Table S1 The content (pg/μL) of twenty-three amino acids in cortex in different groups (n = 6-10).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Amino acids | Group | 3h | 12h | 24h |
| Ala | Sham | 79.33± 8.24 | 101.33±49.38 | 48.55± 12.27 |
| Model | 121.33±13.83\*\*\* | 142.16± 16.43\* | 117.09± 32.41\*\*\* |
| NXT | 126.27±17.65 | 111.86±26.69# | 111.26± 54.16 |
| GABA | Sham | 160.11±22.35 | 194.79± 106.40 | 287.52± 30.55 |
| Model | 185.07±50.40 | 156.32± 93.73 | 148.35± 37.44\*\*\* |
| NXT | 222.76±46.88 | 131.92± 58.59 | 329.67± 65.73### |
| Ser | Sham | 98.03± 27.09 | 129.35± 18.32 | 67.51± 24.81 |
| Model | 96.91± 6.67 | 97.88± 15.56\*\* | 89.54± 28.66 |
| NXT | 97.46± 8.78 | 101.23± 13.61 | 101.82± 48.53 |
| Pro | Sham | 15.37± 3.22 | 18.13± 7.40 | 6.60± 1.85 |
| Model | 27.91± 6.58\*\* | 22.54± 5.54 | 16.53± 5.99\*\*\* |
| NXT | 29.83± 5.83 | 21.51± 4.45 | 10.61±4.94# |
| Val | Sham | 15.10± 0.62 | 18.23± 10.95 | 10.07± 2.81 |
| Model | 26.31± 4.20\*\*\* | 29.56± 5.74\* | 19.19± 7.82\*\*\* |
| NXT | 25.85± 4.92 | 26.36± 5.40 | 20.95± 11.74 |
| Thr | Sham | 64.10± 10.71 | 79.00± 27.70 | 33.58± 9.48 |
| Model | 68.99± 4.55 | 74.95± 11.62 | 49.48± 15.41\*\* |
| NXT | 73.90± 8.77 | 77.85± 11.06 | 47.85± 18.28 |
| Tau | Sham | 432.80± 50.30 | 409.11± 42.64 | 244.75± 79.01 |
| Model | 427.88±54.51 | 337.32± 60.89\*\* | 292.01± 110.83 |
| NXT | 444.16±56.20 | 326.98± 77.60 | 196.46± 56.85 |
| Hyp | Sham | 1.71± 0.66 | 2.43± 0.83 | 0.69± 0.29 |
| Model | 1.63± 0.36 | 2.18± 0.40 | 1.55± 0.35\*\* |
| NXT | 1.61± 0.33 | 2.20± 0.21 | 0.95± 0.37# |
| Ile | Sham | 10.35± 3.83 | 13.43± 6.45 | 5.33± 1.58 |
| Model | 14.74± 2.68 | 19.60± 3.42\* | 11.34± 2.89\*\*\* |
| NXT | 15.08± 3.13 | 16.03± 4.25 | 10.84± 6.02 |
| Asp | Sham | 370.13±119.83 | 336.55± 77.26 | 278.74± 42.62 |
| Model | 117.47±92.51\*\* | 193.23± 64.35\*\* | 116.34± 17.74\*\*\* |
| NXT | 116.85± 28.48 | 137.96± 66.22 | 117.19± 24.79 |
| Gln | Sham | 212.19± 28.70 | 203.28± 36.82 | 96.16±26.8 |
| Model | 203.60± 16.25 | 183.78± 42.55 | 140.5± 33.49\* |
| NXT | 209.78± 28.13 | 185.90± 44.18 | 74.52± 20.91# |
| Glu | Sham | 483.58±75.61 | 516.17± 72.00 | 375.26± 100.07 |
| Model | 436.89± 75.44 | 419.83± 90.32\* | 323.14± 111.41 |
| NXT | 446.71± 86.42 | 396.30± 81.02 | 243.58± 72.51 |
| Met | Sham | 8.35± 2.68 | 9.03± 2.06 | 5.26± 1.65 |
| Model | 10.88± 1.16 | 10.06± 1.36 | 7.54± 2.29\* |
| NXT | 11.15± 1.41 | 9.10± 1.38 | 8.00± 3.59 |
| His | Sham | 37.22± 9.97 | 35.53± 6.64 | 23.40± 2.75 |
| Model | 42.39± 4.69 | 36.03± 2.62 | 32.66±5.60\* |
| NXT | 44.75± 4.10 | 34.43± 2.35 | 26.33±6.5# |
| Phy | Sham | 15.11± 5.14 | 18.77± 6.76 | 8.44± 2.19 |
| Model | 18.86± 2.08 | 21.78± 3.97 | 13.51± 4.47\* |
| NXT | 19.22± 2.92 | 19.07± 3.37 | 14.48± 7.49 |
| Arg | Sham | 26.13± 6.21 | 25.81± 4.68 | 16.91± 3.91 |
| Model | 27.49± 1.97 | 25.11± 3.03 | 23.88± 6.11\* |
| NXT | 29.12± 2.99 | 24.98± 3.52 | 0.56± 0.17### |
| Cit | Sham | 3.16± 0.87 | 3.99± 2.05 | 2.00± 0.83 |
| Model | 4.53± 0.53\* | 7.88±1.52\*\*\* | 7.2± 3.34\*\*\*↑ |
| NXT | 4.95± 1.20 | 6.25± 2.00 | 23.19± 7.98 |
| Try | Sham | 6.43± 2.38 | 7.97± 4.11 | 3.12± 0.90 |
| Model | 8.59± 1.02 | 10.31± 2.75 | 5.89± 1.85\*\* |
| NXT | 9.28± 1.76 | 9.92± 2.22 | 7.28± 4.57 |
| Gly | Sham | 3.98± 0.24 | 4.36± 0.31 | 3.56± 0.28 |
| Model | 4.13± 0.16 | 4.30± 0.24 | 3.62± 0.27 |
| NXT | 4.25± 0.26 | 4.30± 0.29 | 6.28± 3.66 |
| Histamine | Sham | 31.84± 3.57 | 30.45± 2.43 | 27.98± 1.22 |
| Model | 31.41± 2.04 | 28.02± 4.32 | 39.70± 8.87\*\*\* |
| NXT | 33.83± 6.56 | 28.66± 1.97 | 28.34±2.80# |
| Leu | Sham | 17.14± 5.43 | 25.14± 13.62 | 9.36± 2.52 |
| Model | 22.94± 3.55 | 24.72± 5.66 | 20.01± 3.92\*\*\* |
| NXT | 23.14± 4.15 | 24.89± 5.72 | 12.33±6.62### |
| Lys | Sham | 107.16±19.85 | 125.37± 35.99 | 64.76± 10.29 |
| Model | 143.88±31.76 | 135.60± 21.51 | 82.77± 41.11 |
| NXT | 157.91±30.28 | 132.53± 16.36 | 105.02± 39.73 |
| Tyr | Sham | 2.27± 1.00 | 2.49± 1.22 | 1.11± 0.35 |
| Model | 2.86± 0.26 | 2.88± 0.54 | 5.87± 5.49\* |
| NXT | 2.92± 0.41 | 2.47± 0.48 | 1.92± 0.93# |

