

Supporting Information

Interplay between Cocaine, Drug Removal, and Methylphenidate Reversal on Phospholipid Alterations in *Drosophila* Brain Determined by Imaging Mass Spectrometry

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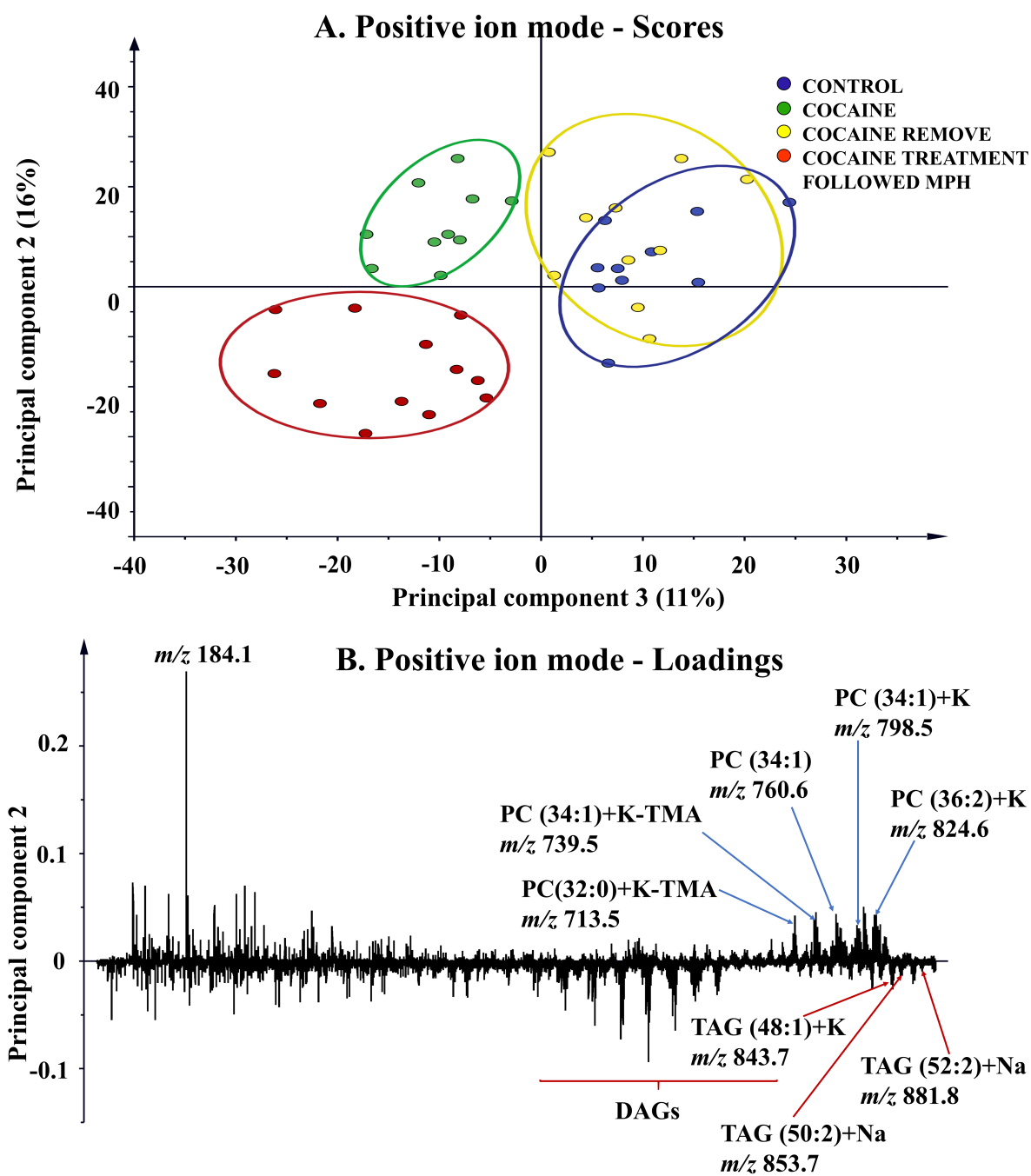
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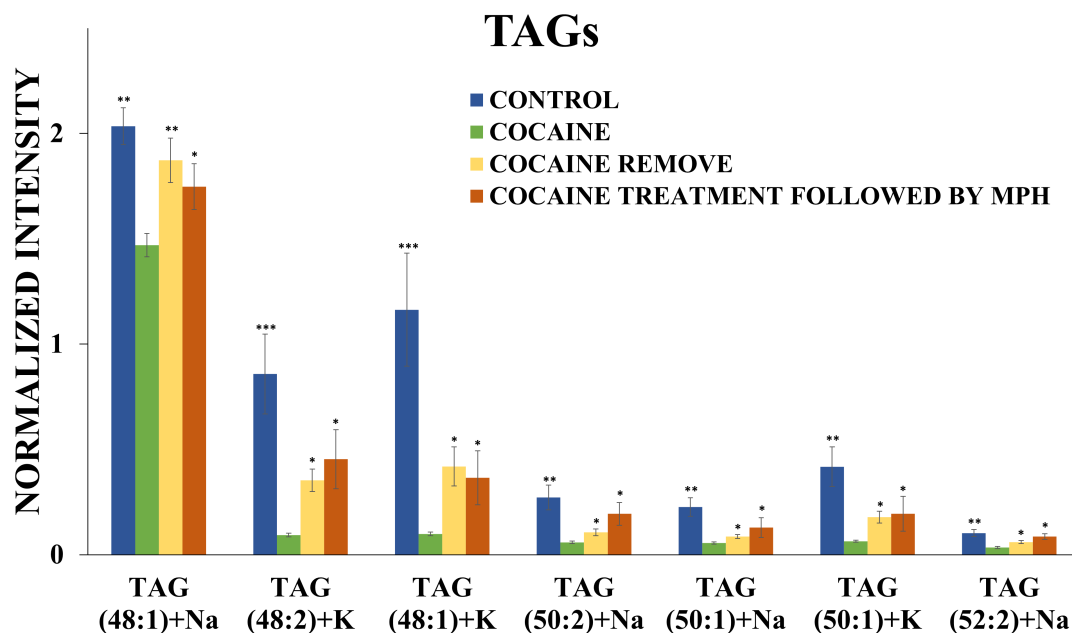
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Supporting Information Table S1. Assignment for selected peaks from fly brain in positive and negative ion modes.

