Dopamine-Modified TiO2 Monolith-Assisted LDI MS Imaging for Simultaneous Localization of Small Metabolites and Lipids in Mouse Brain Tissue with Enhanced Detection Selectivity and Sensitivity

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Supplementary Information

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Additional experimental details

Tissue preparation and sectioning

In all cases, the rodent brains were surgically dissected, frozen in liquid nitrogen, and stored at −80 °C until use. Coronal tissue sections, 14-µm thick, were prepared from frozen mouse cerebrum using a cryostat (3050S, Leica Biosystems Inc., Buffalo Grove, IL) at −19 °C and thaw-mounted onto conductive indium-tin oxide (ITO)-coated glass slides (Delta Technologies, Loveland, CO).

While most samples were analyzed immediately following preparation, some sections were stored at −80 °C for later use. Optical images of the tissues were taken using a flatbed scanner (Epson Perfection V300, Epson America, Inc., Long Beach, CA) with a resolution of 2400 dpi before MSI. The optical images shown in Figure 3 were adjusted to aid in visualization of hippocampal structures using Adobe Photoshop 2014.

Materials characterization

UV-vis absorption spectra detection was performed on an EPOCH T M microplate spectrophotometer (Biotek Instruments, Inc., Winooski, VT) with a scanning range of 200–700 nm. TiO₂ nanoparticle sol solution at the concentration of 0.05 M was measured directly by adding 200 µL solutions into a standard 96-well plate.

Diffuse reflectance UV-vis spectrometry was performed on a Cary 5000 UV-Vis spectrophotometer (Agilent, Santa Clara, CA) with a wavelength scanning range of 200–700 nm. The same amounts of different TiO₂ materials were first coated on quartz slides (1" × 1", Ted Pella, Inc., Redding, CA) with an airbrush (Paasche Airbrush Company, Chicago, IL), and then the slides were used for detection, with bare quartz slides evaluated as blanks.

An environmental scanning electron microscope (ESEM) (Philips XL30 ESEM-FEG, FEI, Hillsboro, OR) was used to investigate the micro-morphology of the TiO₂ materials coated on tissue slices. Before ESEM detection, the samples were coated with gold for 1 min with a sputter coater (Desk-1 TSC, Denton Vacuum, Moorestown, NJ).

Laboratory-constructed system for MALDI matrix sublimation

Sublimation was carried out using a laboratory-constructed system, similar to one previously described, with some modifications. Briefly, an aluminum foil boat was affixed with double-sided conductive copper tape to the inner base of a sublimation chamber. Samples were attached to a copper plate affixed to the bottom face of an ice-filled cold finger. The MALDI matrix-to-sample distance was ~20 mm. For each sublimation deposition, 350 mg of powdered DHB was distributed evenly in the boat. The sublimation chamber and cold finger were assembled together per manufacturer’s instructions, pumped to intermediate vacuum (~10 mTorr), and placed in a heating mantle (Glas-Col LLC, Terre Haute, IN) to equilibrate the vacuum and cool the sample plate. The optimized deposition conditions for derivatized samples included supplying 120 V to the heating mantle for 12 min. After matrix deposition, the chamber was removed from the mantle, vented with room temperature air (25 °C), and the sample promptly removed from the cold finger.

Data analysis

The molecular ion distribution images of the tissue sections were visualized using flexImaging software 4.1 (Bruker Daltonics, Billerica, MA). MALDI MSI data acquired from triplicate or duplicate brain slices from the same mouse, thaw-mounted on three or two separate slides, were used in the statistical analysis.
For method development, samples from the same mouse were used with different sample preparation conditions on different slides.

In the method application experiments, optimized sample preparation conditions were used in the analysis of specimen from 8-month-old (young, n = 3) and 24-month-old (old, n = 4) mice. Slices from different animals were thaw-mounted on the same ITO slide. Statistical comparisons of peak intensities, areas, or S/Ns acquired from different brain regions of different animals, or samples from the same animal but prepared with different sample preparation conditions, were performed by exporting data from manually-defined regions of interest (ROIs). Mass spectra from each ROI were imported into ClinProTools software 3.0 (Bruker Daltonics) with automatic baseline subtraction and total ion count normalization, except for the data presented in Figure 3 and Figure S5. Peaks were picked with an S/N threshold greater than 3 on average spectra. Picked peak parameters were exported as m/z value-peak area tables. To analyze changes in the levels of molecular signals in different subregions of mouse brain, ROIs were chosen. ROIs were outlined using ion maps and optic images aligned to the appropriate Mouse Brain Atlas schematics (http://mouse.brain-map.org/static/atlas). Four ROIs were selected: the CA1, CA3, and dentate gyrus of the hippocampus region, and corpus callosum (shown in Figure 6A). The mass spectra acquired at chosen regions were imported into ClinProTools, set to pick the peaks with an S/N >3. Peak areas were calculated and exported creating a single data set. For the multivariate analysis (principal component analysis, (PCA)) presented in Figure 6, the dataset was imported into OriginPro 8.5 (OriginLab Corporation, Northampton, MA). The mean values of peak areas for signals acquired in selected ROIs were calculated, and the values of technical triplicates or duplicates were used to calculate the relative standard deviation of the replicate measurements. For comparison of the averaged peak areas acquired from different animal groups (old vs. young) described in Figure 5, two way analysis of variance (ANOVA) was performed with OriginPro 8.5 to determine the significance of age effect on compound intensity.
Figure S1. Dependence of TiO$_2$ particle morphology on (A) reaction time for further hydrolysis/condensation (A-1, 0 min; A-2, 10 min; A-3, 3 h; A-4, 12 h). (B) Relation between TiO$_2$ condensation time and relative peak area of intact lipid signals (red lines) and lipid fragment signals (blue lines) acquired from brain tissue. (C) Fragmentation of PE and structures of its fragments. n = 2; the p values of a Tukey test for all conditions are presented in Table S5; $m/z$ 806.5 and $m/z$ 852.6 were identified as [PE (38:4)+K]$^+$ and [PE(42:9)+K]$^+$, respectively, according to FTICR and TOF/TOF MS/MS spectra.
Figure S2. Representative mass spectra acquired from mouse brain tissues using different amounts of TiO\textsubscript{2} nanoparticles. No lipid signals were observed when using the TiO\textsubscript{2} nanoparticles. (top) 100 µg/cm\textsuperscript{2}; (middle) 500 µg/cm\textsuperscript{2}; (bottom) 2000 µg/cm\textsuperscript{2}. The inset shows the enlarged spectra for m/z 300–800.