∗*p*<0.05, ∗∗*p*<0.01, and ∗∗∗*p*<0.001 in the MCAO group versus the sham group; #*p*<0.05, ##*p*<0.01, and ###*p*<0.001 in the NXT group versus the MCAO group.

Table S2 The content (pg/μL) of twenty-three amino acids in hippocampus in different groups (n = 6-10).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Amino acids | Group | 3h | 12h | 24h |
| Ala | Sham | 395.31± 70.03 | 211.00± 24.37 | 314.94± 59.32 |
| Model | 407.24± 28.01 | 238.19± 28.59 | 303.52± 48.19 |
| NXT | 426.11± 48.92 | 205.81± 31.99 | 309.09± 56.22 |
| GABA  | Sham | 1357.14± 248.75 | 929.72± 65.19 | 1138.54± 120.30 |
| Model | 1163.19± 123.30 | 987.54± 137.84 | 1132.93± 215.81 |
| NXT | 1280.95± 128.66 | 1010.42± 142.60 | 1028.82± 171.72 |
| Ser | Sham | 316.83± 61.06 | 247.39± 27.93 | 277.63± 21.81 |
| Model | 312.08± 29.72 | 256.30± 23.55 | 298.01± 35.84 |
| NXT | 302.13± 30.04 | 270.71± 19.51 | 295.55± 43.73 |
| Pro | Sham | 31.70± 3.66 | 18.73± 2.17 | 36.99± 3.17 |
| Model | 35.83± 7.10 | 18.12± 2.23 | 27.76± 6.68\*\* |
| NXT | 42.66± 8.81 | 18.45± 3.96 | 29.54± 4.02# |
| Val | Sham | 30.00± 2.59 | 21.91± 5.13 | 29.95± 2.89 |
| Model | 33.93± 7.36 | 20.55± 2.40 | 28.70± 3.62 |
| NXT | 38.13± 7.56 | 24.34± 4.15 | 29.16± 4.71 |
| Thr | Sham | 171.47± 10.55 | 150.55± 18.96 | 148.09± 6.35 |
| Model | 179.82± 24.34 | 152.29± 16.34 | 152.44± 22.62 |
| NXT | 215.91± 22.74 | 167.55± 27.78 | 159.03± 22.55 |
| Tau | Sham | 1435.06± 235.38 | 1455.51± 200.17 | 1608.14± 111.23 |
| Model | 1453.50± 55.16 | 1461.56± 111.53 | 1526.01± 69.51\* |
| NXT | 1353.04± 244.51 | 1504.67± 120.41 | 1380.06± 158.29 |
| Hyp | Sham | 4.37± 0.82 | 4.99± 0.44 | 3.73± 0.51 |
| Model | 4.32± 0.55 | 6.11± 1.50\* | 5.05± 1.04\*\* |
| NXT | 4.83± 0.65 | 6.06± 0.64 | 4.86± 0.92 |
| Ile | Sham | 20.50± 2.10 | 15.79± 3.08 | 24.20± 1.86 |
| Model | 20.99± 4.25 | 14.17± 1.76 | 19.01± 1.98\*\* |
| NXT | 22.92± 3.76 | 17.10± 3.34 | 22.27± 2.39# |
| Asp | Sham | 643.27± 116.27 | 608.43± 57.06 | 599.00± 72.74 |
| Model | 521.95± 40.51\* | 572.23± 75.71 | 423.93± 59.20\*\*\* |
| NXT | 545.18± 87.74 | 505.73± 88.47 | 491.12±39.03# |
| Gln | Sham | 1927.49± 132.27 | 1733.76± 366.92 | 2063.85± 239.91 |
