Supplementary data

Gender-specific metabolic responses in focal cerebral ischemia of rats and Huang-Lian-Jie-Du decoction treatment

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Table S1 ^{1}H NMR assignment of metabolites in serum samples

No.	Metabolites	Assignments	Chemical shift(ppm)
1	LDL/VLDL	$CH_3(CH_2)n$,$CH_3CH_2CH_2$=	0.87-0.95(m), 1.36-1.38(m)
2	Isoleucine	δCH_3 , γCH_3 , αCH	0.96(t), 1.02(d), 1.46(m)
3	Leucine	δCH_3 , δCH_3, γCH, αCH	0.94(t), 0.96(t), 1.71(m), 3.74(m)
4	Valine	γCH_3, γCH_3, βCH, αCH	0.98(d), 1.03(d), 2.26(m), 3.60(d)
5	3-Hydroxybutyrate	γCH_3, βCH, αCH_2	1.32(d), 2.31(m), 2.41(m), 4.16(m)
6	Lactate	CH_3, CH	1.38(d), 4.16(q)
7	Alanine	βCH_3, αCH	1.53(d), 3.78(q)
8	Lysine	γCH_2, δCH_2, βCH_2, N–CH_2, CH	1.48(m), 1.73(m), 1.91(m), 3.03(t), 3.76(t)
9	Arginine	γCH_2, βCH_2	1.78(m), 1.95(m)
10	Acetone	CH_3	2.26(s)
11	Acetoacetate	CH_3	2.28(s)
12	Glutamate	βCH_2, βCH_2, γCH_2, αCH	2.10(m), 2.14(m), 2.35(m), 2.50(m), 3.77(t)
13	Pyruvate	βCH_3	2.43(s)
14	Succinate	CH_2	2.43(s)
15	Glutamine	βCH_2, γCH_2, αCH	2.46(m), 3.77(t)
16	NAG	CH_3	2.09(s)
17	OAG	$OHCHCH_3$	2.19(s)
18	Citrate	1/2CH_2, 1/2CH_2	2.58(d), 2.80(d)
19	Isocitrate	CH_3, CH, CH	2.88(s)
20	PUFA	HC=$CHCH_2$,CH=CH	2.75-2.95(m)
21	Creatinine	CH_2, N–CH_3	3.04(s), 4.05(s)
22	Creatine	CH_2, N–CH_3	3.08(s), 3.93(s)
23	Phosphocreatine	CH_2, N– CH_3	3.08(s), 3.93(s)
24	Methanol	CH_3	3.67(s)
25	TMAO	CH_3	3.34(s)
26	Taurine	NH_2-CH_2, SO_3-CH_2	3.28(t), 3.46(t)
27	Glycerol	CH_2, CH_2, CH	3.58(ABX), 3.68(ABX), 3.79(ABX)
28	Betaine	$N(CH_3)_3$, CH_2	3.27(s), 3.88(s)
29	β-glucose	CH	3.74-3.84 (m)
30.	α-glucose	CH	5.24(dd), 3.54(dd), 3.73(dd), 3.42(t), 3.84(m)

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets.