Figure S3. Effects of (A) nitric acid and (B) phosphate acid concentrations in TiO\textsubscript{2}-sub-micron particle-containing deposition solutions on intact lipid (red lines) and their fragment (blue and black lines) signals acquired with TiO\textsubscript{2}-assisted LDI (n = 2). The p values of a Tukey’s test for all conditions are listed in Table S3; m/z 806.5 and m/z 852.6 were identified as [PE(38:4)+K]\textsuperscript{+} and [PE(42:9)+K]\textsuperscript{+}, respectively, according to FTICR and TOF/TOF MS/MS spectra.
Figure S4. UV-vis spectra of solutions of different bidentate binding ligands, TiO$_2$ nanoparticles, and bidentate binding ligand-modified TiO$_2$ nanoparticles.
Figure S5. Performance of different chemical modifications of TiO$_2$ sub-micron particles in LDI MS measurements of region I of the hippocampus proper area of the mouse brain. (A) Mass spectra acquired from modified TiO$_2$ sub-micron particle blank samples. (B) Relative peak area ratios of the intact lipid ($m/z$ 844.5) compared to its fragments ($m/z$ 141.0 and $m/z$ 162.9) using the different modified particles. AL, alizarin; SA, salicylic acid; AA, ascorbic acid; DA, dopamine.
Figure S6. Time dependence of UV-vis absorption of TiO₂-DA particle suspensions. UV-vis absorption spectra of DA-modified TiO₂ with (A) different incubation times and (B) its UV absorbance kinetic curve at a wavelength of 355 nm.
Figure S7. Comparison of mass spectra from mouse brain tissue obtained using (blue) TiO$_2$ sub-micron particle-, (yellow) TiO$_2$-DA sub-micron particle-, and (light blue) TiO$_2$-DA monolith-assisted LDI, and mass spectra of blank samples with (dark blue) TiO$_2$ sub-micron particle- and (orange) TiO$_2$-DA monolith-assisted LDI.
**Figure S8.** Effect of water content in reaction solutions for further hydrolysis/condensation on TiO$_2$-DA structure morphology and TiO$_2$-DA materials-assisted LDI MSI measurements. (Left) Representative mass spectra acquired from mouse brain hemisphere sections coated with TiO2-DA materials generated in the presence of (A) 1%, (B) 2.5%, (C) 5%, and (D) 10% water in ethanol solution (reaction time is 60 min each). (Insets) SEM images of corresponding samples coated with TiO2-DA materials. (Right) Ion maps of representative lipid ($m/z$ 844.5) distributions in the sections. Acquisitions were performed in the low spatial resolution mode with a 100-µm raster step size using settings producing a laser footprint ~100-µm in diameter.
Figure S9. Ratios of an intact lipid to its fragment peak areas as determined using different TiO$_2$-based LDI MS methods. The intact lipid ($m/z$ 844.5) to fragment ($m/z$ 141.0) ratios were calculated using data acquired from different subregions of mouse brain using LDI MS assisted with TiO$_2$ sub-micron particles (DA0), TiO$_2$-DA sub-micron particles (DA1), and TiO$_2$-DA monoliths (DA4). CA1, region I of hippocampus proper; CA3, region III of hippocampus proper; CC, corpus callosum.
Figure S10. Scanning electron microscopy image of the TiO2-DA monolith surface with low magnification (×5000).
Figure S11. Repeatability of the unmodified TiO$_2$ sub-micron particle (DA0)-, TiO$_2$-DA sub-micron particle (DA1)-, and TiO$_2$-DA monolith (DA4)-assisted LDI MS measurements. Histograms depict average peak S/Ns of different molecular signals: (A) $m/z$ 100–500, (B) $m/z$ 500–700, and (C) $m/z$ 700–900, acquired from region I of hippocampus (CA1), region III of hippocampus (CA3), and corpus callosum (CC) of different animals prepared and analyzed in different weeks. Average peak S/Ns with standard deviation error bars calculated using data obtained from measurements of adjacent brain slice triplicates collected from the same animals. The slices were deposited on different ITO glass slides and coated with the TiO$_2$ materials. The relative standard deviations of the triplicate data acquired using TiO$_2$-DA monoliths are labeled on the corresponding bar graphs. Columns are positioned and boxed according to the week when samples were collected and analyzed.
Figure S12. MS/MS spectra of different endogenous compounds detected in mouse brain tissues during TiO2-DA monolith-assisted LDI MSI experiments. (A) PE, phosphatidylethanolamine; (B) PC, phosphatidylcholine; (C) M(IP)2C, mannose-(inositol-P)2-ceramide; (D) DAG, diacylglycerol; (E) Cer, ceramide; (F) docosahexaenoic acid; (G) proline-glutamate; (H) glutamate; (I) creatinine; (J) arecaidine.
Figure S13. Results of PCA and ANOVA statistical analyses of data acquired from old and young mice samples. (A) PCA score plots of the TiO$_2$-DA monolith-assisted LDI MSI dataset acquired from different cerebrum regions of young and old mice. (B) Statistical comparison of average peak areas of different identified molecules detected in brain samples of old and young mice. p values were calculated using two-way ANOVA for data sets representing different age groups (see details in Table S4). DG, dentate gyrus; CC, corpus callosum. ***, p value<0.001, **, p value<0.01, *, p value<0.05.
Figure S14. Loading plots of (top) PC1 and (bottom) PC2 of data collected from young and old mice samples using the optimized TiO$_2$-DA-assisted LDI method.
Table S1. Tukey test results for all of the data sets presented in Figure S1 and Figure S3.

| Figure | X axis of two datasets | P values of two data sets determined by Tukey test |
|--------|------------------------|--------------------------------------------------|
|        |                        | m/z 162.9 | m/z 140.9 | m/z 136.1 | m/z 868.5 | m/z 852.5 | m/z 806.5 |
| S1B    | 10 min–0 min           | 0.06076   | 0.0074    | 0.00674   | 0.5422    | 0.0285    | 0.99981    |
|        | 3 h–0 min              | 0.05081   | 0.00253   | 0.00719   | 0.34371   | 0.15104   | 0.99797    |
|        | 3 h–10 min             | 0.99961   | 0.50564   | 0.99997   | 0.98635   | 0.48654   | 0.99991    |
|        | 12 h–0 min             | 0.19164   | 0.0024    | 0.00686   | 0.00151   | 1.62E-04  | 0.05736    |
|        | 12 h–10 min            | 0.78256   | 0.46468   | 1         | 0.00378   | 0.00106   | 0.06669    |
|        | 12 h–3 h               | 0.689     | 0.99998   | 0.99999   | 0.00511   | 4.82E-04  | 0.0757     |
|        | 24 h–0 min             | 0.09771   | 0.00319   | 0.00637   | 0.0021    | 1.43E-04  | 0.0574     |
|        | 24 h–10 min            | 0.98556   | 0.70089   | 0.99999   | 0.00559   | 8.84E-04  | 0.06674    |
|        | 24 h–3 h               | 0.95422   | 0.99314   | 0.99969   | 0.00773   | 4.15E-04  | 0.07576    |
|        | 24 h–12 h              | 0.95818   | 0.98526   | 0.99996   | 0.96667   | 0.99456   | 1          |
| S3A    | 0.0025–0               | 0.99775   | 0.01929   | 0.81613   | 0.00272   | 0.12439   | 0.0153     |
|        | (concentration of HNO₃, M) |          |          |          |          |          |            |
|        | 0.005–0                | 0.81737   | 0.02544   | 0.95645   | 5.36E-04  | 0.02467   | 0.00343    |
|        | 0.005–0.0025           | 0.73214   | 0.97331   | 0.57233   | 0.02932   | 0.31235   | 0.13918    |
|        | 0.01–0                 | 0.47693   | 0.0052    | 0.6885    | 1.55E-04  | 0.01501   | 0.00131    |
|        | 0.01–0.0025            | 0.40543   | 0.23175   | 0.31588   | 0.00199   | 0.15026   | 0.0201     |
|        | 0.01–0.005             | 0.89054   | 0.15635   | 0.91174   | 0.02238   | 0.86676   | 0.20685    |
| S3B    | 0.005–0                | 0.95288   | 0.18956   | 0.29412   | 0.33198   | 0.38204   | 0.27629    |
|        | (concentration of H₃PO₄, M) |          |          |          |          |          |            |
|        | 0.01–0                 | 0.08448   | 0.0992    | 0.09113   | 0.03231   | 0.05654   | 0.07972    |
|        | 0.01–0.005             | 0.10514   | 0.7225    | 0.43322   | 0.09655   | 0.18038   | 0.38979    |
Table S2. Peak list collected from averaged mass spectra acquired from mouse brain tissues and corresponding blanks using TiO₂ sub-micron particle-, TiO₂-DA sub-micron particle-, and TiO₂-DA monolith-assisted LDI MS.

