Electronic Supplementary Material (ESI) for RSC Advances.

Table S1. Linear regression data and lower limit of quantitation (LLOQ) of analytes in serum by LC-MS/MS

Analyte	Regression equation	Linear range (ng/mL)	*r*	LLOQ (ng/mL)
Glu	$y = 0.0009x - 0.0132$	200—20000	0.9977	200
GABA	$y = 0.0011x + 0.0007$	2—200	0.9926	2
Ach	$y = 0.3428x + 0.0001$	2—200	0.9956	2
5-HT	$y = 0.0024x + 0.0115$	2—200	0.9935	2
DA	$y = 0.0207x + 0.0039$	2—200	0.9908	2
NE	$y = 0.0058x - 0.0051$	2—200	0.9929	2
Trp	$y = 0.0308x + 2.6289$	200—20000	0.9945	200
Tyr	$y = 0.0094x - 0.2994$	200—20000	0.9944	200

.

Table S2. Linear regression data and lower limit of quantitation (LLOQ) of analytes in brain by LC-MS/MS

Analyte	Regression equation	Linear range (ng/mL)	*r*	LLOQ (ng/mL)
Glu	$y = 0.0004x + 0.8959$	2000—200000	0.9904	2000
GABA	$y = 0.0011x + 5.4240$	2000—200000	0.9965	2000
Ach	$y = 0.0442x + 1.5267$	20—2000	0.9922	20
5-HT	$y = 0.0015x - 0.0150$	20—2000	0.9925	20
DA	$y = 0.0221x - 0.2020$	20—2000	0.9933	20
NE	$y = 0.0028x + 0.1025$	200—20000	0.9919	200
Trp	$y = 0.0152x + 4.2890$	200—20000	0.9911	200
Tyr	$y = 0.0076x + 0.4154$	200—20000	0.9927	200

Table S3. Summary of precision, accuracy, recovery and matrix effect of analytes in serum by LC-MS/MS (*n*=6)

Analyte	Concentration	Precision		Accuracy (RE, %)	Recovery (%)	Matrix effect (%)
		Intra-day (RSD, %)	Inter-day (RSD, %)			
Glu (ug/ml)	0.5	9.5	6.4	2.2	83.8±9.9	95.9±12.8
	3	8.7	8.2	1.8	89.4±6.6	95.3±9.6
	16	8.5	10.6	0.6	84.6±10.4	97.2±5.8
GABA (ng/ml)	5	9.9	6.5	-0.4	87.9±6.0	99.0±5.6
	30	9.0	6.6	-2.7	83.3±7.3	102.4±6.6
	160	5.6	1.7	-4.6	89.1±4.5	104.3±7.0
Ach (ng/ml)	5	6.3	6.7	-0.3	83.9±6.7	97.8±12.1
	30	8.3	9.8	-1.3	82.4±5.1	94.0±8.1
	160	6.3	3.9	0.3	83.4±1.5	97.4±8.8
5-HT (ng/ml)	5	11.1	3.9	-0.4	84.8±4.3	110.0±11.2
	30	7.6	2.0	-1.3	85.4±7.1	91.1±8.8
	160	7.4	2.4	1.9	85.6±7.8	104.7±2.8
DA (ng/ml)	5	10.9	1.4	-2.1	82.8±5.8	105.7±7.0
	30	9.3	6.6	-0.9	84.7±4.1	100.9±9.5
	160	9.2	6.9	-1.2	83.1±2.8	96.6±1.9
NE (ng/ml)	5	9.6	3.8	-1.8	89.1±7.6	104.4±5.2
	30	8.4	9.8	-2.3	88.2±7.0	102.6±4.3
	160	7.0	3.4	0.2	84.6±5.0	99.8±10.4
Trp (μg/ml)	0.5	8.3	8.3	3.1	83.0±4.1	96.5±9.4
	3	8.9	8.6	-1.2	82.9±6.3	91.0±5.0
	16	9.1	2.3	0.3	82.6±5.1	100.1±11.9
Tyr (μg/ml)	0.5	6.9	3.6	-1.2	82.3±4.4	97.4±5.4
	3	9.0	2.8	1.9	87.8±6.2	101.7±8.5
	16	9.2	7.7	-1.1	86.0±7.0	103.2±8.9