Table S2 ^{1}H NMR assignment of metabolites in cerebrum samples

No.	Metabolite	Assignments	Chemical shift
1	Isoleucine	δCH_3 , γCH_3 , αCH	0.93(t), 1.0(d), 1.46(m)
2	Leucine	δCH_3 , δCH_3, γCH, αCH	0.94(t), 0.96(t), 1.71(m), 3.74(m)
3	Valine	γCH_3, γCH_3	0.98(d), 1.03(d), 2.26(m), 3.60(d)
4	3-Hydroxybutyrate	γCH_3, βCH, αCH_2	1.20(d), 2.31(m), 2.41(m), 4.16(m)
5	Threonine	CH_3, αCH, βCH	1.34(d), 3.59(d), 4.26(m)
6	Lactate	CH_3, CH	1.32(d), 4.11(q)
7	Alanine	βCH_3, αCH	1.47(d), 3.78(q)
8	Lysine	γCH_2, δCH_2, βCH_2, N–CH_2, CH	1.48(m), 1.73(m), 1.91(m), 3.03(t), 3.76(t)
9	Arginine	γCH_2, βCH_2	1.78(m), 1.95(m)
10	GABA	αCH_2, βCH_2, γCH_2	1.91(m), 2.30(t), 3.02(t)
11	Acetate	CH_3	1.91(s)
12	NAA	CH_3, CH_2, CH	2.03(s), 2.51(m), 2.7(m), 4.4(m)
13	Methionine	S-CH_3, β-CH_2, S-CH_2, α-CH	2.14(s), 2.16(m), 2.65(t), 3.86(t)
14	Glutamate	βCH_2, βCH_2, γCH_2, αCH	2.10(m), 2.14(m), 2.36(m), 2.50(m), 3.77(t)
15	Glutathione	S-CH_2, N-CH, N-CH_2, CH_2	2.14(m), 2.55(m), 2.95(m), 3.77(m), 4.56(t)
16	Acetone	CH_3	2.22(s)
17	Pyruvate	CH_3	2.34(s)
18	Succinate	CH_2	2.43(s)
19	Glutamine	βCH_2, γCH_2, αCH	2.46(m), 3.77(t)
20	Aspartate	βCH_2, α-CH	2.68(m), 2.82(m), 3.91(m)
21	Citrate	$1/2CH_2$, $1/2CH_2$	2.68(d), 2.80(d)
22	Isocitrate	CH_2, CH, CH	4.06(s), 3.01(m), 2.52(m)
23	Malate	CH_2,CH	4.30(d), 2.68(m), 2.37(m)
24	TMA	CH_3	2.88(s)
25	Creatinine	N-CH_3, N-CH_2-CO	3.01(s), 4.05(s)
26	Creatine	CH_2, CH_3	3.04(s), 3.93(s)
27	PCr	CH_2, CH_3	3.04(s), 3.93(s)
28	Ethanolamine	O-CH, NH_2-CH_2	3.22(t), 3.97(t)
29	Choline	$N(CH_3)_3$, N-CH_2	3.25(s), 3.51(m)
30	OPC	$N(CH_3)_3$, N-CH_2, O-CH_2	3.21(s), 3.57(t), 4.16(t)
31	Glucose	CH, CH_2	3.65-3.92 (m)
32	Taurine	NH_2-CH_2, SO_3-CH_2	3.25(t), 3.43(t)
33	Betaine	$N(CH_3)_3$, CH_2	3.27(s), 3.90(s)
34	Myo-inositol	CH	3.27 (t), 3.53(dd), 3.62 (t), 4.05 (t)
35	Glycine	CH_2	3.57(s)
36	Ascorbate	CH_2, CH	3.74(d), 3.76(d), 4.03(m), 4.52(d)
37	Inosine	O-CH-N, N-CH=N, N-CH=N	6.10 (d), 8.23 (s), 8.34 (s)
38	Uridine	H5, H6, H1'	5.8(d), 5.82(d), 7.81(d)
39	Adenosine	CH, CH	8.25 (s), 8.34 (s)
40	AMP	N=CH-N, N=CH-N	8.23 (s), 8.56 (s)
41	Fumarate	CH=CH	6.53(s)
42	Tyrosine	H3/H5, C5H/C6H	3.06(m), 3.20(m), 3.94(m), 6.91(d), 7.20(d)

No.	Metabolite	Assignments	Chemical shift
43	Histamine	NH_2-CH_2,CH=C-CH_2, CH=N-CH	3.00(t), 3.29(t), 7.12(s), 7.93(s)
44	Tryptophan	CH=CH	3.49(m), 4.06(m), 7.21(t), 7.29(t), 7.33(s), 7.55(d), 7.74(d)
45	Phenylalanine	CH=CH	3.13(m), 3.28(m), 4.00(m), 7.33(m), 7.38(m), 7.43(m)
46	Nicotinamide	H2/H4/H5/H6	4.44(s), 8.08(t), 8.84(d), 9.12(s)
47	Nicotinurate	NH_2-CH, H2/H4/H5/H6	4.43(s), 8.07(m), 8.83(m), 9.11(s)
48	3-Methylxanthine	NH=CH-N, N-CH_3	3.52(s), 8.02(s)
49	Hypoxanthine	NH=CH-N, N=CH-NH	8.18(s),8.20(s)
50	Glycerol	CH_2,CH	3.6(m),3.8(m)
51	Uracil	CH=CH-N	7.54(d), 5.79(d)
52	Xanthine	NH=CH-N	7.95(s)

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets.

