**Supplementary File**

**Supplementary Methods**

**Immunocytochemistry**

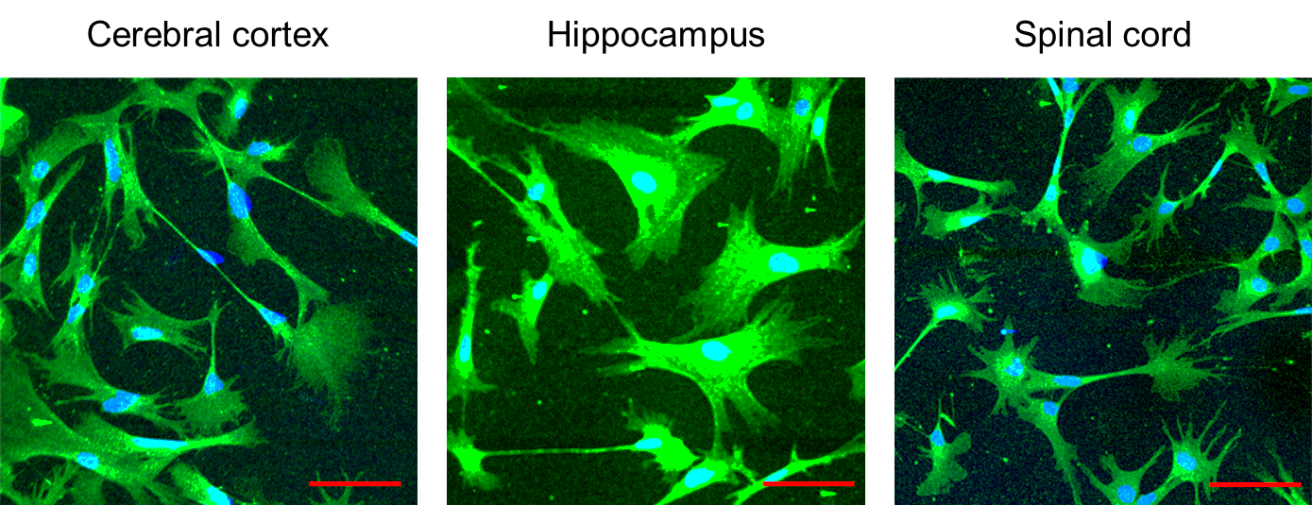
Hippocampal astrocytes on coverslips were fixed with 4% paraformaldehyde for 20 min at room temperature (RT), and then permeabilized and blocked with 10% normal goat serum in PBS containing 0.1% Triton X-100 at RT for 30 min. Cells were then incubated with a primary antibody against GFAP (#11051, 1:50, Immuno-Biological Laboratories, Gunma, Japan) in PBS containing 0.1% Triton X-100 and 1% normal goat serum at 4 °C for at least 12 h. After washing in PBS, cells were incubated with Alexa Fluor 555-conjugated goat anti-mouse secondary antibody (#A21422, 1:500; Thermo Fisher Scientific, Waltham, MA, USA) in the dark at RT for 1 h. Coverslips were mounted onto glass slides with DAPI-Fluoromount G (SouthernBiotech, Birmingham, AL, USA). Fluorescence images were obtained using a laser scanning confocal microscope (LSM 700; Carl Zeiss, Oberkochen, Germany) equipped with a 40× lens objective.

**Supplementary Table S1. The primer sequences used for real-time PCR**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Target gene | Product  size  (bp) | Annealing temperature  (°C) | GenBank accession No. | Sequence  (upper: sense; lower: antisense) |
| *interleukin 6* | 145 | 61 | NM\_012589 | 5'-GATTGTATGAACAGCGATGATGC-3' |
| 5'-AGAAACGGAACTCCAGAAGACC-3' |
| *interleukin 1beta* | 150 | 63 | NM\_031512 | 5'-TTGCTTCCAAGCCCTTGACT-3' |
| 5'-CTCCACGGGCAAGACATAGG-3' |
| *tumor necrosis factor* | 125 | 63 | NM\_012675 | 5'-CATGAGCACGGAAAGCATGA-3' |
| 5'-CCACGAGCAGGAATGAGAAGA-3' |
| *brain-derived　neurotrophic factor* | 55 | 63 | NM\_001270638 | 5'-GGCCCAACGAAGAAAACCAT-3' |
| 5'-AGCATCACCCGGGAAGTGT-3' |
| *nerve growth factor* | 115 | 61 | NM\_001277055 | 5'-CAACAGGACTCACAGGAGCA-3' |
| 5'-GTCCGTGGCTGTGGTCTTAT-3' |
| *fibroblast growth factor 2* | 165 | 63 | NM\_019305 | 5'-ATCACTTCGCTTCCCGCA-3' |
| 5'-TTTGACGTGTGGGTCGCT-3' |
| *glyceraldehyde-3-phosphate dehydrogenase* | 74 | 61 | NM\_017008 | 5'-GCAAGAGAGAGGCCCTCAG-3' |
| 5'-TGTGAGGGAGATGCTCAGTG-3' |

