Environment: p>0.05

Age: p<0.05

Environment×Age: p<0.001

7 weeks

16 weeks

Fig. S1

**(B)**

**(A)**

16 weeks

7 weeks

16 weeks

7 weeks

**(D)**

**(C)**

16 weeks

7 weeks

16 weeks

7 weeks

Fig. S2

**(B)**

**(A)**

16 weeks

7 weeks

16 weeks

7 weeks

Fig. S3

**Table S1**. Effect of microbial environment and age on concentration of amino acids in plasma and brain tissues of mice

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Amino acid concentration (nmol/ml of plasma and pmol/mg of brain tissue) | | | | | | | | ANOVA results | | Results of Benjamini-Hochburg multiple testing | |
|  |  |
|  |  | 7 weeks | | | | 16 weeks | | | |
| Regions | Amino acids | SPF | | GF | | SPF | | GF | | Microbial environment | Age | Microbial environment | Age |
| Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Plasma | L-Asp | 64.2 | 18.0 | 29.0 | 7.5 | 43.3 | 7.8 | 20.5 | 3.5 | p<0.01 | NS | NC | NC |
|  | L-Tyr | 75 | 10 | 69 | 5 | 101 | 8 | 92 | 6 | NS | p<0.005 | NC | NC |
|  | D-Ala | 9.74 | 0.91 | ND | | 8.64a | 0.97 | ND | | p<0.0001 | NS | NC | NC |
|  | L-His | 110 | 10 | 88 | 7 | 98 | 5 | 91 | 4 | p<0.05 | NS | NC | NC |
|  | L-Ile | 97 | 5 | 80 | 5 | 118 | 6 | 105 | 5 | p<0.01 | p<0.0005 | NC | NC |
|  | L-Leu | 174 | 5 | 129 | 8 | 225 | 16 | 167 | 7 | p<0.0001 | p<0.0005 | NC | NC |
|  | L-Phe | 73.4 | 2.5 | 61.0 | 3.6 | 91.6 | 4.8 | 72.6 | 2.8 | p<0.0005 | p<0.0005 | NC | NC |
|  | L-Val | 269 | 9 | 216 | 9 | 312 | 14 | 272 | 12 | p<0.001 | p<0.0005 | NC | NC |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Brainstem | D-Asp | 16.2 | 0.5 | 19.4 | 0.9 | 16.6 | 0.7 | 19.7 | 0.6 | p<0.0001 | NS | q<0.001 | NS |
|  | L-Ala | 604 | 37 | 528 | 54 | 612 | 17 | 591 | 32 | p<0.005 | p<0.05 | q<0.005 | q <0.05 |
|  | L-Ser | 341 | 9 | 397 | 16 | 388 | 2 | 434 | 3 | p<0.0001 | p<0.0001 | q<0.001 | q<0.001 |
|  | L-Arg | 203 | 5 | 155 | 5 | 206 | 9 | 171 | 4 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-Gln | 4501 | 125 | 4202 | 208 | 4487 | 80 | 4121 | 45 | p<0.05 | NS | q<0.05 | NS |
|  | L-Ile | 38.8 | 1.6 | 40.0 | 1.8 | 47.3 | 1.1 | 43.8 | 0.7 | NS | p<0.0001 | NS | q<0.001 |
|  | L-Leu | 77 | 4 | 73 | 4 | 107 | 2 | 96 | 1 | p<0.05 | p<0.0001 | NS | q<0.0005 |
|  | GABA | 3453 | 88 | 3371 | 116 | 3253 | 43 | 3183 | 64 | NS | p<0.05 | NS | q<0.05 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Cerebellum | L-Arg | 232 | 17 | 154 | 35 | 189 | 42 | 162 | 7 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-Gln | 6567 | 146 | 5463 | 361 | 6196 | 240 | 5354 | 109 | p<0.0005 | NS | q<0.0005 | NS |
