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Supplemental Information

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Supplemental Figures

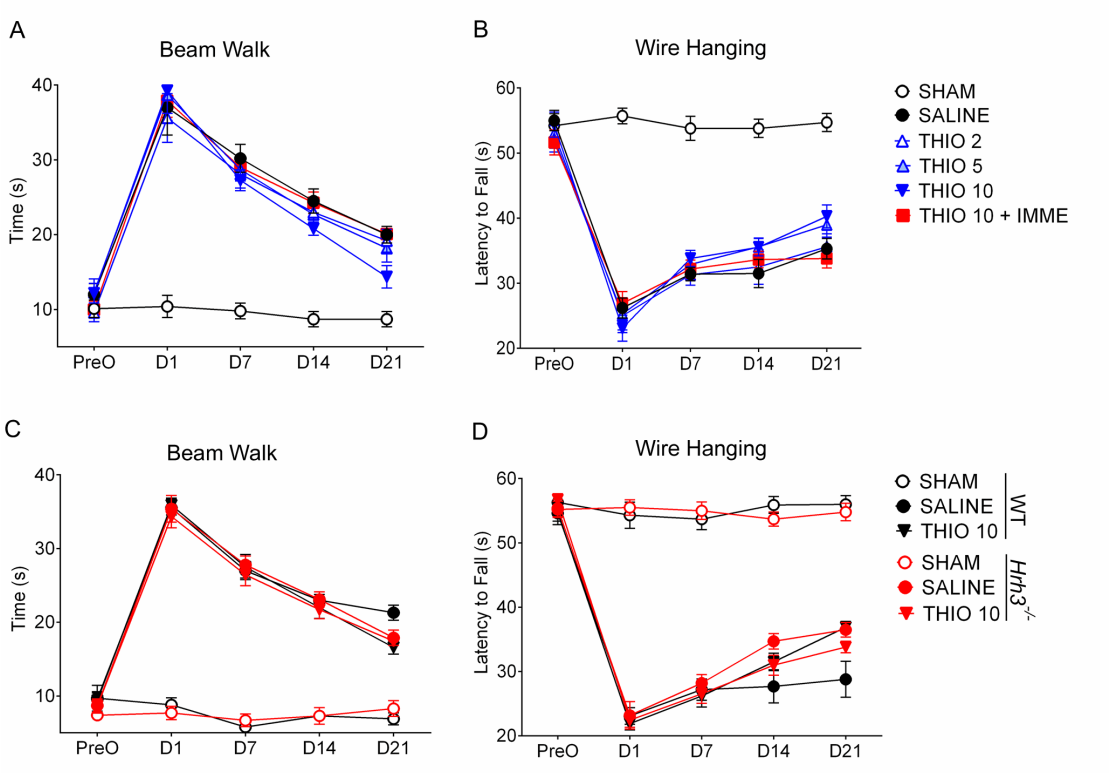

Figure S1. H3R antagonism has no effect on the neurological dysfunction in beam walk and wire hanging tests, related to Figure 1.

WT mice (A, B) or *Hrh3*$^{-/-}$ mice (C, D) were exposed to cryogenic brain lesion and then treated with saline, H3R antagonist thioperamide (THIO, 2, 5 or 10 mg/kg/d, i.p.), or together with H3R agonist immepip (IMME, 1 μg/d, i.c.v.). The neurological function in beam walk test (A, C) or wire hanging test (B, D) was examined before or at 1 d, 7 d, 14 d and 21 d after TBI. n=10-11. Data are represented as means ± SEM.

