Supporting Information

Imaging of Neurotransmitters and Small Molecules in Brain Tissues using Laser Desorption/Ionization Mass Spectrometry Assisted with Zinc Oxide Nanoparticles

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Conditions of Supporting Experiments with LC-MS

Sample Preparations for LC-MS

A frozen rat brain of 112 mg was homogenized in 400 μL of prechilled MeOH/H_2O (1:1) using tissue grinder pestle in a 1.5 mL microtube, followed by centrifugation at 13000 g for 20 min at 4 °C. Aliquots of 100 μL of the supernatant were transferred into microtubes and then spun in a vacuum concentrator for 3 h at 43 °C until dry. The aqueous extracts of the tissue samples were finally reconstituted in 200 μL of solvent mixture of MeOH/H_2O (1:1), after centrifugation for 20 min at 13000 g and 4 °C. Following aqueous extraction, the residual pellet was homogenized in 400 μL of prechilled $CHCl_3$/MeOH (3:1) using tissue grinder pestle in 1.5 mL microtube, followed by centrifugation at 13000 g for 20 min at 4 °C. Aliquots of 150 μL of the supernatant were transferred into another microtube and were allowed to evaporate at room temperature in fume hood overnight. The organic extracts of the tissue samples were reconstituted in 25 μL of the solvent mixture of H_2O /ACN/isopropanol (ISP) (1:1:2), after centrifugation for 10 min at 5000 g and 4 °C.

LC-MS analysis of rat brain

LC/MS/MS analysis was performed with an Agilent 1290 HPLC system coupled to a Q-Exactive Quadrupole Orbitrap mass spectrometer (Thermo Scientific). Chromatographic conditions consisted of an Eclipse Plus C18 RRHD column (2.1 × 100 mm, 1.8 μm; Agilent Technologies) maintained at 35 °C using an established gradient program. The mobile phase consisted of water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B) (Optima grade, Fisher Scientific). Mobile phase B was held at 0% B for 0.5 min before increasing to 100% over 3.5 min, held at 100% for 2.5 min, before returning to 0% B over 0.5 min. Brain metabolite aqueous and organic extracts were injected with a volume of 5 μL each and separated at a flow rate of 0.3 mL/min.

Heated electrospray ionization was used in both positive and negative ion mode with the following settings: capillary voltage, 3.9 kV and 3.5 kV respectively in positive and negative ion mode; capillary temperature, 400 °C; sheath gas, 17 units; auxiliary gas, 8 units; probe heater temperature, 450 °C; S-Lens RF level, 50%. MS data were acquired using untargeted DDA that included a full MS scan at 35 000 resolution, with a scan range of 70–1000 *m/z*; automatic gain control target, 1×10^6; and a maximum injection time of 128 ms. The five highest intensity ions were selected from each full scan for MS/MS analysis using a 1.2 Da isolation window and were analyzed using the following conditions: resolution, 17 500; automatic gain control target, 1×10^6; max IT, 64 ms; normalized stepped collision energy, 20/40; intensity threshold, 2×10^5; dynamic exclusion, 7 s. Raw data files were imported into MZmine. The *m/z* peak list was matched against the theoretical *m/z* of tentatively identified molecules from MALDI MSI using 5 ppm.

Table S1. Summary of Common Neurotransmitters Imaged by LDI MSI in the Literature.

analyte	detection mode	derivatizaion	matrix	tissue type	spatial resolution	reference
glycine	+	yes	DHB	mouse brain	125 µm	[28]
		no	ZnO TiO_2	mouse brain	70 µm	This work
aspartate	+	yes	DHB	mouse brain	125 µm	[28]
		no	TiO_2	mouse brain	70 µm	[55]
		no	ZnO TiO_2	mouse brain	70 µm	This work
	−	no	9-AA	mouse brain	50 µm	[18]
		no	NEDC	mouse brain	100 µm	[20]
		no	PNA	rat brain	200 µm	[21]
		no	BNDM	rat brain	100 µm	[23]
serine	+	yes	DHB	mouse brain	125 µm	[28]
		no	TiO_2	mouse brain	70 µm	[55]
		no	TiO_2 ZnO	mouse brain mouse brain Rat brain	70 µm 70 µm 80 µm	This work
GABA	+	yes	DHB	mouse brain	125 µm	[28]
		yes	CHCA	rat brain	100 µm	[29]
		yes	CHCA	rat brain pig adrenal gland	50 µm 200 µm	[30]
		yes	TPP	rat brain	120 µm	[33]
		no	TiO_2	mouse brain	70 µm	[55]
		no	TiO_2 ZnO	mouse brain mouse brain rat brain	70 µm 70 µm 80 µm	This work
		no	modified-TiO_2	mouse brain	30 µm	[58]
glutamate	+	yes	DHB	mouse brain	125 µm	[28]
		yes	CHCA	brain	-	[29]
		yes	CHCA	rat brain	50 µm	[30]
		no	TiO_2	mouse brain	70 µm	[55]
		no	TiO_2 ZnO	mouse brain mouse brain rat brain	70 µm 70 µm 80 µm	This work
		no	nodified-TiO_2	mouse brain	30 µm	[58]
	−	no	9-AA	mouse brain	50 µm	[18]
		no	NEDC	mouse brain	100 µm	[20]
		no	PNA	rat brain	200 µm	[21]
		no	BNDM	rat brain	100 µm	[23]
alanine	+	yes	DHB	mouse brain	125 µm	[28]
		no	TiO_2 ZnO	mouse brain mouse brain rat brain	70 µm 70 µm 80 µm	This work
taurine	+	yes	DHB	mouse brain	125 µm	[28]
		no	ZnO	mouse brain rat brain	70 µm 80 µm	This work
	−	no	NEDC	mouse kidney	50 µm	[20]
		no	PNA	rat brain	200 µm	[21]
cysteine	+	no	TiO_2	mouse brain	70 µm	[55]
		no	ZnO	mouse brain	70 µm	This work

