Supporting Information

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

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In this Supporting Information, we present the image PCA of the chemical distribution for control and cocaine-exposed fly brain, the 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. A table of mass accuracy of all assigned lipid species and changes in phospholipid molecules is also provided.

SUPPLEMENTARY INFORMATION

RESULTS AND DISCUSSION

PCA image analysis.

PCA limited to fly brain was carried out in the mass range 170-900 for positive ion mode and 100-950 for negative ion mode. The ion images of both control and cocaine-treated brains were combined in order to obtain the same principal components. The highest variance of biomolecular distribution in the central brain is captured in principal component 5 and 4 in the positive and negative modes, respectively (Figure S1). The scores images, Figure 2 (inset), are displayed such that the positive scoring pixels are colored green and the negative scoring pixels red. Pixels displaying no variance on the specified principal component are black. For clarity the positive and negative loading peaks are color coded similarly. Variations caused by principal components 1 to 9 show the differences in molecular localization in the control and treated samples (Figure S2 below).

The following ions are considered (all $[M+H]^+$ unless otherwise specified). In positive mode, PCs, particularly PC (32:1) at *m/z* 732.56, and PC (34:1) at *m/z* 760.58, and their salt adducts such as $[PC (32:0)+K]^+$ at *m/z* 772.52, $[PC (34:1)+Na]^+$ at *m/z* 782.56, and $[PC (34:1)+K]^+$ at *m/z* 798.54, in the salivary and proboscis of the control sample were detected (Figure S1A). For the cocaine treated samples, the localization of those species changed and they were found to be distributed in the entire brain. The peaks $[M+K-TMA]^+$ (trimethylamine) for potassium salt adduct of PC also shows the same trend, such as $[PC (32:0)+K]^+$ at m/z 713.45 and $[PC (34:1)+K]^+$ at m/z 739.47. Likewise, we also found changes for other PCs, particularly PC (36:1) at *m/z* 788.62, PC (38:5) at *m/z* 808.59, $[PC (36:2)+K]^+$ at *m/z* 824.55, as well as $[PC (36:2)+K-TMA]^+$ at m/z 765.50. In these cases, they are higher in relative intensity in the control versus cocaine-treated brain.

The following ions are considered (all $[M-H]^-$ unless otherwise specified). In negative mode, principal component 4 shows that cocaine induces significant changes of PEs and PIs in the fly brain tissue (Figure S1B). FA (24:0) at *m/z* 367.36 is localized mainly in the proboscis of control brain as well as treated-brain. FA (18:3) at *m/z* 277.22, FA (22:0) at *m/z* 339.33, PE (36:2) at *m/z* 742.54, PE (36:3) at *m/z* 740.52, and PI (36:4) at *m/z* 857.51 are more dominant in the central brain and optical lobes of the cocaine treated brain, whereas those peaks are observed only in the proboscis

of the control brain. On the other hand, the PI head group at *m/z* 241.01, FA (16:1) at *m/z* 253.22, FA (18:1) at 281.25, PE (32:1) at *m/z* 688.49, PE (34:2) at *m/z* 714.51, PE (34:1) at *m/z* 716.52, PI (32:1) at *m/z* 807.50, PI (34:2) at *m/z* 833.51, and PI (42:1) at *m/z* 947.66 distribute in the whole control brain, however only in the proboscis of cocaine-treated brain.