| Model | 2045.08± 153.42 | 2060.94±244.34\* | 2353.87± 266.82\* |
| NXT | 1853.62± 286.29 | 1902.69± 194.69 | 2351.81± 251.00 |
| Glu | Sham | 2873.53± 403.14 | 2728.10± 229.15 | 2849.94± 107.86 |
| Model | 3274.32± 285.72 | 2842.90± 297.95 | 2773.05± 356.24 |
| NXT | 3027.37± 229.52 | 2990.83± 213.89 | 2651.17± 223.02 |
| Met | Sham | 11.05± 0.75 | 9.05± 0.66 | 13.39± 2.06 |
| Model | 14.04± 2.12\* | 8.31± 2.02 | 12.92± 1.91 |
| NXT | 15.63± 3.16 | 9.13± 1.51 | 13.30± 1.83 |
| His | Sham | 56.50± 2.35 | 53.06± 3.24 | 71.58± 5.59 |
| Model | 62.60± 10.17 | 49.98± 2.38 | 69.08± 17.72 |
| NXT | 71.12± 12.07 | 53.68± 4.53 | 56.07± 6.68 |
| Phe | Sham | 2.70± 0.27 | 2.11± 0.50 | 3.00± 0.48 |
| Model | 3.27± 0.65 | 1.90± 0.26 | 3.03± 0.65 |
| NXT | 4.01± 0.65 | 2.25± 0.29# | 3.07± 0.73 |
| Arg | Sham | 66.98± 7.92 | 55.88± 4.07 | 88.75±7.54 |
| Model | 70.33± 8.02 | 55.52± 7.58 | 101.69±13.81\* |
| NXT | 74.00± 10.49 | 58.52± 11.82 | 77.11± 10.75## |
| Cit | Sham | 5.57± 1.27 | 9.19± 2.99 | 5.03± 0.72 |
| Model | 5.87± 1.24 | 7.80± 2.04 | 10.84± 3.75\*\*\* |
| NXT | 9.80± 1.82 | 9.22± 1.73 | 6.64± 1.36## |
| Try | Sham | 11.37± 0.44 | 12.93± 2.82 | 12.49± 1.02 |
| Model | 15.30± 2.54\*\* | 12.52± 1.16 | 13.99± 1.42\* |
| NXT | 17.92± 3.51 | 14.53± 1.52 | 12.41±0.76# |
| Gly | Sham | 269.35± 32.42 | 215.36± 19.49 | 269.91± 45.58 |
| Model | 287.69± 32.19 | 213.18± 23.80 | 218.11± 16.70\*\* |
| NXT | 292.55± 27.42 | 218.95± 27.09 | 217.90± 39.27 |
| Histamine | Sham | 0.57± 0.06 | 0.36± 0.04 | 0.79± 0.09 |
| Model | 0.57± 0.03 | 0.33± 0.04 | 0.81± 0.25 |
| NXT | 0.62± 0.13 | 0.36± 0.06 | 0.62± 0.09 |
| Leu | Sham | 35.65± 4.58 | 38.97± 9.15 | 33.93± 4.92 |
| Model | 36.47± 7.13 | 42.73± 13.59 | 36.79± 9.33 |
| NXT | 41.00± 8.33 | 38.88± 5.82 | 34.01± 6.26 |
| Lys | Sham | 100.55± 6.04 | 77.19± 12.29 | 140.06± 18.49 |
| Model | 100.78± 26.74 | 80.90± 17.95 | 98.46± 18.3\*\* |
| NXT | 101.62± 15.66 | 82.98± 15.63 | 125.81±14.28# |
| Tyr | Sham | 2.60± 0.24 | 2.89± 0.67 | 3.04± 0.43 |
| Model | 3.58±0.49\*\* | 2.64± 0.42 | 3.50± 0.72 |
| NXT | 3.65± 0.65 | 2.96± 0.23 | 3.29± 0.87 |