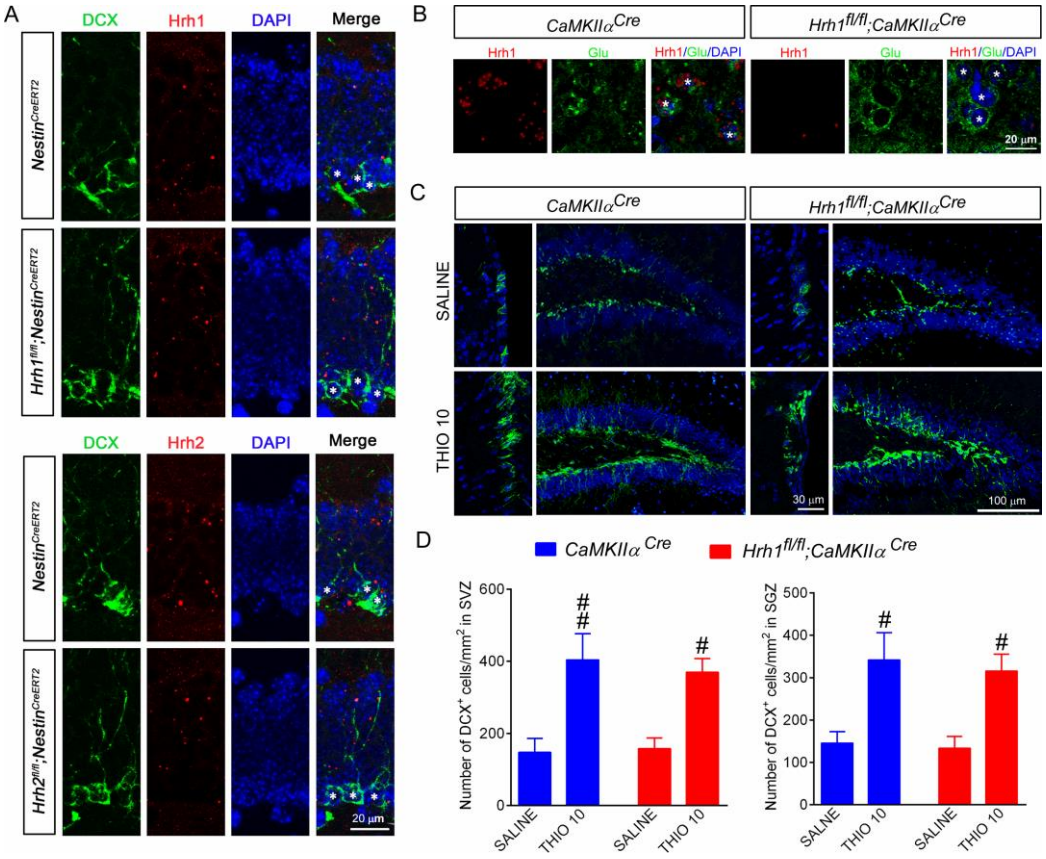

Figure S2. Deletion of *Hrh1* and *Hrh2* in neuroblasts examined by *in situ* hybridization and deletion of *Hrh1* in glutamatergic neurons has no effect on the promotion of neurogenesis conferred by H3R antagonism, related to Figure 3.

(A) In situ hybridization of *Hrh1* or *Hrh2* mRNA together with immunostaining of DCX and visualization of nuclei by DAPI to confirm the deletion of H1R or H2R in neuroblasts (labeled by *) in $Hrh1^{fl/fl}$; $Nestin^{CreERT2}$ or $Hrh2^{fl/fl}$; $Nestin^{CreERT2}$ mice. (B) In situ hybridization of *Hrh1* mRNA together with immunostaining of glutamate and visualization of nuclei by DAPI to confirm the deletion of H1R in glutamatergic neurons (labeled by *) in $Hrh1^{fl/fl}$; $CaMKII\alpha^{Cre}$ mice. (C, D) The number of DCX^+ neuroblasts in the SVZ and SGZ at 14 d after TBI are shown in D with representative photographs in C. n = 6. Data are represented as means ± SEM. *#P* <0.05, *##P* <0.01 *vs.* the SALINE group in the same species.