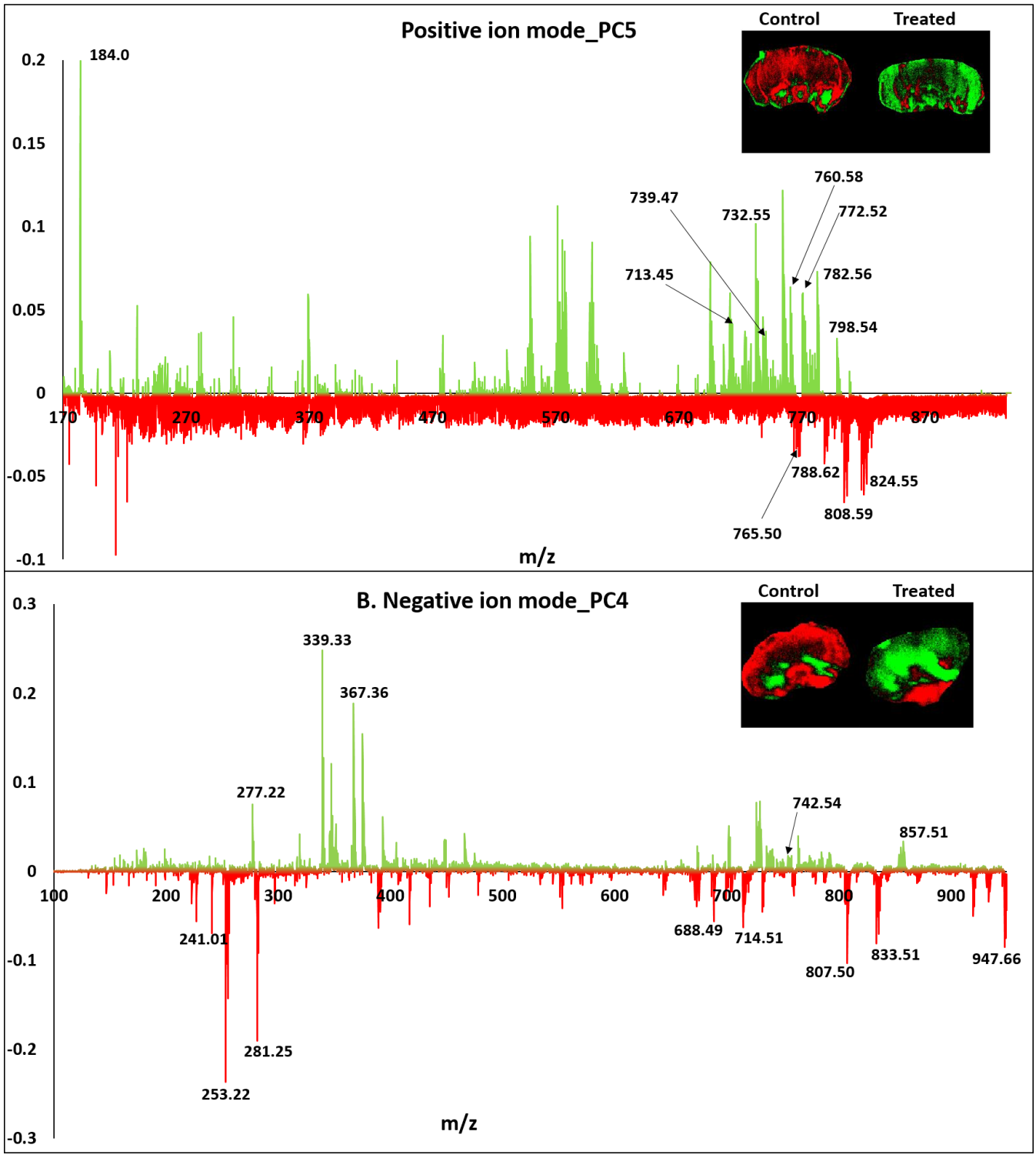

Figure S1. Loading of image PCA for (A) principal component 5 in positive mode, and (B) principal component 4 in negative mode generated from the spectra of all brain region of the control and cocaine-treated samples. The significant signals of intact phospholipids are labelled. The red and green peaks correspond to the red and green regions in the fly brain images (insets), respectively. These were evaluated in Matlab.