| m/z   | TiO₂ sub-micron particles | TiO₂-DA sub-micron particles | TiO₂-DA monolith | Analyte identity | Molecular mass error (Δppm) | TiO₂ sub-micron particles | TiO₂-DA sub-micron particles/monolith |
|-------|---------------------------|-----------------------------|------------------|-----------------|--------------------------|---------------------------|--------------------------------------|
| 104.26| 1.135                     | 1.642                       | 1.668            |                 |                          |                           |                                       |
| 109.08| 3.944                     | 4.231                       | 7.517            |                 |                          |                           |                                       |
| 111.15| 1.954                     | 0.742                       | 0.325            |                 |                          |                           |                                       |
| 113.02| 5.818                     | 20.998                      | 51.533           |                 |                          |                           |                                       |
| 115.01| 1.905                     | 8.061                       | 22.988           | *               | *                        |                           |                                       |
| 117.01| 0.358                     | 0.899                       | 2.216            |                 |                          |                           |                                       |
| 121.07| 1.362                     | 0.928                       | 1.525            |                 |                          |                           |                                       |
| 123.12| -                         | -                           | -                |                 |                          |                           |                                       |
| 125.01| 11.659                    | 10.706                      | 20.918           | *               |                          |                           |                                       |
| 126.92| -                         | -                           | -                |                 |                          |                           |                                       |
| 127.01| 0.735                     | 0.577                       | 0.912            |                 |                          |                           |                                       |
| 136.11| 0.365                     | 0.689                       | 1.799            |                 |                          |                           |                                       |
| 137.102| -                         | -                           | -                |                 |                          |                           |                                       |
| 138.96| 0.502                     | 0.382                       | 0.668            |                 |                          |                           |                                       |
| 140.95| 12.574                    | 15.461                      | 35.571           |                 |                          |                           |                                       |
| 142.08| 0.345                     | 0.216                       | 0.339            |                 |                          |                           |                                       |
| 142.94| 1.615                     | 1.809                       | 3.709            |                 |                          |                           |                                       |
| 143.895| -                         | -                           | -                | *               | *                        |                           |                                       |
| 145.07| -                         | 3.781                       | 4.521            | *               | *                        |                           |                                       |
| 147.03| 2.099                     | 2.692                       | 4.223            |                 |                          |                           |                                       |
| 152.05| 0.327                     | 0.820                       | 2.905            |                 |                          |                           |                                       |
| 154.05| 0.245                     | 0.191                       | 0.332            |                 |                          |                           |                                       |
| 154.9 | 0.434                     | 0.614                       | 1.362            |                 |                          |                           |                                       |
| 156.9 | 6.866                     | 9.316                       | 26.446           |                 |                          |                           |                                       |
| 158.9 | 1.531                     | 1.700                       | 3.949            |                 |                          |                           |                                       |
| 161.01| 0.521                     | 1.300                       | 1.356            |                 |                          |                           |                                       |
| 162.97| 0.744                     | 0.978                       | 1.854            |                 |                          |                           |                                       |
| 164.01| 0.766                     | 0.736                       | 0.773            | *               |                          |                           |                                       |
| 167.02| 0.203                     | 0.083                       | 0.029            |                 |                          |                           |                                       |
| 167.98| 0.270                     | 0.435                       | 0.622            |                 |                          |                           |                                       |
| 168.95| 0.312                     | 0.330                       | 0.522            |                 |                          |                           |                                       |
| 169.98| 0.241                     | 0.508                       | 0.947            | *               |                          |                           |                                       |
| 170.84| 0.469                     | 0.873                       | 2.294            |                 |                          |                           |                                       |
| 172.02| 0.166                     | 0.360                       | 0.557            |                 |                          |                           |                                       |
|       |       |       |       |                                |   |   |
|-------|-------|-------|-------|---------------------------------|---|---|
| 172.84 | 0.504 | 0.882 | 2.202 |                               |   |   |
| 174.053 | 0.504 | 0.668 | 1.272 | Dopamine quinone+Na            | 3 | * |
| 176.0686 | 0.504 | 2.225 | 2.756 | Dopamine +Na                   | 2 | * |
| 179.97 | 0.587 | 0.812 | 0.844 |                               |   |   |
| 184.04 | 7.996 | 6.440 | 5.110 |                               |   |   |
| 184.928 |       |       |       | *                               |   |   |
| 185.94 | 0.294 | 0.920 | 2.150 | *                               |   |   |
| 186.8 | 0.342 | 0.573 | 1.246 |                               |   |   |
| 188.0 | 0.310 | 0.451 | 1.027 |                               |   |   |
| 189.98 | 0.270 | 0.735 | 1.122 | *                               |   |   |
| 192 |       | 0.342 | 0.573 | 1.246                           |   |   |
| 195.95 |       | 0.112 | 0.143 |                               |   |   |
| 198.03 |       | 0.710 | 0.843 | 1.250                           |   |   |
| 201.89 |       | 0.851 | 2.969 | 2-Thiothiazolidine-4-carboxylic acid+K | 24  |
| 202.99 | 1.136 | 0.763 | 0.546 |                               |   |   |
| 206.766 |       |       |       | *                               |   |   |
| 214.78 |       | 0.526 | 0.543 | 1.259                           |   |   |
| 215.88 |       | 0.163 | 0.143 | 0.216                           |   |   |
| 218.02 |       | 0.773 | 0.418 |                               |   |   |
| 218.96 |       | 0.771 | 0.547 | 0.295                           |   |   |
| 223.717 |       |       |       | *                               |   |   |
| 224.89 | 0.087 | 0.489 | 0.369 |                               |   |   |
| 233.99 |       | 0.449 | 0.201 |                               |   |   |
| 240.91 |       | 0.335 | 0.216 |                               |   |   |
| 241.91 |       | 0.299 | 0.216 |                               |   |   |
| 257.88 |       | 0.349 | 0.349 |                               |   |   |
| 258.77 |       | 0.709 | 0.783 | 2.059                           |   |   |
| 260.97 |       | 1.216 | 1.046 | 1.539                           |   |   |
| 263.92 | 0.577 | 0.401 | 0.489 |                               |   |   |
| 264.8 |       |       |       | *                               |   |   |
| 274.72 | 0.501 | 0.391 | 1.022 |                               |   |   |
| 279.1 |       | 0.549 | 0.348 | 0.165                           |   |   |
| 280.92 |       | 0.292 | 0.349 | 0.865                           |   |   |
| 282.95 | 0.562 | 1.024 | 2.270 | Glutamyl-Asparagine+Na         | 21|
| 289.892 |       |       |       | *                               |   |   |
| 292.86 |       | 0.242 | 0.189 | 0.135                           |   |   |
| 294.9 |       | 0.232 | 0.253 | 0.279                           |   |   |
| 296.91 |       | 0.314 | 0.417 | 0.898                           |   |   |
| 298.92 | 1.288 |       | 2.787 | Glutamyl-Asparagine+K          | 27|

