Supporting Information

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SI Materials and Methods

Nonselectively Bred Rats (Outbred). All Sprague–Dawley rats were bred and maintained at the University of Michigan animal facilities in accordance with University Committee Use and Care of Animals. The animals were maintained under conditions of constant temperature and a 12 h/12 h light–dark cycle with free access to food and water in 43 × 21.5 × 25.5-cm Plexiglas cages. Animals were weaned on postnatal day 21 (PND21), and only males were used and housed two per cage for the remainder of the experiment. At PND23 and -67, animals were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4). Brains were postfixed overnight at 4 °C and placed in 30% sucrose in 100 mM phosphate buffer until saturated. Brains were frozen in isopentane for immunohistochemistry. The brains were then stored at −80 °C until processed.

Selectively Bred Rats High-Responder and Low-Responder Rats. One set of selectively bred high-responder rats (bHR) and selectively bred low-responder (bLR) adult males was screened for locomotor activity on PND58 and killed on PND72; brains were removed, snap frozen in isopentane, and processed for in situ hybridization to assess basal fibroblast growth factor-2 (FGF2) mRNA expression. In a separate experiment, bLR and bHR pups were injected with either FGF2 or vehicle (VEH) on PND2 as described above. A separate set of bLR and bHR pups were injected with either FGF2 or vehicle on PND2 and tested in the light–dark box on PND62. All behavioral testing was performed between 0800 and 1130 h. Animals were killed 1 wk after the forced swim test, and brains were collected for gene expression analyses.

Locomotor Activity. Briefly, rats were transported to a testing room separate from animal housing and placed in a cage outfitted with a wire mesh floor. Horizontal and rearing activity was monitored in 5-min intervals over a period of 60 min by computer. An animal's total locomotor score was determined by summing total rearing and horizontal movements over the entire 60-min test period.

Elevated Plus Maze. The test was conducted under dim light (~40 lx), and the rat was placed in the central square facing a closed arm to start the test. The latency to enter the open arm and the total time that the animals spent in the open arms, closed arms, and center square were recorded by a computerized video tracking system (Noldus).

Forced Swim Test. Swimming consisted of horizontal movement in the swim chamber. Climbing consisted of a vertically directed movement with the forepaws typically above water along the walls of the swim chamber. Immobility consisted of the minimal amount of movement necessary to keep the head above water level. Percent total duration of climbing, swimming, and immobility episodes was scored using The Observer software (Noldus Information Technology).

Light–Dark Box. The light–dark box is a 30 × 60 × 30-cm Plexiglas shuttle box with a translucent cover. The floor is composed of stainless steel bars suspended above corncob bedding. Each box is divided into two equal-sized compartments by a wall with a 12-cm-wide open door. One compartment is white and brightly lit, and the other is black and dimly lit. The total time spent in each compartment as well as the latency to enter the light compartment were monitored by photocells located 2.5 cm above the grid floor of each compartment and recorded with a microprocessor.

Immunohistochemistry. For BrdU, K_i-67, and NeuN, sections were rinsed in 0.05% Triton X-100/TBS solution three times for 10 min. For BrdU and K_i-67, sections were subsequently incubated in 90 °C water baths for 1 h in 10% Na-citrate buffer (pH 6.0). All sections were then incubated in 0.05% Triton X-100/Tris-buffered saline (TBS) containing 0.6% H_2O_2 for 30 min. For BrdU, sections were incubated in 50% formamide in 2× SSC at 65 °C for 2 h, rinsed in 2× SSC, incubated for 30 min in 2N HCl at 37 °C, and then rinsed for 10 min in 0.1 M boric acid (pH 8.5). All sections were rinsed again in TBS and then preincubated in blocking solution (3% goat serum in 0.05% Triton X-100 in TBS) for 1 h. Sections were incubated with primary antibodies for either BrdU (1:1,000, rat monoclonal; Accurate Chemicals), K_i-67 (1:1,000, rabbit monoclonal; Santa Cruz), or NeuN (1:500, mouse monoclonal; Chemicon) in blocking solution overnight at 4 °C. Sections were then rinsed with TBS and incubated in secondary antibodies (1:300, goat anti-rabbit IgG for K_i-67, Vectastain Elite ABC kit; Vector Labs, 1:1,000, goat anti-mouse IgG for NeuN; Chemicon, or 1:300, goat anti-rat IgG for BrdU; Jackson Labs) for 1 h at room temperature, incubated with avidin–biotin complex for 1 h at room temperature (Vectastain Elite ABC kit; Vector Labs), and visualized by diaminobenzidine staining. Sections were washed with TBS and H_2O, dehydrated through graded alcohols, immersed in xylene for 20 min, and coverslipped with Permount mounting medium. Cresyl violet staining was also performed.

