SUPPLEMENTARY METHODS

Animals

Sixty-four female 129Sv/Ev mice, 10 weeks old, were obtained from Taconic Farms, Germantown, NY. Animals were maintained on a 12 hr light/dark cycle throughout the course of the experiment. Behavioral testing (anxiety-like behavior and spatial learning) was performed during the light phase. The procedures described herein were conducted in accordance with National Institutes of Health regulations and approved by the Institutional Animal Care and Use Committees of Columbia University and the New York State Psychiatric Institute.

X-Irradiation

Mice received fractionated low dose x-irradiation to the head as described previously\textsuperscript{15}. This consisted of anesthetizing all animals with a mix of ketamine and xylazine in saline solution (1.5:0.5:8.0 mixture) and then placing them in a stereotaxic frame. Half the animals (n = 32) then received irradiation. Animals were placed 30 cm below the x-ray source (Stabilipan, Siemens Medical Systems Inc., Avon, CT), which delivered 1.8 gy per minute. Animals were irradiated for 2 minutes and 47 seconds to total 5 gy per exposure. Exposures were performed on days 15, 19 and 22 for a total of 15 gy. A lead shield (produced in house) covered the entire animal but left unshielded an area (3.22x11 mm) above the hippocampus.

Housing

Standard housing (n = 32) consisted of 4 mice, 2 x-irradiated and 2 shams, in a clear plastic cage (29.2x19x12.7 cm, N10 cage, Ancare, Bellmore, NY) with food (Prolab
IsoPro RMH3000, PMI Nutrition International LLC., Brentwood, MO) and water (autoclaved tap water) *ad libitum.*

Enriched housing (n = 32) consisted of 8 mice, 4 x-irradiated and 4 shams, in a cage made of two large clear plastic cages (48.3x26.7x15.5 cm, N40 cage, Ancare, Bellmore, NY) connected by a red plastic tube (10 cm long x 5 cm diameter, #K3322, Bio-Serv, Frenchtown, NJ). Holes were cut in the sides of cages to allow the tube to connect the cages. In each housing unit there was a wooden house (11.4x11.4x6.4 cm, Triangle House, Small Animal Kingdom, PetCo., USA), hollow wooden log (15.2 cm long, #60122 Fun Log Tube, T-Rex Products Inc., PetCo., USA), amber plastic igloo (10.2 cm diameter x 5 cm high, #K3328, Bio-Serv, Frenchtown, NJ), paper tube (11.4 cm long x 4.5 cm diameter, Alamo Paper Tube Co., San Antonio, TX), 4 running wheels (12.7 cm diameter, Rolf C. Hagen Inc., Montreal, Canada) and 2 paper towels (25.4x24.1 cm, Windsoft, USA). Animals shredded the paper towels and used shreds for nesting. Enriched animals had access to food and water *ad libitum.*

**Timeline/Overview**

On day 0, all animals (n = 64) arrived and were randomly distributed into standard housing, 4 per cage. On day 15, animals began the irradiation procedure. Irradiation was performed on half of all animals; each cage of 4 then consisted of 2 sham and 2 x-irradiated mice. On day 85, two months after irradiation, environmental enrichment began. Half of all animals were placed in enriched housing. In order to do this, 2 standard cages of 4 mice (2 sham, 2 x-irradiated) were combined into one large enriched cage to consist of 8 mice (4 sham, 4 x-irradiated). This experimental strategy resulted in even distribution of mice throughout the 4 experimental groups (n = 16 per group): standard sham, standard x-irradiated, enriched sham, enriched x-irradiated. On
day 117, a behavioral observation of mice in the enrichment was performed (see below). On day 126, after six weeks of enrichment, behavioral testing began (see below). On days 175-181, a wheel-running distance assessment was performed (see below). On days 187-189, animals were injected with 5-bromo-2’-deoxyuridine (BrdU, Roche, Indianapolis, IN), and on day 190 they were sacrificed (see below).

Novelty Suppressed Feeding

Mice that had been food deprived for 24 hours were gently placed in a corner of a brightly lit arena (~650 lux at floor of arena, 50x50x20 cm) with white sides for a maximum of 6 minutes. The floor was covered with approximately 2 cm of bedding (Bed O’ Cobs, The Andersons Inc., Maumee, OH). Two pellets of food were attached by wire to a circle of Whatman paper (Whatman Inc., Florham Park, NJ) at the center of the arena. When the animal was observed to be sitting on its haunches eating food with its forepaws, the latency to feed was recorded. The experimenter was blind to housing condition and irradiation treatment.