Table S3 ^{1}H NMR assignment of metabolites in cerebellum samples

No.	Metabolite	Assignments	Chemical shift
1	Isoleucine	δCH_3 , γCH_3 , αCH	0.93(t), 1.0(d), 1.46(m)
2	Leucine	δCH_3 , δCH_3, γCH, αCH	0.94(t), 0.97(t), 1.71(m), 3.74(m)
3	Valine	γCH_3, γCH_3	0.98(d), 1.04(d), 2.26(m), 3.60(d)
4	3-Hydroxybutyrate	γCH_3, βCH, αCH_2	1.20(d), 2.31(m), 2.41(m), 4.16(m)
5	Threonine	CH_3, αCH, βCH	1.34(d), 3.59(d), 4.26(m)
6	Lactate	CH_3, CH	1.33(d), 4.12(q)
7	Alanine	βCH_3, αCH	1.48(d), 3.78(q)
8	Lysine	γCH_2, δCH_2, βCH_2, N–CH_2, CH	1.48(m), 1.73(m), 1.91(m), 3.03(t), 3.76(t)
9	Arginine	γCH_2, βCH_2	1.78(m), 1.95(m)
10	GABA	αCH_2, βCH_2, γCH_2	1.91(m), 2.31(t), 3.01(t)
11	Acetate	CH_3	1.92(s)
12	NAA	CH_3, CH_2, CH	2.02(s), 2.51(m), 2.7(m), 4.4(m)
13	Methionine	S-CH_3, β-CH_2, S-CH_2, α-CH	2.14(s), 2.16(m), 2.65(t), 3.86(t)
14	Glutamate	βCH_2, βCH_2, γCH_2, αCH	2.10(m), 2.14(m), 2.36(m), 2.50(m), 3.77(t)
15	Glutathione	S-CH_2, N-CH, N-CH_2, CH_2	2.14(m), 2.55(m), 2.95(m), 3.77(m), 4.56(t)
16	Acetone	CH_3	2.21(s)
17	Pyruvate	CH_3	2.35(s)
18	Succinate	CH_2	2.41(s)
19	Glutamine	βCH_2, γCH_2, αCH	2.46(m), 3.76(t)
20	Aspartate	βCH_2, α-CH	2.68(m), 2.82(m), 3.91(m)
21	Citrate	1/2CH_2, 1/2CH_2	2.52(d), 2.70(d)
22	Isocitrate	CH_2, CH, CH	4.06(s), 3.01(m), 2.52(m)
23	Malate	CH_2,CH	4.30(d), 2.66(m), 2.37(m)
24	TMA	CH_3	2.89(s)
25	Creatinine	N-CH_3, N-CH_2-CO	3.03(s), 4.05(s)
26	Creatine	CH_2, CH_3	3.04(s), 3.93(s)
27	PCr	CH_2, CH_3	3.04(s), 3.93(s)
28	Ethanolamine	O-CH, NH_2-CH_2	3.14(t), 3.82(t)
29	Choline	$N(CH_3)_3$, N-CH_2	3.20(s), 3.51(m)
30	OPC	$N(CH_3)_3$, N-CH_2, O-CH_2	3.21(s), 3.57(t), 4.16(t)
31	Glucose	CH, CH_2	3.65-3.92 (m)
32	Taurine	NH_2-CH_2, SO_3-CH_2	3.25(t), 3.43(t)
33	Betaine	$N(CH_3)_3$, CH_2	3.25(s), 3.90(s)
34	Myo-inositol	CH	3.27 (t), 3.53(dd), 3.62 (t), 4.05 (t)
35	Glycine	CH_2	3.56(s)
36	Ascorbate	CH_2, CH	3.74(d), 3.76(d), 4.03(m), 4.52(d)
37	Inosine	O-CH-N, N-CH=N, N-CH=N	6.10 (d), 8.23 (s), 8.34 (s)
38	Uridine	H5, H6, H1'	5.8(d), 5.82(d), 7.81(d)
39	Adenosine	CH, CH	8.24 (s), 8.34 (s)
40	AMP	N=CH-N, N=CH-N	8.23 (s), 8.54 (s)
41	Fumarate	CH=CH	6.54(s)
42	Tyrosine	H3/H5, C5H/C6H	3.06(m), 3.20(m), 3.94(m), 6.91(d), 7.20(d)