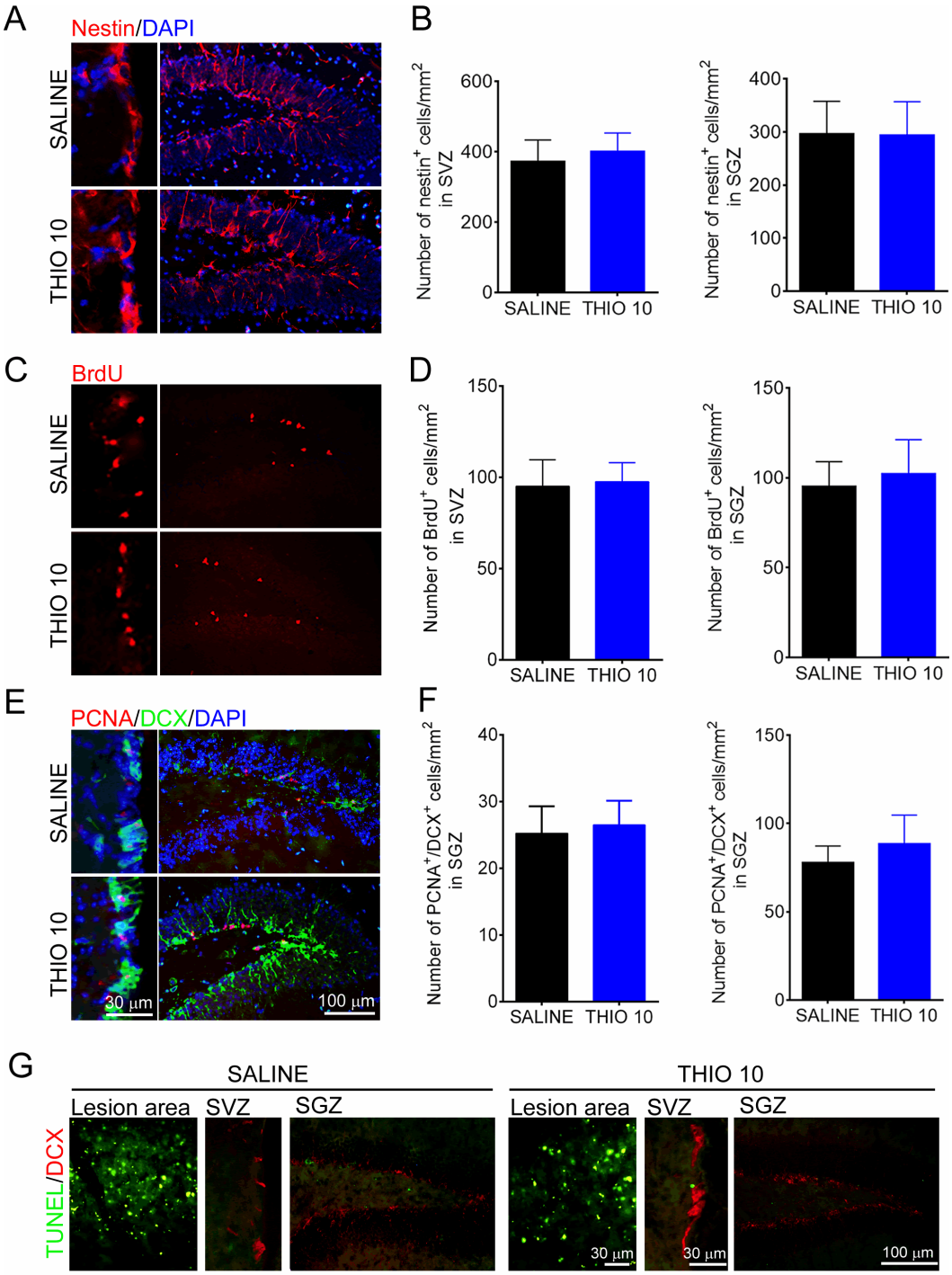

Figure S3. H3R antagonism shows no effect on neural proliferation and apoptosis following TBI, related to Figure 4.

WT mice were exposed to cryogenic brain lesions and then treated with saline or thioperamide (THIO, 10 mg/kg/d). The number of Nestin$^+$ NSCs in the SVZ and SGZ at 14 d after TBI are shown in B with representative photographs in A. The number of BrdU$^+$ proliferating cells in the SVZ and SGZ at 14 d after TBI are shown in D with representative photographs in C. The number of PCNA$^+$/DCX$^+$ proliferating neuroblasts in the SVZ and SGZ at 14 d after TBI are shown in F with representative photographs in E. The apoptosis in lesion areas, SVZ and SGZ examined by TUNEL assay are shown in G. n = 6-8. Data are represented as means ± SEM.

