

Supporting Information

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SI Experimental Procedures

Synthesis of Riboprobes. The riboprobes for nerve growth factor inducible clone A, dopamine D1, and D2 receptor were synthesized as described (1, 2). For BDNF and DARPP-32, new probes were prepared by amplifying a unique region of BDNF and DARPP-32 (GenBank accession no. AK017559 nucleotides 360–641, and GenBank accession no. AY601872 nucleotides 719–1243, respectively) from a rat brain cDNA library. The amplified cDNA fragment was subcloned into a pCR1III-TOPO vector (Invitrogen) and confirmed by nucleotide sequencing. Linearized subclones were used to synthesize ^{35}S UTP-labeled riboprobes. In vitro transcription was carried out by using the MAXIsript SP6/T7 kit (Applied Biosystems) and $[\alpha^{35}\text{S}]$ UTP (NEG039H; Perkin-Elmer) according to the manufacturer's instructions. The transcripts were purified by using NucAway spin columns (Applied Biosystems).

Quantification. Films were scanned with an Epson Perfection 4990 scanner as grayscale film, using 800 pixels and saved as high-quality JPEGs. Optical density values were quantified by using appropriate software (NIH Image J version 1.29, National Institutes of Health). A ^{14}C step standard (GE Healthcare) was included to calibrate optical density readings and convert measured values into nCi/g. All comparisons between groups were made on sections hybridized together under identical conditions and exposed for the same time of period to β -Max film (VWR). Anatomical regions were identified and subdivided for densitometric analysis according to the stereotaxic atlas of ref. 3. Statistical analysis of mRNA expression was performed by using one-way ANOVA, and individual comparisons were made by using Bonferroni/Dunn test.

1. Ronnback A, Dahlqvist P, Bergstrom SA, Olsson T (2005) Diurnal effects of enriched environment on immediate early gene expression in the rat brain. *Brain Res* 1046: 137–144.
2. Diaz R, Sokoloff P, Fuxe K (1997) Codistribution of the dopamine D3 receptor and glucocorticoid receptor mRNAs during striatal prenatal development in the rat. *Neurosci Lett* 227:119–122.

3. Paxinos G, Franklin K (2007) *The Mouse in Stereotaxic Coordinates* (Academic Press, Burlington, MA).

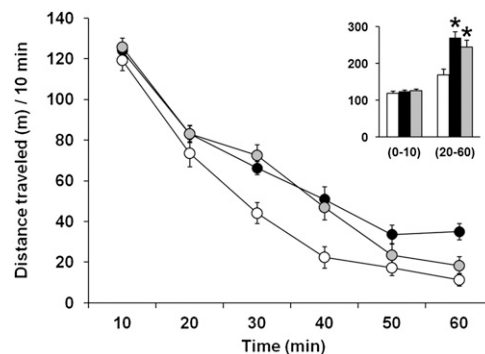


Fig. S1. Spontaneous locomotor activity in the open-field test. Average distance traveled (meters) measured in 10-min time bins across a 60-min session in an open field/activity box. (Inset) Bars show cumulative distance traveled (meters) during the initial 10 min of testing and during the 20–60 min time interval. All data are mean (\pm SEM; $n = 7$ –14 per group). White bars/circles, SPF mice. Black bars/circles, GF mice. Light gray bars/circles, CON mice. Lines connecting cumulative data were drawn for clarity only. * $P < 0.05$ compared with SPF mice.

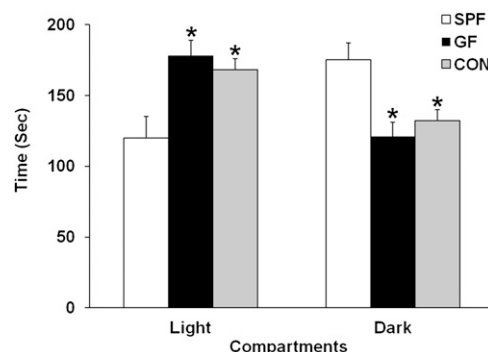


Fig. S2. Anxiety-like behavior in the light–dark box test. Bars show time (seconds) spent in the light and dark compartments during a 5 min light–dark box test by the SPFC, GF, and CON mice. All data are mean (\pm SEM; $n = 7$ –8 per group). * $P < 0.05$ compared with SPF mice.

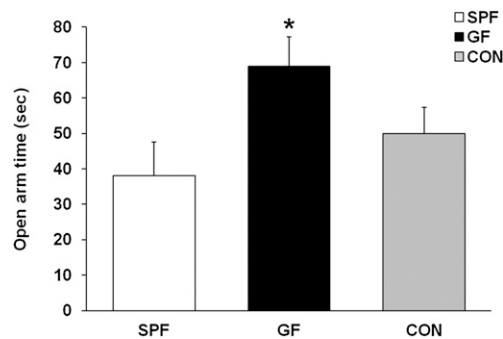


Fig. S3. Anxiety-like behavior in the elevated plus maze. Bars show time (seconds) spent in the open arm of the elevated plus maze by the SPF, GF, and CON mice during a 5-min test session. All data are mean (\pm SEM; $n = 7-9$ per group). * $P < 0.05$ compared with SPF mice.