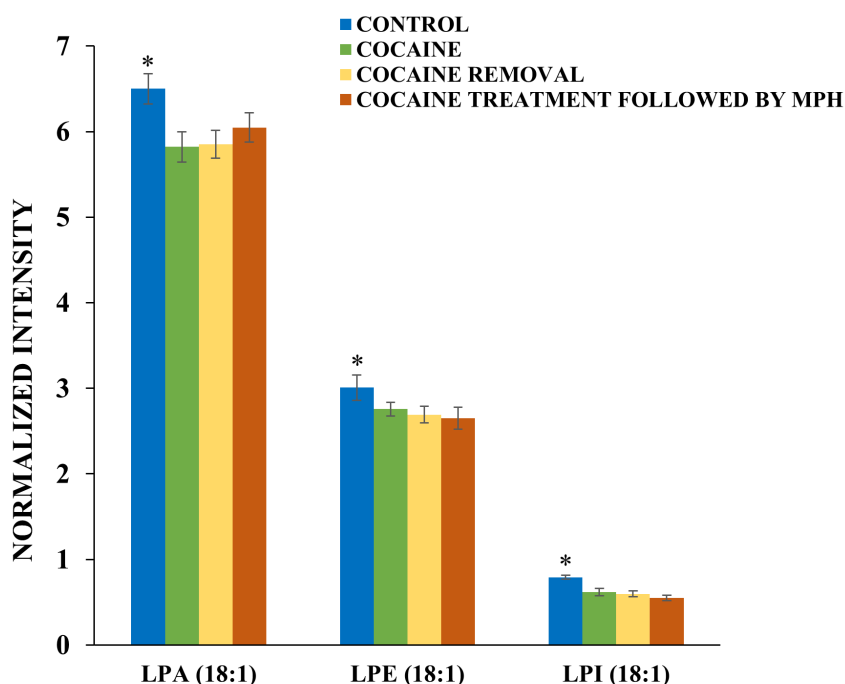
	Exact m/z	Measured m/z	Δ ppm	Label	Species
Positive ion mode	713.4482	713.4501	2.7	PC(32:0)	[M+K-TMA] ⁺
	721.4779	721.4802	3.2	PC(34:2)	[M+Na-TMA] ⁺
	732.5560	732.5485	-2.0	PC(32:1)	[M+H] ⁺
	734.5655	734.5621	-4.6	PC(32:0)	[M+H] ⁺
	739.4714	739.4668	-6.2	PC(34:1)	[M+K-TMA] ⁺
	749.5135	749.5154	2.5	PC(36:2)	[M+Na-TMA] ⁺
	758.5652	758.5626	-3.4	PC(34:2)	[M+H] ⁺
	760.5846	760.5809	-4.9	PC(34:1)	[M+H] ⁺
	765.4753	765.4693	-7.8	PC(36:2)	[M+K-TMA] ⁺
	770.5129	770.5096	-4.3	PC(32:1)	[M+K] ⁺
	772.5243	772.5218	-3.2	PC(32:0)	[M+K] ⁺
	780.5514	780.5464	-6.4	PC(34:2)	[M+Na] ⁺
	782.5612	782.5563	-6.3	PC(34:1)	[M+Na] ⁺
	784.5606	784.5568	-4.8	PC(34:0)	[M+Na] ⁺
	786.5809	786.5768	-5.2	PC(36:2)	[M+H] ⁺
	788.6209	788.6139	-5.9	PC(36:1)	[M+H] ⁺
	794.5063	794.5024	-4.9	PC(34:3)	[M+K] ⁺
	796.5218	796.5186	-4.0	PC(34:2)	[M+K] ⁺
	798.5396	798.5474	-2.7	PC(34:1)	[M+K] ⁺
	822.5410	822.5398	-1.4	PC(36:3)	[M+K] ⁺
	824.5513	824.5487	-3.1	PC(36:2)	[M+K] ⁺
	826.5702	826.5687	-1.8	PC(36:1)	[M+K] ⁺
	827.7099	827.7054	-5.4	TAG(48:1)	[M+Na] ⁺
	841.7256	841.7208	-5.7	TAG(48:2)	[M+K] ⁺
	843.7412	843.7439	3.2	TAG(48:1)	[M+K] ⁺
	853.7256	853.7208	-5.6	TAG(50:2)	[M+Na] ⁺
	855.7412	855.7358	-6.3	TAG(50:1)	[M+Na] ⁺
	871.7225	871.7186	-4.5	TAG(50:1)	[M+K] ⁺
	879.7412	879.7376	-4.1	TAG(52:3)	[M+Na] ⁺
	881.7569	881.7535	-3.8	TAG(52:2)	[M+Na] ⁺
	883.7725	883.7697	-3.2	TAG(52:1)	[M+Na] ⁺
Negative ion mode	253.2173	253.2185	4.7	FA(16:1)	[M-H] ⁻
	255.2330	255.2347	6.7	FA(16:0)	[M-H] ⁻
	279.2330	279.2346	5.7	FA(18:2)	[M-H] ⁻
	281.2486	281.2497	3.9	FA(18:1)	[M-H] ⁻
	283.2643	283.2657	4.9	FA(18:0)	[M-H] ⁻
	339.3269	339.3287	5.3	FA(22:0)	[M-H] ⁻
	367.3582	367.3602	5.4	FA(24:0)	[M-H] ⁻
	435.2517	435.2533	3.7	LPA(18:1)	[M-H] ⁻
	478.2939	478.2965	5.4	LPE(18:1)	[M-H] ⁻
	597.3045	597.3081	6.0	LPI(18:1)	[M-H] ⁻
	660.4567	660.4534	-5.0	PE(30:1)	[M-H] ⁻
	688.4878	688.4831	-6.8	PE(32:1)	[M-H] ⁻
	714.5053	714.4998	-7.7	PE(34:2)	[M-H] ⁻
	716.5223	716.5201	-3.1	PE(34:1)	[M-H] ⁻
	740.5236	740.5195	-5.5	PE(36:3)	[M-H] ⁻
	742.5382	742.5334	-6.5	PE(36:2)	[M-H] ⁻
	833.5149	833.5125	-2.9	PI(34:2)	[M-H] ⁻
	835.5298	835.5298	-6.0	PI(34:1)	[M-H] ⁻
	837.6070	837.5989	-2.4	PI(34:0)	[M-H] ⁻
	855.5041	855.5015	-3.0	PI(36:5)	[M-H] ⁻
	857.5148	857.5098	-5.8	PI(36:4)	[M-H] ⁻
	859.5342	859.5295	-5.5	PI(36:3)	[M-H] ⁻