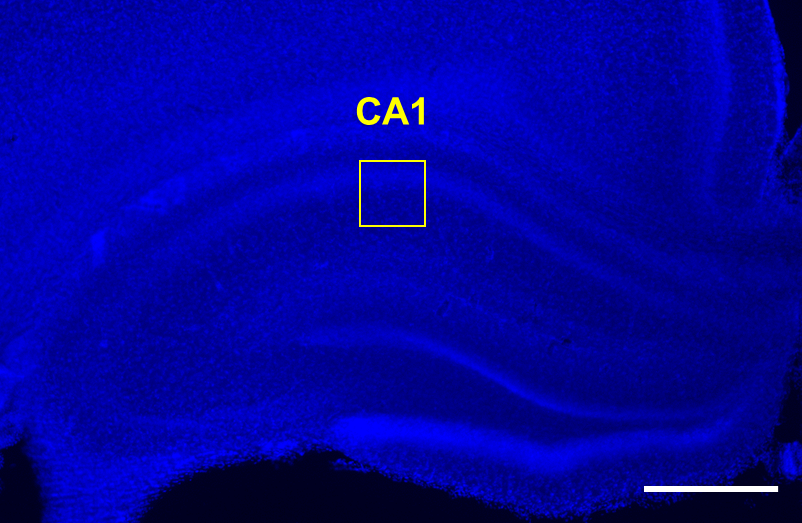
**Supplementary Table S2. Primer sequences used for non-quantitative PCR**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Target gene | Product  size  (bp) | Annealing temperature  (°C) | GenBank accession No. | Sequence  (upper: sense; lower: antisense) |
| *dopamine receptor D1* | 136 | 68 | NM\_012546 | 5'-GTCTGTCCTTATATCCTTCATCCC-3' |
| 5'-ATACGTCCTGCTCAACCTTG-3' |
| *dopamine receptor D2* | 141 | 64 | NM\_012547 | 5'-TGCCATTGTTCTCGGTGTGTTC-3' |
| 5'-TTGACGGCACTGTTGACATAGC-3' |
| *dopamine receptor D3* | 270 | 68 | NM\_017140 | 5'-TCTGCTCCATCTCCAACCCTGA-3' |
| 5'-TGTGCTCCATTTGTCCTGTGGC-3' |
| *dopamine receptor D4* | 151 | 68 | NM\_012944 | 5'-GGTGCTGGTGTTGCCTCTCTTTG-3' |
| 5'-AGCCACAAACCTGTCCACGCTG-3' |
| *dopamine receptor D5* | 192 | 68 | NM\_012768 | 5'-CGTGGAGCCTATGAACCTGACC-3' |
| 5'-GCTGACACAAGGGAAGCCAGTC-3' |
| *adrenoceptor  beta 1* | 248 | 60 | NM\_012701 | 5'-GCTCTGGACTTCGGTAGACG-3' |
| 5'-ACTTGGGGTCGTTGTAGCAG-3' |
| *adrenoceptor  beta 2* | 208 | 60 | NM\_012492 | 5'-AGCCACCTACGGTCTCTGAA-3' |
| 5'-GTCCCGTTCCTGAGTGATGT-3' |
| *adrenoceptor  beta 3* | 150 | 60 | NM\_013108 | 5'-TCGTCTTCTGTGCAGCTACG-3' |
| 5'-ATGGTCCTTCATGTGGGAAA-3' |
| *beta-actin* | 280 | 60 | NM\_031144 | 5'-TGTCACCAACTGGGACGATA-3' |
| 5'-ACCCTCATAGATGGGCACAG-3' |



**Supplementary Figure S1. GFAP expression in cerebral cortical, hippocampal, and spinal cord astrocytes.**

Representative images of multicolor immunofluorescence staining for DAPI (blue) and GFAP (green) in cerebral cortical, hippocampal, and spinal cord astrocytes. Scale bars = 100 µm.



