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Mass Spectrometry Imaging Shows Cocaine and Methylphenidate Have Opposite Effects on Major Lipids in *Drosophila* Brain

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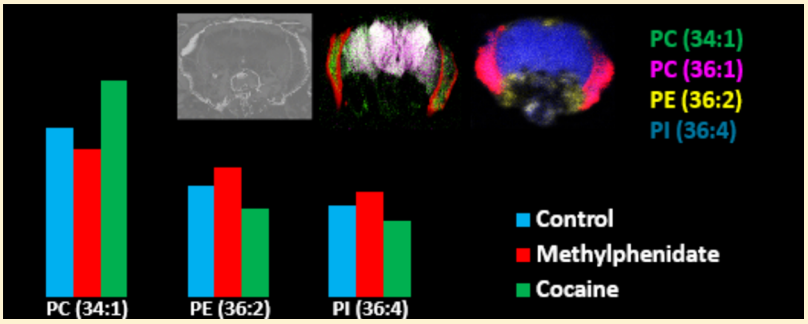
Supporting Information



ABSTRACT: Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to study the effects of cocaine versus methylphenidate administration on both the localization and abundance of lipids in *Drosophila melanogaster* brain. A J105 ToF-SIMS with a 40 keV gas cluster primary ion source enabled us to probe molecular ions of biomolecules on the fly with a spatial resolution of ∼3 μm, giving us unique insights into the effect of these drugs on molecular lipids in the nervous system. Significant changes in phospholipid composition were observed in the central brain for both. Principal components image analysis revealed that changes occurred mainly for phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositols. When the lipid changes caused by cocaine were compared with those induced by methylphenidate, it was shown that these drugs exert opposite effects on the brain lipid structure. We speculate that this might relate to the molecular mechanism of cognition and memory.

KEYWORDS: *Mass spectrometry imaging, phospholipids, Drosophila, cocaine, methylphenidate*

INTRODUCTION

The alteration of major brain lipids caused by cocaine was compared to that induced by methylphenidate (MPH), both psychostimulants but having opposite effects on learning, to evaluate the patterns of their effects on the chemical structure of the brain. Cocaine abuse is associated with cognitive deficits in attention, learning, and working memory, impulsivity, and behavior flexibility.[1−4] Many studies have demonstrated that cocaine can induce changes in phospholipid profiles due to alterations in dopaminergic signaling, which have been implicated in a number of human neurodegenerative diseases.[5,6]

Phospholipids that are highly enriched in the central nervous system are involved in numerous functions, including neurotransmission, neuronal signal reduction, and membrane fusion during exocytosis and endocytosis.[7] Phospholipid translocation during endo- and exocytosis results in the bending and unfolding of the plasma membrane.[8,9] Phospholipids also function in the clustering and regulation of neurotransmitter receptors.[9−11] Alterations in lipid concentration, location, and metabolism have been shown to affect exocytosis and neurotransmitter release that might be associated with various psychiatric and neurodegenerative disorders.[12,13] There are various reasons for the changes in brain lipid structures and composition such as stress, depression, long-term diet, and drugs, and it has been suggested that the cognitive impairment caused by cocaine might be related to lipids.[14,15] In addition, several studies have accessed the effects of cocaine abuse on lipid metabolism in human brains; however, the specific lipid species altered following drug exposure are unclear. It is possible that the change in phospholipid content in the brain following repeated cocaine use might be an important factor in the impairment of learning and memory.

MPH, a drug used to treat hyperactive children, also causes significant changes to certain classes of lipids in the fly brain.[16] This drug calms children, but its mechanism is cloudy, and in contrast to cocaine, it clearly increases focus and cognitive

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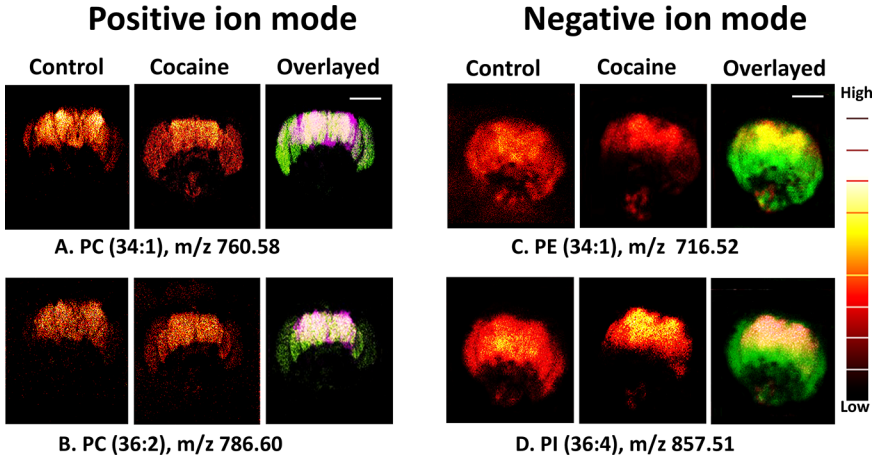

