Mapping the Lipid composition in mouse brain by single and multiple step micro extraction analyses

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Introduction

Lipids can perform structural and functional roles within the body and are known to be important mediators of cell signaling. One of the most lipid-rich organs is the brain, and lipids account for approximately 50% dry weight. Deregulation in lipid metabolism in the brain is commonly associated with many disorders and diseases including, bipolar disorders, schizophrenia and Alzheimer's, and Parkinson's diseases. Mass Spectrometry Imaging techniques permit the direct scanning of tissue slices without losing the precise anatomical localization, which is of importance to understand the physiopathology of the different lipid species in central nervous system.
Here we demonstrate the analysis of mouse brain tissue by Liquid Extraction Surface Analyses improving the lipid coverage and number of quantified lipid species.

Materials and Methods

Frozen Mice brain tissue, horizontally sliced in 15 um thickness and placed onto a glass slide, was analyzed on a LTQ Orbitrap XL mass spectrometer equipped with a TriVersa Nanomate ion source.
Lipids were automatically micro extracted from a 0.03mm^2 brain area using 0.5ul extraction solvent spiked with Lipid standards, and infused at a flow rate of 40nL/min. MS Spectra were acquired by multiple overlapping segment range acquisition with a target mass resolution of R = 100,000 at m/z 400 in both polarity modes. MSMS fragmentation was performed by data-dependent selection of the 10 most intense peaks in the segment range using dynamic exclusion. The total acquisition cycle took 7min.

Workflow

Solvent Delivery
Tip picks up solvent

Analyte Extraction
aspirate/dispense cycles for analyte extraction

Analyte Ionization
Electrospray at using 400 nozzle nESI chip

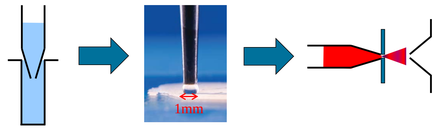


Figure 1 LESA Workflow

LTQ Orbitrap acquisition method								
Segment 1				Segment 2				
FTMS(-) $R_{m/z400}$=100000				FTMS(+) $R_{m/z400}$=100000				
SIM 200-500	SIM 450-700	SIM 650-1000	SIM 950-2000	SIM 200-700	SIM 650-900	SIM 850-2000		
Top10 MS^2	Top10 MS^2	Top10 MS^2	Top10 MS^2	Top10 MS^2	Top10 MS^2	Top10 MS^2		
Neg. composite full spectrum m/z 200-2000				Pos. composite full spectrum m/z 200-2000				
Nanomate Relay State								
Open				Close				
Nanomate Method								
Spray Voltage: - 1.25 kV Pressure: 0.95 psi				Spray Voltage: +1.25 kV Pressure: 0.95 psi				

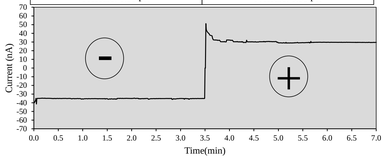
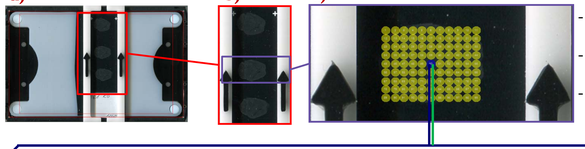


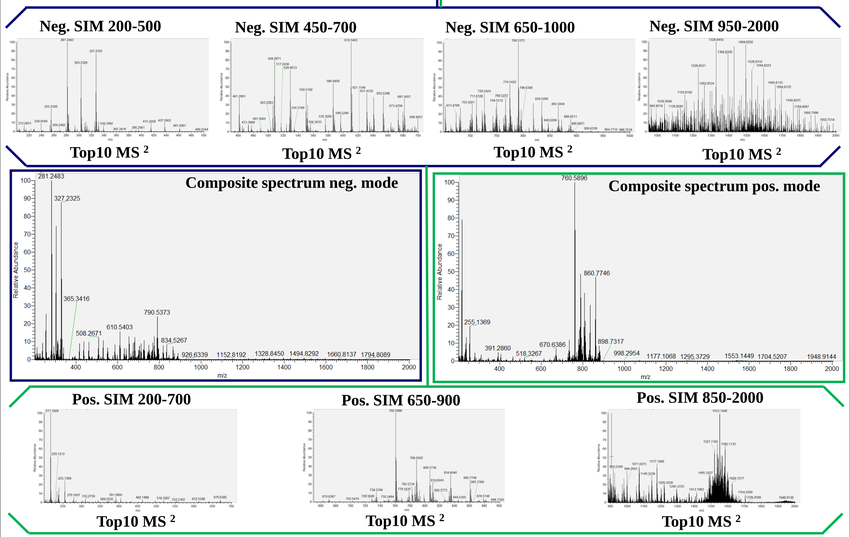
Figure 2 Experimental Set up

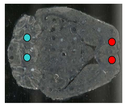
Results

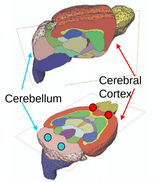
Figure 3 Experimental workflow for positional selection during LESA analyses



- 15 um thick slices Mice brain tissue, placed onto a glass slide (a)
- Image scanned using LESA point software (a,b)
- 100 Positions are selected for LESA extraction analyses (c)







PS ⇓ PE ⇓ ⇓ Plasmenyl-PE

Cerebellum
Cerebral Cortex

Figure 4 Lipid molecular species distribution in different areas of brain

Summary and discussions

- LESA uses 500 nL solvent for in situ extraction
- Polarity switching combined with DDA improves lipidome coverage
- Lipid confirmation by additional Top10 MS^2 experiment
- SIM scanning improves dynamik range during shotgun Lipidomics
- LESA can be used to study brain lipid metabolism

Literature and Acknowledgments

Schuhmann K, Almeida R, Baumert M, Herzog R, Bornstein SR, Shevchenko A: Shotgun Lipidomics on a LTQ Orbitrap mass spectrometer by successive switching between acquesition polarity modes. Journal of Mass Spectrometry **2012**;47(1):96-104

Lu A, Waanders LF,Almeida R,Li G, Allen M, Cox J, Olsen JV, Bonaldi T, Mann M: Nanoelectrosprayt peptide mapping reviseted: Composite survey spectra allow high dynamic range protein characterization without LCMS on an orbitrap mass spectrometer. International Journal of Mass Spectrometry **2007**;(268)2-3;158-167

Kertesz V, VanBerkel GJ: Fully automated liquid extraction-based surface sampling and ionization using a chip-based robotic nanoelectrospray platform. Journal of Mass Spectrometry **2009** 45(3) 252-60