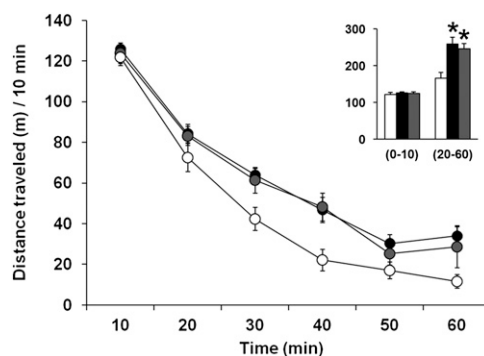


Fig. S4. Spontaneous locomotor activity in the open field test. Average distance traveled (meters) measured in 10-min time bins across a 60-min session in an open field/activity box. (Inset) Bars show cumulative distance traveled (meters) during the initial 10 min of testing and during the 20- to 60-min time interval. All data are mean (\pm SEM; $n = 7-14$ per group). White bars/squares, SPF mice. Black bars/squares, GF mice. Dark gray bars/squares, CON mice. Lines connecting cumulative data were drawn for clarity only. * $P < 0.05$ compared with SPF mice.

Table S1. Monoamine levels and major metabolite/neurotransmitter ratios in various brain regions of SPF and GF mice

Monoamine	Frontal cortex		Striatum		Hippocampus	
	SPF	GF	SPF	GF	SPF	GF
NA	0.207 (0.009)	0.185 (0.008)	0.065 (0.010)	0.070 (0.004)	0.251 (0.007)	0.270 (0.008)
MHPG	0.032 (0.001)	0.030 (0.002)	0.010 (0.000)	0.017 (0.002)	0.030 (0.001)	0.032 (0.001)
MHPG/NA	0.154 (0.004)	0.163 (0.005)	0.162 (0.010)	0.244 (0.022)	0.121 (0.002)	0.119 (0.003)
DA	0.022 (0.002)	0.021 (0.001)	7.513 (0.340)	8.468 (0.378)	0.022 (0.004)	0.020 (0.003)
DOPAC	0.039 (0.003)	0.039 (0.003)	0.757 (0.049)	1.066 (0.036)	0.017 (0.001)	0.020 (0.002)
HVA	0.059 (0.004)	0.054 (0.004)	0.743 (0.027)	0.876 (0.039)	0.021 (0.001)	0.022 (0.001)
DOPAC/DA	1.854 (0.108)	1.840 (0.046)	0.100 (0.002)	0.126 (0.004)	0.868 (0.092)	1.094 (0.132)
HVA/DA	2.810 (0.157)	2.562 (0.065)	0.099 (0.002)	0.104 (0.001)	1.050 (0.108)	1.226 (0.158)
5-HT	0.473 (0.028)	0.507 (0.022)	0.310 (0.011)	0.368 (0.017)	0.404 (0.018)	0.414 (0.017)
5-HIAA	0.185 (0.009)	0.195 (0.020)	0.165 (0.004)	0.245 (0.019)	0.242 (0.009)	0.256 (0.012)
5-HIAA/5-HT	0.394 (0.015)	0.382 (0.029)	0.535 (0.017)	0.662 (0.021)	0.600 (0.012)	0.620 (0.027)

All values represent means \pm SEM. The concentration of NA, DA, and 5-HT and their major metabolites in each brain region are expressed as nanograms per milligram of tissue wet weight. Bold denotes where GF mice differ significantly ($P < 0.01$; $n = 6$ per group) from SPF mice according to post hoc comparisons made with Bonferroni/Dunn test.

Table S2. Quantitative gene expression analysis in hippocampus using TaqMan real-time PCR assay

