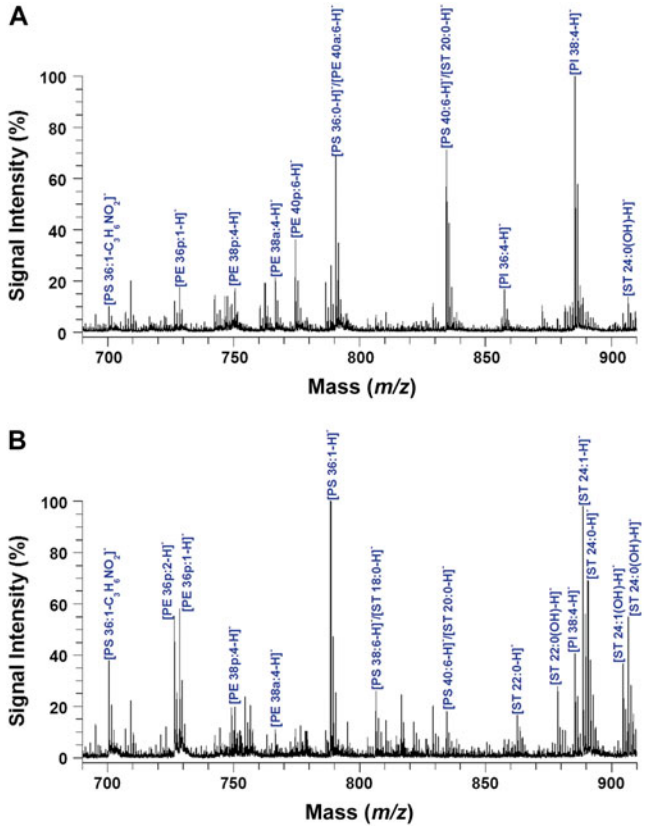

Figure 3. MALDI mass spectra of PE, PI, PS, and ST species in the (A) cerebellar cortex and (B) cerebellar peduncle regions in rat brain with DHA matrix in negative-ion mode.

Mass spectra of the cerebellar cortex in negative ion mode are dominated by mass peaks attributed to PI 38:4 and PS species with less intense signals observed for peaks associated with ST and ST(OH) species, while mass spectra of the cerebellar peduncle in negative ion mode is dominated by mass peaks assigned to PS, ST and ST(OH) species with a weak signal observed for the peak associated with PI 38:4. Previous distribution profiles of phosphatidylinositol species have measured PI 38:4 as the dominant specie, which is in agreement with our results.[27,28] Furthermore, the detection of sulfatides in negative mode is expected since they constitute ~6% of the total lipid in adult brain and contain a negatively charged sulfate group, which aids ionization in negative mode.[27] In addition, sulfatides are known to be concentrated in white matter, while phospholipids such as phosphatidylinositols are more widely represented in gray matter than in white matter, which also agrees with our mass spectral results.[8]

Gangliosides are complex sphingoglycolipids that contain one or more negatively charged sialic acids and have been implicated in brain development, neuritogenesis, memory formation, synaptic transmission, and aging.[29] The main gangliosides in the central nervous system of higher vertebrates are GM1, GD1a, GD1b, and GT1b, which account for ~80% to 90% of the total gangliosides and are illustrated in Figure 4.[30] Figure 5 shows MALDI mass spectra of the cerebellar cortex and cerebellar peduncle regions in the mass range of the most common brain gangliosides in negative ion mode with DHA matrix. The mass spectrum shown in Figure 5A was recorded from the cerebellar cortex, while the mass spectrum shown in Figure 5B was acquired from the cerebellar peduncle. In the cerebellar cortex, mass peaks assigned to GM1, GD1, and GT1 gangliosides were detected, while in the cerebellar peduncle only mass peaks associated with GM1 were observed. Although GD1 gangliosides were detected, we were unable to distinguish between GD1a and GD1b gangliosides because they are structural isomers differing only in the position of one sialic acid. Recent studies using immunostaining techniques have shown similar results in the rat cerebellum in which GM1 is concentrated in white matter regions, while GD1a and GD1b are more prevalent in gray matter regions.[31,32]

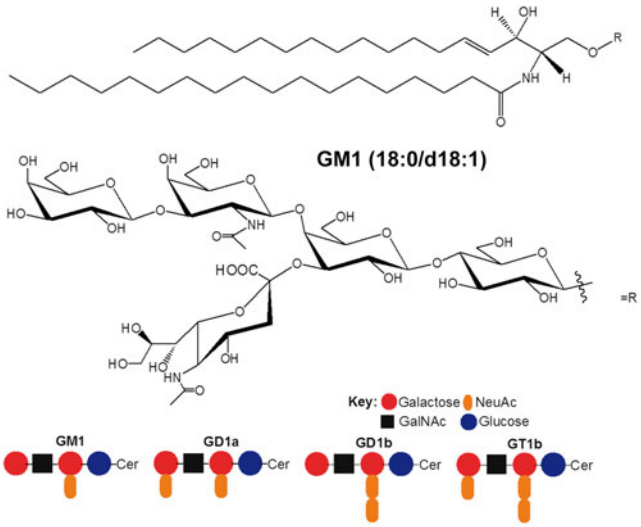

Figure 4. Major brain gangliosides.