adenosine	+	No	TiO_2	mouse brain	70 µm	this work
			ZnO	mouse brain	70 µm	
				rat brain	80 µm	
acetylcholine	+	Yes	CHCA	rat brain	15 µm	[29]
				mouse brain	100 µm	
dopamine	+	Yes	DHB	mouse brain	125 µm	[28]
		Yes	CHCA	rat brain	100 µm	[29]
		Yes	CHCA	pig adrenal gland	200 µm	[30]
		Yes	TPP	rat brain	120 µm	[33]
		No	TiO_2	mouse brain	70 µm	
			ZnO	mouse brain	70 µm	this work
				rat brain	80 µm	
norepinephrine	+	Yes	CHCA	pig adrenal gland	200 µm	[30]
		No	TiO_2	mouse brain	70 µm	this work
			ZnO	mouse brain	70 µm	
			TiO_2	rat brain	80 µm	
epinephrine	+	Yes	CHCA	pig adrenal gland	200 µm	[30]
		No	TiO_2	mouse brain	70 µm	this work
			ZnO	rat brain	80 µm	
serotonin	+	Yes	CHCA	-	-	[29]
tyramine	+	Yes	CHCA	brain	-	[29]
tryptamine	+	Yes	CHCA	brain	-	[29]

Table S2. A List of the Small Molecules Detected in Mouse Brain Tissues by LDI MSI with TiO_2 NP or Dopamine-Modified TiO_2 Monolith.

m/z	compd	detected ions			classification
		TiO_2 in this work	TiO_2[54]	dopamine-modified TiO_2[57]	
99.00 113.97	glycine	$[M + Na]^+$ $[M + K]^+$			amino acids
122.03	cysteine		$[M + H]^+$		amino acids
127.98	alanine	$[M + K]^+$			amino acids
133.08	ornithine		$[M + H]^+$		amino acids
134.05 172.01	aspartic acid	 $[M + K]^+$	$[M + H]^+$		amino acids
132.07 170.03 170.03	creatine	 $[M + K]^+$	$[M + H]^+$	 $[M + K]^+$	amino acids
143.99	serine	$[M + K]^+$			amino acids
154.00 154.02	proline	$[M + K]^+$		 $[M + K]^+$	amino acids
156.02	valine	$[M + K]^+$			amino acids
120.06 158.01	threonine	 $[M + K]^+$	$[M + H]^+$		amino acids
166.08	phenylalanine		$[M + H]^+$		amino acids
167.01 167.02	2-amino-4-cyano-butanoic acid	$[M + K]^+$		 $[M + K]^+$	amino acids
168.00 168.01	pyroglutamic acid	$[M + K]^+$		 $[M + K]^+$	amino acids
169.04 169.06 185.02	glutamine	$[M + Na]^+$ $[M + K]^+$		 $[M + Na]^+$	amino acids
148.06 186.00 186.02	glutamic acid	 $[M + K]^+$	$[M + H]^+$	 $[M+K]^+$	amino acids
150.06 188.00	methionine	 $[M + K]^+$	$[M + H]^+$		amino acids
194.02	histidine	$[M + K]^+$			amino acids
196.00 196.01	amino-muconic acid	 $[M + K]^+$		$[M + K]^+$	amino acids
198.09	amino-octanoic acid			$[M + K]^+$	amino acids
204.03	phenylalanine	$[M + K]^+$			amino acids
175.11 213.06	arginine	 $[M + K]^+$	$[M + H]^+$		amino acids
234.07	methoxytyrosine			$[M + Na]^+$	amino acids
89.10 111.09	putrescine	 $[M + Na]^+$	$[M + H]^+$		alkaloids
123.06 160.99	nicotinamide	 $[M + K]^+$	$[M + H]^+$		alkaloids
168.13 184.09	spermidine	$[M + Na]^+$ $[M + K]^+$			alkaloids
180.04	arecaidine			$[M + K]^+$	alkaloids
241.17	spermine	$[M + K]^+$			alkaloids
136.06 174.01	adenine	 $[M + K]^+$	$[M + H]^+$		purine
137.05 159.00 174.98	hypoxanthine	 $[M + Na]^+$ $[M + K]^+$	$[M + H]^+$		purine
113.03 150.96	uracil	 $[M + K]^+$	$[M + H]^+$		pyrimidine
104.06 126.02 142.00 142.03	γ-aminobutyric acid (GABA)	 $[M + Na]^+$ $[M + K]^+$	$[M + H]^+$	 $[M + K]^+$	neurotransmitters
191.03	dopamine	$[M + K]^+$			neurotransmitters
208.02	norepinephrine	$[M + K]^+$			neurotransmitters
222.05	epinephrine	$[M + K]^+$			neurotransmitters
306.06	adenosine	$[M + K]^+$			neurotransmitters
409.34 409.36 425.32 425.34	cholesterol	 $[M + Na]^+$ $[M + K]^+$		$[M + Na]^+$ $[M + K]^+$	sterol lipids
429.24	hydroxy-oxo-cholan-oic acid			$[M + K]^+$	sterol lipids
439.30 439.31	OH-7-dehydrocholesterol	 $[M + K]^+$		$[M + K]^+$	sterol lipids
465.33	cholesta-6,8(14)-dien-3beta,5alpha-diol			$[M + K]^+$	sterol lipids
243.08 259.05	Met-Ala	$[M + Na]^+$		 $[M + K]^+$	dipeptides
277.06	Gly-Tyr			$[M + Na]^+$	dipeptides
283.07	Pro-Glu			$[M + K]^+$	dipeptides