Figure 1. Distribution of biomolecules in the *Drosophila* brain before and after cocaine treatment by ToF-SIMS in positive and negative ion modes. A 40 keV Ar_{4000}^+ beam with primary ion dose density 1.7×10^{13} ions/cm^2 was used. Image area: 800 × 800 μm^2 and 256 × 256 pixels; pixel size, ~3 μm. Overlayed images: purple, control brain; green, cocaine-treated brain (positive ion mode) (A, B); green, control; red, treated brain (negative ion mode) (C, D). Scale bar is 200 μm. A color "thermal" scale is shown.

ability. MPH appears to increase the high curvature lipids like phosphatidylethanolamine and inositol and to decrease the lipids associated with flat membrane curvature, like phosphatidylcholine, in the fly brain.

The development of mass spectrometry technology opened a new opportunity to characterize and quantify lipids in complex biological samples. Cumming et al. has shown the effects of cocaine exposure on the abundance of phospholipid species in rat brain and blood using electrospray ionization-mass spectrometry.[14] Mass spectrometric imaging can take this a step further, allowing spatial localization of species. Simultaneous detection of all secondary ions from the samples with low detection limit, high lateral resolution, and sufficient mass resolution can be obtained with time-of-flight secondary ion mass spectrometry (ToF-SIMS), and the sample preparation is label-free and simple. Furthermore, the recently developed gas cluster ion beam (GCIB) has provided a chance to image the localization of intact molecules such as intact lipids as a result of the reduced fragmentation by large size clusters.[17,18]

Drosophila has become an important system to study various human genetic diseases, learning and memory, and characteristics of addictive drugs.[19,20] In this paper, we used ToF-SIMS imaging to investigate the effect of cocaine on levels of specific phospholipids in the nervous system of the fly. ToF-SIMS was performed with a J105-3D Chemical Imager equipped with a quasi-continuous 40 keV GCIB with clusters generated using 8% CO_2 in argon.[21] Positive and negative ions were analyzed to maximize the coverage of different lipid species. We then investigated the changes in lipid composition induced by MPH in more detail and side by side with those of cocaine to investigate if the contrast in behavior is also observed as a change in chemical structure.

RESULTS AND DISCUSSION

Effect of Cocaine on Lipid Composition. Control and cocaine-treated fly brain sections were kept frozen hydrated during the ToF-SIMS analysis to prevent chemical delocalization.[22] Phospholipid species, particularly phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and phosphatidylinisitols (PIs), were found abundant in *Drosophila* brain, examples of which are shown in Figure 1 (larger list included in Table S1). Most of these molecules localize in the central brain and optical lobe areas.

Lipid species that make positive ions, like the phosphatidylcholine pseudomolecular ions ($[M + H]^+$), such as PC (34:1) at m/z 760.58 and PC (36:2) at m/z 786.60 are mainly found in the central brain as shown in Figure 1A, B. These species became visually more abundant in the central brain and the optical lobes following oral cocaine administration. In negative ion mode, PEs (e.g., PE (34:1), $[M - H]^-$ at m/z 716.52) and PIs (e.g., PI (36:4), $[M - H]^-$ at m/z 857.51) are observed distributed over the whole brain in the control samples (Figure 1C, D). After cocaine exposure, their overall intensity decreased, and they were relocated to the anterior part of the central brain and partially to the optical lobe. On the basis of these data, it is clear that cocaine has significant effects on the chemical structure of the brain, as it induces changes in the spatial lipid organization of the fly brain, especially the central brain.

Principal components analysis (PCA)[22] was carried out on the image data to identify the differences in the chemical distribution after cocaine treatment (discussed in Figure S1). A list of mass accuracies for all identified peaks is in Table S1. Principal component 5 (Figure S1A) shows the most significant changes for many of the PC species and their salt adducts located in the salivary region and proboscis of the control brain. Cocaine changes the localization of those species and their distribution in the entire cocaine-treated brain (Figure S2).

Analyzing lipids that make negative ions, principal component 4 shows that cocaine induces significant changes of some fatty acids, PEs, and PIs in the fly brain tissue (Figure S1B). These species are more dominant in the central brain and optical lobes of the cocaine-treated brain, whereas they are mainly observed only in the proboscis of the control brain. In contrast, the PI headgroup (m/z 241.01), several other fatty acids, and PE and PI species all distribute over the whole control brain; however, they are only found in the proboscis of the cocaine-treated brain. Again, cocaine exposure causes significant distributional changes of these phospholipids in the central region of the fly brain, which correlates with the observed ion images (Figure 1 and Figure S1B).