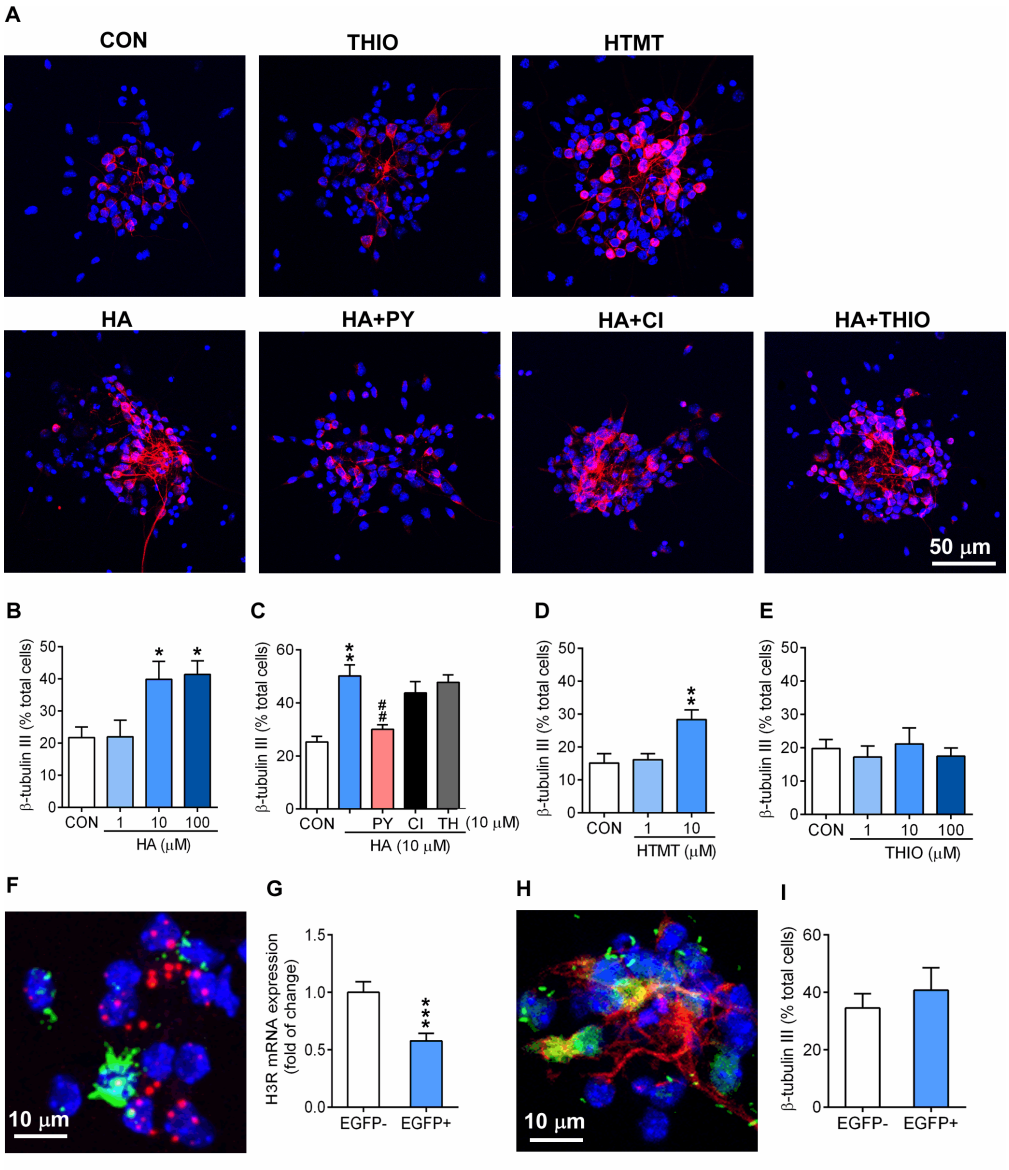

Figure S4. H1R in NSCs promotes their differentiation *in vitro*, related to Figure 4.
(A) The representative photographs of NSC differentiation under different treatment. (B) Effect of histamine (HA) on NSC differentiation. (C) Effect of H1R antagonist pyrilamine (PY), H2R antagonist cimetidine (CI) or H3R antagonist thioperamide (THIO) on the promotion of NSC differentiation conferred by HA. (D) Effect of H1 agonist HTMT on NSC differentiation. (E) Effect of H3 antagonist THIO on NSC differentiation. (F, G) The NSCs from *Hrh3*$^{fl/fl}$ mice were transfected with adeno-associated virus *AAV-CAG-EGFP-Cre* to knock down H3R expression. The decrease of H3R expression was found in EGFP^{+} NSCs examined by in situ hybridization of *Hrh3* mRNA, with representative photographs in F. (H, I) The NSC differentiation was analyzed in EGFP^{+} and EGFP^{-} NSCs, with representative photographs in H. Data are represented as means ± SEM. **P* <0.05, ***P* <0.01, ****P* <0.001 *vs.* the CON group or EGFP^{-} group; ##*P* <0.01 *vs.* the HA group.

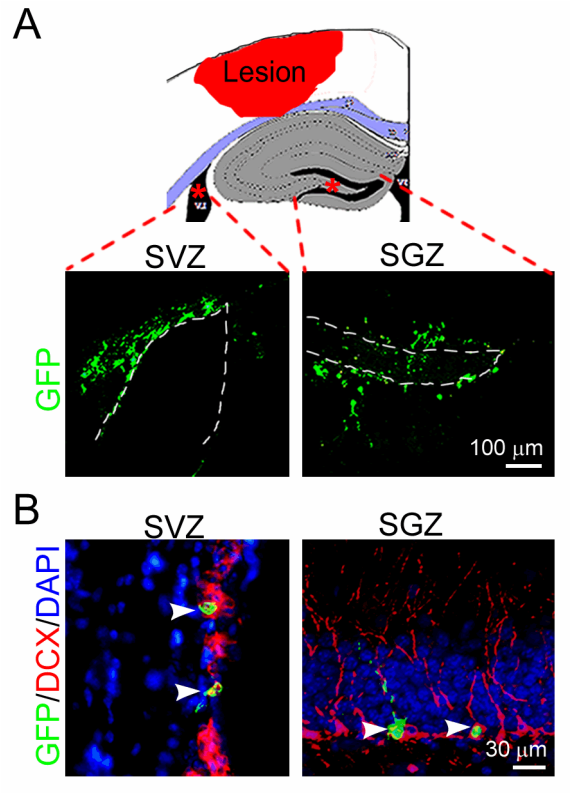

Figure S5. Illustration of GFP+ retrovirus transfection, related to Figure 5.

A pROV-EF1a-GFP retrovirus was used to trace newly generated cells by direct injection of the virus into the SVZ or SGZ areas 5 d before TBI. Representative photographs for GFP^+ retrovirus transfection in SVZ and SGZ at 14 d after TBI are shown in A. Lesion sites are marked in red in A. GFP^+ /DCX^+ double staining shows GFP+ neuroblasts in SVZ or SGZ in B.

Supplemental Experimental Procedures

Animals