Immediately after the test, mice were individually placed in their home cage for 5 minutes and allowed to feed. Food pellets were weighed before and after the 5 minutes, and the amount of food consumed was calculated (see Supplementary Data). Animals that did not feed within this 5-minute period were removed from all analyses. This decision was made a priori and assures that only hungry animals are included in the analysis. Ten mice were removed, resulting in n’s of 12 to 14 per group. Mouse weights were recorded before food deprivation and upon completion of the test. Percent weight loss over the 24-hour food deprivation period was calculated (see Supplementary Data). To mitigate possible effects of enrichment on hunger levels, four days prior to testing, all
enriched mice were placed in standard laboratory housing. One day after completion of the test, enriched mice were returned to their enriched cages.

**Habituation of Activity**

Mice were placed into an enclosed rectangular chamber (one side of a Med-Associates shuttle box ENV-010MC; 20x16x20.5 cm), with Plexiglas and metal walls and a floor made of metal bars (1 cm between each bar). Each chamber was illuminated by a CM1820 bulb (28v, 100mA) located near the ceiling. Mice were placed into the chamber for 10 minutes, once per day for two consecutive days. Sessions were video recorded and scored offline. Minutes 1-2, 5-6, and 9-10 of each session were scored by a blind observer. During each two-minute epoch the total duration of activity was recorded using a stopwatch.

**Morris Water Maze**

Water maze pretraining took place in a plastic bucket (43x37x30.5 cm) filled to a depth of 19 cm containing a square, clear Plexiglas platform (10x10 cm) submerged 0.5 cm below the water surface. Visible and hidden platform training took place in a white plastic tank (1.7 m internal diameter, 56 cm deep) filled with water to a depth of 51 cm. A circular, clear Plexiglas platform (14.6 cm diameter) was submerged 0.5 cm below the surface of the water in the middle of one quadrant of the maze. To provide traction the surface of the platform was covered with clear, rubber “non-slip” coating. For both visible and hidden training, the water was made opaque with white, non-toxic tempera paint so that the computerized tracking system could easily identify the location of the mice (#2910, Pearl Paint, NY). For visible training, the platform location was identified by a cylindrical, multicolored plastic tube (12 cm high x 1 cm diameter) fixed atop the
submerged platform. Water temperature was maintained at 21°C at all times. Data from visible and hidden training were collected using the Chromotrack system (San Diego Instruments, San Diego, CA). Using a contrast-detection algorithm, the system recorded the location of the mouse every 0.055 seconds. The location data were then analyzed offline using in-house software to calculate the following measures: total distance and (on the probe trial) percent time in each quadrant and number of target crossings. The mean distance to the platform was computed for each training day by taking the mean across the trials for that day.

Water maze training comprised three phases, executed in the following order: pretraining (2 days), visible platform training (2 days), and hidden platform training (5 days). The purpose of pretraining was to habituate mice to the water and to train mice to climb onto a submerged platform and to remain there until being removed by the experimenter. On each pretraining trial a mouse was placed into the bucket and allowed to find the platform. After reaching the platform (all mice found the platform within 30 seconds), the mouse was required to remain there for 20 seconds, at which time it was removed and placed into a holding cage containing dry paper towels. If the mouse jumped off the platform, the clock was restarted and the mouse was not removed from the bucket until it returned to the platform and remained there for 20 consecutive seconds. The 4 pretraining trials were administered over days (2 trials per day) with an intertrial interval of 3-5 minutes. Data were not collected during pretraining.

Visible platform training commenced on the day following pretraining. There were 2 days of visible platform training, followed by 5 days of hidden platform training. In both the visible platform and hidden platform training phases, trials were administered at the rate of 3 trials per day with an intertrial interval of approximately 45 minutes. Visible platform and hidden platform trials were identical with two exceptions: on visible
trials the platform location was indicated by a marker visible above the water line, and the platform location was changed between the visible platform and hidden platform training phases. Within each training phase the platform location was constant. At the start of each trial, the mouse was gently placed into the water with its heading facing the outside of the tank. The start location was varied semi-randomly between trials, with the exception that a single start location was not used on consecutive trials. The eight start locations were spaced evenly around the maze. The maximum trial duration was 2 minutes. If a mouse did not find the platform by that time, it was either led there or placed there by the experimenter. After remaining on the platform for 10-15 seconds the mouse was removed to a warmed holding cage (Zoo Med under cage heater, #RH-5) containing dry paper towels.

The final trial of hidden training (trial 3 of day 5) was a probe trial in which the platform was removed and mice were placed into the maze tank for 60 seconds.

**Behavioral Observation of Wheel Interactions**

To assess whether x-irradiation affected mouse interactions with the enriched environment, a behavioral observation was conducted. Mice were virtually always either within the toys or running on the wheels, and the time spent in transit between the two was negligible. Therefore, quantification of wheel interaction alone was sufficient to assess interaction with all the enrichment stimuli. To avoid redundancy only wheel interaction, and not toy interaction, was recorded.