Cocaine does not significantly affect the lipid content of the optical lobes and proboscis; however, it dramatically alters the

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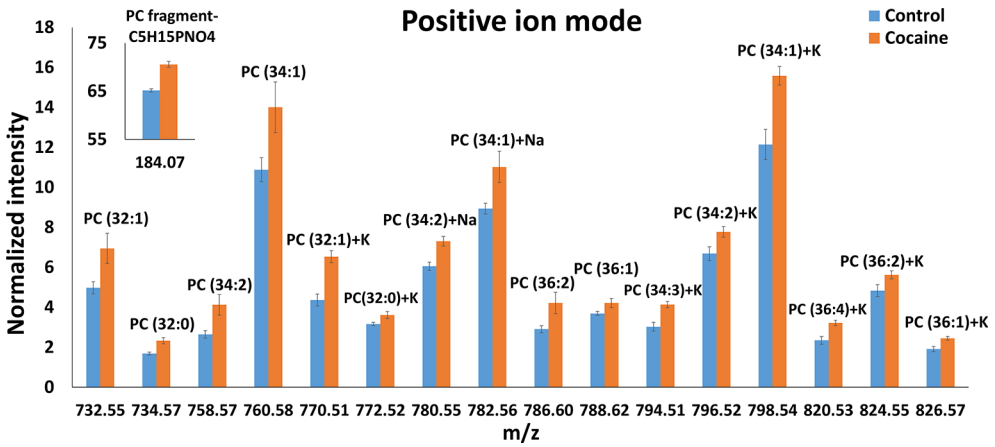

Figure 2. Effect of cocaine on lipid abundance in the central brain of the fly using ToF-SIMS/40 keV Ar_{4000}^+ GCIB (positive ions). Peak intensities normalized to the number of pixels and total peak intensities. Error bars: standard deviation of 11 controls (blue bars) and 7 cocaine-treated brains (red bars). Lipids were detected as $[M + H]^+$ ions unless specified as +K or +Na.

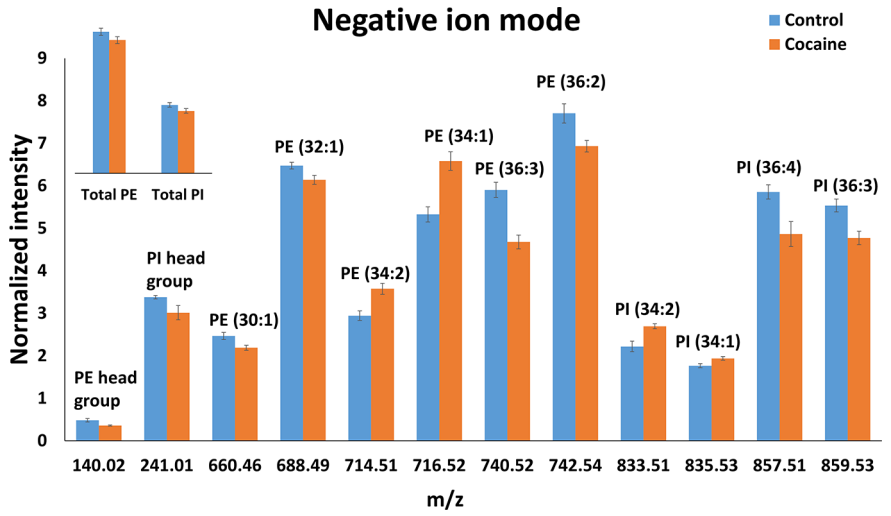

Figure 3. Cocaine effect on lipids in the central brain of the fly brain using ToF-SIMS/40 keV Ar_{4000}^+ GCIB (negative ions). Peak intensities normalized to the number of pixels and total peak intensities. Seven controls and 6 cocaine-treated brains were used. Total contents of PE and PI species were calculated by summing the molecular ion intensity of all their species. All species were detected as $[M - H]^-$ ions.

abundances of numerous intact phospholipids in the central brain. Chemical species that showed changes in the central brain in the image PCA were then compared across multiple fly samples for further statistical analysis. In positive ion mode, many PC species are significantly changed ($p < 0.05$, probability value) (Figure 2 and Table S2) after cocaine administration. The relative amounts of PC species and their salt adducts significantly increased in the cocaine-treated brain, which is correlated with the increased intensity of the PC headgroup signal at m/z 184.07.