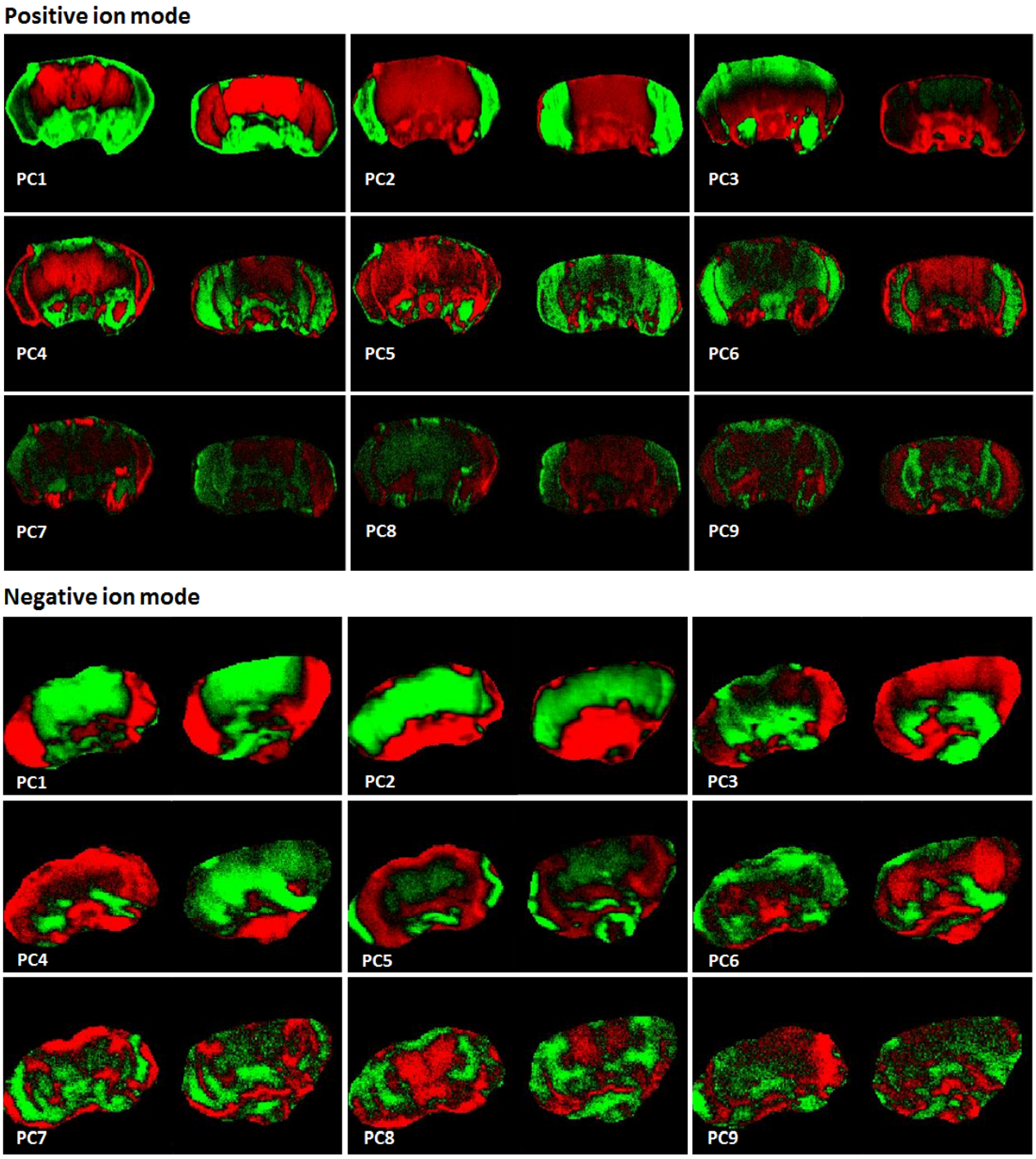

Figure S2. Image PCA to compare the alteration of molecular localization in the control and cocaine-treated samples analysed by 40 keV Ar_{4000}^{+} GCIB in positive and negative ion modes. Left, control brains; right, cocaine-treated brains.