|  | L-Ile | 39.8 | 0.9 | 32.7 | 1.8 | 40.2 | 1.3 | 38.1 | 1.1 | p<0.005 | p<0.05 | q<0.001 | NS |
|  | L-Leu | 89 | 1 | 68 | 3 | 108 | 4 | 96 | 3 | p<0.0001 | p<0.0001 | q<0.0005 | q<0.0005 |
|  | L-Phe | 71.0 | 2.11 | 59.7 | 2.57 | 69.2 | 2.6 | 63.9 | 1.6 | p<0.001 | NS | q<0.005 | NS |
|  | L-Val | 111 | 4 | 89 | 5 | 111 | 7 | 100 | 2 | p<0.005 | NS | q<0.01 | NS |
|  | GABA | 2148 | 119 | 1780 | 169 | 2073 | 296 | 2047 | 87 | p<0.01 | NS | q<0.05 | NS |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Cerebral | D-Asp | 41.0 | 1.7 | 45.7 | 1.8 | 68.7 | 2.6 | 73.2 | 1.6 | p<0.05 | p<0.0001 | NS | q<0.0005 |
| cortex | D-Ser | 223 | 5 | 281 | 11 | 292 | 8 | 323 | 4 | p<0.0001 | p<0.0001 | q<0.001 | q<0.0005 |
|  | L-Ser | 492 | 64 | 603 | 37 | 636 | 58 | 664 | 30 | p<0.001 | p<0.0001 | q<0.005 | q<0.0005 |
|  | L-Ala | 705 | 30 | 714 | 29 | 867 | 30 | 816 | 11 | NS | p<0.0001 | NS | q<0.0005 |
|  | L-Arg | 220 | 10 | 172 | 12 | 162 | 9 | 146 | 5 | p<0.005 | p<0.0001 | q<0.005 | q<0.0005 |
|  | L-Gln | 5500 | 110 | 4590 | 254 | 5372 | 127 | 4526 | 123 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-Leu | 97 | 2 | 80 | 6 | 114 | 6 | 105 | 4 | p<0.05 | p<0.0005 | q<0.05 | q<0.0005 |
|  | L-Val | 134 | 3 | 122 | 6 | 114 | 5 | 119 | 1 | NS | p<0.05 | NS | q<0.05 |
|  | GABA | 3357 | 127 | 3247 | 191 | 2313 | 89 | 2240 | 84 | NS | p<0.0001 | NS | q<0.001 |
|  | Tau | 8013 | 271 | 8185 | 555 | 9450 | 310 | 10498 | 156 | NS | p<0.0001 | NS | q<0.0005 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Hippocampus | D-Asp | 55.7 | 1.7 | 53.7 | 0.79 | 45.6 | 1.5 | 42.5 | 1.3 | NS | p<0.0001 | NS | q<0.0005 |
|  | D-Ser | 296 | 11.2 | 335 | 25.9 | 284 | 34.4 | 269 | 34.0 | NS | p<0.001 | NS | q<0.0005 |
|  | L-Ser | 715 | 46 | 770 | 38 | 678 | 79 | 639 | 42 | NS | p<0.0005 | NS | q<0.001 |
|  | L-Ala | 1255 | 45 | 1079 | 48 | 1057 | 50 | 890 | 17 | p<0.0005 | p<0.0005 | q<0.005 | q<0.0005 |
|  | L-Arg | 192 | 7 | 148 | 7 | 149 | 7 | 126 | 4 | p<0.0001 | p<0.0001 | q<0.0005 | q<0.0005 |
|  | L-Gln | 5582 | 103 | 4620 | 125 | 4953 | 237 | 4113 | 262 | p<0.0005 | p<0.05 | q<0.0005 | NS |
|  | L-His | 740 | 22 | 758 | 20 | 715 | 38 | 648 | 16 | NS | p<0.05 | NS | q<0.05 |
|  | L-Ile | 47.0 | 1.7 | 40.5 | 2.3 | 39.3 | 2.0 | 33.6 | 1.0 | p<0.005 | p<0.0005 | q<0.01 | q<0.005 |