**Supplementary Figure S2. The CA1** **region of the hippocampus was used for the fluorescence intensity measurements and evaluation of astrocytic morphology.**

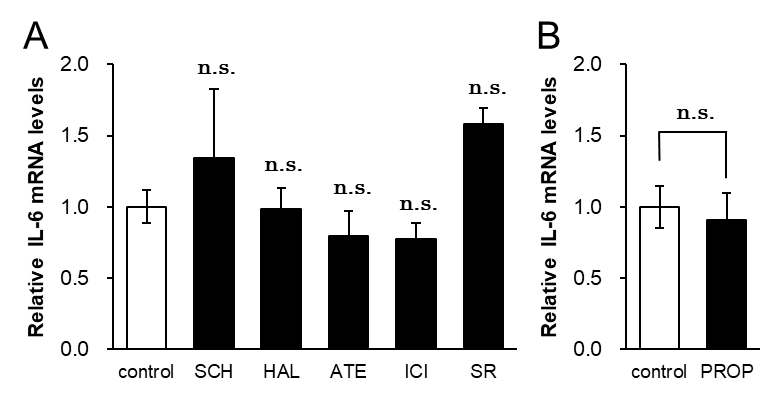
The hippocampal slice was stained with DAPI. The CA1 area used for the fluorescence intensity measurements and evaluation of astrocytic morphology is shown (yellow frame). Scale bars = 500 µm.

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自動的に生成された説明

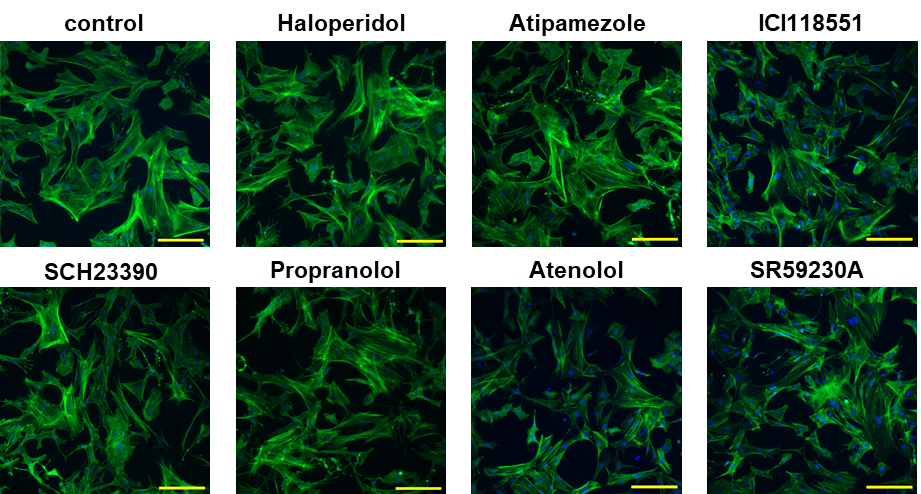
**Supplementary Figure S3. The differences in GFAP-staining intensity between the hippocampus and cerebral cortex.**

The cerebral cortex (upper) was not stained with GFAP and the hippocampus (lower) was well stained with GFAP. Scale bars = 500 µm.



**Supplementary Figure S4. The** **effects of dopamine receptor and adrenoceptor antagonists on IL-6 mRNA levels.**

(A) IL-6 mRNA levels in cerebral cortical astrocytes treated with the D1-like receptor antagonist SCH23390 (SCH, 10 µM), D2-like receptor antagonist haloperidol (HAL, 10 µM), β1-adrenoceptor antagonist atenolol (ATE, 10 µM), β2-adrenoceptor antagonist ICI118551 (ICI, 1 µM), and β3-adrenoceptor antagonist SR59230A (SR, 1 µM) for 2 h. n.s.: not significant (Dunnett’s test vs. control), n = 6. (B) IL-6 mRNA levels in astrocytes treated with the β-adrenoceptor antagonist (PROP, 10 µM) for 2 h. These data were extracted from Figure. 3A. n.s.: not significant (unpaired Student’s t-test). All data are presented as means ± S.E.M. The cytokine mRNA levels were normalised to those of the control, which was arbitrarily set to a value of “1.0”.



**Supplementary Figure S5. The** **effects of dopamine receptor and adrenoceptor antagonists on** **astrocytic morphology.**

Representative images of F-actin (green) and DAPI (blue) in hippocampal astrocytes treated with the D1-like receptor antagonist SCH23390 (10 µM), D2-like receptor antagonist haloperidol (10 µM), β-adrenoceptor antagonist propranolol (10 µM), α2-adrenoceptor antagonist atipamezole (10 µM), β1-adrenoceptor antagonist atenolol (10 µM), β2-adrenoceptor antagonist ICI118551 (1 µM), and β3-adrenoceptor antagonist SR59230A (1 µM) for 4 h. Scale bars = 100 μm.