Supporting Information

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SI Methods

Experimental Procedures. To investigate the effect of irradiation on cell proliferation in the young postnatal mouse, pups (n = 4 in each group) were subjected to irradiation or sham-irradiation on postnatal day 9 (P9). Proliferating cells were labeled at P10 (24 h after irradiation) with a single injection of BrdU (100 mg/kg). The animals were then killed 12 h later at P11. To investigate the effect of voluntary running on neurogenesis in early postnatal irradiated mice, 24 mice (n = 6 per group) were randomly divided into sham-irradiated nonrunning, irradiated nonrunning, sham-irradiated running, and irradiated running groups. After cranial irradiation, animals were returned to their biological dams, weaned at P21 and maintained in their groups. Seven weeks after irradiation, the animals were transferred to individual cages for acclimatization to individual housing (P55). Mice in the running groups had free access to a running wheel that was mounted in the cage, and wheel revolutions were automatically registered with customized computer software. At P62, the running wheels were unlocked and all groups received 50 mg/kg BrdU once per day for 5 days to P67. Animals were then left in their relevant housing conditions before open-field behavioral testing (P90). Behavior was recorded for 1 h on 3 consecutive days. 108 variables, describing different aspects of motor activity, movement path shape, and exploratory activity were generated and summarized into 10-min bins using EthoVision Color-Pro 3.1.16 (Noldus Information Technologies). Animals were killed immediately after testing (P93).

Microscopy and Immunohistochemistry. Consecutive 25-μm-thick free-floating sections were used. For BrdU, DNA denaturation was conducted by incubation for 30 min in 2 N hydrochloric acid at 37°C followed by 10 min in 0.1 N borate buffer (pH 8.5). After washing, sections were incubated for 30 min in 0.6% H_2O_2, blocked with 3% normal donkey serum in 0.1% Triton X-100, and then incubated with monoclonal anti-BrdU (1:500; Nordic) overnight at 4°C. Sections were then washed in TBS, placed in the secondary antibody (1:1,000 biotinylated donkey anti-mouse; Jackson ImmunoResearch Laboratories) followed by amplification with avidin–biotin complex (Vectastain ABC Elite; Vector Laboratories) and then visualized using a detection solution (0.25 mg/ml diaminobenzidine; Saveen Biotech). For doublecortin (DCX), after antigen retrieval (sodium citrate, pH 9.0), sections were washed, incubated for 30 min in 0.6% H_2O_2, blocked with 3% normal donkey serum in 0.1% Triton X-100, and then incubated in polyclonal goat-anti DCX (1:250; Santa Cruz Biotechnology) overnight at 4°C. Sections were then washed in TBS, placed in the secondary antibody (1:1,000 biotinylated donkey anti-goat; Jackson ImmunoResearch Laboratories) followed by amplification with avidin–biotin complex (Vectastain ABC Elite), and then visualized using a detection solution (0.25 mg/ml diaminobenzidine; Saveen Biotech). For double-immunolabeling, free-floating sections were incubated in a mixture of primary antibodies, 1:250 anti-BrdU and 1:125 anti-NeuN (Chemicon) or glial–fibrillary acidic protein (GFAP) (1:500 mouse anti-GFAP; Chemicon International) and Sox-2 (1:200 rabbit anti Sox-2; Cemicon International) or DCX (1:125 goat anti-DCX; Santa Cruz Biotechnology) and phosphohistone H3 (1:400 rabbit anti-H3; Upstate) for 48 h at 4°C. Sections were then washed and visualized using appropriate Alexa Fluor-conjugated secondary antibodies (1:1,000; Molecular Probes). Sections were mounted on slides in fluorescent medium containing DAPI (DAPI Pro-Long Gold anti-fade reagent; Molecular Probes).

Stereological Quantification of Cells. Every 8th (P11) and 12th (P93) section throughout the hippocampus was used to determine the total number of BrdU- and DCX-labeled cells in the dentate gyrus (DG) [subgranular zone (SGZ) and granule cell layer (GCL)] under light microscopy in each animal. The percentage number of newborn neurons (60 BrdU-positive cells per animal) was assessed using a confocal microscope (Leica TCS SP2; Leica Microsystems). The resulting percentages of NeuN-positive cells were multiplied with the absolute number of BrdU-positive cells to give the absolute number of newly generated neurons. Double-labeled GFAP/Sox-2 and DCX/phosphohistone H3-positive cells were counted exhaustively in every 12th serial section containing dorsal hippocampus. Cell counts were then multiplied with the series factor (12) and represent the total number of cells per dentate gyrus. The volume of the GCL in P93 mice was measured in every 6th section throughout the hippocampus, and the total sum of the area traced was multiplied by section thickness and series number to give the volume of the entire GCL.