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|     |   |   |   |   |
|-----|---|---|---|---|
| 303.907 | - | - | - | - |
| 305.13 | 0.366 | 0.293 | 0.278 | * |
| 306.892 | - | - | - | * |
| 307.13 | 0.443 | 0.397 | 0.203 | |
| 314.9 | 0.280 | 0.696 | 1.487 | |
| 320.9 | 0.305 | 0.211 | 0.226 | |
| 322.87 | 0.150 | 0.155 | 0.175 | |
| 327.07 | 0.295 | 0.405 | 0.569 | |
| 335.11 | 0.289 | 0.250 | 0.169 | |
| 336.95 | 0.217 | 0.199 | 0.141 | |
| 340.88 | 0.140 | 0.069 | 0.004 | |
| 343.06 | 0.145 | 0.306 | 0.214 | |
| 351.08 | 0.343 | 0.259 | 0.367 | |
| 355.14 | 0.165 | 0.213 | 0.139 | |
| 363.14 | 0.287 | 0.150 | 0.080 | |
| 367.06 | 0.242 | 0.125 | 0.047 | |
| 369.83 | - | - | - | * |
| 393.175 | - | - | - | * |
| 407.18 | 1.696 | 0.360 | 0.582 | |
| 409.21 | 15.746 | 6.786 | 7.994 | |
| 410.21 | 4.020 | 1.577 | 1.928 | |
| 413.13 | 0.094 | 0.042 | 0.027 | |
| 415.11 | 0.178 | 0.067 | 0.089 | |
| 417.13 | 0.178 | 0.065 | 0.018 | |
| 425.18 | 1.090 | 0.553 | 0.617 | |
| 429.12 | 0.126 | 0.065 | 0.019 | |
| 431.12 | 0.171 | 0.045 | 0.058 | |
| 439.18 | 0.253 | 0.093 | 0.051 | |
| 441.12 | 0.494 | 0.243 | 0.190 | |
| 453.08 | 0.254 | 0.094 | 0.078 | |
| 457.19 | 0.521 | 0.148 | 0.057 | |
| 459.14 | 0.210 | 0.071 | 0.006 | |
| 465.13 | 0.259 | 0.198 | 0.195 | |
| 469.02 | 0.189 | 0.092 | 0.078 | |
| 481.11 | 0.116 | 0.067 | 0.085 | |
| 495.19 | 0.118 | 0.043 | 0.042 | |
| 497.07 | 0.126 | 0.105 | 0.052 | |
| 502.17 | 0.203 | 0.112 | 0.065 | |
| 551.25 | 0.151 | 0.098 | 0.089 | |
| 564.16 | 0.121 | 0.107 | 0.055 | |
| 586.45 | 0.277 | 0.144 | 0.110 | |
|      |     |     |     |
|------|-----|-----|-----|
| 588.46 | 0.439 | 0.339 | 0.346 |
| 592.18 | 0.272 | 0.283 | 0.220 |
| 604.43 | 0.101 | 0.079 | 0.064 |
| 617.43 | 0.616 | 0.270 | 1.650 |
| 630.18 | 0.109 | 0.156 | 0.037 |
| 633.42 | 0.200 | 0.115 | 0.245 |
| 655.4 | 0.233 | 0.152 | 0.238 |
| 667.52 | 1.483 | 0.897 | 4.515 |
| 683.46 | 0.393 | 0.259 | 0.562 |
| 691.48 | 0.205 | 0.140 | 0.364 |
| 697.45 | 0.176 | 0.128 | 0.137 |
| 701.38 | 0.178 | 0.160 | 0.127 |
| 707.47 | 0.173 | 0.103 | 0.114 |
| 711.46 | 0.198 | 0.140 | 0.122 |
| 713.42 | 0.174 | 0.141 | 0.218 |
| 723.51 | 0.527 | 0.297 | 0.201 |
| 740.47 | 0.444 | 0.416 | 0.260 |
| 766.61 | 2.754 | 2.969 | 1.924 |
| 772.54 | 0.388 | 0.710 | 0.831 |
| 774.58 | 0.250 | 0.410 | 0.490 |
| 778.49 | 0.764 | 1.014 | 1.120 |
| 782.58 | 0.942 | 1.350 | 0.946 |
| 784.55 | 0.588 | 0.775 | 0.527 |
| 788.57 | 0.690 | 1.747 | 2.049 |
| 790.56 | 0.607 | 1.604 | 1.797 |
| 792.57 | 0.695 | 0.748 | 0.772 |
| 794.67 | 2.612 | 2.330 | 1.173 |
| 802.53 | 0.322 | 0.523 | 0.546 |
| 804.59 | 0.520 | 1.319 | 1.485 |
| 806.6 | 1.311 | 2.246 | 3.058 |
| 807.55 | 0.357 | 0.872 | 1.214 |
| 808.58 | 0.813 | 1.076 | 1.521 |
| 809.49 | 0.770 | 1.000 | 1.013 |
| 810.57 | 0.974 | 1.602 | 1.269 |
| 811.6 | 0.715 | 0.833 | 0.601 |
| 812.58 | 0.804 | 1.405 | 1.595 |
| 814.55 | 0.469 | 0.831 | 0.752 |
| 816.57 | 0.501 | 1.028 | 0.956 |
| 818.62 | 0.609 | 0.953 | 0.710 |
| 820.69 | 1.250 | 1.191 | 0.326 |
| 822.72 | 20.355 | 22.901 | 16.706 |
| m/z  | Intensity | Charge 1 | Charge 2 |
|------|-----------|----------|----------|
| 824.55 | 3.655     | 4.443    | 3.970    |
| 826.49 | 0.518     | 0.839    | 0.892    |
| 828.59 | 0.368     | 1.286    | 2.329    |
| 830.55 | 0.776     | 1.178    | 1.030    |
| 832.75 | 5.830     | 11.035   | 7.467    |
| 834.65 | 0.656     | 1.412    | 0.877    |
| 836.73 | 11.053    | 13.412   | 10.642   |
| 837.58 | 3.880     | 5.202    | 4.485    |
| 838.67 | 7.797     | 10.591   | 8.178    |
| 840.53 | 1.806     | 1.984    | 0.950    |
| 844.55 | 0.375     | 1.110    | 1.871    |
| 846.57 | 0.306     | 0.341    | 0.472    |
| 848.73 | 8.926     | 16.396   | 11.044   |
| 849.73 | 2.612     | 5.512    | 3.708    |
| 850.73 | 7.836     | 10.570   | 7.178    |
| 851.74 | 3.461     | 4.427    | 2.865    |
| 852.59 | 4.499     | 6.522    | 6.640    |
| 856.59 | 0.086     | 0.152    | 0.211    |
| 864.7 | 2.824     | 4.656    | 3.450    |
| 865.72 | 0.954     | 1.687    | 1.185    |
| 866.72 | 3.205     | 4.463    | 3.413    |
| 867.75 | 0.749     | 1.285    | 0.884    |
| 868.6 | 0.689     | 0.919    | 1.094    |
| 884.61 | 0.179     | 0.149    | 0.167    |
| 1268.44 | 0.093   | 0.095    | 0.058    |
| 1336.53 | 0.092  | 0.089    | 0.034    |