Table S4. Summary of precision, accuracy, recovery and matrix effect of analytes in brain by LC-MS/MS (*n*=6)

Analyte	Concentration	Precision		Accuracy (RE, %)	Recovery (%)	Matrix effect (%)
		Intra-day (RSD, %)	Inter-day (RSD, %)			
Glu (μg/ml)	5	10.2	13.9	0.8	79.4±8.8	94.3±4.4
	30	7.6	2.0	-0.9	77.9±4.3	104.1±9.0
	160	7.2	2.3	5.6	78.0±5.5	107.3±5.9
GABA (μg/ml)	5	7.2	4.9	-2.2	85.8±4.0	101.4±9.4
	30	6.4	8.0	3.4	81.9±5.9	96.6±9.5
	160	5.6	9.4	-0.3	82.1±1.0	106.2±7.7
Ach (μg/ml)	0.05	8.2	3.0	1.0	77.1±11.1	92.1±8.9
	0.3	7.3	2.3	0.4	74.4±2.6	98.3±11.9
	1.6	5.7	5.1	-0.1	80.5±5.7	99.9±6.6
5-HT (μg/ml)	0.05	8.4	13.1	-4.6	81.7±5.4	92.2±2.4
	0.3	5.0	10.7	2.7	81.0±4.7	92.5±0.7
	1.6	3.8	4.0	0.3	83.9±6.0	96.3±4.7
DA (μg/ml)	0.05	10.0	6.5	2.6	80.7±4.2	104.0±13.2
	0.3	6.9	3.2	1.7	73.2±2.6	101.1±6.4
	1.6	3.6	1.3	3.3	80.9±1.1	99.3±3.0
NE (μg/ml)	0.5	6.0	10.7	-0.6	82.1±3.7	98.8±8.9
	3	7.5	7.9	4.1	87.3±5.7	98.6±10.7
	16	8.8	3.9	1.2	83.5±6.7	100.6±8.7
Trp (μg/ml)	0.5	6.8	8.7	2.9	85.8±6.2	102.7±7.1
	3	3.9	7.1	2.8	90.6±4.9	105.6±7.5
	16	5.3	1.3	-2.4	79.3±3.6	106.2±7.3
Tyr (μg/ml)	0.5	7.6	13.1	-1.4	85.6±5.8	95.5±6.1
	3	8.1	6.9	0.2	83.5±6.5	99.5±4.0
	16	3.1	2.1	4.2	83.2±4.8	101.1±6.9

Table S5. Stability of analytes in serum by LC-MS/MS (n = 6)

Analyte	Concentration	freeze thaw		Room temperature		long-term		post-preparative	
		(RE, %)	(RSD, %)	(RE, %)	(RSD, %)	(RE, %)	(RSD, %)	(RE, %)	(RSD, %)
Glu (μg/ml)	0.5	3.6	6.9	-8.8	2.6	-6.7	7.5	-2.5	9.2
	3	3.8	12.3	-2.0	5.3	2.2	9.8	1.5	10.8
	16	4.4	6.7	7.4	3.7	-3.4	6.5	-9.3	2.0
GABA (ng/ml)	5	-4.4	6.0	-0.4	6.4	8.2	0.9	0.4	12.7
	30	0.4	13.9	-5.7	2.8	2.9	14.8	2.1	11.5
	160	-1.3	9.2	5.8	3.2	-4.8	5.9	-7.8	5.3
Ach (ng/ml)	5	5.2	8.0	-6.4	11.0	-0.5	12.3	-5.0	11.0
	30	-6.6	9.5	-8.8	5.2	1.3	7.3	-5.3	8.5
	160	2.2	1.1	-5.5	7.2	-2.3	11.5	-3.0	6.9
5-HT (ng/ml)	5	-8.1	1.0	2.8	8.4	-1.1	13.5	-2.0	7.7
	30	2.1	11.3	-6.3	8.7	8.8	2.1	1.3	10.2
	160	-1.8	5.5	10.3	2.2	-2.8	12.6	1.3	9.8
DA (ng/ml)	5	0.8	8.7	1.6	14.2	-3.5	7.8	0.6	11.7
	30	3.3	8.6	-3.3	9.5	-3.4	6.3	2.4	10.4
	160	-3.6	9.9	3.4	9.8	2.3	8.1	-2.6	5.7
NE (ng/ml)	5	1.1	8.6	7.3	2.7	-2.8	6.9	-3.7	8.9
	30	-2.3	11.3	-7.5	7.7	2.8	6.5	3.0	8.9
	160	6.7	8.3	4.1	5.3	5.8	5.7	0.3	9.6
Trp (μg/ml)	0.5	-4.7	1.7	-6.1	8.9	-1.4	12.1	-2.6	13.8
	3	-11.2	4.2	1.4	0.8	0.4	5.1	4.8	3.6
	16	-2.7	13.2	8.9	3.7	-0.5	11.0	-6.8	7.3
Tyr (μg/ml)	0.5	-1.0	10.1	-0.2	7.7	-3.1	10.1	-6.0	8.6
	3	-7.0	1.7	3.3	10.2	-1.5	6.1	-0.2	6.7
	16	-4.9	5.4	1.5	12.3	-6.5	3.3	1.4	5.8