Observations were performed during the dark phase between days 117 and 118. A red light (40 watt, Verilux, Stamford, CT) was used to illuminate the housing room during data collection. Animals’ tails were marked with a black marker for identification. The time sampling method of behavioral observation was used. Approximately every 30
seconds, one set of two wheels was observed for 5 seconds. If an animal was using the wheel set during that period, the experimental group to which it belonged, sham or x-irradiated, was recorded. Each wheel set was observed 16 times. Two separate series of observations were performed during the night. From the observational data we calculated the mean probability of a sham mouse or x-irradiated mouse using the wheels during a 5 second period (see Supplementary Data).

**Wheel Running Distance**

To assess whether x-irradiation affected the wheel running, a wheel running distance assessment was performed (see Supplementary Data). Sixteen enriched (8 sham, 8 x-irradiated) mice were singly housed with a wheel for 48 hours then returned to their enriched housing. Each wheel was outfitted with an odometer (BC 401, Sigma Sport, Germany) to measure running distance.

**Histology Preparation**

Animals were deeply anesthetized with ketamine and transcardially perfused with cold saline for 1 minute and cold 4% paraformaldehyde dissolved in 0.1M phosphate buffer for 3 minutes. Brains were then removed from the skull and postfixed for 24 hours. After postfixing, brains were cryoprotected at 4°C in 15 ml of 30% sucrose for 72 hours. After cryoprotection, brains were imbedded in TissueTek medium and stored at -20°C until sectioning. Serial sections (35 μm) were made of the entire hippocampus using a cryostat (CM3050S, Leica, Germany) and stored at 4°C in PBS with 0.1% NaN₃.

**BrdU Immunohistochemistry**

Animals (n = 4 per group) were injected once a day for 3 days with BrdU
(150mg/kg dissolved in saline; intraperitoneal). Twenty-four hours after the last injection animals were sacrificed (see Histology Preparation above). Every 6th section of the entire hippocampus was mounted on slides (Superfrost Plus, Fisher Scientific, USA) in PBS. After drying, slides were boiled in citric acid (pH = 6), for 5 minutes, rinsed with PBS and then subjected to 0.3% hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) for 30 minutes at room temperature. Sections were then washed with PBS and treated with 0.01% trypsin (Sigma-Aldrich, St. Louis, MO) in tris/CaCl₂ for 10 minutes. Following a rinse with PBS, sections were treated with 2N HCl for 30 minutes, rinsed in PBS and then blocked with 3% Normal Goat Serum (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). Sections were then left overnight at room temperature with anti-mouse BrdU (1:100, Becton-Dickinson, San Jose, CA). The next day slides were washed with PBS and then incubated for one hour with biotinylated goat anti-mouse secondary antibody (1:200, Jackson ImmunoResearch Laboratories Inc., West Grove, PA), followed by a rinse with PBS and then amplification with an avidin-biotin complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA). Sections were then rinsed in PBS and exposed to 3,3-Diaminobenzidine (SigmaFast, Sigma-Aldrich, St. Louis, MO) for visualization. Sections were then counterstained with Nuclear Fast Red (Vector Laboratories, Burlingame, CA), dehydrated with an ethanol series and covered using Permount (Biomed, Foster City, CA) and cover glass (FisherFinest, Fisher Scientific, USA).

**Doublecortin Immunohistochemistry**

All steps were performed on a shaker (Lab-Line Instruments Inc., Melrose Park, IL). Serial free-floating sections consisting of every 6th section of the entire hippocampus (n = 2-3 per group) were rinsed at room temperature with TBS, treated with 0.3%
hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) for 30 minutes, rinsed with TBS, blocked with 10% Normal Goat Serum (Jackson ImmunoResearch Laboratories Inc., West Grove, PA), and incubated overnight at 4°C with Doublecortin antibody (1:3500, Doublecortin C-18, Santa Cruz Biotechnology, Santa Cruz, CA). Sections were then rinsed at room temperature in TBS, exposed to biotinylated donkey anti-goat secondary antibody (1:500, Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for one hour, rinsed with TBS, and then amplified with an avidin-biotin complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA). Sections were then rinsed in TBS and exposed to 3,3-Diaminobenzidine (DAB Substrate Kit, Vector Laboratories, Burlingame, CA) for visualization. After a final rinse with TBS, sections were mounted on slides (Superfrost Plus, Fisher Scientific, USA) with Aqua Poly/Mount (Polysciences Inc., Warrington, PA) and cover glass (FisherFinest, Fisher Scientific, USA).