|  | L-Leu | 102 | 4 | 82 | 4 | 113 | 5 | 96 | 4 | p<0.0001 | p<0.005 | q<0.0005 | q<0.01 |
|  | L-Phe | 78.5 | 1.9 | 70.6 | 3.0 | 63.0 | 2.9 | 53.5 | 2.1 | p<0.005 | p<0.0001 | q<0.005 | q<0.0005 |
|  | L-Val | 143 | 5 | 126 | 6 | 115 | 6 | 103 | 3 | p<0.01 | p<0.0001 | q<0.05 | q<0.001 |
|  | GABA | 2918 | 95 | 2580 | 117 | 2568 | 126 | 2214 | 97 | p<0.005 | p<0.005 | q<0.05 | q<0.05 |
|  | Tau | 11190 | 242 | 10979 | 367 | 9601 | 316 | 9731 | 278 | NS | p<0.0001 | NS | q<0.0005 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Hypothalamus | D-Asp | 35.0 | 4.4 | 28.9 | 3.0 | 24.3 | 0.6 | 25.4 | 0.7 | NS | p<0.01 | NS | q<0.05 |
|  | L-Ala | 635 | 33 | 552 | 50 | 496 | 14 | 455 | 13 | NS | p<0.001 | NS | q<0.005 |
|  | L-Arg | 262 | 16 | 177 | 11 | 242 | 10 | 197 | 4 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-Gln | 6950 | 376 | 5084 | 371 | 6895 | 105 | 6116 | 140 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-Ile | 70.1 | 7.1 | 48.8 | 3.9 | 62.9 | 1.9 | 55.2 | 0.9 | p<0.005 | NS | q<0.01 | NS |
|  | L-Leu | 128 | 10 | 94 | 7 | 152 | 5 | 132 | 3 | p<0.0005 | p<0.0001 | q<0.0005 | q<0.0005 |
|  | L-Phe | 94.1 | 8.8 | 66.5 | 4.6 | 85.7 | 1.6 | 78.1 | 1.0 | p<0.005 | NS | q<0.005 | NS |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Striatum | L-Tyr | 119 | 11 | 102 | 8 | 86 | 2 | 93 | 3 | NS | p<0.005 | NS | q<0.05 |
|  | L-Gln | 6424 | 148 | 5198 | 235 | 6234 | 113 | 5917 | 171 | p<0.0005 | NS | q<0.0005 | NS |
|  | L-Ile | 51.0 | 7.5 | 41.6 | 3.0 | 41.1 | 5.1 | 40.5 | 3.1 | p<0.01 | p<0.005 | q<0.05 | q<0.01 |
|  | L-Leu | 103 | 2 | 88 | 2 | 98 | 3 | 87 | 2 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-Phe | 81.2 | 2.1 | 74.9 | 2.7 | 73.5 | 1.0 | 71.9 | 1.5 | p<0.05 | p<0.01 | NS | q<0.05 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Thalamus | D-Asp | 41.0 | 1.7 | 45.7 | 1.8 | 52.8 | 1.2 | 57.1 | 1.3 | p<0.01 | p<0.0001 | q<0.05 | q<0.0005 |
|  | D-Ser | 223 | 5 | 281 | 11 | 238 | 3 | 274 | 7 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-Ser | 492 | 14 | 603 | 24 | 557 | 10 | 629 | 12 | p<0.0001 | p<0.01 | q<0.0005 | q<0.05 |
|  | L-Arg | 220 | 10 | 172 | 12 | 216 | 9 | 192 | 4 | p<0.0005 | NS | q<0.0005 | NS |
|  | L-Gln | 5500 | 110 | 4590c | 254 | 5582 | 70 | 5098 | 103 | p<0.0001 | NS | q<0.0005 | NS |
|  | L-His | 867 | 27 | 933 | 20 | 830 | 10 | 826 | 14 | NS | p<0.001 | NS | q<0.005 |
|  | L-Leu | 97b | 2 | 80 | 6 | 123 | 3 | 114 | 2 | p<0.005 | p<0.0001 | q<0.005 | q<0.0005 |