Male mice from the C57BL/6 strain, 8 to 12-week-old, including wild type (WT), *Hrh3*$^{-/-}$, *Nestin*^{CreERT2}, *Hrh1*$^{fl/fl}$; *Nestin*^{CreERT2}, *Hrh2*$^{fl/fl}$; *Nestin*^{CreERT2}, *Hrh1*$^{fl/fl}$;*CaMKII*α^{Cre} and *Hrh3*$^{fl/fl}$ genotypes were used. The *Hrh3*$^{-/-}$ mice were supplied by Johnson and Johnson Pharmaceutical Research and Development, LLC (La Jolla, CA), bred and maintained by the Jackson Laboratory. *Nestin*^{CreERT2} were kindly provided by Professor Mengsheng Qiu, College of Life Sciences, Hangzhou Normal University. *Hrh1*$^{fl/fl}$*; Nestin*^{CreERT2} and *Hrh2*$^{fl/fl}$*; Nestin*^{CreERT} mice were obtained by breeding *Hrh1*$^{fl/fl}$ mice or *Hrh2*$^{fl/fl}$ mice with *Nestin*^{CreERT2} mice. *Hrh1*$^{fl/fl}$ mice or *Hrh2*$^{fl/fl}$ mice were generated by standard homologous recombination commercially at the Nan Jing Biomedical Research Institute of Nanjing University, Nanjing, China. Exon 3, encoding the core region of *Hrh1*, and exon 2, encoding the core region of *Hrh2*, were both flanked on either side by *loxP* sequences. *Hrh1*$^{fl/fl}$*; Nestin*^{CreERT2} and *Hrh2*$^{fl/fl}$*; Nestin*^{CreERT} mice were injected intraperitoneally with 2 mg of tamoxifen (Sigma-Aldrich, USA) once per day for five consecutive days before the surgery, to induce the selective deletion of *Hrh1* or *Hrh2* in Nestin-expressing NSCs. *Hrh1*$^{fl/fl}$;*CaMKII*α^{Cre} mice were generated by crossing *Hrh1*$^{fl/fl}$ mice with *CaMKII*α^{Cre} mice. *Hrh3*$^{fl/fl}$ mice were generated by CRISPR-Cas9-mediated editing at the Shanghai Model Organisms, Shanghai, China. Exon 2, encoding the core region of *Hrh3* was flanked on either side by *loxP* sequences. All experiments and protocols were approved by the Zhejiang University Animal Experimentation Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Throughout the traumatic brain injury (TBI) performance, behavioral testing and histological analysis, experimenters were blinded to the experimental conditions of the animals.