(table continued on next page)

m/z	compd	detected ions			classification
		TiO_2 in this work	TiO_2[54]	dopamine-modified TiO_2[57]	
413.12	Cys Gly Pro Val			$[M + K]^+$	peptides
453.17	Gly Glu Pro Ile			$[M + K]^+$	peptides
457.21	Met Leu Ala Thr			$[M + Na]^+$	peptides
459.22	Thr Leu Gly Phe			$[M + Na]^+$	peptides
469.15	Glu Trp Pro			$[M + K]^+$	peptides
481.21	Asp Val Leu Pro			$[M + K]^+$	peptides
495.16	Gly Phe Ala Tyr			$[M + K]^+$	peptides
497.18	Asn Asn Pro Met			$[M + Na]^+$	peptides
126.99	butyric acid	$[M + K]^+$			fatty acids
168.98	5-oxohexanoic acid	$[M + K]^+$			fatty acids
277.18 293.19	FA(16:1)	$[M + Na]^+$		 $[M + K]^+$	fatty acids
279.23 295.20	FA(16:0)			$[M + Na]^+$ $[M + K]^+$	fatty acids
305.24 321.23	FA(18:1)	 $[M + K]^+$		$[M + Na]^+$	fatty acids
307.26 323.23 323.23	FA(18:0)	 $[M + K]^+$		$[M + Na]^+$ $[M + K]^+$	fatty acids
327.23 343.20 343.21	FA(20:4)	 $[M + K]^+$		$[M + Na]^+$ $[M + K]^+$	fatty acids
337.21	hydroxy-oleic acid			$[M + K]^+$	fatty acids
341.19	FA(20:5)			$[M + K]^+$	fatty acids
351.22 351.23 367.20 367.21	FA(22:6)	$[M + Na]^+$ $[M + K]^+$		 $[M + Na]^+$ $[M + K]^+$	fatty acids
355.26	docosatetraenoic acid (22:4)			$[M + Na]^+$	fatty acids
363.27	FA(21:1)			$[M + K]^+$	fatty acids
139.00	fumaric acid	$[M + Na]^+$			organic acids
144.98	glyceric acid	$[M + K]^+$			organic acids
153.00	citraconic acid	$[M + Na]^+$			organic acids
154.99	levulinic acid	$[M + K]^+$			organic acids
156.98	succinic acid	$[M + K]^+$			organic acids
159.00	threonic acid	$[M + Na]^+$			organic acids
187.01	cinnamic acid	$[M + K]^+$			organic acids
189.03	3-phenylpropionic acid	$[M + K]^+$			organic acids
212.97	aconitic acid	$[M + K]^+$			organic acids
217.02	glucuronic acid	$[M + Na]^+$			organic acids
104.09	choline	M^+			others
110.03	hypotaurine		$[M + H]^+$		others
112.01	oxamic acid	$[M + Na]^+$			others
112.06 150.00	cytosine	 $[M + K]^+$	$[M + H]^+$		others
130.08	pipecolic acid		$[M + H]^+$		others
136.04 152.01 152.02	creatinine	 $[M + K]^+$		$[M + Na]^+$ $[M + K]^+$	others
139.08	aminopentanamide			$[M+Na]^+$	others
143.08	ectoine		$[M + H]^+$		others
146.98 162.96	phosphono-acetaldehyde	$[M + Na]^+$ $[M + K]^+$			others
164.07	S-methylmethionine		M^+		others
165.04	lumazine		$[M + H]^+$		others
184.07	phosphocholine		M^+		others
192.98	dihydroxyacetone phosphate	$[M + Na]^+$			others
203.04 219.01	myo-inositol	$[M + Na]^+$ $[M + K]^+$			others
207.03 223.01	3-methoxy-4-hydroxyphenylglycol	$[M + Na]^+$ $[M + K]^+$			others
214.00	N-acetylaspartic acid	$[M + K]^+$			others
228.01	N-acetylglutamic acid	$[M + K]^+$			others
260.05	N-acetylglucosamine	$[M + K]^+$			others
266.05	cytidine	$[M + Na]^+$			others
307.05	guanosine	$[M + K]^+$			others
375.20 391.20	prostaglandin E2	$[M + Na]^+$ $[M + K]^+$			others