* Peak is observed in this m/z.

a Peak identities are assigned using the precise molecular masses determined by FTICR MS.
### Table S3. Tukey test results for all of the data points presented in Figure 2.

| X axis of two data sets | P values of two data sets by Tukey test |
|------------------------|----------------------------------------|
|                        | m/z 152.1 | m/z 588.5 | m/z 298.9 | m/z 822.5 | m/z 409.2 | m/z 828.5 | m/z 667.5 | m/z 804.5 | m/z 136.1 |
| CA1                    |           |           |           |           |           |           |           |           |           |
| DA1-DA0               | 0.2818    | 1         | 0.9176    | 1         | 1         | 0.9049    | 0.9851    | 0.9285    | 0.9981    |
| DA4-DA0               | 0.0002    | 0.00005   | 0.002     | 0.8236    | 0.0457    | 0.00005   | 0.5138    | 0.0168    | 0.9957    |
| DA4-DA1               | 0.0342    | 0.00007   | 0.0272    | 0.896     | 0.0807    | 0.0007    | 0.0128    | 0.1885    | 0.4655    |
| CA3                    |           |           |           |           |           |           |           |           |           |
| DA4-DA0               | 0.0011    | 0.00009   | 0.00004   | 0.9555    | 0.02      | 0.000002  | 0.456     | 0.0322    | 0.8943    |
| DA4-DA1               | 0.06      | 0.000003  | 0.001     | 0.9782    | 0.0481    | 0.00003   | 0.5973    | 0.2278    | 0.9995    |
| DA1-DA0               | 0.5885    | 0.9968    | 0.7131    | 1         | 0.9997    | 0.85      | 0.9951    | 0.9728    | 0.2956    |
| CC                     |           |           |           |           |           |           |           |           |           |
| DA1-DA0               | 0.91      | 1         | 0.1188    | 0.0831    | 0.9996    | 0.9938    | 1         | 0.2639    | 0.6837    |
| DA4-DA0               | 0.1057    | 0.8239    | 0.00002   | 0.00002   | 0.004     | 0.3171    | 0.00000005| 0.0006    | 0.1856    |
| DA4-DA1               | 0.6924    | 0.8657    | 0.004     | 0.0105    | 0.0125    | 0.7871    | 0.0000006 | 0.1171    | 0.4367    |

Abbreviations: CA1, region I of hippocampus proper; CA3, region III of hippocampus proper; CC, corpus callosum. TiO$_2$ sub-micron particles, (DA0); TiO$_2$-DA sub-micron particles, (DA1); and TiO$_2$-DA monoliths (DA4).
Table S4. List of identified or putatively identified analytes detected in mouse brain using TiO$_2$-DA monolith-assisted LDI MS, DHB-assisted MALDI MS, and 9-AA assisted MALDI MS. Precise m/z and mass errors (Δppm) of measurements are reported. Due to the use of precise molecular masses and/or molecular fragmentation patterns for characterization of the listed analytes, different levels of confidence in their identification are stated (see footnotes for the table).