Table S6. Stability of analytes in brain by LC-MS/MS (n = 6)

Analyte	Concentration	freeze thaw		Room temperature		long-term		post-preparative	
		(RE, %)	(RSD, %)	(RE, %)	(RSD, %)	(RE, %)	(RSD, %)	(RE, %)	(RSD, %)
Glu (μg/ml)	5	-4.8	13.9	-3.7	5.5	-1.4	12.6	-7.0	3.1
	30	0.5	12.8	2.3	2.0	-8.2	6.4	5.2	6.6
	160	5.4	7.5	-1.1	5.4	3.8	7.9	5.5	9.0
GABA (μg/ml)	5	0.8	7.1	5.3	5.6	3.6	9.1	4.2	6.1
	30	0.7	10.9	-2.8	4.9	-0.9	8.9	-3.6	1.0
	160	-6.3	4.9	8.4	3.7	11.9	2.5	7.2	3.7
Ach (μg/ml)	0.05	-2.7	8.9	-4.8	11.1	3.5	8.9	0.0	12.2
	0.3	-3.6	11.1	0.8	4.6	-7.7	7.0	-3.9	6.5
	1.6	3.6	7.5	1.0	1.8	0.1	6.7	-8.7	5.8
5-HT (μg/ml)	0.05	7.0	6.3	0.3	9.7	-5.2	9.8	-2.6	7.4
	0.3	3.6	3.5	-6.5	6.5	-11.6	5.2	-2.9	4.2
	1.6	2.2	2.8	-6.7	1.8	-2.7	4.4	-2.2	1.5
DA (μg/ml)	0.05	7.2	7.6	-11.1	11.2	-1.8	6.8	-2.1	3.9
	0.3	-1.7	6.9	-2.4	8.8	7.8	3.0	2.6	5.0
	1.6	6.6	4.5	6.8	6.3	1.8	4.8	3.0	5.7
NE (μg/ml)	0.5	-1.6	6.5	-11.8	14.5	-2.0	12.1	1.3	5.7
	3	1.7	7.0	-1.5	10.7	-1.8	6.8	2.1	8.6
	16	6.0	6.7	-7.9	2.2	-4.7	4.6	0.6	9.5
Trp (μg/ml)	0.5	7.2	10.6	-12.2	3.7	5.9	2.1	3.3	6.1
	3	7.6	2.0	3.6	5.3	8.5	5.7	5.0	5.0
	16	6.4	3.4	-8.3	7.1	-3.1	4.2	-0.6	1.7
Tyr (μg/ml)	0.5	-2.8	11.3	4.6	10.6	5.3	9.3	-2.3	12.4
	3	-1.0	8.0	3.5	4.9	3.8	6.5	3.4	2.6
	16	6.7	2.3	4.0	2.7	2.1	1.4	2.1	1.5

Table S7. The levels of analytes in serum samples by LC-MS/MS (n = 6)