In negative ion mode, statistically significant changes were observed ($p < 0.05$) in PE and PI signals (Figure 3 and Table S3). The intensity of most PE peaks decreased after cocaine exposure, although some were found to increase. For example, the amounts of PE (36:3) and PE (36:2) in cocaine-treated brains were ~20% lower than the controls. The intensities of PE (30:1) and PE (32:1) slightly decreased. Conversely, the amounts of PE (34:1) and PE (34:2) were ~20% higher in the cocaine-treated brains. Overall, there is a small drop in the total amount of PE species associated with the decreased signal of PE headgroup at m/z 140.02. The change in the content of PIs is similar to that of PEs. After cocaine exposure, the signal for PI (36:4) at m/z 857.51 and PI (36:3) at m/z 859.53 decreased ~15%, whereas the intensity of PI (34:2) at m/z 833.51 and PI (34:1) at m/z 835.53 increased slightly. However, the general trend of total PI intensities is a decrease after cocaine administration, as represented by the slightly decreased intensity of the PI headgroup at m/z 241.01. The chemical structures of PCs and their salt adducts in *Drosophila* brain were previously confirmed based on the fragmented PC headgroup m/z 184.07 using MS^2 on the same ToF-SIMS instrument.[23] From the same literature, the structures of PEs and PIs and their corresponding fatty acid fragments were also verified. Here, we found that several 16 and 18 carbon fatty acids increased in abundance correlating to the increases for the PEs and PIs mentioned above (Figure S3). This could be explained by the fact that these fatty acids are the structural components of these PEs and PIs, for instance, PE (16:0_18:1 or 16:1_18:0) and PI (16:0_18:1 or 16:1_18:0).

Cocaine Might Broadly Affect Cell Function via Lipid Changes. The syntheses of PC, PE, and PI, the main phospholipids altered by cocaine in the central brain, are related via many biological synthesis pathways. In eukaryotes, most of the PCs are synthesized by the CDP-choline pathway.[24]

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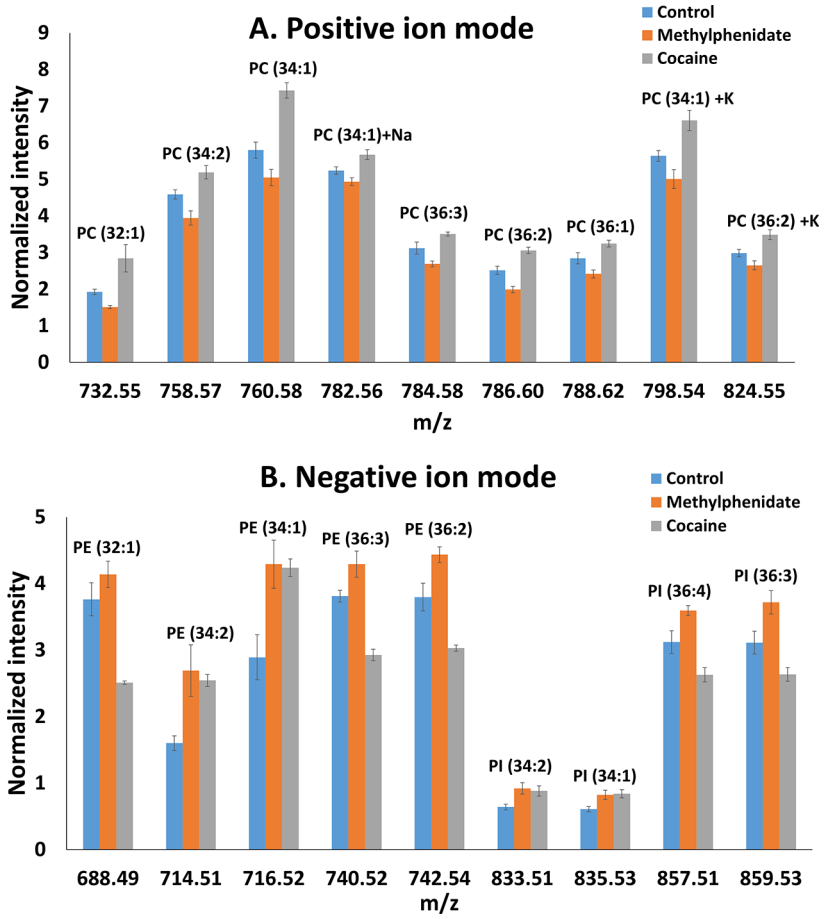

Figure 4. Relative quantification of biomolecular changes in the central brain of *Drosophila* following cocaine or methylphenidate administration using ToF-SIMS/40 keV Ar_{4000}^{+} GCIB in positive (A) and negative ion mode (B) for 6 control, 5 methylphenidate central brains, and 5 cocaine central brains.