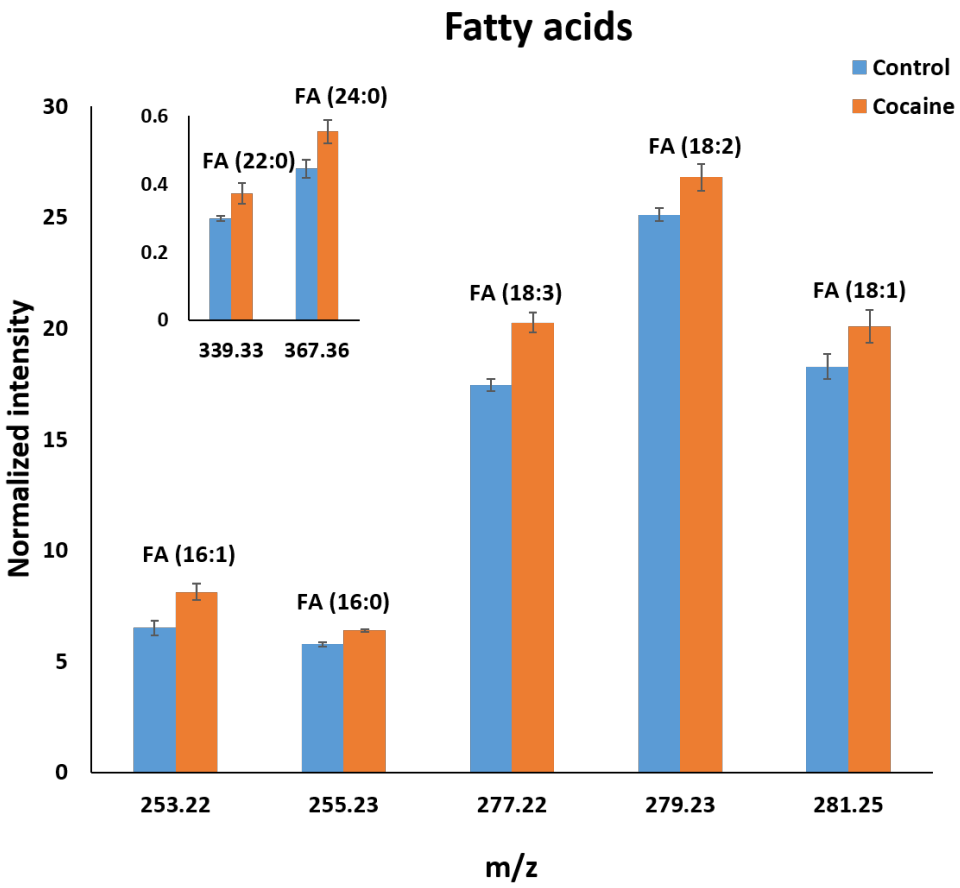

Figure S3. Change in abundance of fatty acids in central brain analysed by ToF-SIMS/40 keV Ar_{4000}^{+} GCIB in negative ion mode. Peak intensities were normalized to the number of pixels and total ion intensities. The error bar is the standard deviation of the 7 control brains (blue bar) and 6 cocaine brains (red bar).