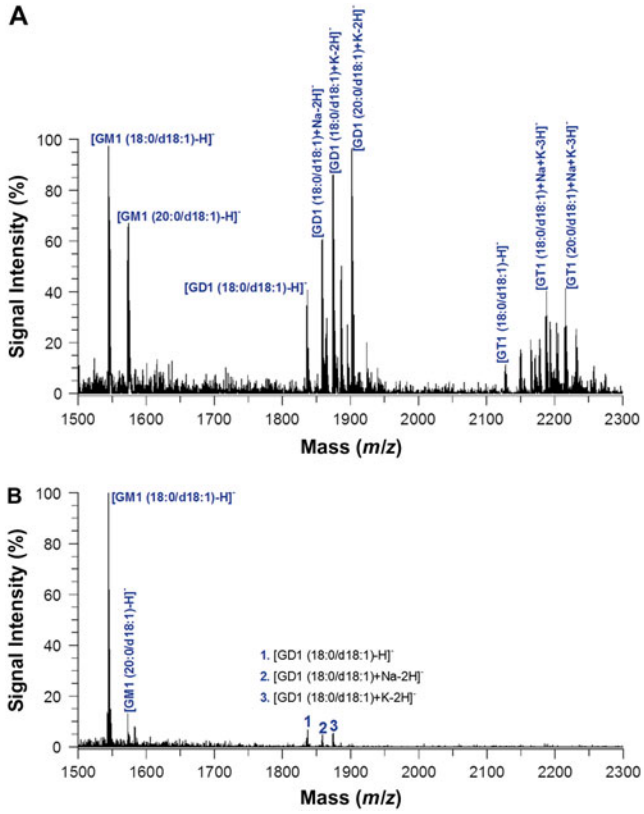

Figure 5. MALDI mass spectra of gangliosides in the (A) cerebellar cortex and (B) cerebellar peduncle regions in rat brain with DHA matrix in negative-ion mode.

Future Outlook

The outlook for in situ lipid analysis using MALDI is highly dependent on 2 factors. One factor is the ability to deposit small amounts of matrix solution (nanoliters), hence highly localizing the area to be probed. In our work, the major limitation to spatial resolution is the size of the initial wet matrix spot size, not the dry crystalline matrix spot. We have observed that the presence of organic solvents in the matrix will cause extraction of biomolecules from the tissue sections causing them to migrate, thus losing spatial resolution. The advent of chemical inkjet printing technology to spot matrix is one possible solution. The other is the use of UV lasers, which have highly focused spots able to probe minute sites. MALDI analysis with submicron lateral resolution is currently being developed.[33] Nevertheless, direct probing of tissues in general and brain tissue in particular has proven to be a very successful technique for studying the lipid makeup of various brain regions.

REFERENCES

1. Fujiwaki T, Yamaguchi S, Sukegawa K, Taketomi T. Application of delayed extraction matrix-assisted laser desorption ionization time-of-flight mass spectrometry for analysis of sphingolipids in tissues from sphingolipidosis patients. *J Chromatogr B Biomed Sci Appl*. 1999;731:45-52.

2. He X, Chen F, McGovern MM, Schuchman EH. A fluorescence-based, high-throughput sphingomyelin assay for the analysis of Niemann-Pick disease and other disorders of sphingomyelin metabolism. *Anal Biochem*. 2002;306:115-123.

3. Han X, Jr, Holtzman DM, Jr, McKeel DW, Jr, Kelley J, Morris JC. Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. *J Neurochem*. 2002;82:809-818.

4. Murphy EJ, Schapiro MB, Rapoport SI, Shetty HU. Phospholipid composition and levels are altered in Down syndrome brain. *Brain Res*. 2000;867:9-18.

5. Woods AS, Moyer SC, Wang H-YJ, Wise RA. Interaction of chlorisondamine with the neuronal nicotinic acetylcholine receptor. *J Proteome Res*. 2003;2:207-212.

6. Woods AS, Ugarov M, Egan T, et al. Lipid/peptide/nucleotide separation with MALDI-ion mobility-TOF MS. *Anal Chem*. 2004;76:2187-2195.

7. Woods AS. The mighty arginine, the stable quaternary amines, the powerful aromatics, and the aggressive phosphate: their role in the noncovalent minuet. *J Proteome Res*. 2004;3:478-484.

8. Suzuki K. Chemistry and metabolism of brain lipids. In: Albers RW, ed. *Basic Neurochemistry.* Boston, MA: Little, Brown, and Company; 1972:207-227.

9. Joyce S, Woods AS, Yewdell JW, et al. Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol. *Science*. 1998;279:1541-1544.