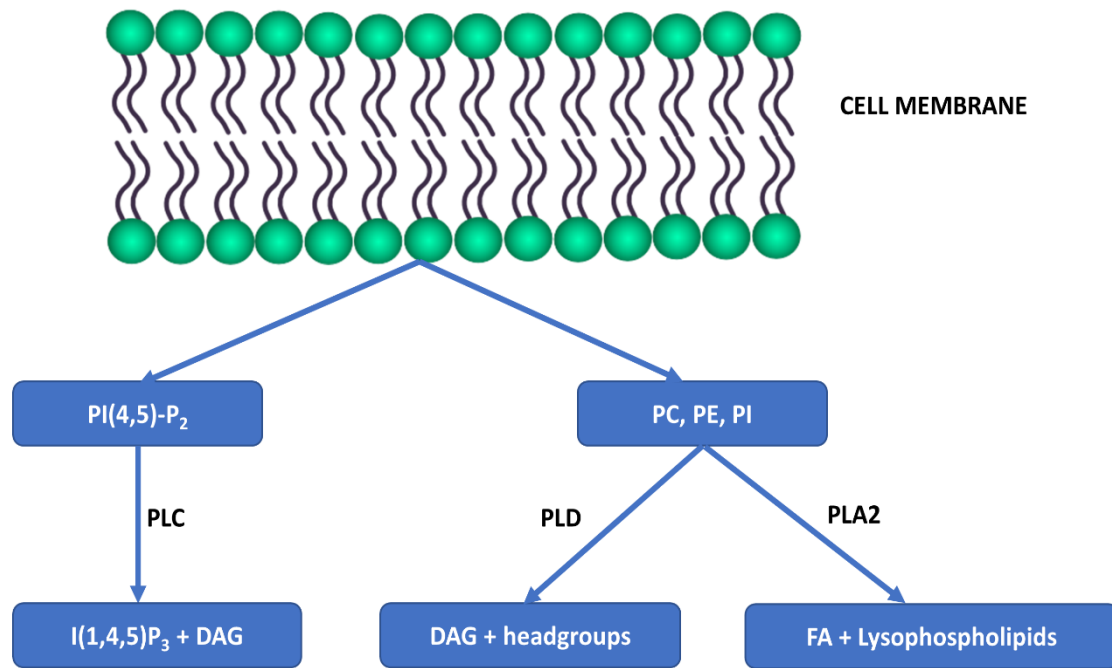
Supporting Information Figure S1. PCA analysis for ToF-SIMS data in positive mode; (A) Scores plot of the second principal component versus the third principal component; (B) Corresponding loadings for the second principal component for ToF-SIMS data in positive mode.