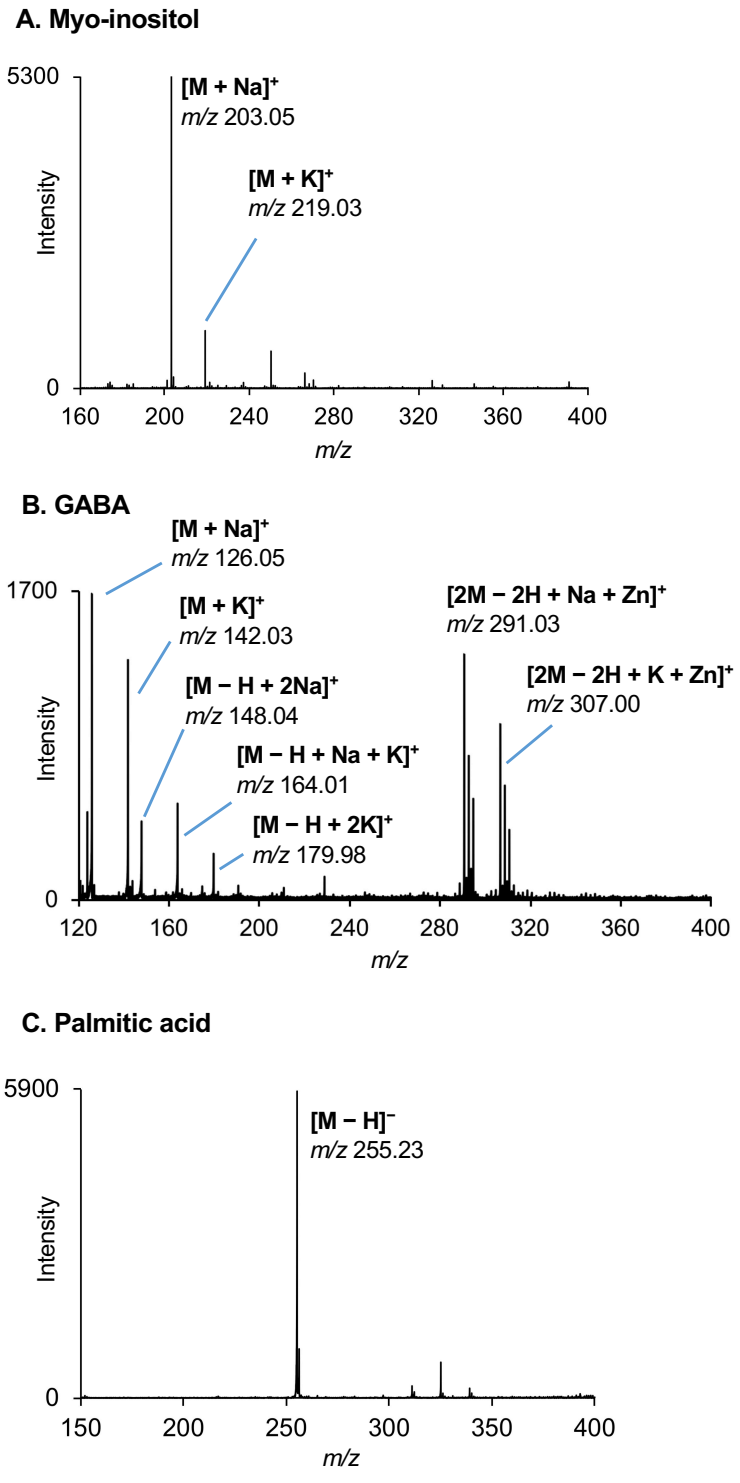


Figure S1. ZnO NP-assisted LDI mass spectra of standards: 30 ng of **(A)** myo-inositol and **(B)** GABA in positive ion mode and **(C)** 300 ng of palmitic acid in negative ion mode.