Assessment of DCX-positive Cell Orientation. To determine the orientation of the DCX-positive cells in the SGZ of the DG, we used a compass mapping system. The orientation of the leading process from the DCX-positive cell body was used to determine the angle of the cell in relation to its position within the SGZ. A normal leading cell process from a DCX-positive cell in the SGZ protrudes directly through the GCL into the perforant pathway of the molecular layer. We defined the angle of a perfect process leaving the cell body perpendicular to the SGZ (defined as 0°). We then measured the leading process on at least 60 DCX-positive cells in each animal in several random sections of the hippocampus (or to the maximum number available if necessary) and calculated a mean average degree of the orientation of all of the cells.

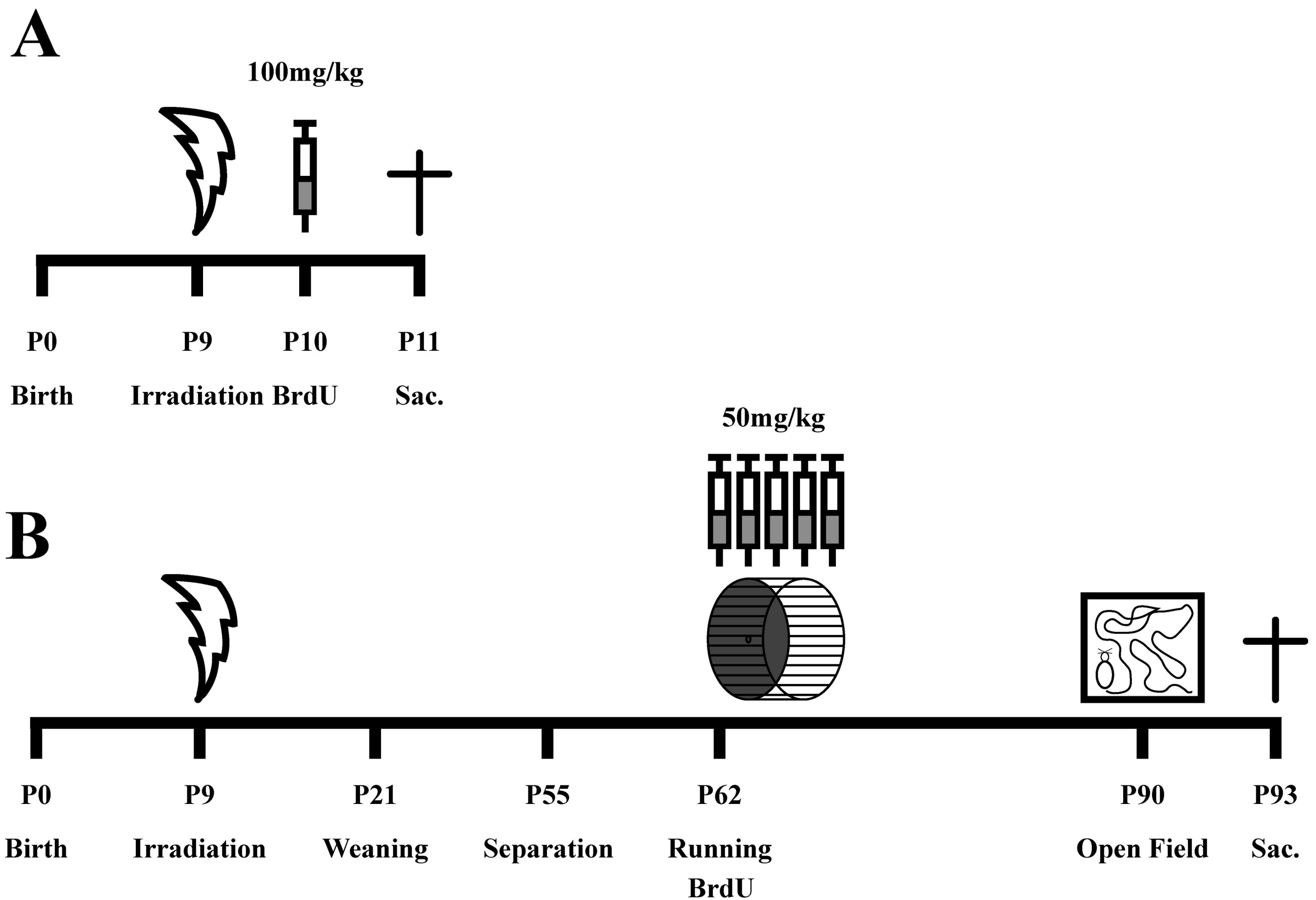

Fig. S1. Schematic diagram of the experimental procedures. (*A*) To investigate the immediate effects of irradiation on progenitor proliferation, mice were irradiated (n = 4) or sham-irradiated (n = 4) on P9. Twenty-four hours later, all mice were injected i.p. with BrdU (100 mg/kg; P10) and then 12 h later were killed and brains removed for immunohsitochemistry. (*B*) To investigate the effect of voluntary running on neurogenesis in early postnatal irradiated mice, 24 mice were randomly divided into sham-irradiated nonrunning, sham-irradiated running, irradiated nonrunning, or irradiated running (n = 6 per group). Mice were irradiated or sham-irradiated at P9 and then allowed to reach adulthood. At P55, mice were separated into individual cages, and then at P62, running mice were allowed free access to the wheels until P93. In addition, BrdU (50 mg/kg) was injected daily for 5 consecutive days at P62. At P90, mice were subjected to the open-field test daily for 3 days, and then at P93 all animals were killed and brains perfusion fixed for immunohsitochemistry.