Supporting Information Figure S2. Alteration of TAG levels in the central fly brain after cocaine, cocaine removal, and flies with cocaine treatment followed MPH oral administration in positive ion mode analyzed using a 40 keV $(\text{CO}_2)_{6000}^+$ GCIB. Peak intensity is normalized to number of pixels selected and total peak intensity. Error bars is the standard error of the mean of 33 controls (blue bars), 28 cocaine-treated flies (green bars), 27 flies with cocaine withdrawal (yellow bars), and 27 flies with MPH feeding after cocaine withdrawal (red bars). *t-test* is applied to identify the statistic difference between cocaine-treated group and others (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).



Supporting Information Figure S3. The relative amounts of lysophospholipids in the central fly brain after cocaine, cocaine removal, and flies with cocaine treatment followed MPH oral administration in negative ion mode analyzed using a 40 keV $(\text{CO}_2)_{6000}^+$ GCIB. (A) PE levels; (B) PI levels. Peak intensity is normalized to number of pixels selected and total peak intensity. Error bars is the standard error of the mean of 32 controls (blue bars), 24 cocaine-treated flies (green bars), and 26 flies with cocaine withdrawal (yellow bars), and 24 flies fed with cocaine followed by MPH. *t-test* is applied to identify the statistic difference between cocaine-treated group and others (* $p < 0.05$). There is no statistic difference between cocaine group and cocaine removal group, as well as cocaine-treated followed by MPH group ($p > 0.05$).



Supporting Information Figure S4. Scheme showing the degradation of membrane phospholipids by phospholipase A₂, C, and D (PLA₂, PLC, and PLD). PI(4,5)P₂, phosphatidylinositol 4,5-bisphosphate; I(1,4,5)P₃, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.