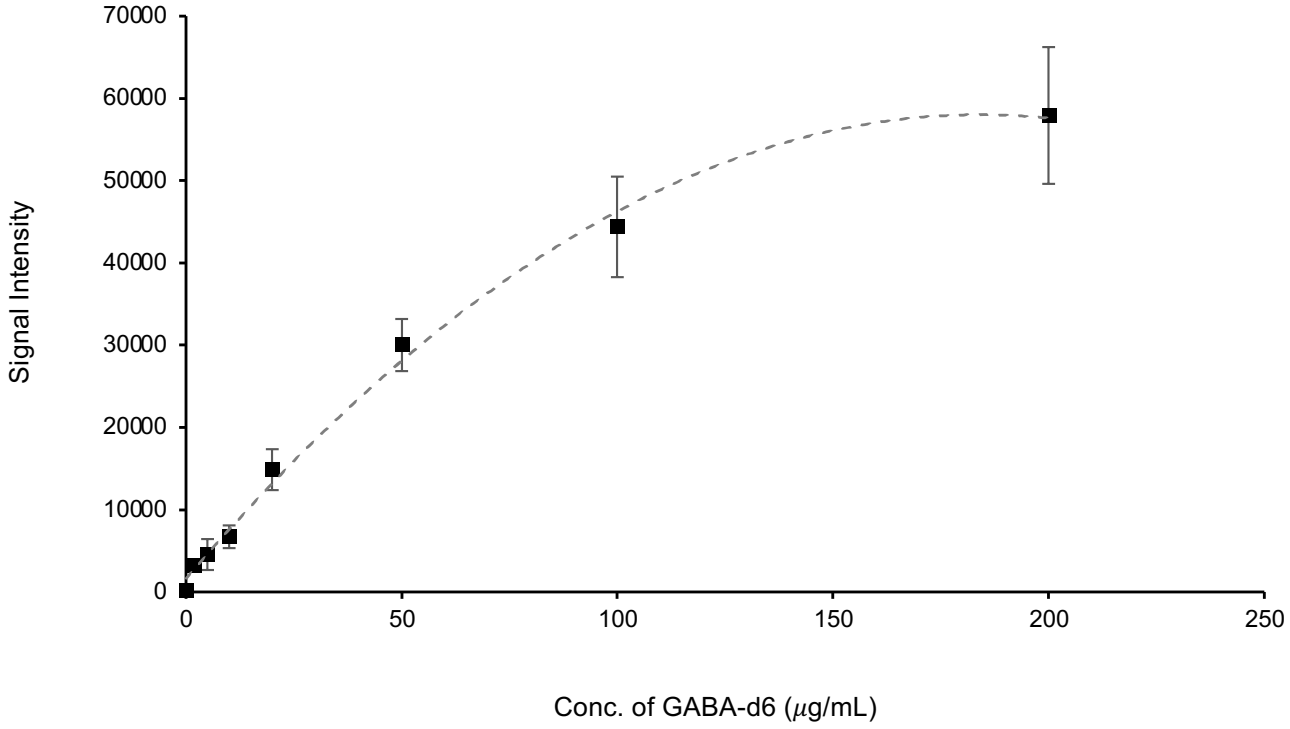


Figure S2. Calibration curve of GABA-d6 signals measured by ZnO NP-assisted LDI MS. The GABA-d6 concentration refers to that of the standard solution spiked on rat brain tissue sections. Error bars show +/– the standard deviation from 9 replicate measurements.

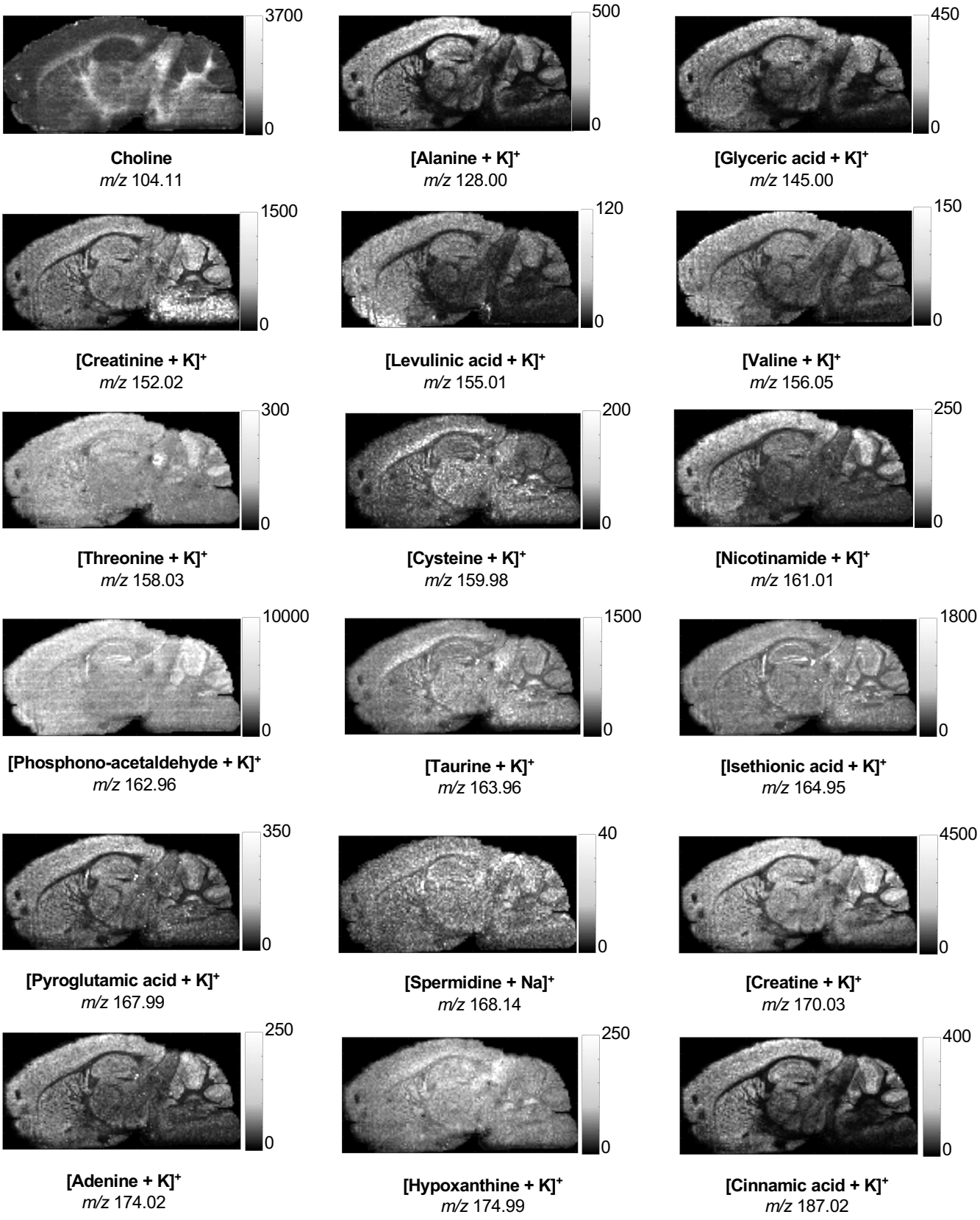
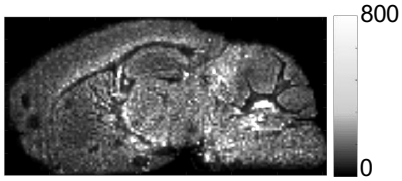
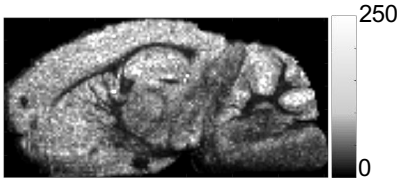