Fig. S2. Assessment of cell proliferation in the dentate gyrus of the young irradiated mouse. (*A*) Proliferating cells, labeled with a single injection of BrdU at P10, 24 h after irradiation and examined at P11, were decreased by 85% in irradiated compared with sham-irradiated animals. **, $P < 0.01$ (*B* and *C*) The representative micrographs show large numbers of BrdU-labeled cells dispersed over the GCL of the dentate gyrus in sham-irradiated animals with much fewer cells in the irradiated animals. Data are represented as group means ± SEM. (Scale bars, 100 μm.)

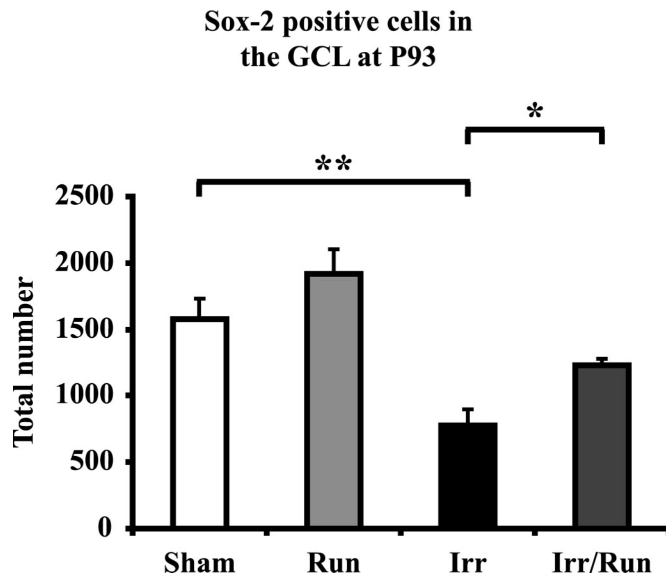

Fig. S3. Voluntary running increases the number of Sox-2-positive cells after irradiation. Confocal microscopic analysis revealed a significant decrease in the number of Sox-2-positive cells in the DG of irradiated non-running compared with sham-irradiated nonrunning mice. After voluntary running in irradiated animals, there was a significant increase in the number of Sox-2-positive cells compared with irradiated nonrunning mice. Group means ± SEM. *, $P < 0.05$; **, $P < 0.01$.

Fig. S4. Progenitor cell differentiation in the mouse dentate gyrus. (*A*) At P93, 4 weeks after BrdU injection, the survival of newly generated cells was analyzed. The whole dentate gyrus of the hippocampus was analyzed on approximately eight sagittal sections per animal in all groups. There was a significant increase in BrdU-labeled cells in sham-irradiated running mice compared with sham-irradiated non-running mice. There was also a significant increase in BrdU-labeled cells in the irradiated running mice compared with irradiated nonrunning mice. (*B*) Measurement of the GCL showed a significant decrease in the irradiated nonrunning mice compared with sham-irradiated nonrunning and sham-irradiated running mice. (*C* and *D*) Representative micrographs show few BrdU-labeled cells in the irradiated mice and a greater number of BrdU-labeled cells after voluntary running. (Scale bars, 100 μm.) Data are represented as group means $\pm$ SEM. *, $P < 0.05$; **, $P < 0.01$.