Analyte	Group	15th day	16th day	18th day	23rd day	30th day
	Blank	15.10±1.23	15.31±1.45	15.35±1.09	14.91±0.81	14.16±1.23
Glu	Positive	15.38±1.20	15.44±1.27	15.73±1.41	15.67±0.97	15.86±1.28*
(μg/ml)	SAE	15.17±1.08	14.83±0.88	15.40±0.65	15.23±1.30	16.33±0.96**
	TGP	15.39±0.80	15.34±1.07	15.52±1.49	14.97±1.12	15.18±1.02
	Blank	103.10±14.42	97.56±7.92	104.86±15.28	104.93±9.91	99.26±8.91
GABA	Positive	98.63±9.73	102.97±6.19	103.72±17.15	104.16±15.03	88.43±9.50
(ng/ml)	SAE	102.78±15.86	98.21±6.71	98.27±9.69	97.78±13.62	95.35±11.28
	TGP	93.57±10.39	95.18±12.72	101.86±15.16	105.35±9.70	102.44±11.92
	Blank	31.86±2.61	31.05±4.55	30.30±2.44	30.65±2.01	31.43±1.52
Ach	Positive	29.35±2.63	32.78±5.15	33.39±2.06	32.98±2.11	34.13±5.85
(ng/ml)	SAE	31.10±4.57	32.12±3.44	35.20±3.35*	38.25±3.20**	40.99±5.04**
	TGP	32.96±3.52	32.01±3.57	31.18±5.71	34.38±2.57$^{*\#}$	37.23±2.31*
	Blank	50.14±6.36	48.54±8.09	49.47±5.39	46.90±3.46	50.26±3.25
5-HT	Positive	46.71±5.05	49.13±6.67	52.90±3.99	56.65±4.74**	65.10±8.49**
(ng/ml)	SAE	46.03±3.08	48.97±4.69	53.81±5.20	58.39±5.96**	62.80±7.67**
	TGP	49.20±4.42	48.68±5.05	49.54±7.17	49.41±7.11$^{\#}$	51.87±5.44$^{\#}$
	Blank	12.38±1.26	11.76±0.63	11.96±0.68	12.39±1.18	11.56±0.72
DA	Positive	12.15±1.45	12.10±1.39	11.68±1.37	11.91±0.92	9.61±0.94*
(ng/ml)	SAE	12.08±1.39	11.29±0.74	11.07±1.13	10.63±1.51*	9.42±1.25*
	TGP	10.94±1.70	11.41±1.40	11.37±2.27	10.95±1.46	10.94±1.94
	Blank	17.21±1.19	17.13±0.77	17.67±1.18	18.19±1.10	16.89±0.83
NE	Positive	16.63±1.77	17.11±1.37	16.23±1.14	16.13±0.94*	14.70±0.86**
(ng/ml)	SAE	17.02±1.31	16.01±0.86	16.00±1.22	15.15±2.30**	14.73±1.13**
	TGP	16.88±1.61	16.56±2.04	16.13±2.10	15.40±1.21	16.06±0.64$^{\#}$
	Blank	13.94±1.18	13.74±1.75	13.83±0.50	13.85±0.78	13.68±1.45
Trp	Positive	13.51±1.52	14.17±2.05	14.70±1.08	15.61±1.02*	15.75±1.03*
(μg/ml)	SAE	13.99±1.88	14.24±1.38	14.65±1.52	15.47±1.90*	15.99±1.26**
	TGP	14.10±1.07	13.41±1.07	13.63±1.46	13.96±0.78$^{\#}$	13.79±1.54$^{\#}$
	Blank	1.72±0.12	1.67±0.18	1.71±0.17	1.80±0.09	1.62±0.10
Tyr	Positive	1.64±0.18	1.60±0.20	1.56±0.14	1.64±0.13*	1.40±0.19**
(μg/ml)	SAE	1.59±0.15	1.55±0.17	1.55±0.16	1.50±0.18**	1.44±0.09*
	TGP	1.58±0.06	1.54±0.07	1.51±0.18*	1.49±0.11**	1.45±0.08*

a) ** $p < 0.01$ and * $p < 0.05$ (compared with blank group rats);

b) $^{\#\#}$ $p < 0.01$ and $^{\#}$ $p < 0.05$ (compared with SAE group rats).