Figure S3. Ion images of sagittal sections of mouse brain acquired with ZnO NP-assisted LDI-MSI at a raster size of 70 µm in the positive ion mode (figure continued on next page).

[3-Phenylpropionic acid + K]$^+$
m/z 189.05

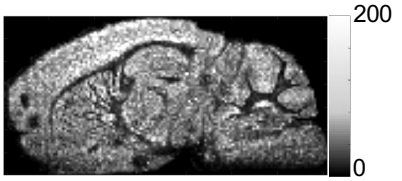


[Dopamine + K]$^+$
m/z 192.03



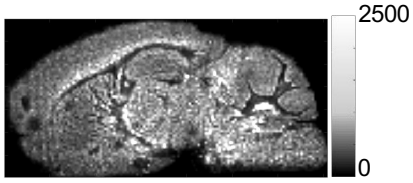
[Dihydroxyacetone phosphate + Na]$^+$
m/z 192.98

[Histidine + K]$^+$
m/z 194.01

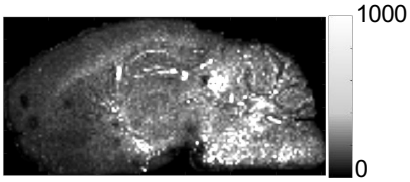


[Amino-muconic acid + K]$^+$
m/z 196.00

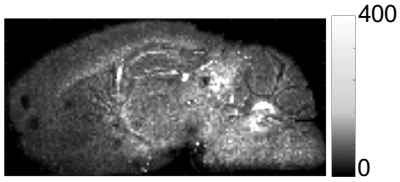
[Carnitine + K]$^+$
m/z 200.08



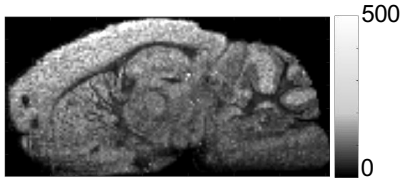
[Norepinephrine + K]$^+$
m/z 208.01



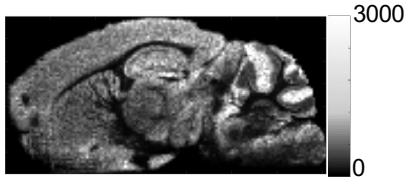
[Aconitic acid + K]$^+$
m/z 212.98



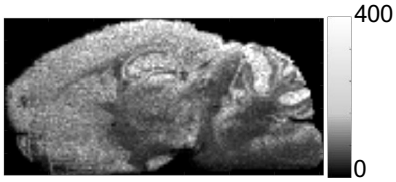
[Arginine + K]$^+$
m/z 213.06



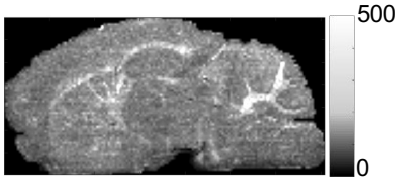
[N-Acetylaspartic acid + K]$^+$
m/z 214.00



[Myo-inositol + K]$^+$
m/z 219.02

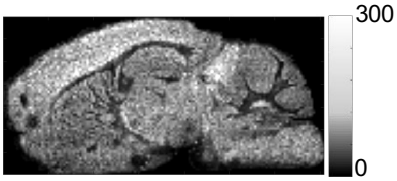


[Tyrosine + K]$^+$
m/z 220.02

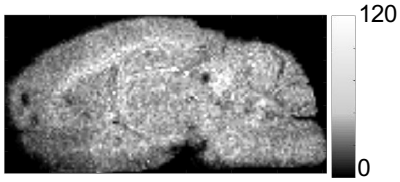


[Epinephrine + K]$^+$
m/z 222.05

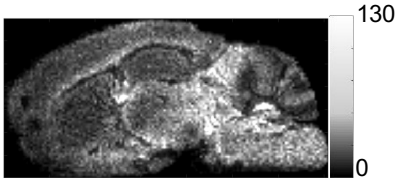
[Metanephrine + K]$^+$
m/z 236.08



[3-Indoxyl sulfuric acid + K]$^+$
m/z 251.98



[Gly-Tyr + K]$^+$
m/z 277.04



[Glycerophosphoryl-choline + K]$^+$
m/z 296.07

[Fructose 6-phosphate + K]$^+$
m/z 298.97

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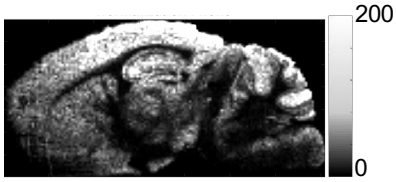
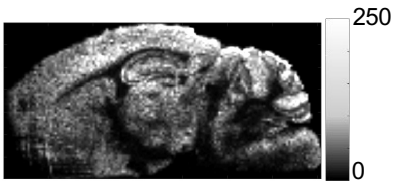
[Sorbitol 6-phosphate + K]$^+$
m/z 301.00