| Mass to charge ratio (m/z) | Mass error (Δppm) | Compounds | Detected ions | Analyte chemical class | Ref. |
|--------------------------|-------------------|-----------|--------------|------------------------|------|
| TiO$_2$-DA-assisted LDI MS |                   |           |              |                        |      |
| 170.03272                | 0.7               | Creatine  | [M+K]$^+$    | Alkaloid               | 3,5  |
| 172.037                  | 0.6               | 5,6-dihydroxyindole | [M+Na]$^+$  | Alkaloid               | 6,7c |
| 180.0422$^a$            | 0.5               | Arecaidine | [M+K]$^+$    | Alkaloid               | 3c   |
| 295.07237                | 0.2               | 5-S-Cysteinyldopamine | [M+Na]$^+$  | Alkaloid               | 9,10c|
| 136.04855                | 3                 | Creatinine | [M+Na]$^+$   | Alkaloid               | 11c  |
| 152.02295$^a$           | 5                 | Creatinine | [M+K]$^+$    | Alkaloid               | 11c  |
| 216.042$^a$             | 0.8               | 5-Hydroxytryptophol | [M+K]$^+$   | Alkaloid               | 12c  |
| 225.20538$^a$           | 1                 | Spermine  | [M+Na]$^+$   | Alkaloid               | 3,13 |
| 241.17888$^a$           | 3                 | Spermine  | [M+K]$^+$    | Alkaloid               |      |
| 139.08317$^a$           | 7                 | Aminopentanamide | [M+Na]$^+$  | Amino acids            | 14d  |
| 142.02682                | 2                 | Aminobutanoic acid | [M+K]$^+$   | Amino acids            | 3,15 |
| 154.02049                | 38                | Proline   | [M+K]$^+$    | Amino acids            | 16,17|
| 167.0219                 | 1                 | 2-amino-4-cyano-butanolic acid | [M+K]$^+$ | Amino acids            | 18,21|
| 168.0057$^a$            | 0.6               | Pyroglutamic acid | [M+K]$^+$   | Amino acids            | 21,22c|
| 169.0585                 | 0.6               | Glutamine  | [M+Na]      | Amino acids            | 16,23|
| 186.01661$^a$           | 1                 | Glutamate  | [M+K]$^+$    | Amino acids            | 16   |
| 196.00076                | 0.3               | Amino-muconic acid | [M+K]$^+$   | Amino acids            | 24d  |
| 198.08906                | 0.2               | Amino-octanoic acid | [M+K]$^+$   | Amino acids            | 25d  |
| 234.07355$^a$           | 0.6               | Methoxytyrosine | [M+Na]$^+$  | Amino acids            | 15   |
| 586.4919                 | 42                | Cer(d36:2) | [M+K]$^+$    | Cer                    | 26   |
| 598.53436$^a$           | 2                 | Cer(d36:1) | [M+Na]$^+$   | Cer                    | 26   |
| 604.5066                 | 7                 | Cer(d36:1) | [M+K]$^+$    | Cer                    | 26   |
| 429.23852                | 3                 | Hydroxy-oxo-cholan-oic Acid | [M+K]$^+$ | CL                     | 27,28c|
| 439.29546                | 4                 | OH-7-Dehydrocholesterol | [M+K]$^+$ | CL                     | 29   |
| 465.33124                | 5                 | Cholesta-6,8(14)-dien-3beta,5alpha-diol | [M+K]$^+$ | CL                     | d    |
| 691.58617$^a$           | 19                | 18:0 Cholesteryl ester | [M+K]$^+$  | CL                     | 30,31|
| 409.34237$^a$           | 4                 | Cholesterol | [M+Na]$^+$  | CL                     | 32,36|
| 425.31629                | 5                 | Cholesterol | [M+K]       | CL                     |      |
| 667.53166                | 10                | DAG(38:4) | [M+Na]$^+$   | DAG                    | 37c  |
| 683.49536$^a$           | 8                 | DAG(38:4) | [M+K]$^+$    | DAG                    |      |
| 711.5751$^a$            | 20                | DAG(41:3) | [M+Na]$^+$   | DAG                    | d    |
| 617.51259$^a$           | 7                 | DAG(34:1) | [M+Na]$^+$   | DAG                    | 38   |
| 633.48768                | 3                 | DAG(34:1) | [M+K]$^+$    | DAG                    |      |
| 655.46536$^a$           | 6                 | DAG(36:4) | [M+K]$^+$    | DAG                    | d    |
| 259.05220                | 10                | Met-Ala   | [M+K]$^+$    | Di-peptides            | 39c  |
| 277.0617                | 1                 | Gly-Tyr   | [M+Na]$^+$   | Di-peptides            | 40c  |
| 283.06917                | 0.2               | Pro-Glu   | [M+K]$^+$    | Di-peptides            | 36c  |
| m/z       | Adduct | Molecule                                      | Intensity | Type |
|-----------|--------|-----------------------------------------------|-----------|------|
| 299.04309 | Glu-Asn | [M+K]$^+$ Di-peptides                         | 27        |      |
| 279.22797 | Palmitic acid | [M+Na]$^+$ FA                               | 1         |      |
| 293.18727 | FA16:1   | [M+K]$^+$ FA                                 | 1         |      |
| 295.20288 | FA14:0   | [M+K]$^+$ FA                                 | 1         |      |
| 337.21264 | Hydroxy-oleic acid | [M+K]$^+$ FA                           | 3         |      |
| 341.1872  | FA20:5   | [M+K]$^+$ FA                                 | 1         |      |
| 355.25968 | Docosatetraenoic acid (22:4) | [M+Na]$^+$ FA                           | 2         |      |
| 363.2657  | FA21:1   | [M+K]$^+$ FA                                 | 0.8       |      |
| 351.4999  | FA36:4   | [M+Na]$^+$ FA                                 | 36        |      |
| 305.244   | FA18:1   | [M+Na]$^+$ FA                                 | 3         |      |
| 321.2177  | FA18:1   | [M+K]$^+$ FA                                 | 4         |      |
| 307.26049 | Stearic acid (fa18:0) | [M+Na]$^+$ FA                           | 1         |      |
| 323.23377 | Stearic acid (fa18:0) | [M+K]$^+$ FA                           | 2         |      |
| 327.22878 | Arachidonic acid | [M+Na]$^+$ FA                           | 2         |      |
| 343.20437 | Arachidonic acid | [M+K]$^+$ FA                                 | 2         |      |
| 351.22846 | Docosahexaenoic acid | [M+Na]$^+$ FA                           | 3         |      |
| 367.202   | Docosahexaenoic acid | [M+K]$^+$ FA                                 | 3         |      |
| 774.59036 | GalCer(d38:3) | [M+Na]$^+$ GlcCer                           | 6         |      |
| 810.58796 | GlcCer(d38:1) | [M+K]$^+$ GlcCer                           | 2         |      |
| 848.62954 | GlcCer(d42:2) | [M+K]$^+$ GlcCer                           | 9         |      |
| 1268.718  | M(IP)2C(d36:0) | [M+K]$^+$ M(IP)2C                          | 69        |      |
| 1336.818  | M(IP)2C(d42:0) | [M+K]$^+$ M(IP)2C                          | 82        |      |
| 417.23857 | PA(P-16:0) | [M+Na]$^+$ PA                              | 2         |      |
| 441.2377  | CPA(18:1) | [M+Na]$^+$ PA                              | 0.3       |      |
| 701.44678 | PA(33:0)   | [M+K]$^+$ PA                              | 7         |      |
| 707.49624 | PA(O-36:3) | [M+Na]$^+$ PA                              | 3         |      |
| 713.44677 | PA(34:1)   | [M+K]$^+$ PA                              | 7         |      |
| 723.48923 | PA(36:2)   | [M+Na]$^+$ PA                              | 5         |      |
| 415.22086 | PA(16:0)   | [M+Na]$^+$ PA                              | 2         |      |
| 431.1935  | PA(16:0)   | [M+K]$^+$ PA                              | 5         |      |
| 782.56108 | PC(34:1)   | [M+Na]$^+$ PC                              | 7         |      |
| 826.56495 | PC(36:1)   | [M+K]$^+$ PC                              | 8         |      |
| 844.46889 | PC(38:6)   | [M+K]$^+$ PC                              | 66        |      |
| 864.62704 | PC(P-41:2) | [M+K]$^+$ PC                              | 3         |      |
| 866.64113 | PC(O-40:2) | [M+K]$^+$ PC                              | 1         |      |
| 784.5217  | PC(33:1)   | [M+K]$^+$ PC                              | 4         |      |
| 832.66687 | PC(38:4)   | [M+Na]$^+$ PC                              | 4         |      |
| 848.55262 | PC(38:4)   | [M+K]$^+$ PC                              | 4         |      |
| 740.47886 | PE(34:1)   | [M+K]$^+$ PE                              | 1         |      |
| 772.52012 | PE(35:0)   | [M+K]$^+$ PE                              | 6         |      |
| 778.4763  | PE(36:4)   | [M+K]$^+$ PE                              | 3         |      |
| 790.52275 | PE(p-38:4) | [M+K]$^+$ PE                              | 10        |      |
| 802.47473 | PE(38:6)   | [M+K]$^+$ PE                              | 4         |      |
| 804.4907  | PE(38:6)   | [M+K]$^+$ PE                              | 4         |      |
| 806.50434 | PE(38:4)   | [M+K]$^+$ PE                              | 6         |      |
| 814.51051 | PE(P-40:6) | [M+K]$^+$ PE                              | 5         |      |
| 828.48788 | PE(40:7)   | [M+K]$^+$ PE                              | 7         |      |