Table S8. The levels of analytes in brain samples by LC-MS/MS (n = 6)

Analyte	Group	16th day	18th day	23rd day	30th day
	Blank	307.59±17.51	327.58±14.84	331.27±24.54	304.45±20.47
Glu	Positive	318.45±24.36	316.54±32.59	328.25±23.47	333.08±15.55
(μg/g)	SAE	322.45±22.08	328.76±17.33	338.02±28.43	338.94±19.45*
	TGP	318.33±18.32	301.35±14.76	327.58±23.14	301.54±44.78
	Blank	535.07±80.00	522.12±77.54	544.63±53.22	524.04±44.62
GABA	Positive	536.10±59.45	481.08±63.87	526.34±61.14	504.18±45.19
(μg/g)	SAE	519.58±45.70	512.59±53.55	508.86±51.17	480.93±37.45
	TGP	504.92±57.24	514.25±40.59	536.89±39.48	544.01±44.49$^{\#}$
	Blank	1.87±0.24	1.96±0.21	1.94±0.18	1.89±0.21
Ach	Positive	1.98±0.13	1.97±0.06	2.00±0.13	2.12±0.20
(μg/g)	SAE	2.03±0.06	2.13±0.23	2.21±0.12	2.29±0.16**
	TGP	1.84±0.22	1.83±0.30$^{\#}$	2.05±0.07$^{\#}$	2.01±0.27$^{\#}$
	Blank	0.79±0.07	0.75±0.07	0.79±0.011	0.71±0.06
5-HT	Positive	0.81±0.06	0.81±0.09	0.86±0.07	0.84±0.08**
(μg/g)	SAE	0.79±0.05	0.82±0.08	0.83±0.06	0.85±0.09**
	TGP	0.72±0.06	0.80±0.07	0.77±0.06	0.8±0.04*
	Blank	2.23±0.17	2.24±0.31	2.21±0.27	2.18±0.25
DA	Positive	2.12±0.12	2.00±0.13	1.90±0.12**	1.81±0.13**
(μg/g)	SAE	1.96±0.16*	1.97±0.18*	1.90±0.14**	1.84±0.12**
	TGP	2.12±0.24	2.12±0.16	2.07±0.13	2.06±0.20$^{\#}$
	Blank	5.51±0.49	5.52±0.34	5.62±0.42	5.32±0.41
NE	Positive	5.38±0.61	5.15±0.43	5.17±0.58	4.82±0.23*
(μg/g)	SAE	5.24±0.26	5.12±0.28*	5.05±0.36*	4.86±0.25*
	TGP	5.47±0.43	5.26±0.21	5.24±0.25	5.13±0.40
	Blank	17.62±1.32	18.52±1.69	18.55±1.04	17.03±1.56
Trp	Positive	18.78±1.44	19.05±1.11	21.26±1.46**	21.08±1.57**
(μg/g)	SAE	18.96±1.59	18.94±1.43	20.75±1.58*	21.40±1.37**
	TGP	18.20±0.59	18.55±0.72	18.13±1.76$^{\#\#}$	20.81±1.23**
	Blank	33.81±0.114	35.01±3.19	33.93±2.46	33.83±1.49
Tyr	Positive	34.06±2.61	32.17±1.84	31.27±2.59	29.77±1.90**
(μg/g)	SAE	33.66±2.21	30.91±3.01*	30.23±2.75*	29.38±2.48**
	TGP	35.79±2.14	33.59±2.74	31.35±2.72	34.20±2.37$^{\#\#}$

a) ** $p < 0.01$ and * $p < 0.05$ (compared with blank group rats);

b) $^{\#\#}$ $p < 0.01$ and $^{\#}$ $p < 0.05$ (compared with SAE group rats).