Cell Counting. The Cavalieri estimator (StereoInvestigator; MicroBrightField) was used to estimate volume on a Zeiss Axiophot microscope interfaced with a CCD color video camera and displayed on a high-resolution video monitor at a final magnification of 250×. Total cell counts were estimated by applying an optical dissector and an x and y step size of 225 μm. The cells were counted when they first came into focus on the microscope described above using StereoInvestigator. Estimates were obtained by multiplying the sum of the neurons that were counted by the reciprocal of the fraction that was sampled (derived from the section sampling interval, x and y step size, and section thickness). Estimates were calculated from 300 to 400 cells in 100–200 dissectors per animal. For K_i-67 and BrdU cell counts, a Leica DMR light microscope was used to focus through the thickness of the section using a 63× oil objective.

Laser Capture Microdissection. Sections containing the dorsal hippocampus were processed through the following dehydration protocol: room temperature for 30 s, 75% ethanol for 30 s, dH_20 for 30 s, 75% alcohol for 30 s, two 95% alcohol washes for 30 s, 100% alcohol for 30 s, two xylene washes for 5 min each, and air dry for 20 min. The left dentate gyrus and subgranular zone of two consecutive slices were identified by their distinct morphology and were captured using the AutoPix laser capture microdissection (LCM) system (Arcturus) onto CapSure Macro LCM caps (Arcturus). Laser settings were set to 70 mW and 1.5 ms. The bregma level was chosen, because neurogenesis is greater in the rostral hippocampus, and the region was easy to identify by the morphology of the dorsal and ventral blades of the dentate gyrus.

RNA Isolation and Amplification. Cell preparations were stored at −80 °C between RNA extraction and isolation, and an optional

DNase treatment was used in the isolation process. The total volume of RNA sample was 11 μL in elution buffer. This process yielded 5–10 ng total RNA. Isolated RNA was amplified by the RiboAmp Plus 1.5-round RNA Amplification kit (Molecular Devices). Quality and quantity of cRNA were determined before amplification on the Agilent 2100 Bioanalyzer using the picochip. After amplification, the quantity was determined on the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc).

Microarray Analysis. Each chip encodes 22,523 gene probes corresponding to 21,910 genes. The samples were quantile-normalized using BeadStudio and analyzed with Illumina's error model (Illumina). Fold changes and *P* values were calculated from the average array signal. Samples that correlated less than 94% with any other given sample were omitted from the analysis (n = 4–5 per group). Data were also analyzed by Ingenuity Pathways Analysis Software version 8.0 (Ingenuity Systems, www.ingenuity.com). The Functional Analysis tool identified biological functions and/or diseases that were most significant to the dataset. Molecules from the dataset that had a fold-change cutoff of 1.1, had a *P* value less than 0.05, and were associated with biological functions and/or diseases in Ingenuity's Knowledge Base were considered for the analysis. Right-tailed Fisher's exact test was used to calculate a *P* value determining the probability that each biological function and/or disease assigned to that dataset is caused by chance alone.

Quantitative RT-PCR. Samples with less than 30 ng cDNA were omitted from the study. In a preliminary study, biological replicates exhibited less than 2% variation. Therefore, samples were run as singletons in this experiment. The TaqMan Low Density Array cards were prepared with TaqMan Universal PCR Master Mix (2×) with AmpErase UNG according to the manufacturer's instructions and processed on the 7900 HT Sequence Detection System (Applied Biosystems). Cycle threshold (Ct) values were determined, and quality control of the samples was performed in SDS v2.3. Wells that were not amplified or leaked were omitted from the study. The data were then analyzed by the relative quantification method in RQ Manager v1.2 (Applied Biosystems).