Mean values with standard errors (n=7-9).

Mean values within a row with different superscript letters were significantly different (P<0·05).

NC: not calculated.

ND: not detected, the detection limit of D-alanine was 5 nmol/ml.

NS: not significant.

SPF: specific pathogen-free.

GF: germ-free.

**Table S2**. Effects of microbial environment and age on plasma and concentration of amino acids in brain tissues of mice

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Amino acid concentration (nmol/ml of plasma and pmol/mg of brain tissue) | | | | | | | |
|  |  | 7 weeks | | | | 16 weeks | | | |
| Regions | Amino acid | SPF | | GF | | SPF | | GF | |
| Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Plasma | L-Ser | 235 | 19 | 223 | 21 | 212 | 13 | 194 | 5.5 |
|  |  |  |  |  |  |  |  |  |  |
| Brainstem | L-Asp | 3863 | 101 | 3536 | 196 | 3718 | 116 | 3700 | 122 |
|  | D-Ser | 54.1 | 3.0 | 56.3 | 2.6 | 58.4 | 1.7 | 60.9 | 0.7 |
|  | L-Tyr | 66.0 | 5.0 | 71.1 | 3.5 | 75.5 | 1.9 | 76.7 | 1.3 |
|  | L-His | 866 | 26 | 890 | 48 | 874 | 6 | 845 | 17 |
|  | L-Val | 106 | 5 | 104 | 5 | 116 | 4 | 109 | 2 |
|  | Tau | 4375 | 146 | 4541 | 219 | 4182 | 91 | 4697 | 28 |
|  |  |  |  |  |  |  |  |  |  |
| Cerebellum | L-Ser | 580 | 29 | 569 | 15 | 564 | 29 | 627 | 11 |
|  | L-Tyr | 84.3 | 4.6 | 79.5 | 4.2 | 85.1 | 5.2 | 90.4 | 2.7 |
|  | L-His | 978 | 51 | 915 | 44 | 925 | 27 | 929 | 12 |
|  |  |  |  |  |  |  |  |  |  |
| Cerebral | L-Asp | 3063 | 135 | 2736 | 129 | 2605 | 138 | 2609 | 97 |
| cortex | L-Tyr | 84.7 | 5.5 | 90.8 | 8.5 | 84.1 | 2.0 | 86.8 | 2.0 |
|  | L-Ile | 46.5 | 2.3 | 40.5 | 3.6 | 44.4 | 2.0 | 41.3 | 0.9 |
|  | L-Phe | 69.8 | 2.6 | 67.9 | 3.1 | 69.1 | 2.4 | 66.6 | 1.4 |
|  |  |  |  |  |  |  |  |  |  |
| Hippocampus | L-Tyr | 89.1 | 2.9 | 86.5 | 3.9 | 83.3 | 7.2 | 72.3 | 3.1 |
|  |  |  |  |  |  |  |  |  |  |
| Hypothalamus | L-Asp | 3345 | 182 | 2779 | 210 | 3344 | 138 | 3221 | 126 |
|  | D-Ser | 148 | 9 | 158 | 15 | 141 | 4 | 154 | 6 |
|  | L-Ser | 486 | 24 | 488 | 32 | 473 | 10 | 512 | 12 |
|  | L-Tyr | 106 | 8 | 100 | 9 | 103 | 6 | 101 | 3 |
|  | L-His | 1174 | 81 | 1031 | 67 | 1136 | 24 | 1123 | 33 |
|  | L-Val | 168 | 12 | 140 | 12 | 158 | 7 | 147 | 4 |
|  | GABA | 5968 | 315 | 5023 | 387 | 6207 | 200 | 5954 | 178 |
|  | Tau | 5601 | 218 | 5570 | 584 | 5245 | 184 | 5781 | 183 |
|  |  |  |  |  |  |  |  |  |  |
| Striatum | D-Asp | 60.3 | 4.1 | 59.6 | 4.4 | 58.3 | 2.0 | 60.7 | 2.4 |
|  | D-Ser | 286 | 15 | 286 | 17 | 255 | 2 | 284 | 8 |
|  | L-His | 939 | 40 | 867 | 55 | 847 | 24 | 853 | 20 |
|  | GABA | 5338 | 90 | 4866 | 368 | 4670 | 198 | 4941 | 173 |
|  |  |  |  |  |  |  |  |  |  |
| Thalamus | L-Asp | 3063 | 134 | 2736 | 129 | 2785 | 80 | 2856 | 124 |
|  | L-Tyr | 84.7 | 5.5 | 90.8 | 8.5 | 91.7 | 2.5 | 93.6 | 1.9 |
|  | L-Ala | 705 | 30 | 714 | 29 | 718 | 13 | 695 | 15 |
|  | L-Ile | 46.5 | 2.3 | 40.5 | 3.6 | 47.9 | 2.0 | 44.6 | 0.47 |
|  | L-Phe | 69.8 | 2.6 | 67.9 | 3.1 | 74.0 | 1.3 | 67.6 | 1.1 |
|  | L-Val | 134 | 2.5 | 122 | 5.7 | 128 | 4.3 | 128 | 2.0 |
|  | GABA | 3357 | 127 | 3247 | 191 | 3430 | 90 | 3492 | 99 |
|  | Tau | 8013 | 271 | 8185 | 555 | 7694 | 190 | 8943 | 174 |