Nonetheless, ~30% of PCs are produced from the *N*-methylation of PEs using enzyme phosphatidylethanolamine methyltransferase.[25,26] After cocaine, the PC level is increased (Figure 2), and the PE level is decreased (Figure 3). It is possible that the methylation process, where PE is converted to PC, is elevated in the cocaine-treated flies.

Sharma et al. showed that dopamine could stimulate the phospholipid methylation pathway by activation of the D4 dopamine receptor.[27] The enhancement in phospholipid methylation of PE could cause a decrease in phospholipid packing density in the plasma membrane and thus change the conformational dynamics of membrane proteins. Thus, an increase of extracellular dopamine stimulated by cocaine might enhance the methylation pathway and result in a larger amount of PC and decreasing PE concentration. A reduction of PE content in the brain tissue of a *Drosophila* epilepsy model and human Alzheimer's disease has been reported.[28−30] Therefore, the decrease in PE composition (Figure 3) in our data caused by cocaine might similarly be associated with cell membrane dysfunction and perhaps also with neurodegenerative diseases.

The cellular plasma membrane is a lipid bilayer that consists of asymmetrical outer and inner leaflets of lipids. The outer leaflet is enriched in cylindrical-shaped PC molecules, whereas the inner one is concentrated with PE and PI. PE has an overall conical shape with a smaller polar headgroup than that of PC. Because of the high concentration of PE in the inner leaflet, a small difference in lipid shapes supports the changes in lipid bilayer topology into negative curvature.[31] The alteration of PC and PE abundance caused by cocaine influences the asymmetry of the two membrane leaflets and consequently affects the biophysical properties of the membrane, including bilayer curvature, elasticity, and viscosity. Dawaliby et al. found that the reduction in the ratio of PE/PC decreases the fluidity of the membrane in insect cells.[32] The modification in membrane fluidity might cause impairment in many cellular processes that are associated with brain disorders.[33,34]

Cocaine versus Methylphenidate. We have compared the alteration of brain lipid structure induced by cocaine to methylphenidate. MPH and cocaine similarly block the reuptake of the dopamine transporter and increase the level of dopamine outside the synapse.[35] Although having similar effects on the neurotransmitter system, cocaine produces cognitive deficiency regarding attention, learning and memory, and working memory,[2,3,36,37] whereas MPH appears to enhance cognition, memory, and behavior.[38,39] MPH improves the cognitive function of the prefrontal cortex, where task-relevant information is encoded into working memory, a form of short-term memory.[40] MPH also significantly alters the lipid composition of *Drosophila* brain.[16] The opposite cognitive effects of cocaine and MPH suggest a second molecular mechanism for their action in the brain in addition to blocking the dopamine transporter. We therefore performed experiments in which three groups of flies were treated in parallel with

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cocaine, MPH, or a yeast-fed control and report the data in Figure 4.

The effects of cocaine and MPH on the lipids of the central area of the fly brain are strikingly opposite and statistically different, whereas no significant difference in lipid content is observed in the optical lobes (Figure 4). A downward trend of ∼10% in the levels of PCs and their salt adducts is observed after MPH administration (Figure 4A). On the contrary, the levels of PCs increase moderately after cocaine treatment. MPH increased the PE and PI content (Figure 4B), whereas cocaine decreased the levels of the same species. PEs and PIs with a 34 carbon chain appear exceptional as their signal intensities are significantly higher following both MPH and cocaine administration. Cocaine causes an increase of ∼15% of total phospholipid content, which is similar to that of other studies using different models.[14,41]

In summary, MPH and cocaine have opposite effects on the lipid structure of the fly brain, possibly implicating the involvement of the brain lipids in learning and memory. The cognitive-enhancing MPH causes the abundance of lamellar-shaped lipids such as PC to decrease by 13% and the conically shaped lipids PE and PI to increase by 20% (total PE and PI). In contrast, cognitive-inhibiting cocaine increases the lamellar-shaped PC by 30% and decreases the conically shaped PE and PI by 9%. It is clearly possible that the morphological shapes of the lipids relate to their molecular mechanism in neurological effects caused by these drugs. In addition, the changes of the brain lipids reported here occur following 3 days of drug administration, suggesting that these lipid alterations might also serve as markers of drug use.

METHODS

Materials. Cocaine hydrochloride, methylphenidate, and gelatin were purchased from Sigma-Aldrich (Germany). The deionized water was collected from a Milli-Q water system (Millipore, Merck, Darmstadt, Germany).