mRNA in Situ Hybridization. The sequence of rat mRNA used for generating the probes was complementary to the following RefSeq database numbers: FGF2 (NM_019305 and 716–994), neurotrophic tyrosine kinase receptor type 3 (ntrk3; S62924 and 1,900–2,670), and glutamic acid decarboxylase 1 (gad1; M34445 and 1,145–2,045). All cDNA segments were extracted (Qiaquick Gel Extraction Kit; Qiagen), subcloned in Bluescript SK (Stratagene), and confirmed by nucleotide sequencing. After appropriate exposure times for FGF2 (7 d), gad1 (6 d), and ntrk3 (12 d), the films were developed (Kodak D-19; Eastman Kodak). Brain-section images were captured from film using a ScanMaker 9800XL scanner (Microtek Lab Inc.) with SilverFast v6.6.0r2 software (LaserSoft Imaging Inc.), and relative ODs were determined for each section. Radioactive signals were quantified using computer-assisted optical densitometry software (Scion Image Beta 4.03; Scion). Integrated optical densities were found by outlining the region of interest throughout the rostrocaudal extent of the dentate gyrus.

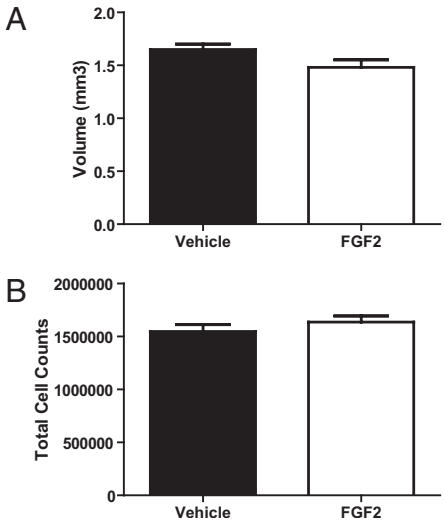

Fig. S1. Cresyl violet histochemistry of the adult dentate gyrus after early-life treatment with FGF2 or vehicle. (*A*) There was no significant difference in dentate gyrus volume in animals treated with early-life FGF2 (n = 4) compared with vehicle (n = 4). (*B*) There was no significant difference in the number of total cells in the dentate gyrus in animals treated with early-life FGF2 compared with vehicle.

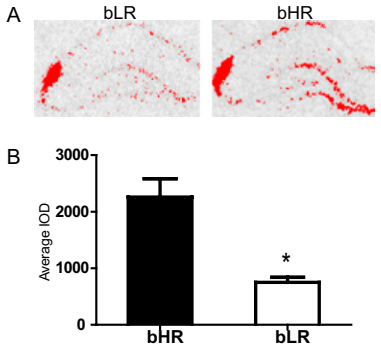

Fig. S2. FGF2 gene expression in bHR and bLR rats by mRNA in situ hybridization in the dentate gyrus. (*A*) Representative FGF2 mRNA expression in the hippocampus of bLR (*Left*) and bHR (*Right*) animals. Red denotes a high level of gene expression. (*B*) Average integrated optical density (IOD) values of FGF2 mRNA expression for bLR and bHR rats in the hippocampus. Adult bHR (n = 4) rats exhibited higher levels of FGF2 gene expression in the dentate gyrus than adult bLR (n = 4) rats ($t_{(27)}$ = 4.31, $P < 0.001$). All values are mean ± SEM.

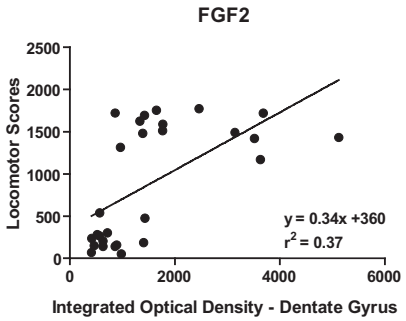

Fig. S3. Correlation between locomotor activity and FGF2 gene expression in the adult dentate gyrus. There was a moderate positive correlation (r^2 = 0.37, $P <$ 0.001) between locomotor scores over 1 h and the IOD of FGF2 mRNA in the dentate gyrus of bHRs (n = 14) and bLRs (n = 14).

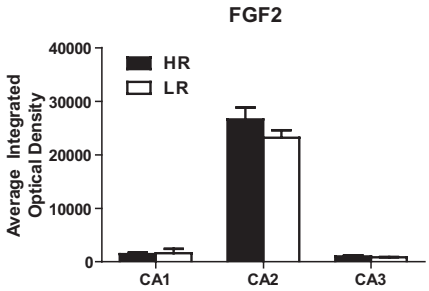

Fig. S4. FGF2 gene expression was unaltered in other hippocampal subfields. There was no significant difference in FGF2 gene expression in CA1, CA2, or CA3 between bHR (n = 4) and bLR (n = 4) animals, which was assessed by mRNA in situ hybridization.