**CD68 Immunohistochemistry**

Serial free-floating sections consisting of every 12th section of the entire hippocampus (n = 5-7 per group) were incubated for one hour in 0.1M TBS with 0.5% Triton X-100 (Tx) and 10% normal donkey serum (NDS). Sections were then exposed to rat anti-CD68 primary antibody (1:500, Serotec, Oxford, UK) in TBS/Tx overnight at 4°C, then treated with biotinylated donkey anti-rat secondary antibody (1:1,000, Jackson ImmunoResearch Laboratories Inc., West Grove, PA) in TBS/NDS, and amplified using an avidin-biotin complex, both for one hour at room temperature. Sections were exposed to DAB for visualization.

**Fluorescent Double Labeling for BrdU and Doublecortin**

Serial free-floating sections consisting of every 6th section of the entire
hippocampus (n = 3 per group) were rinsed at room temperature with TBS, treated in a 1:1 solution of formamide and SSC for two hours at 65°C, rinsed in SSC at room temperature, treated with 2N HCL for 30 minutes at 37°C, and immediately placed in 0.1M Boric Acid for 10 minutes at room temperature. The Boric Acid step and all subsequent steps were performed on a shaker. Slices were then rinsed in TBS at room temperature, blocked with 10% Normal Goat Serum, and then incubated overnight at 4°C with rat anti-BrdU antibody (1:75, Serotec, Oxford, UK) and goat anti-Doublecortin antibody (1:500, Doublecortin C-18, Santa Cruz Biotechnology, Santa Cruz, CA). Sections were then rinsed at room temperature in TBS, exposed to donkey anti-rat Cy3 (1:400, Jackson ImmunoResearch Laboratories Inc., West Grove, PA) and biotinylated donkey anti-goat secondary antibody (1:500, Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for two hours, rinsed in TBS at room temperature, and exposed to Avidin-Cy2 (1:200, Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for one hour. After a final rinse with TBS, sections were mounted on slides with Gel/Mount (BioMeda Co., Foster City, CA) and cover glass. The cover glass was then permanently sealed with clear nail polish.

**Imaging and Quantification of BrdU, Doublecortin and CD68**

Quantification was performed on a Zeiss Axiovert 200 microscope (Carl Zeiss, Thornwood, NY). A modified unbiased stereology protocol was used that had previously been reported to successfully quantify BrdU labeling\textsuperscript{15}. To ensure that immunoreactive cells would not be counted twice and that the area in each section would be consistent, every 6\textsuperscript{th} section (BrdU, DCX) or 12\textsuperscript{th} section (CD68) of the entire hippocampus was included in the quantification. A researcher, unaware of the experimental group to which the sample belonged, counted all positively stained cells in the dentate gyrus (granule cell
layer), omitting cells in the outermost focal plane. A cell was counted as being in the subgranular zone of the dentate gyrus if it was touching or in the subgranular zone. Cells that were located more than two cells away from the subgranular zone were not included in the count. For CD68 quantification, the granule cell layer and the hilus were included in the area counted. For fluorescent double labeling, if a cell was observed to be positive for BrdU, the filter on the microscope was immediately switched to assess if the cell was positive for DCX. Images were taken with a Retiga EXi camera and QCapture Pro Software (both from QImaging Inc., Burnaby, BC, Canada). For images of BrdU and DCX visualized with DAB see Supplementary Fig. 2. For quantification of DCX that was visualized with DAB see Fig. 2a. For quantification of BrdU and double staining with BrdU and DCX visualized with fluorescence see Fig. 2b,c. For images of DCX, BrdU and double staining with BrdU and DCX visualized with fluorescence see Fig. 2d.

To confirm BrdU/DCX double labeling, confocal z-stacks were taken of cells characterized as double-labeled. Confocal images were acquired using a Zeiss LSM 510 NLO scanning module with an Axioskop2 FS microscope (Carl Zeiss Microimaging, inc.). Sections were scanned at 1μm intervals using a Plan-Neofluar 40x oil-immersion (NA = 1.30) objective and dual-channel excitation with argon (488nm) and helium-neon (543nm) lasers. Z-stacks were taken of 40 cells previously characterized as double-labeled using the quantification procedure described above. The cells were randomly selected from the standard sham (n=20) and enriched sham (n=20) groups (double-labeled cells were not observed in the irradiated groups). Eighteen of 20 cells sampled from the standard sham group were confirmed as double labeled, and 17 of 20 cells sampled from the enriched sham group were confirmed as double-labeled.
Data Analysis

For analysis of novelty-suppressed feeding and histological data, 2 (housing: standard or enriched) x 2 (treatment: sham or x-irradiated) ANOVAs were performed. For habituation of activity data, 2 (housing: standard or enriched) x 2 (treatment: sham or x-irradiated) x 3 (minute) ANOVAs were performed. For the water maze data, repeated measures ANOVA was used, with day as the repeated measure. The wheel interaction behavioral observation and wheel running distance analyses were performed with the unpaired Student’s *t*-Test. An α level of 0.05 was adopted for all analyses, which were conducted using StatView software (SAS Institute, Cary, NC).