10. Ceppi P, Colombo S, Francolini M, Raimondo F, Borgese N, Masserini M. Two tail-anchored protein variants, differing in transmembrane domain length and intracellular sorting, interact differently with lipids. *Proc Natl Acad Sci USA*. 2005;102:16269-16274.

11. Fisar Z. Interactions between tricyclic antidepressants and phospholipid bilayer membranes. *Gen Physiol Biophys*. 2005;24:161-180.

12. Siuzdak G. Ion sources and sample introduction. In: *Mass Spectrometry for Biotechnology.* San Diego, CA: Academic Press; 1996:11-13.

13. Harvey DJ. Matrix-assisted laser desorption/ionization mass spectrometry of phospholipids. *J Mass Spectrom*. 1995;30:1333-1346.

14. Harvey DJ. Matrix-assisted laser desorption/ionization mass spectrometry of sphingo- and glycosphingo-lipids. *J Mass Spectrom*. 1995;30:1311-1324.

15. Al-Saad KA, Siems WF, Hill HH, Zabrouskov V, Knowles NR. Structural analysis of phosphatidylcholines by post-source decay matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Am Soc Mass Spectrom*. 2003;14:373-382.

16. Schiller J, Sub R, Arnhold J, et al. Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research. *Prog Lipid Res*. 2004;43:449-488.

17. Estrada R, Yappert MC. Alternative approaches for the detection of various phospholipid classes by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Mass Spectrom*. 2004;39:412-422.

18. Juhasz P, Costello CE. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry of underivatized and permethylated gangliosides. *J Am Soc Mass Spectrom*. 1992;3:785-796.

19. Caprioli RM, Farmer TB, Gile J. Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. *Anal Chem*. 1997;69:4751-4760.

20. Rubakhin SS, Li L, Moroz TP, Sweedler JV. Characterization of the aplysia californica cerebral ganglion F cluster. *J Neurophysiol*. 1999;81:1251-1260.

21. Jackson SN, Wang H-YJ, Ugarov M, Egan T, Schultz JA, Woods AS. Direct tissue analysis of phospholipids in rat brain using MALDI-TOFMS and MALDI-ion mobility-TOFMS. *J Am Soc Mass Spectrom*. 2005;16:133-138.

22. Jackson SN, Wang H-YJ, Woods AS. Direct profiling of lipid distribution in brain tissue using MALDI-TOF MS. *Anal Chem*. 2005;77:4523-4527.

23. Jackson SN, Wang H-YJ, Woods AS. In situ structural characterization of phosphatidylcholines in brain tissue using MALDI/TOF-TOF. *J Am Soc Mass Spectrom*. 2005;16:2052-2056.

24. Rujoi M, Estrada R, Yappert MC. In situ MALDI-TOF MS regional analysis of neutral phospholipids in lens tissue. *Anal Chem*. 2004;76:1657-1663.

25. Touboul D, Piednoel H, Voisin V, et al. Changes of phospholipid composition within the dystrophic muscle by matrix-assisted laser desorption/ionization mass spectrometry and mass spectrometry imaging. *Eur J Mass Spectrom (Chichester, Eng)*. 2004;10:657-664.

26. Schwartz SA, Reyzer ML, Caprioli RM. Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: practical aspects of sample preparation. *J Mass Spectrom*. 2003;38:699-708.

27. Agranoff BW, Benjamins JA, Hajra AK. Lipids. In: Siegel GJ, [Include other editors; use "et al" after 3 if there are more than 6.]et al, eds. *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects.* 6th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 1999:47-67.

28. Ma Y-C, Kim H-Y. Development of the on-line high-performance liquid chromatography/thermospray mass spectrometry method for the analysis of phospholipid molecular species in rat brain. *Anal Biochem*. 1995;226:293-301.

29. Sonnino S, Chigorno V. Ganglioside molecular species containing C18- and C20-sphingosine in mammalian nervous tissues and neuronal cell cultures. *Biochim Biophys Acta*. 2000;1469:63-77.

30. Schwarz A, Futerman AH. The localization of gangliosides in neurons of the central nervous system: the use of anti-ganglioside antibodies. *Biochim Biophys Acta*. 1996;1286:247-267.

31. Heffer-Lauc M, Lauc G, Nimrichter L, Fromholt SE, Schnaar RL. Membrane redistribution of gangliosides and glycosylphosphatidylinositol-anchored proteins in brain tissue sections under conditions of lipid raft isolation. *Biochim Biophys Acta*. 2005;1686:200-208.

32. Molander M, Berthold C-H, Persson H, Fredman P. Immunostaining of ganglioside GD1b, GD3 and GM1 in rat cerebellum: cellular layer and cell type specific associations. *J Neurosci Res*. 2000;60:531-542.

33. Spengler B, Hubert M. Scanning microprobe matrix-assisted laser desorption ionization (SMALDI) mass spectrometry: instrumentation for sub-micrometer resolved LDI and MALDI surface analysis. *J Am Soc Mass Spectrom*. 2002;13:735-748.