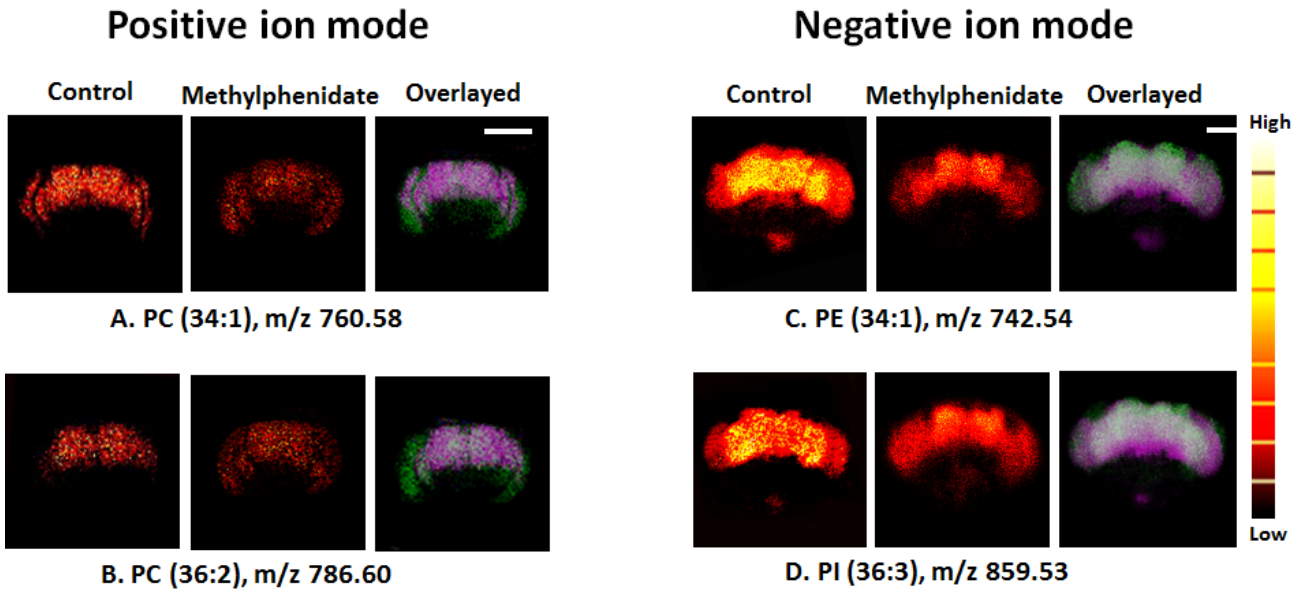

Figure S4. Distribution of phospholipids in the *Drosophila* brain before and after methylphenidate treatment by ToF-SIMS in positive (A, B) and negative (C, D) ion modes. A 40 keV Ar_{4000}^{+} beam was used. Image area: 800 × 800 μm² and 128 × 128 pixels. Overlayed images: control brain (purple), methylphenidate-treated brain (green). Scale bar is 200 μm.