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| m/z          | Intensity | Charge State | Mass/Charge | Description                        |
|-------------|-----------|--------------|-------------|------------------------------------|
| 830.50526   | 5         | M+K          | PE(40:6)    |                                    |
| 834.53751   | 4         | M+K          | PE(40:4)    |                                    |
| 836.66568 a | 13        | M+Na         | PE(42:9)    |                                    |
| 852.48229   | 13        | M+K          | PE(42:9)    |                                    |
| 884.49514   | 69        | M+K          | PE(44:7)    |                                    |
| 782.5631 a  | 21        | M+K          | PE(O-37:2)  |                                    |
| 794.61321 a | 5         | M+Na         | PE(P-39:1)  |                                    |
| 810.58796   | 2         | M+K          | PE(P-39:1)  |                                    |
| 824.46241 a | 36        | M+Na         | PE(40:9)    |                                    |
| 822.64786   | 60        | M+Na         | PE(40:2)    |                                    |
| 838.6233 a  | 60        | M+K          | PE(40:2)    |                                    |
| 697.47287   | 6         | M+K          | PE-Cer(d34:2)| Cer-PE                            |
| 413.12415   | 3         | M+K          | Cys Gly Pro Val | Peptides                          |
| 453.17582   | 2         | M+K          | Gly Glu Pro Ile | Peptides                          |
| 457.20921   | 0.2       | M+Na         | Met Leu Ala Thr | Peptides                          |
| 459.22234   | 2         | M+Na         | Thr Leu Gly Phe | Peptides                          |
| 469.14905   | 1         | M+K          | Glu Trp Pro    | Peptides                          |
| 481.20686   | 1         | M+K          | Asp Val Leu Pro | Peptides                          |
| 495.1643    | 0.6       | M+K          | Gly Phe Ala Tyr | Peptides                          |
| 497.17951   | 1         | M+Na         | Asn Asn Pro Met | Peptides                          |
| 502.2659    | 4         | M+Na         | Ser Phe Val Lys | Peptides                          |
| 564.2442    | 2         | M+Na         | Val Tyr Asn Phe | Peptides                          |
| 592.23942   | 2         | M+Na         | Phe Glu Phe Gln | Peptides                          |
| 630.19389   | 6         | M+K          | Phe Cys Trp His | Peptides                          |
| DHB-assisted MALDI MS |
| 606.848     | 19        | M+H          | Cer(d39:2)   | Cer                               |
| 369.35762   | 13        | M+H-2H2O     | 5beta-Cholestane-3alpha,12alpha-diol | CL                           |
| 391.29314/390.887 | 2 | M+H-2H2O | (23S)-3α,7β,23-Trihydroxy-5β-cholan-24-oic Acid | CL                           |
| 551.51348   | 1         | M+H-H2O      | 12:0 Cholesteryl ester | CL                    |
| 577.52959   | 9         | M+H-H2O      | 14:1 Cholesteryl ester | CL                    |
| 790.55310   | 7         | M+K          | GalCer(d38:3) | GalCer                            |
| 958.59544 a | 9         | M+H-H2O      | MIPC(d34:0(2OH)) | MIPC             |
| 734.58424   | 16        | M+H          | PC(32:0)     | PC                               |
| 756.56601   | 21        | M+H          | PC(34:3)     | PC                               |
| 760.6013 a  | 20        | M+H          | PC(34:1)     | PC                               |
| 762.61858 a | 14        | M+H          | PC(34:0)     | PC                               |
| 782.58283   | 19        | M+H          | PC(36:4)     | PC                               |
| 784.59648 a | 21        | M+H          | PC(36:3)     | PC                               |
| 788.63513 a | 2         | M+H          | PC(O-37: 1)  | PC                               |
| 804.56972   | 20        | M+H          | PC(38:7)     | PC                               |
| 806.52966 a | 23        | M+K          | PC(P-36:3)   | PC                               |
| 808.60317   | 17        | M+Na         | PC(P-37:1)   | PC                               |
| 810.6161    | 19        | M+H          | PC(38:4)     | PC                               |
| 812.62343   | 23        | M+H          | PC(38:3)     | PC                               |
| 814.55575 a | 20        | M+K          | PC(35:0)     | PC                               |
| 820.54448   | 21        | M+Na         | PC(36: 4)    | PC                               |
| Mass     | Charge | Type            | Confirmation | Supplemental Notes |
|----------|--------|-----------------|--------------|--------------------|
| 826.59089 | 8      | PC(P-37:0) [M+K]^+ | PC           | 55                 |
| 828.57068 | 18     | PC(36:0) [M+K]^+  | PC           |                     |
| 830.53114 | 33     | PC(P-38:5) [M+K]^+ | PC           |                     |
| 832.60197 | 22     | PC(38:4) [M+Na]^+ | PC           | 55                 |
| 836.53845 | 20     | PC(37:3) [M+K]^+  | PC           |                     |
| 844.54491 | 19     | PC(38:6) [M+K]^+  | PC           |                     |
| 846.55926 | 17     | PC(38:5) [M+K]^+  | PC           |                     |
| 856.60328 | 20     | PC(38:0)[U] [M+K]^+ | PC           |                     |
| 872.57700 | 20     | PC(40:0) [M+K]^+  | PC           |                     |
| 896.50257 | 18     | PC(41:1) [M+K]^+  | PC           |                     |
| 672.43388  | 22     | PC(26:0) [M+Na]^+ | PC           |                     |
| 688.42946  | 20     | PC(26:0) [M+K]^+  | PC           |                     |
| 734.58424  | 10     | PC(32:0) [M+H]^+  | PC           | 55                 |
| 772.54151  | 6      | PC(32:0) [M+K]^+  | PC           |                     |
| 798.55817  | 16     | PC(34:1) [M+K]^+  | PC           |                     |
| 800.56994  | 20     | PC(34:0) [M+K]^+  | PC           |                     |
| 848.57717  | 21     | PC(38:4) [M+K]^+  | PC           |                     |
| 852.51184  | 14     | PI-Cer(34:0(2OH)) [M+K]^+ | Cer-PI   |                     |
| 351.15263  | 4      | Ser Thr Ser Gly [M+H]^+ Peptide |                     |                     |
| 383.14284  | 8      | Pro Asp Gly Met [M+H-2H2O]^+ Peptide |                     |                     |
| 511.27947  | 5      | Cys Tyr Cys Thr [M+Na]^+ Peptide |                     |                     |
| 518.31127  | 2      | Lys Asp Lys Lys [M+H]^+ Peptide |                     |                     |
| 731.62011  | 5      | TG(43:3)[iso3] [M+H]^+ TG |                     |                     |
| 753.60379  | 8      | TG(43:3)[iso6] [M+Na]^+ TG |                     |                     |
| 769.57901  | 6      | TG(39:3)[iso3] [M+K]^+ TG |                     |                     |
| 932.58000  | 15     | TH-Cer (d30:1) [M+H-2H2O]^+ TH-Cer |                     |                     |
| 199.00045  | 23     | 3-Phospho-D-erythronate [M+H-H2O]^+ Lipid fragments |                     |                     |
| 206.05814  | 1      | N-Acetylpseudophosphothricin [M+H-H2O]+ Lipid fragments |                     |                     |
| 222.03249  | 14     | Phosphocholine [M+K]^+ Lipid fragments |                     |                     |
| 230.94900  | 10     | 2,3-Bisphospho-D-glycerate [M+H-2H2O]^+ Lipid fragments |                     |                     |
| 329.01119  | 20     | D-glycero-D-manno-Heptose 1-phosphate [M+K]^+ Lipid fragments |                     |                     |