[Adenosine + K]$^+$
m/z 306.07



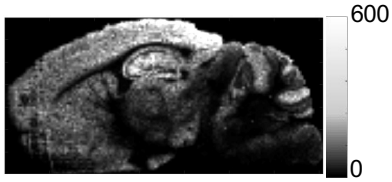
[FA(16:0) + K]$^+$
m/z 295.19



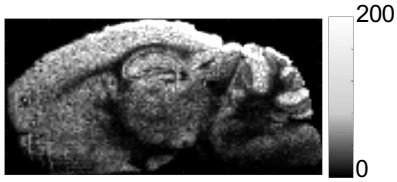
[FA(18:1) + K]$^+$
m/z 321.22

[FA(18:0) + K]$^+$
m/z 323.24

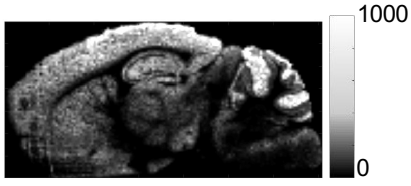
[Hydroxy-oleic acid + K]$^+$
m/z 337.23



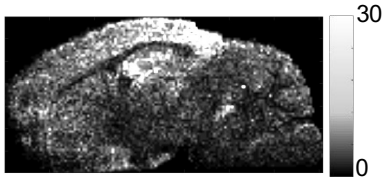
[FA(20:4) + K]$^+$
m/z 343.20



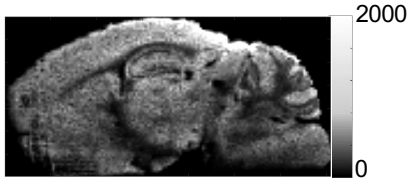
[FA(21:1) + K]$^+$
m/z 363.29



[FA(22:6) + K]$^+$
m/z 367.21



[FA(22:4) + K]$^+$
m/z 371.24



[Cholesterol + Na]$^+$
m/z 409.35

[OH-7-Dehydrocholesterol + Na]$^+$
m/z 423.31

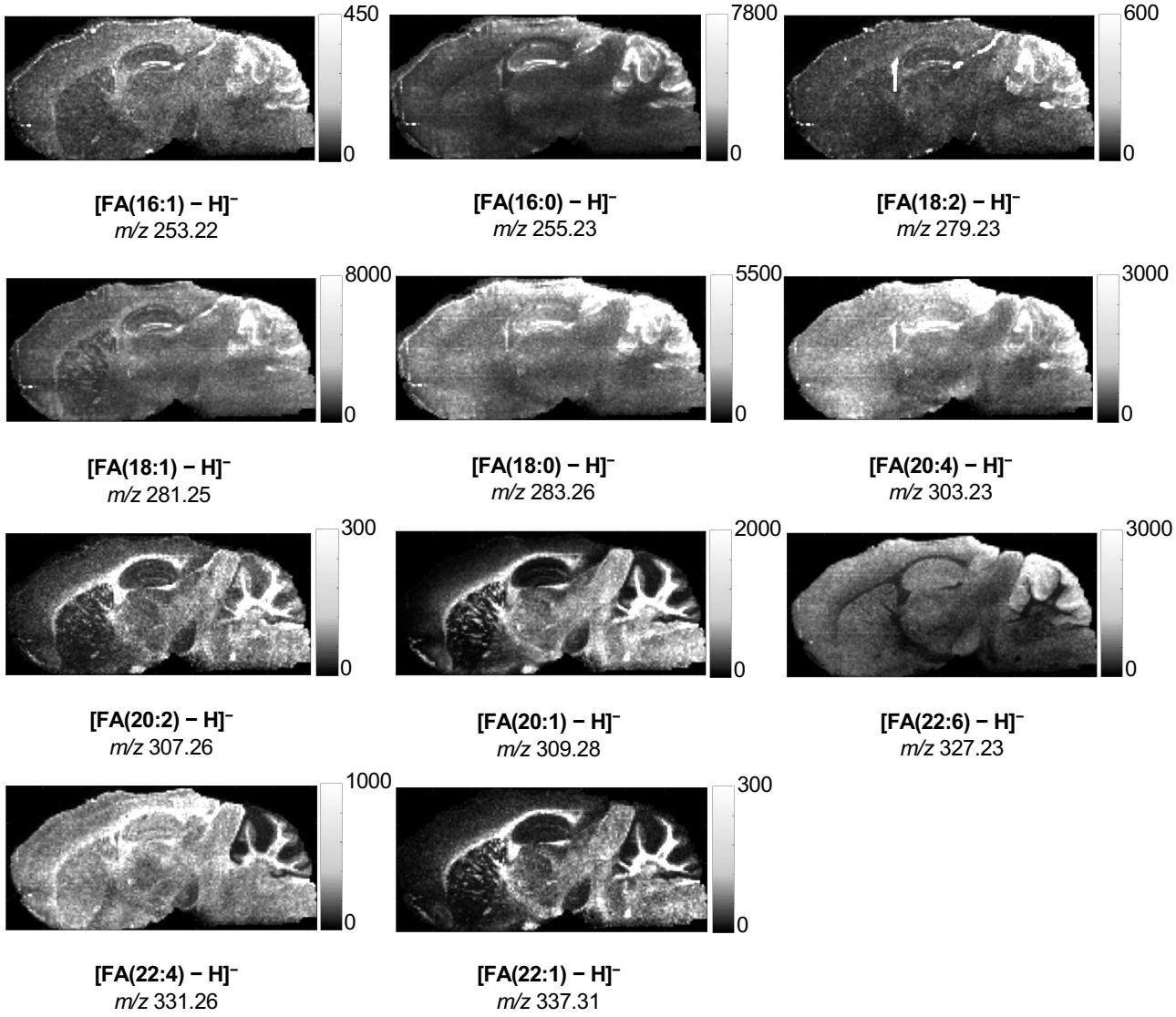