Sample Preparation. Canton S flies, wild-type *Drosophila* strain, were used for experiments. Three- to four-day-old male flies were chosen and transferred to yeast paste containing 15 mM cocaine. The flies were fed with the food containing cocaine for 3 days. The food was replaced daily to maintain the concentration of cocaine. For all of the fly heads to be kept in the same orientation, flies were placed into a fly collar (4 M Instrument and Tool LLC) for analysis, which was then put in a mold containing 10% gelatin solution. The gelatin mold was frozen at −20 °C and then further cooled in liquid nitrogen. The frozen block containing fly heads was detached and cryo-sectioned using a cryo-microtome (Cryostat Leica CM 1520) at −20 °C to obtain thin sections with 12 μm thickness. Sectioning and handling was carried out under an argon atmosphere to avoid water condensation on the sample surface. The fly brain sections were thaw-mounted on indium tin oxide (ITO)-coated glass slides. The frozen hydrated samples were rapidly transported in liquid nitrogen to the argon-filled glovebox of the ToF-SIMS instrument and then inserted into the vacuum chamber. The experiments were performed using frozen-hydrated samples, and the temperature was kept below −170 °C during measurements. The experiments were performed with three different generations of flies treated with the drug.

For comparison experiments between cocaine and MPH, *Drosophila* were treated with 15 mM of cocaine and 50 mM of MPH,[16] respectively. A higher dose of cocaine causes fly death. The control flies (flies fed with yeast only), cocaine-treated, and MPH-treated flies were sequentially loaded on the same fly collar so that the embedding, cryo-sectioning, and analysis were carried out under the same conditions to be able to compare the results obtained from ToF-SIMS.

ToF-SIMS Imaging. ToF-SIMS analysis was performed using the J105-3D Chemical Imager (Ionoptika Ltd., Southampton, UK). The principle of this instrument has been described in detail elsewhere.[21] In this study, a 40 keV Ar_{4000}^+ GCIB was used as a primary ion beam to sputter the sample surface with a cluster size of 4000 Ar atoms (8% CO_2 in Ar). High energy clusters were used to provide enhanced yields for intact lipid signals in the biological samples. The spectra were acquired over a m/z range of 80−950. The images were recorded by scanning the 40 keV Ar_{4000}^+ primary ions over an area of 800 × 800 μm^2 with 128 × 128 pixels or with 256 × 256 pixels (stated in more detail in the figures). The total ion dose density was 1.3×10^{13} ions/cm^2 in both positive and negative ion modes for the 128 × 128 pixel images and 1.7×10^{13} ions/cm^2 for the 256 × 256 pixel images.

Data Analysis. The data obtained from the J105 instrument were analyzed using Ionoptika Image Analyzer software (Ionopika Ltd., Southampton, U.K.). Multivariate analysis, such as principal components analysis (PCA), on the ToF-SIMS images was performed using MatLab (The MathWorks Inc., Massachusetts, US). Regions of interests within the ToF-SIMS images of the fly brain area were selected in order to remove the interferences of inorganic species from the ITO-coated glass substrate. The spectra were binned down to 0.1 Da within the mass range of 80−900 Da. The data were also normalized to the number of pixels in the selected central brain area and the total ion counts and mean centered before PCA analysis. Moreover, further statistical analysis using t-tests was performed to confirm the significant changes in molecular composition compared between control- and drug-treated brain tissues.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.8b00046.

Image PCA of the chemical distribution for control and cocaine-exposed fly brain, ToF-SIMS ion images of different phospholipids in the fly brain before and after MPH treatment in positive and negative ion modes, and overlayed images of SEM and ToF-SIMS of the fly head section along with a table of mass accuracies of all assigned lipid species and changes in phospholipid molecules (PDF)

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

ToF-SIMS, time-of-flight secondary ion mass spectrometry; GCIB, gas cluster ion beam; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PCA, principal component analysis; MPH, methylphenidate; ITO, indium tin oxide

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REFERENCES

(1) Gould, R. W., Gage, H. D., and Nader, M. A. (2012) Effects of chronic cocaine self-administration on cognition and cerebral glucose utilization in Rhesus monkeys. *Biol. Psychiatry 72*, 856−863.

(2) Tomasi, D., Goldstein, R. Z., Telang, F., Maloney, T., Alia-Klein, N., Caparelli, E. C., and Volkow, N. D. (2007) Widespread disruption in brain activation patterns to a working memory task during cocaine abstinence. *Brain Res. 1171*, 83−92.

(3) Hester, R., and Garavan, H. (2004) Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. *J. Neurosci. 24*, 11017−11022.

(4) Vonmoos, M., Hulka, L. M., Preller, K. H., Minder, F., Baumgartner, M. R., and Quednow, B. B. (2014) Cognitive Impairment in Cocaine Users is Drug-Induced but Partially Reversible: Evidence from a Longitudinal Study. *Neuropsychopharmacology 39*, 2200−2210.

(5) Rangel-Barajas, C., Coronel, I., and Floran, B. (2015) Dopamine Receptors and Neurodegeneration. *Aging Dis 6*, 349−368.