∗*p*<0.05, ∗∗*p*<0 01, and ∗∗∗*p* < 0.001 in the MCAO group versus the sham group; #*p* < 0.05, ##*p*<0.01, and ###*p*<0.001 in the NXT group versus the MCAO group.

Table S3 Pathway enrichment analysis of perturbed metabolites in MCAO rats based on MetaboAnalyst databases

|  |  |  |  |
| --- | --- | --- | --- |
|  | Pathway name | *P*-value | Related compounds |
| Cortex | Valine, leucine and isoleucine biosynthesis | 4.7679E-6 | Leu, Ile, Val |
| Valine, leucine and isoleucine degradation | 7.7886E-4 | Leu, Ile, Val |
| Alanine, aspartate and glutamate metabolism | 0.0079908 | Ala, GABA |
| Arginine and proline metabolism | 0.013048 | GABA, Pro |
| Hippocampus | Arginine biosynthesis | 2.1913E-4 | Asp, Cit |
| Valine, leucine and isoleucine biosynthesis | 0.01517 | Ile |
| Nicotinate and nicotinamide metabolism | 0.028318 | Asp |
| Histidine metabolism | 0.030187 | Asp |
| Pantothenate and CoA biosynthesis | 0.037637 | Asp |
| beta-Alanine metabolism | 0.039494 | Asp |

Table S4 Pathway enrichment analysis of neuroprotective effects of NXT based on MetaboAnalyst database

|  |  |  |  |
| --- | --- | --- | --- |
|  | Pathway name | *P*-value | Related compounds |
| Corex | Valine, leucine and isoleucine biosynthesis | 0.020182 | Leu |
| Arginine and proline metabolism | 0.0029624 | GABA, Pro |
| Hippocampus | Arginine biosynthesis | 2.1913E-4 | Asp, Cit |
| Valine, leucine and isoleucine biosynthesis | 0.01517 | Ile |
| Nicotinate and nicotinamide metabolism | 0.028318 | Asp |
| Histidine metabolism | 0.030187 | Asp |
| Pantothenate and CoA biosynthesis | 0.037637 | Asp |
| beta-Alanine metabolism | 0.039494 | Asp |