Cryogenic brain lesion model

Mice were executed by a cryogenic brain lesion after anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg). A cryogenic brain lesion was inflicted using a 3-mm diameter metal rod supercooled with liquid nitrogen. The metal rod was placed on the skull with the center of the rod at 1 mm behind the Bregma and 1.5 mm right lateral. The rod was left in place for 15 s causing focal injury. Uninjured control mice were surgically prepared in a similar manner, but not subjected to injury. After the surgery, the scalp was sutured and mice were allowed to recover in a recovery chamber at 37 ℃.

Assessment of functional outcome

Neurological function was evaluated 1 d before the surgery, and at 1, 7, 14 and 21 days after surgery using a beam walk test and a wire hanging test. Beams are horizontal and 50 cm above the table. A bright light illuminates the start area. An enclosed escape box, 20 cm^3, is at the end of the beam. The time to traverse the 1 cm wide beam is averaged from three trails on the indicated test days. For the wire hanging test, we used a standard wire cage lid that measured the balance and grip strength by the animal while gripping the wire cage lid. Briefly, the mouse is placed on the top of a wire cage lid. The investigator shakes the lid lightly to cause the mouse to grip the wires, and then turns the lid upside down. The upside-down lid is high enough to prevent the mouse from easily climbing down but not to cause harm in the event of a fall. The investigator uses a stopwatch to quantitate the latency to fall off the wire lid. A 60 s cutoff time is used for both behavioral tests.

Drug administration

Animals were intraperitoneally injected with the H3R antagonist thioperamide (2, 5, 10 mg/kg/d, Sigma-Aldrich, USA) within 1 h, and then once per day after TBI. H3R agonist immepip (IMME, 1 μg/d in saline, Sigma-Aldrich, USA, i.c.v.) were given at 15 min before treatment with thioperamide.

5-bromo-2-deoxyuridine (BrdU, 50 mg/kg/d, Roche, USA) was intraperitoneally injected for five consecutive days starting at the day after surgery.

Retrovirus Injection

A pROV-EF1a-GFP retrovirus (Neuron Biotech, China) was used to trace newborn cells, by direct injection of the virus in the ipsilateral subventricular zones (SVZ, AP= -1.5 mm, RL=2.0 mm, H= -2.2 mm) or subgranular zones (SGZ, AP= -1.5 mm, RL=1.0 mm, H= -2.2 mm). A volume of 1 μl of GFP-retrovirus solution (titer $4x10^8$ cfu/ml) was carefully drawn up into a sterile Hamilton syringe at day 5 before TBI.