Mean values with standard errors (n=7-9).

SPF: specific pathogen-free.

GF: germ-free.

**Table S3**. Effect of microbial environment and age on concentration of D-aspartic acid as a proportion of the total amount of D- and L-aspartic acids

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | D/L proportion of aspartic acid | | | | | | | | Results of ANOVA | |
|  | 7 weeks | | | | 16 weeks | | | |
| Regions | SPF | | GF | | SPF | | GF | | Microbial environment | Age |
| Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Brainstem | 0.417 | 0.010 | 0.532 | 0.011 | 0.446 | 0.016 | 0.532 | 0.011 | p<0.0001 | NS |
| Cerebellum | 0.599 | 0.009 | 0.738 | 0.025 | 0.568 | 0.021 | 0.689 | 0.009 | p<0.05 | p<0.0001 |
| Cerebral cortex | 1.25 | 0.03 | 1.67 | 0.08 | 2.53 | 0.05 | 2.81 | 0.06 | p<0.0001 | p<0.0001 |
| Hypothalamus | 0.86 | 0.07 | 1.02 | 0.04 | 0.70 | 0.03 | 0.76 | 0.01 | p<0.05 | p<0.0001 |

Mean values with standard errors (n=7-9).

SPF: specific pathogen-free.

GF: germ-free.

**Table S4**. Effect of microbial environment and age on concentration of D-serine as a proportion of the total amount of D- and L-serine

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | D/L proportion of serine | | | | | | | | | Results of ANOVA | |
|  | 7 weeks | | | | 16 weeks | | | | |
| Regions | SPF | | GF | | SPF | | GF | | | Microbial environment | Age |
| Mean | SE | Mean | SE | Mean | SE | Mean | | SEM |
| Brainstem | 13.7 | 0.8 | 12.4 | 0.3 | 13.0 | 0.3 | 12.3 | 0.1 | | p<0.05 | NS |
| Cerebral cortex | 31.1 | 0.2 | 31.7 | 0.3 | 31.5 | 0.3 | 32.6 | 0.3 | | p<0.01 | p<0.05 |
| Hippocampus | 29.0 | 0.1 | 30.8 | 0.3 | 29.2 | 0.1 | 30.4 | 0.3 | | p<0.0001 | NS |
| Hypothalamus | 22.2 | 0.6 | 22.9 | 0.9 | 22.5 | 0.4 | 23.1 | 0.3 | | NS | NS |
| Thalamus | 31.1 | 0.2 | 31.7 | 0.3 | 29.7 | 0.2 | 30.5 | 0.1 | | p<0.005 | p<0.0001 |

Mean values with their standard errors (n=7-9).

SPF: specific pathogen-free.

GF: germ-free.