Supporting Information

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SI Materials and Methods

In Vivo Brain Magnetic Resonance Imaging (MRI). In vivo brain MRI was performed in 6-month-old *Tph2*$^{-/-}$ and control male mice (C57BL/6 genetic background). For the MRI experiments anesthesia was induced using isoflurane (1.0–2.0 ppm in oxygen) keeping the animals free breathing at a rate between 50 and 70 per minute. Body temperature and breathing rate were monitored during the whole experiment.

Images were acquired on a 9.4T MRI system with the use of a 4-element surface coil dedicated for mouse brain imaging (Biospect, Bruker). A set of coronal and axial slices were acquired in each animal using a T2 weighted turbo spin echo MRI sequence with the following imaging parameters: field of view (FOV) 2.5 × 2.5 cm; matrix 384 × 512; echo time (TE) 33ms; repetition time (TR) 2200 (coronal slices) and 3800 (axial slices), slice thickness 0.3 mm, resulting in an in-plane resolution of 49 × 65 μm. Images were analyzed using ImageJ software (Rasband, WS, ImageJ, National Institutes of Health, http://rsb.info.nih.gov/ij/, 1997–2008).

Recording and Analysis of Ultrasonic Vocalization. Ultrasonic vocalization was measured in *Tph2*$^{-/-}$ and their control littermates (FVB/N-F7 genetic background) at day 1 and day 2 after birth (day of birth was counted as day 0), before the test pups were visually examined for the presence of milk in the stomach. To induce ultrasound vocalization, pups were one by one carefully isolated from their mother and transferred to a new cage for 8 min at room temperature (20.5–23.0 °C). Ultrasound vocalizations were recorded during 8 min using a condenser ultrasound microphone (CM16/CMPA, Avisoft Bioacoustics) sensitive to frequencies of 10–200 kHz. It was connected via an Avisoft UltraSoundGate 116 Hbm device (Avisoft Bioacoustics) to a personal computer, where acoustic data were displayed in real time by an Avisoft Recorder (version 3.4, Avisoft Bioacoustics) and were recorded with a sampling rate of 250,000 Hz in 16-bit format. For acoustical analysis, recordings were transferred to SASLabPro (version 4.51, Avisoft Bioacoustics) and a fast Fourier transform was conducted (512 FFT length, 100% frame, Hamming window, and 75% time window overlap). Spectrograms were produced at 488 Hz of frequency resolution and 0.512 ms of time resolution.

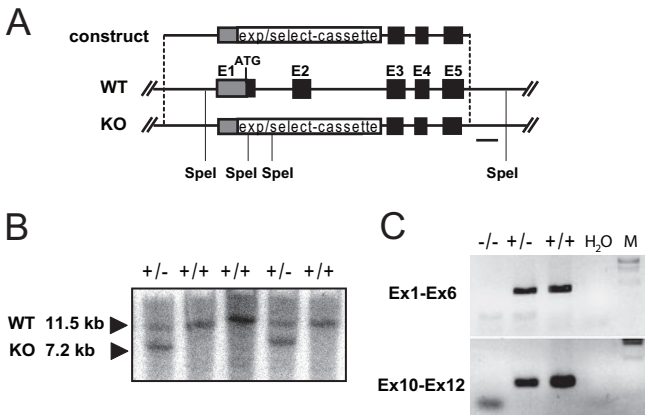


Fig. S1. Generation of *Tph2*-deficient mice. (*A*) The coding part of exon 1 (E1), including the first ATG and the whole exon 2 (E2) of the mouse *Tph2* gene were exchanged with a expression/selection cassette by homologous recombination in embryonic stem (ES) cells. WT, wild type; KO, knockout allele; black boxes, coding sequence; shaded box, 5′-UTR; bold line, Southern blot probe. (*B*) Representative Southern blot analysis of ES cell DNA digested with SpeI. (*C*) *Tph2* expression in the brain analyzed by RT-PCR with primers TPH2Ex1.5 and TPH2Ex6.3 (*Upper* panel) and TPH2Ex10.5 and TPH2Ex12.3 (*Lower* panel). M, DNA size marker (λ HindIII/EcoRI); +/+, $Tph2^{+/+}$; +/−, $Tph2^{+/-}$; −/−, $Tph2^{-/-}$ mice.