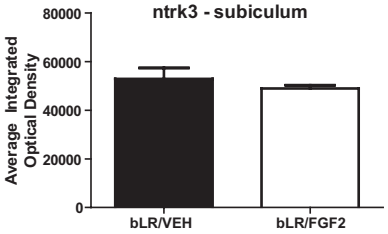

Fig. S5. ntrk3 gene expression was unaltered in the subiculum. There was no difference in ntrk3 gene expression, which was assessed by mRNA in situ hybridization, in bLR animals treated with either vehicle (n = 4) or FGF2 (n = 4) early in life.

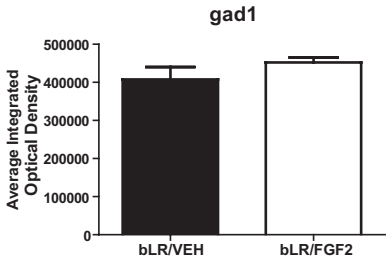

Fig. S6. gad1 gene expression was unaltered in the dentate gyrus. There was no significant difference in gad1 gene expression, which was assessed by mRNA in situ hybridization, in bLR animals treated with either vehicle (n = 4) or FGF2 (n = 4) early in life.

Table S1. Top five functions of differentially expressed genes in the dentate gyrus above threshold by Ingenuity analysis in bLR/FGF2 compared with bLR/VEH rats

Function	GI_	Gene name
Cellular assembly and organization (7 genes)		
Up	5426176	ras homology gene family, member A
Up	62662407	Microtubule-associated protein 1S
Up	13592114	Thyroid hormone receptor-α, transcript variant 2
Down	13929021	LIM motif-containing protein kinase 1
Down	11560084	Connective tissue growth factor
Down	14389298	Vimentin
Down	6981709	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta
Cellular movement (8 genes)		
Up	5426176	ras homology gene family, member A
Up	62648154	Inhibitor of growth family, member 4
Up	6981667	Tenascin R
Down	13929021	LIM motif-containing protein kinase 1
Down	14389298	Vimentin
Down	11560084	Connective tissue growth factor
Down	13929143	Trophoblast glycoprotein
Down	8393405	Glutamic acid decarboylase 1
Cellular compromise (4 genes)		
Up	5426176	ras homology gene family, member A
Down	11560084	Connective tissue growth factor
Down	14389298	Vimentin
Down	13929021	LIM motif-containing protein kinase 1
Neurological disorders (20 genes)		
Up	62655720	Adenylate cyclase 9
Up	13929025	Potassium voltage-gated channel, Shal-related family, member 2
Up	62640415	Kruppel-like factor 13
Up	16758421	Membrane-associated guanylate kinase, WW and PDZ domain containing 2
Up	62647237	Membrane protein, palmitoylated 6
Up	21955258	Olfactomedin 3
Up	47059192	Prefoldin 6
Up	26080405	Protein phosphatase 1, regulatory (inhibitor) subunit 14c
Up	13592114	Thyroid hormone receptor-α, transcript variant 2
Down	13676852	CDP-diacylglycerol synthase 1
Down	11560084	Connective tissue growth factor
Down	8393405	Glutamic acid decarboylase 1
Down	17865328	Gap junction protein, beta 6
Down	13929021	LIM motif-containing protein kinase 1
Down	51036656	Proline 4-hydroxylase, α-polypeptide 1
Down	61557231	PQ loop repeat containing 1
Down	6978788	SPARC-like 1
Down	14389298	Vimentin
Down	42627868	Vitamin K epoxide reductase complex, subunit 1-like 1
Down	6981709	Trosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta
Cell morphology (9 genes)		
Up	16758421	Membrane-associated guanylate kinase, WW and PDZ domain containing 2
Up	5426176	ras homology gene family, member A
Up	13592114	Thyroid hormone receptor-α, transcript variant 2
Up	62648154	Inhibitor of growth family, member 4
Up	47059192	Prefoldin 6
Down	13929021	LIM motif-containing protein kinase 1
Down	14389298	Vimentin
Down	6981709	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta
Down	62646388	Sulfatase

Other Supporting Information Files

Dataset S1 (XLSX)