Transcript ID	Gene name	TaqMan assay ID (ABI)	Q-PCR	
			Fold change	P value
NM_008312.4	5-HT2C	Mm00434127_m1	1.54	0.03
XM_194020.3	<i>Acvr1c</i>	Mm01331057_m1	1.67	0.01
NM_029267.1	<i>Boll</i>	Mm01255489_m1	1.45	0.05
NM_138304.1	<i>Calml4</i>	Mm00460360_m1	3.14	0.03
NM_033037.2	<i>Cdo1</i>	Mm00473573_m1	1.91	0.009
NM_145827.1	<i>Cias1</i>	Mm00840904_m1	1.79	0.01
NM_016674.2	<i>Cldn1</i>	Mm00516701_m1	3.57	0.02
NM_018827.1	<i>Crlf1</i>	Mm00517026_m1	1.42	0.05
NM_007789.2	<i>Cspg3</i>	Mm00484007_m1	1.81	0.008
NM_010307.2/NM_177137.4	<i>Gnal</i>	Mm00622607_m1	1.80	0.02
NM_181748.1	<i>Gpr120</i>	Mm00725193_m1	2.14	0.04
XM_144986.2	<i>Grm7</i>	Mm01189424_m1	1.91	0.02
NM_023422.1	<i>Hist1h2bc</i>	Mm00517807_m1	1.79	0.04
NM_008287.2	<i>Hrsp12</i>	Mm00476177_m1	1.92	0.01
NM_008324.1	<i>Indo</i>	Mm01218007_m1	1.95	0.002
NM_010601.2	<i>Kcnh3</i>	Mm01310216_m1	1.81	0.02
NM_013823.1	<i>Ki</i>	Mm00502002_m1	3.21	0.04
NM_013532.1	<i>Lilrb4</i>	Mm01614371_m1	2.46	0.0003
NM_001011758.1	<i>Olfr1016</i>	Mm02345429_s1	3.15	0.01
NM_146632.1	<i>Olfr116</i>	Mm00837370_s1	1.64	0.04
NM_001011795.1	<i>Olfr1275</i>	Mm00528474_s1	2.11	0.05
NM_146381.1	<i>Olfr1284</i>	Mm00526561_s1	2.24	0.03
NM_001001809.1	<i>Olfr218</i>	Mm00844445_s1	1.90	0.02
NM_146672.1	<i>Olfr816</i>	Mm00837547_s1	1.95	0.02
NM_008806.1	<i>Pde6b</i>	Mm00476679_m1	1.81	0.04
NM_011868.1	<i>Peci</i>	Mm00478725_m1	1.88	0.02
NM_011123.1	<i>Plp</i>	Mm01297210_m1	1.99	0.03
NM_027869.1	<i>Pnpt1</i>	Mm00466286_m1	1.90	0.01
NM_009052.1	<i>Rex3</i>	Mm00784371_s1	1.39	0.02
NM_025589.1	<i>Rpl36al</i>	Mm01623038_g1	1.47	0.03
NM_023374.3	<i>Sdhb</i>	Mm00458268_m1	1.76	0.008
NM_013657.4	<i>Sema3c</i>	Mm00443129_m1	1.93	0.02
NM_029949.1	<i>Snpc3</i>	Mm00712813_m1	1.62	0.03
NM_019879.1	<i>Suclg1</i>	Mm00451244_m1	1.84	0.006
NM_030682.1	<i>Tlr1</i>	Mm00446095_m1	1.91	0.03

Table S3. Quantitative gene expression analysis in frontal cortex using TaqMan real-time PCR assay

Transcript ID	Gene name	TaqMan assay ID (ABI)	Q-PCR	
			Fold change	P value
XM_194020.3	<i>Acvr1c</i>	Mm01331057_m1	-1.53	0.01
NM_016674.2	<i>Cldn1</i>	Mm00516701_m1	3.26	0.03
NM_010307.2/NM_177137.4	<i>Gnal</i>	Mm00622607_m1	-1.78	0.02
NM_008324.1	<i>Indo</i>	Mm01218007_m1	-1.65	0.004
NM_010601.2	<i>Kcnh3</i>	Mm01310216_m1	-1.51	0.0004
NM_019394	<i>Mia1</i>	Mm00444563_m1	1.77	0.03
NM_010807.2	<i>Mlp</i>	Mm00456784_m1	2.08	0.02
NM_001011758.1	<i>Olfr1016</i>	Mm02345429_s1	1.85	0.05
NM_009052.1	<i>Rex3</i>	Mm00784371_s1	1.44	0.03
NM_029949.1	<i>Snpc3</i>	Mm00712813_m1	1.23	0.03
NM_030682.1	<i>Tlr1</i>	Mm00446095_m1	-1.64	0.02

Table S4. Quantitative gene expression analysis in striatum using TaqMan real-time PCR assay

Transcript ID	Gene name	TaqMan assay ID (ABI)	Q-PCR	
			Fold change	P value
NM_010307.2/NM_177137.4	<i>Gnal</i>	Mm00622607_m1	-1.68	0.008
NM_008324.1	<i>Indo</i>	Mm01218007_m1	-1.38	0.03
NM_019911.2	<i>Tdo2</i>	Mm01218212_m1	-1.56	0.02
NM_030682.1	<i>Tlr1</i>	Mm00446095_m1	-1.85	0.001



Movie S1. The elevated plus maze is a sensitive test for the degree of anxiety in rodents. A GF mouse illustrates representative recording of the behavior pattern presented in Fig. 2B. In addition, we have included a frozen image of the GF mouse. The behavior of the animal was recorded with a video camera and later scored by two independent observers. The following behaviors were scored: open and closed arm entries, time spent in the closed and open arms as well as in the center and exploration of open arm ends.

[Movie S1](#)



Movie S2. The elevated plus maze is a sensitive test for the degree of anxiety in rodents. A specific pathogen-free mouse illustrates representative recording of the behavior pattern presented in Fig. 2B. In addition, we have included a frozen image of the specific pathogen-free mouse. The behavior of the animal was recorded with a video camera and later scored by two independent observers. The following behaviors were scored: open and closed arm entries, time spent in the closed and open arms as well as in the center and exploration of open arm ends.

[Movie S2](#)