**9-AA assisted MALDI MS**

| Mass     | Charge | Type            | Confirmation | Supplemental Notes |
|----------|--------|-----------------|--------------|--------------------|
| 716.09b  | 1      | PE(34:1) [M-H]^- PE | 60            |                     |
| 744.100b | 1      | PE(36:1) [M-H]^- PE | 60            |                     |
| 746.96b  | 1      | PE(P-38:6) [M-H]^- PE | 60            |                     |
| 762.89b  | 1      | PE(38:6) [M-H]^- PE | 60            |                     |
| 766.93b  | 1      | PE(38:4) [M-H]^- PE | 60            |                     |
| 794.80b  | 1      | PE(40:4) [M-H]^- PE | 60            |                     |
| 774.91b  | 1      | PS (p36:0) [M-H]^- PS | 60            |                     |
| 788.86b  | 1      | PS(36:1) [M-H]^- PS | 60            |                     |
| 834.75 b | 1      | PS(40:6) [M-H]^- PS | 60            |                     |
| 806.81b  | 1      | ST(d18:1/18:0) [M-H]^- ST | 61            |                     |
| 822.76b  | 1      | ST(d18:1/18:0OH) [M-H]^- ST | 61            |                     |
| m/z     | Molecular Formula | Charge State | Abbreviation | Reference |
|---------|-------------------|--------------|--------------|-----------|
| 850.72  | ST(d18:1/h20:0OH) | [M-H]        | ST           | 60        |
| 860.71  | ST(d18:1/22:1)    | [M-H]        | ST           |           |
| 862.73  | ST(d18:1/22:0)    | [M-H]        | ST           | 61        |
| 874.68  | ST(d18:1/22:2OH)  | [M-H]        | ST           |           |
| 878.70  | ST(d18:1/h22:0OH) | [M-H]        | ST           | 60        |
| 888.70  | ST(d18:1/24:1)    | [M-H]        | ST           | 60,61     |
| 904.60  | ST(d18:1/24:1OH)  | [M-H]        | ST           | 60        |

a Molecules in the m/z column that had their precise molecular masses determined by FTICR MS and their fragmentation patterns acquired using MALDI-TOF/TOF MS/MS.

b Molecules in the m/z column that did not have their precise molecular masses determined by FTICR MS but their fragmentation patterns were acquired using MALDI-TOF/TOF MS/MS.

Molecules not marked in the m/z column had their precise molecular masses determined by FTICR MS. However, their fragmentation patterns were not acquired.

c Labeled references in the Ref. column report detection of the listed molecules in either rat or mouse brains with methods other than MALDI MS or LDI MS.

d Labeled references in the Ref. column describe listed molecules, but these molecules are not detected in the rodent brains.

References without labeling in the Ref. column describe detection of the listed molecules in either the rat or mouse brain with MALDI MS or LDI MS.

e The marked peptides in the Ref. column have structures of representative isomer of all the possible candidates for this precise m/z shown.

Abbreviations:

PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PA, Phosphatidic acid; CPA, cyclic phosphatidic acid; Cer-PE, Ceramide phosphoethanolamine; Cer-PI, Ceramide phosphoinositol; DAG, Diacylglycerol; TG, Triglyceride; Cer, Ceramide; GlcCer, Glucosylceramide; GalCer, Galactoceramide; THCer, Trihexosylceramide; M(IP)2C, Mannose-(inositol-P)2-ceramide; MIPC, Mannose-inositol-P-ceramide; FA, Fatty acid; DHA, Docosahexaenoic acid; AA, Arachidonic acid; CL, Cholesterol; PS, Phosphatidylinerine; ST, Sulfatide. **Amino Acids:** Ala, Alanine; Asn, Asparagine; Asp, Aspartate; Cys, Cysteine; Glu, Glutamate; Gln, Glutamine; Gly, Glycine; His, Histidine; Ile, Isoleucine; Leu, Leucine; Lys, Lysine; Met, Methionine; Phe, Phenylalanine; Pro, Proline; Ser, Serine; Thr, Threonine; Trp, Tryptophan; Tyr, Tyrosine; Val, Valine
Table S5. ANOVA results for data presented in Figure 5C and Figure S12B.

| Hippocampal region | Factors        | p values determined by ANOVA for different compounds |
|--------------------|----------------|------------------------------------------------------|
|                    | arecaidine     | creatinine   | cholesterol | DAG(34:1) | DAG(38:4) | PC(38:6) |
| CC                 | Interaction    | 0.1         | 0.62        | 0.81      | 0.13      | 0.1      | 6.41E-04 |
|                    | age            | 0.02        | 0.002       | 0.37      | 0.01      | 0.005    | 7.04E-04 |
| DG                 | interaction    | 0.66        | 0.83        | 0.89      | 0.62      | 0.24     | 0.04     |
|                    | age            | 0.025       | 0.008       | 0.83      | 0.28      | 0.4      | 0.01     |
| CA3                | interaction    | 0.36        | 0.1476      | 1.33E-04 | 1.39E-05 | 0.0026   | 0.0012   |
|                    | age            | 0.000428    | 3.33E-05    | 0.000122 | 3.77E-05 | 0.001    | 2.84E-06 |
| CA1                | interaction    | 0.52        | 0.2         | 0.11      | 0.91      | 0.42     | 0.62     |
|                    | age            | 0.0061      | 0.0035      | 0.25      | 0.05      | 0.09     | 0.88     |

a Interaction is a change in the simple main effect of age over levels of paired comparison. If the value is <0.05, the interaction between age and paired comparison batch is significant.

b Effect of aging on the compound’s average peak area. If the value is <0.05, the effect of aging is significant.

Abbreviations: CC, corpus callosum; DG, dentate gyrus; CA3, region III of hippocampus proper; CA1, region I of hippocampus proper.
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