Figure S4. Ion images of sagittal sections of mouse brain acquired with ZnO NP-assisted LDI-MSI at a raster size of 70 μm in the negative ion mode.

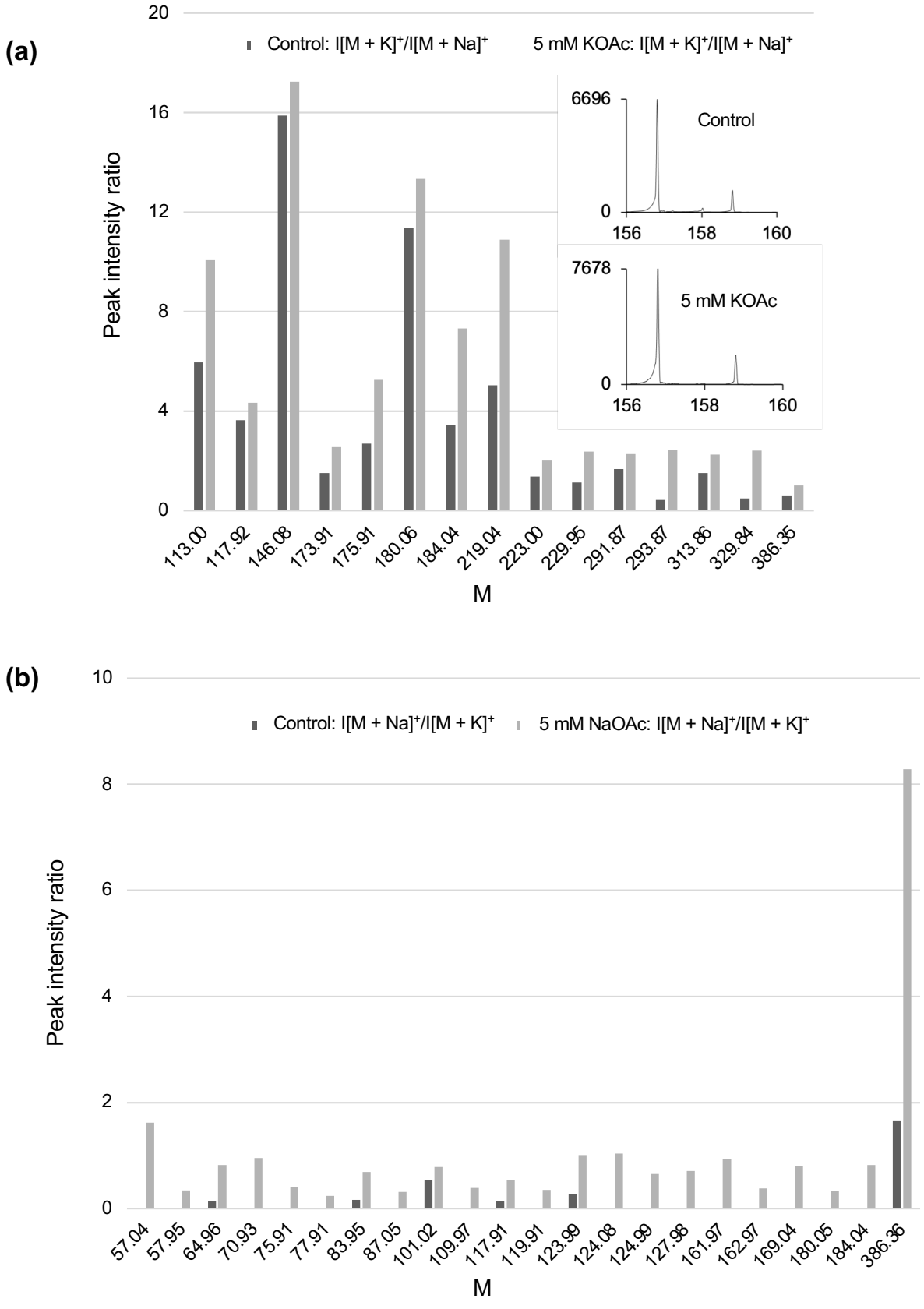


Figure S5. Peak height intensity ratios of the sodiated and potassiated ions from two adjacent mouse brain tissue sections without (control) and with the application of 5 mM potassium acetate **(a)** or 5 mM sodium acetate **(b)** by automated sprayer. M represent the molecular weight. The figure inset shows the isotope patterns of $[M + K]^+$ at *m/z* 156.88 without and with the application of 5 mM potassium acetate.