Immunohistochemistry staining

Mice were transcardially perfused with a solution containing 0.9% NaCl at 4℃, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PBS, pH 7.4). Brains were removed and postfixed by incubation in 4% paraformaldehyde at 4℃ overnight. Brains were cryoprotected by incubation in 30% sucrose in saline for 48 h. Coronal sections of brain tissue 20-μm-thick were obtained using a cryostat (CM 3050S, Leica) and were thaw-mounted onto coated glass slides. Nonspecific staining was blocked by 5% donkey serum. Slices were first incubated with primary antibodies at 4℃ overnight and then with Alexa Fluor-conjugated secondary antibodies (#711-545-142, #715-585-150, #715-585-152 from Jackson ImmunoResearch Laboratories, USA or #A11016 from Invitrogen, USA) at room temperature. 4', 6'-diamidino-2-phenylindole (DAPI), 1:1000 (Sigma-Aldrich, USA) was used as a nuclear stain. Primary antibodies used in this study were as follows: rabbit anti-DCX (1:500, CST, USA, #4604s); mouse anti-Nestin (1:200, Millipore, USA, MAB353); mouse anti-PCNA (1:200, Gene, China, #GM087902); sheep anti-BrdU (1:400, Abcam, USA, #ab1893). The apoptosis was examined by TUNEL assay (Roche, USA). All sections are observed under a confocal microscope (FV1000, Olympus, Japan). Single- and merge-channel views were acquired with the associated FV10-ASW 2.1 Viewer software. All quantifications of labeled cells were carried out at a similar coronal position.

Neural stem cell culture

E12~14 embryos were extracted from C57 mice to obtain multipotent neural stem cells (NSCs). NSCs were grown in DMEM/F12 medium supplemented with 1% N2 and 40 ng/mL bFGF for 5 days and then subjected to passage. 5×10^4 cells/cm^2 was seeded onto 15 ng/mL poly-L-ornithine and 1 ng/ml human fibronectin coated coverslips. Differentiation was promoted by removing bFGF and keeping the cells in N2 medium. One day after subculture, histamine, H1R agonist HTMT, H1R antagonist pyrilamine, H2R antagonist cimetidine, or H3R antagonist thioperamide was administrated at indicated concentration. Twenty-four hours later, cells were fixed with 4% paraformaldehyde and stained with anti-β-tubulin III (1:200, CST, USA, 4466S). Nuclei were visualized by DAPI staining (blue). The differentiation of NSCs was then quantified by the percentage of the number of β-tubulin III^+ neuron from at least 3 independent experiments.

The NSCs from *Hrh3*$^{fl/fl}$ mice were transfected with adeno-associated virus *AAV-CAG-EGFP-Cre* (OBiO, China) one day before the subculture to knock down H3R expression. Forty-eight hours after subculture, cells were fixed with 4% paraformaldehyde and stained with anti-β-tubulin III. The decrease of H3R expression was found in $EGFP^+$ NSCs examined by in situ hybridization of *Hrh3* mRNA (Advanced Cell Diagnostics, U.S.A.). The NSC differentiation was analyzed in $EGFP^+$ and $EGFP^-$ NSCs.

In situ hybridization

In situ hybridization experiments were performed according to manufacturer's instructions for fresh frozen tissue or cultured cells (Advanced Cell Diagnostics, U.S.A.). RNAscope® Multiplex

Fluorescent Reagent Kit (Advanced Cell Diagnostics, #323100) was used. Sections or cells were permeabilized with pretreatment reagent containing Protease III for 30 min and incubated with mouse *Hrh1* probe, *Hrh2* probe or *Hrh3* probe (Advanced Cell Diagnostics, #491141, #517751 or #428481) for 2 hr at 40 ℃ in the hybridization oven. After that, 3 amplification steps and 1 detection step were performed as per manufacturer instruction. The slices were then incubated with anti-DCX conjugated with Alexa Flour 647 (1:100, Santa Cruz, USA, #sc-271390) or rabbit anti-glutamate (1:1000, Sigma-Aldrich, USA, #G6642) and Alexa Fluor-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, USA, #711-545-152). DAPI (Sigma-Aldrich, USA) was applied to visualize the cell nuclei.

Statistical analysis

Data are presented as mean ± S.E.M. Multiple comparisons in pathological examination were analyzed by One-way ANOVA followed by Bonferroni's test, while two tailed-Student's t-test was applied for other comparisons between two groups. Behavioral tests were analyzed by two-way ANOVA. For all analyses, the tests were two-sided and a $P < 0.05$ was considered statistically significant. "n" stands for the number of mice used in the experiment.