(6) Ross, B. M., Moszczynska, A., Peretti, F. J., Adams, V., Schmunk, G. A., Kalasinsky, K. S., Ang, L., Mamalias, N., Turenne, S. D., and Kish, S. J. (2002) Decreased activity of brain phospholipid metabolic enzymes in human users of cocaine and methamphetamine. *Drug Alcohol Depend. 67*, 73−79.

(7) van Meer, G., Voelker, D. R., and Feigenson, G. W. (2008) Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol. 9*, 112−124.

(8) Devaux, P. F. (2000) Is lipid translocation involved during endo- and exocytosis? *Biochimie 82*, 497−509.

(9) Adibhatla, R. M., and Hatcher, J. F. (2007) Role of lipids in brain injury and diseases. *Future Lipidol. 2*, 403−422.

(10) Ritz, M. C., Lamb, R. J., Goldberg, S. R., and Kuhar, M. J. (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science 237*, 1219−1223.

(11) Lauwers, E., Goodchild, R., and Verstreken, P. (2016) Membrane Lipids in Presynaptic Function and Disease. *Neuron 90*, 11−25.

(12) Berger, G. E., Smesny, S., and Amminger, G. P. (2006) Bioactive lipids in schizophrenia. *International Review of Psychiatry 18*, 85−98.

(13) Mapstone, M., Cheema, A. K., Fiandaca, M. S., Zhong, X., Mhyre, T. R., MacArthur, L. H., Hall, W. J., Fisher, S. G., Peterson, D. R., Haley, J. M., Nazar, M. D., Rich, S. A., Berlau, D. J., Peltz, C. B., Tan, M. T., Kawas, C. H., and Federoff, H. J. (2014) Plasma phospholipids identify antecedent memory impairment in older adults. *Nat. Med. 20*, 415−418.

(14) Cummings, B. S., Pati, S., Sahin, S., Scholpa, N. E., Monian, P., Trinquero, P. M., Clark, J. K., and Wagner, J. J. (2015) Differential effects of cocaine exposure on the abundance of phospholipid species in rat brain and blood. *Drug Alcohol Depend. 152*, 147−156.

(15) Muller, C. P., Reichel, M., Muhle, C., Rhein, C., Gulbins, E., and Kornhuber, J. (2015) Brain membrane lipids in major depression and anxiety disorders. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids 1851*, 1052−1065.

(16) Phan, N. T. N., Fletcher, J. S., and Ewing, A. G. (2015) Lipid structural effects of oral administration of methylphenidate in Drosophila brain by secondary ion mass spectrometry imaging. *Anal. Chem. 87*, 4063−4071.

(17) Ninomiya, S., Ichiki, K., Yamada, H., Nakata, Y., Seki, T., Aoki, T., and Matsuo, J. (2009) Precise and fast secondary ion mass spectrometry depth profiling of polymer materials with large Ar cluster ion beams. *Rapid Commun. Mass Spectrom. 23*, 1601−1606.

(18) Angerer, T. B., Blenkinsopp, P., and Fletcher, J. S. (2015) High energy gas cluster ions for organic and biological analysis by time-of-flight secondary ion mass spectrometry. *Int. J. Mass Spectrom. 377*, 591−598.

(19) Keene, A. C., and Waddell, S. (2007) Drosophila olfactory memory: single genes to complex neural circuits. *Nat. Rev. Neurosci. 8*, 341−354.

(20) Pandey, U. B., and Nichols, C. D. (2011) Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. *Pharmacol Rev. 63*, 411−436.

(21) Fletcher, J. S., Rabbani, S., Henderson, A., Blenkinsopp, P., Thompson, S. P., Lockyer, N. P., and Vickerman, J. C. (2008) A new dynamic in mass spectral imaging of single biological cells. *Anal. Chem. 80*, 9058−9064.

(22) Phan, N. T. N., Fletcher, J. S., Sjovall, P., and Ewing, A. G. (2014) ToF-SIMS imaging of lipids and lipid related compounds in Drosophila brain. *Surf. Interface Anal. 46*, 123−126.

(23) Phan, N. T. N., Munem, M., Ewing, A. G., and Fletcher, J. S. (2017) MS/MS analysis and imaging of lipids across Drosophila brain using secondary ion mass spectrometry. *Anal. Bioanal. Chem. 409*, 3923−3932.

(24) Sohlenkamp, C., Lopez-Lara, I. M., and Geiger, O. (2003) Biosynthesis of phosphatidylcholine in bacteria. *Prog. Lipid Res. 42*, 115−162.

(25) DeLong, C. J., Shen, Y. J., Thomas, M. J., and Cui, Z. (1999) Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway. *J. Biol. Chem. 274*, 29683−29688.