No.	Metabolite	Assignments	Chemical shift
43	Histamine	NH_2-CH_2,CH=C-CH_2, CH=N-CH	3.00(t), 3.28(t), 7.12(s), 7.93(s)
44	Tryptophan	CH=CH	3.49(m), 4.06(m), 7.20(t), 7.29(t), 7.33(s), 7.55(d), 7.74(d)
45	Phenylalanine	CH=CH	3.13(m), 3.28(m), 4.00(m), 7.33(m), 7.38(m), 7.43(m)
46	Nicotinamide	H2/H4/H5/H6	4.44(s), 8.08(t), 8.84(d), 9.12(s)
47	Nicotinurate	NH_2-CH, H2/H4/H5/H6	4.43(s), 8.07(m), 8.73(m), 9.11(s)
48	3-Methylxanthine	NH=CH-N, N-CH_3	3.52(s), 8.02(s)
49	Hypoxanthine	NH=CH-N, N=CH-NH	8.18(s),8.20(s)
50	Glycerol	CH_2,CH	3.6(m),3.8(m)
51	Uracil	CH=CH-N	7.54(d), 5.79(d)
52	Xanthine	NH=CH-N	7.95(s)

s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets.

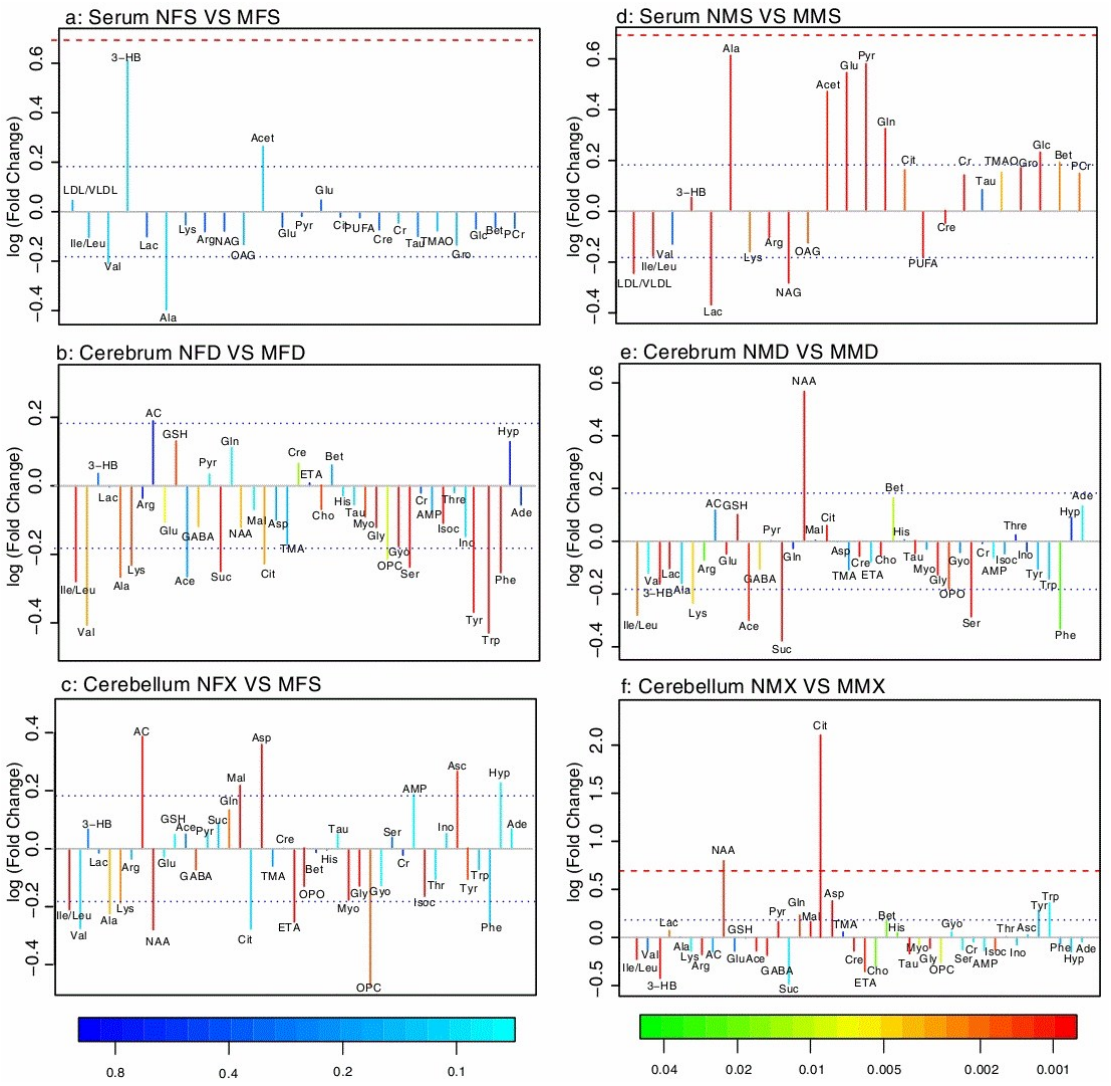

Fig. S1 color-coded fold change plots adjusted by Benjamini-Hochberg method indicating significance of altered metabolites in serum (a and d),cerebrum (b and e) and cerebellum (c and f) of female sham-operated rats versus female MCAO rats (a, b and c) and male sham-operated rats versus male MCAO rats (d, e and f). The blue dotted lines and red dashed lines representing an increase or decrease of 20% and 100%, respectively.

123 **Table S4** Results of altered pathways in MCAO rats

Metabolism	Impact[a]	Raw p[b]	Holm p[c]	FDR[d]
Aminoacyl-tRNA biosynthesis	1.13E-01	9.53E-10	7.62E-08	7.62E-08
Glycine, serine and threonine metabolism	4.44E-01	3.94E-08	3.11E-06	1.58E-06
Taurine and hypotaurine metabolism	3.85E-01	1.27E-05	9.88E-04	3.38E-04
Alanine, aspartate and glutamate metabolism	1.60E-01	3.31E-05	2.51E-03	5.29E-04
Valine, leucine and isoleucine biosynthesis	6.15E-02	6.06E-05	4.54E-03	8.07E-04
Propanoate metabolism	4.45E-02	2.21E-04	1.63E-02	2.52E-03
Citrate cycle (TCA cycle)	2.26E-01	2.62E-04	1.91E-02	2.62E-03
Glycolysis or Gluconeogenesis	9.58E-02	1.49E-03	1.07E-01	1.32E-02