Table S9. The levels of analytes in serum samples by ELISA ($n = 6$)

Analyte	Group	15th day	16th day	18th day	23rd day	30th day
TRH (μIU/ml)	Blank	2.74±0.13	2.81±0.14	3.08±0.17	2.85±0.17	2.83±0.36
	Positive	2.47±0.38	2.13±0.44**	1.60±0.19**	1.52±0.38**	1.49±033**
	SAE	2.40±0.43	1.97±0.52**	1.48±0.24**	1.61±0.15**	1.54±0.26**
	TGP	2.60±0.61	2.36±0.30	1.92±0.32**#	2.09±0.39**#	1.90±0.47**
CRH (ng/L)	Blank	29.47±3.58	28.97±2.76	30.96±2.30	30.38±2.73	30.01±1.37
	Positive	24.84±2.08	21.64±2.10**	18.65±3.33**	18.49±5.88**	17.28±3.54**
	SAE	27.37±2.08	21.30±2.67**	19.51±1.50**	19.60±1.54**	20.24±1.15**
	TGP	27.23±3.45	28.11±3.92#	27.39±2.50#	29.40±1.40##	26.10±2.73*#
ADH (ng/L)	Blank	35.99±4.38	32.27±2.71	36.45±4.56	33.94±3.04	30.82±1.96
	Positive	41.09±4.71	25.39±2.84**	23.96±3.44**	26.81±5.78	21.11±4.17**
	SAE	38.28±3.03	26.48±1.75**	28.63±4.52**	21.34±2.53**	22.68±2.37**
	TGP	35.23±2.55	32.60±2.07##	31.01±4.35	31.67±4.91##	33.22±1.52##
PRL (ng/L)	Blank	178.07±16.55	188.66±12.72	185.17±16.19	178.47±8.82	193.47±9.70
	Positive	203.96±19.81	142.58±14.30**	133.61±7.29**	119.46±16.92*	125.59±26.23**
	SAE	189.11±12.49	149.73±12.66**	134.87±7.29**	147.58±29.87	131.40±6.80**
	TGP	178.72±29.04	188.51±18.36##	180.24±21.75##	168.39±31.59	158.71±18.83**#

a) ** $p < 0.01$ and * $p < 0.05$ (compared with blank group rats);

b) ## $p < 0.01$ and # $p < 0.05$ (compared with SAE group rats).

Table S10. The levels of analytes in brain samples by ELISA (n = 6)

Analyte	Group	16th day	18th day	23rd day	30th day
	Blank	21.06±3.86	24.33±3.71	25.65±1.57	23.52±1.84
TRH	Positive	17.91±3.08	19.48±0.62*	16.90±2.38**	16.35±1.09**
(μIU/g)	SAE	19.70±3.76	19.12±1.81*	18.58±0.53**	18.46±2.15**
	TGP	20.62±3.92	24.69±6.02$^{\#}$	19.46±2.57**	24.30±0.98$^{\#\#}$
	Blank	250.93±16.23	239.89±18.83	276.99±27.46	256.94±16.27
CRH	Positive	216.32±15.80**	193.55±13.07*	191.42±22.71**	186.99±15.70**
(ng/g)	SAE	213.33±12.04**	207.79±31.83*	188.86±25.85**	195.43±12.17**
	TGP	261.15±9.25$^{\#\#}$	245.41±7.35$^{\#\#}$	231.91±23.69$^{**\#\#}$	243.10±22.68$^{\#\#}$
	Blank	355.34±27.42	324.47±28.02	327.35±34.41	335.94±17.51
ADH	Positive	261.33±25.46**	235.84±34.12**	208.85±25.51**	220.41±42.43**
(ng/g)	SAE	272.07±17.10**	248.96±37.39*	223.23±29.57**	249.52±13.70**
	TGP	305.88±20.69$^{*\#}$	381.58±51.78$^{*\#\#}$	300.63±18.67$^{\#\#}$	307.22±27.14$^{\#\#}$
	Blank	1982.64±213.59	1952.27±203.02	2030.75±127.61	1864.65±55.88
PRL	Positive	1550.68±194.20**	1530.83±97.39*	1294.57±284.05**	1219.12±260.49**
(ng/g)	SAE	1424.69±151.56**	1016.43±277.20**	1422.19±75.59**	1485.10±77.25**
	TGP	1784.49±197.19$^{\#}$	1717.13±374.77$^{\#\#}$	1873.59±213.67$^{\#}$	1669.22±96.41

a) ** $p < 0.01$ and * $p < 0.05$ (compared with blank group rats);

b) $^{\#\#}$ $p < 0.01$ and $^{\#}$ $p < 0.05$ (compared with SAE group rats).