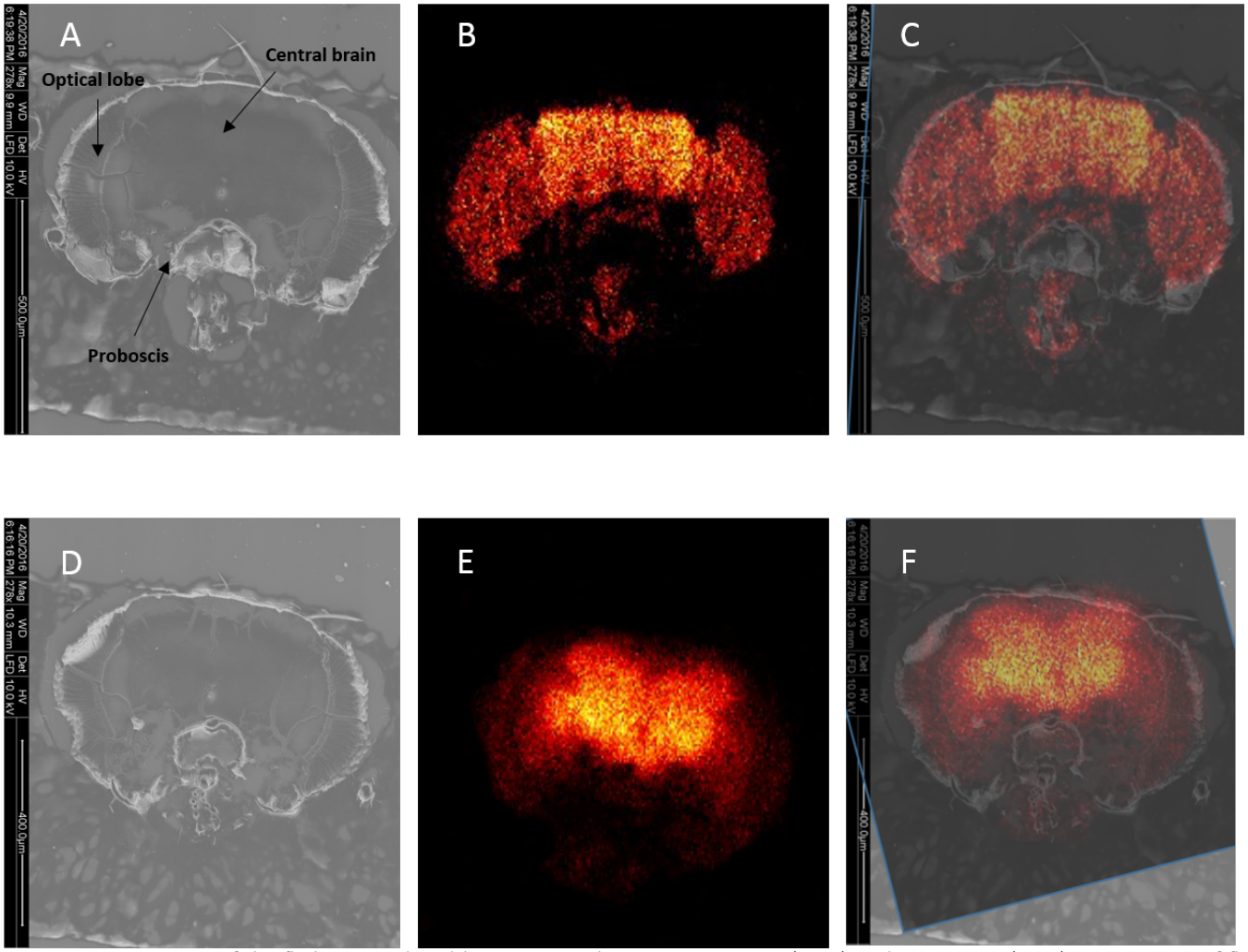

Figure S5. Structure of the fly brain analyzed by scanning electron microscopy (SEM) and ToF-SIMS. (A, D) SEM images of fly sections. (B) ToF-SIMS ion image of PC (34:1) at m/z 760.58 in positive ion modes. (C) Overlayed image of (A) and (B). (E) ToF-SIMS ion image of PE (36:3) at m/z 740.52. (F) Overlayed image of (D) and (E).

Table S1. Lipid species assigned from ToF-SIMS profile spectra in positive and negative ion modes.

	Identity	Measured m/z	Calculated m/z	Δppm
Positive ion mode Detected ions are $[M+H]^+$ unless specified +Na/K	PC (32:0)+K-TMA	713.4482	713.4518	-5.05
	PC (32:1)	732.556	732.5538	3
	PC (32:0)	734.5655	734.5694	-5.31
	PC (34:1)+K-TMA	739.4714	739.4675	5.27
	PC (32:1)+Na	754.531	754.5357	-6.23
	PC (32:0)+Na	756.5538	756.5514	3.17
	PC (34:2)	758.5652	758.5694	-5.54
	PC (34:1)	760.5846	760.5851	-0.66
	PC (36:2)+K-TMA	765.4951	765.4988	-4.83
	PC (32:1)+K	770.5129	770.5097	4.15
	PC (32:0)+K	772.5243	772.5253	-1.29
	PC (34:2)+Na	780.5488	780.5514	-3.33
	PC (34:1)+Na	782.5612	782.567	-7.41
	PC (36:3)	784.5809	784.5851	-5.35
	PC (36:2)	786.5953	786.6007	-6.86
	PC (36:1)	788.6209	788.6164	5.71
	PC (34:3)+K	794.5063	794.5097	-4.28
	PC (34:2)+K	796.5218	796.5253	-4.39
	PC (34:1)+K	798.5396	798.541	-1.75
	PC (36:2)+Na	808.587	808.5827	5.32
	PC (36:1)+Na	810.5929	810.5983	-6.66
	PC (36:4)+K	820.5277	820.5253	2.92
	PC (36:2)+K	824.5513	824.5566	-6.43
	PC (36:1)+K	826.5702	826.5723	-2.54
Negative ion mode $[C_nH_{2n+2-2m}O_2]^-$ [a]	FA (16:1)	253.2171	253.2173	-0.79
	FA (16:0)	255.2319	255.233	-4.31
	FA (18:3)	277.2176	277.2173	1.08
	FA (18:2)	279.2344	279.233	5.01
	FA (18:1)	281.2496	281.2486	3.56
	FA (18:0)	283.2654	283.2643	3.88
	FA (22:0)	339.3254	339.3269	-4.42
	FA (24:0)	367.3564	367.3582	-4.9
Negative ion mode $[M-H]^-$	PE (30:1)	660.4567	660.461	-6.51
	PE (32:1)	688.4878	688.4923	-6.54
	PE (34:2)	714.5053	714.5079	-3.64
	PE (34:1)	716.5223	716.5236	-1.81
	PE (36:3)	740.5236	740.5199	-5
	PE (36:2)	742.5382	742.5392	-1.35
	PI (30:0)	781.482	781.4873	-6.78
	PI (32:1)	807.4991	807.5029	-4.71
	PI (34:2)	833.5149	833.5186	-4.44
	PI (34:1)	835.5298	835.5342	-5.27
	PI (36:5)	855.5041	855.5029	1.4
	PI (36:4)	857.5148	857.5186	-4.43
	PI (36:3)	859.5301	859.5342	-4.77