Cysteine and methionine metabolism	1.63E-01	2.01E-03	1.43E-01	1.61E-02
Sulfur metabolism	6.61E-02	2.92E-03	2.04E-01	2.07E-02
Glutathione metabolism	2.45E-01	3.21E-03	2.22E-01	2.07E-02
Synthesis and degradation of ketone bodies	7.00E-01	3.87E-03	2.63E-01	2.07E-02
Valine, leucine and isoleucine degradation	2.23E-02	3.88E-03	2.63E-01	2.07E-02
Butanoate metabolism	1.54E-01	3.88E-03	2.63E-01	2.07E-02
Phenylalanine metabolism	1.19E-01	5.96E-03	3.88E-01	2.98E-02
Arginine and proline metabolism	5.71E-02	8.05E-03	5.07E-01	3.58E-02
Glyoxylate and dicarboxylate metabolism	2.82E-02	8.68E-03	5.38E-01	3.59E-02
Phenylalanine, tyrosine and tryptophan biosynthesis	8.00E-03	9.44E-03	5.76E-01	3.59E-02
Pyruvate metabolism	4.20E-01	1.51E-02	8.91E-01	5.49E-02
Galactose metabolism	2.76E-03	2.93E-02	1.00E+00	9.77E-02
Tyrosine metabolism	4.72E-02	3.55E-02	1.00E+00	1.14E-01
Ascorbate and aldarate metabolism	1.30E-01	3.73E-02	1.00E+00	1.15E-01
Selenoamino acid metabolism	3.21E-03	5.05E-02	1.00E+00	1.50E-01
Purine metabolism	9.82E-02	6.40E-02	1.00E+00	1.83E-01
Vitamin B6 metabolism	2.71E-02	9.77E-02	1.00E+00	2.61E-01
Methane metabolism	1.75E-02	1.08E-01	1.00E+00	2.79E-01
Glycerophospholipid metabolism	6.69E-02	1.36E-01	1.00E+00	3.40E-01
D-Glutamine and D-glutamate metabolism	3.26E-01	1.69E-01	1.00E+00	3.97E-01
Primary bile acid biosynthesis	1.64E-02	1.83E-01	1.00E+00	4.06E-01
Lysine degradation	1.47E-01	1.83E-01	1.00E+00	4.06E-01
Lysine biosynthesis	9.99E-02	4.17E-01	1.00E+00	8.33E-01
Ubiquinone and other terpenoid-quinone biosynthesis	1.37E-01	4.83E-01	1.00E+00	8.98E-01
Nicotinate and nicotinamide metabolism	1.70E-02	5.71E-01	1.00E+00	9.93E-01
Pentose and glucuronate interconversions	1.09E-01	7.40E-01	1.00E+00	1.00E+00

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