(26) Li, Z., Agellon, L. B., Allen, T. M., Umeda, M., Jewell, L., Mason, A., and Vance, D. E. (2006) The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab. 3*, 321−331.

(27) Sharma, A., Kramer, M. L., Wick, P. F., Liu, D., Chari, S., Shim, S., Tan, W., Ouellette, D., Nagata, M., DuRand, C. J., Kotb, M., and Deth, R. C. (1999) D4 dopamine receptor-mediated phospholipid methylation and its implications for mental illnesses such as schizophrenia. *Mol. Psychiatry 4*, 235−246.

(28) Guan, Z. Z., Wang, Y. A., Cairns, N. J., Lantos, P. L., Dallner, G., and Sindelar, P. J. (1999) Decrease and structural modifications of phosphatidylethanolamine plasmalogen in the brain with Alzheimer disease. *J. Neuropathol. Exp. Neurol. 58*, 740−747.

(29) Pettegrew, J. W., Panchalingam, K., Hamilton, R. L., and McClure, R. J. (2001) Brain membrane phospholipid alterations in Alzheimer's disease. *Neurochem. Res. 26*, 771−782.

(30) Kliman, M., Vijayakrishnan, N., Wang, L., Tapp, J. T., Broadie, K., and McLean, J. A. (2010) Sructural mass spectrometry analysis of lipid changes in a Drosophila epilepsy model brain. *Mol. BioSyst. 6*, 958−966.

(31) McMahon, H. T., and Boucrot, E. (2015) Membrane curvature at a glance. *J. Cell Sci. 128*, 1065−1070.

(32) Dawaliby, R., Trubbia, C., Delporte, C., Noyon, C., Ruysschaert, J. M., Van Antwerpen, P., and Govaerts, C. (2016) Phosphatidylethanolamine Is a Key Regulator of Membrane Fluidity in Eukaryotic Cells. *J. Biol. Chem. 291*, 3658−3667.

(33) Farooqui, A. A., Horrocks, L. A., and Farooqui, T. (2000) Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. *Chem. Phys. Lipids 106*, 1−29.

(34) Sameni, S., Malacrida, L., Tan, Z., and Digman, M. A. (2018) Alteration in Fluidity of Cell Plasma Membrane in Huntington Disease Revealed by Spectral Phasor Analysis. *Sci. Rep. 8* (734), 1−10.

(35) Volkow, N. D., Fowler, J. S., Gatley, S. J., Dewey, S. L., Wang, G. J., Logan, J., Ding, Y. S., Franceschi, D., Gifford, A., Morgan, A., Pappas, N., and King, P. (1999) Comparable changes in synaptic dopamine induced by methylphenidate and by cocaine in the baboon brain. *Synapse 31*, 59−66.

(36) Jovanovski, D., Erb, S., and Zakzanis, K. K. (2005) Neurocognitive deficits in cocaine users: a quantitative review of the evidence. *J. Clin Exp Neuropsychol 27*, 189−204.

(37) Goldstein, R. Z., Tomasi, D., Rajaram, S., Cottone, L. A., Zhang, L., Maloney, T., Telang, F., Alia-Klein, N., and Volkow, N. D. (2007) Role of the anterior cingulate and medial orbitofrontal cortex in processing drug cues in cocaine addiction. *Neuroscience 144*, 1153−1159.

(38) Arnsten, A. F. T., and Dudley, A. G. (2005) Methylphenidate improves prefrontal cortical cognitive function through alpha 2

DOI: 10.1021/acschemneuro.8b00046
ACS Chem. Neurosci. 2018, 9, 1462−1468

adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav Brain Funct 1*, 1.
(39) Dommett, E. J., Henderson, E. L., Westwell, M. S., and Greenfield, S. A. (2008) Methylphenidate amplifies long-term plasticity in the hippocampus via noradrenergic mechanisms. *Learn. Mem. 15*, 580−586.
(40) Baddeley, A. (2003) Working memory: looking back and looking forward. *Nat. Rev. Neurosci. 4*, 829−839.
(41) Nassogne, M. C., Lizarraga, C., N'Kuli, F., Van Bambeke, F., Van Binst, R., Wallemacq, P., Tulkens, P. M., Mingeot-Leclercq, M. P., Levade, T., and Courtoy, P. J. (2004) Cocaine induces a mixed lysosomal lipidosis in cultured fibroblasts, by inactivation of acid sphingomyelinase and inhibition of phospholipase A1. *Toxicol. Appl. Pharmacol. 194*, 101−110.

DOI: 10.1021/acschemneuro.8b00046
ACS Chem. Neurosci. 2018, 9, 1462−1468