[a] They are might not be free fatty acids. They could come from fragmentation of lipid species.

Table S2. Changes in phospholipids and their related compounds observed in positive ion mode between control and cocaine-treated samples. All species were detected as $[M+H]^+$ ions unless specified, assignments are putative based on peak position and isotopic distributions compared with literature data.

Identity	Measured m/z	% change between control and cocaine-treated samples[a]	P value
PC (32:1)	732.6	39.5	0.01701
PC (32:0)	734.6	37.9	0.00093
PC (32:1)+Na	754.5	21.0	0.00001
PC (32:0)+Na	756.6	45.1	0.00023
PC (34:2)	758.6	55.9	0.00901
PC (34:1)	760.6	28.7	0.03021
PC (32:1)+K	770.5	49.7	0.00017
PC (32:0)+K	772.5	14.2	0.02199
PC (34:1)+Na	782.6	23.3	0.01271
PC (36:3)	784.6	53.8	0.00093
PC (36:2)	786.6	45.0	0.0188
PC (36:1)	788.6	14.4	0.03661
PC (34:3)+K	794.5	36.5	0.00228
PC (34:2)+K	796.5	16.2	0.04301
PC (34:1)+K	798.5	28.3	0.0045
PC (36:2)+Na	808.6	25.3	0.01134
PC (36:1)+Na	810.6	21.8	0.03692
PC (36:4)+K	820.5	37.1	0.00583
PC (36:2)+K	824.6	16.3	0.07817
PC (36:1)+K	826.6	28.1	0.01236

[a] (+) and (-) values were the increase and decrease of lipid content of cocaine-treated samples compared to the control, respectively.
n = 18

Table S3. Changes in phospholipids observed in negative ion mode between control and cocaine-treated samples. All species were detected as $[M-H]^-$ ions assignments are putative based on peak position and isotopic distributions compared with literature data.

Identity	Measured m/z	% change between control and cocaine-treated samples[a]	P value
PE (30:1)	660.5	-11.2	0.0461
PE (32:1)	688.5	-5.2	0.0353
PE (34:2)	714.5	21.5	0.0067
PE (34:1)	716.5	23.5	0.0018
PE (36:3)	740.5	-20.7	0.001
PE (36:2)	742.5	-13	0.0348
PI (30:0)	781.5	-15.5	0.0004
PI (32:1)	807.5	-15.2	0.0152
PI (34:2)	833.5	21.5	0.0169
PI (34:1)	835.5	9.8	0.0414
PI (36:5)	855.5	-21.2	0.0017
PI (36:4)	857.5	-16.9	0.0162
PI (36:3)	859.5	-13.8	0.0097

[a] (+) and (-) values were the increase and decrease of lipid content of cocaine-treated samples compared to the control, respectively.
n = 18