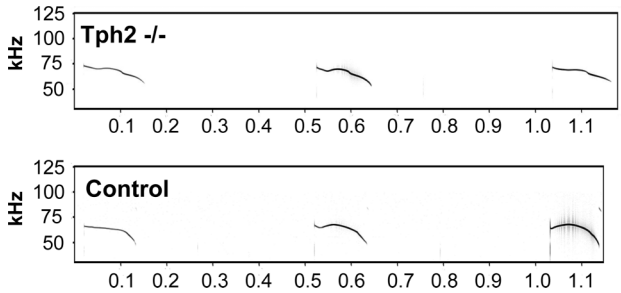


Fig. S2. Representative examples of ultrasonic vocalizations emitted by a *Tph2*$^{-/-}$ and a control pup (FVB/N-F7 genetic background) at postnatal day 1 after isolation from mother and nest.

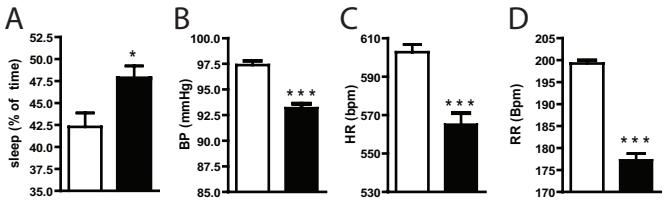
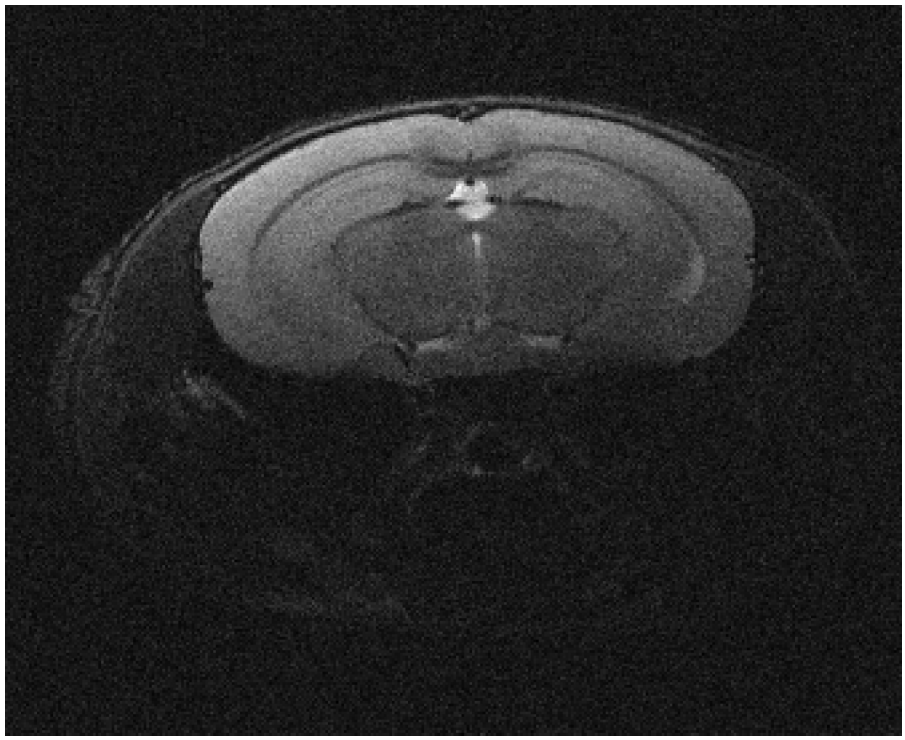
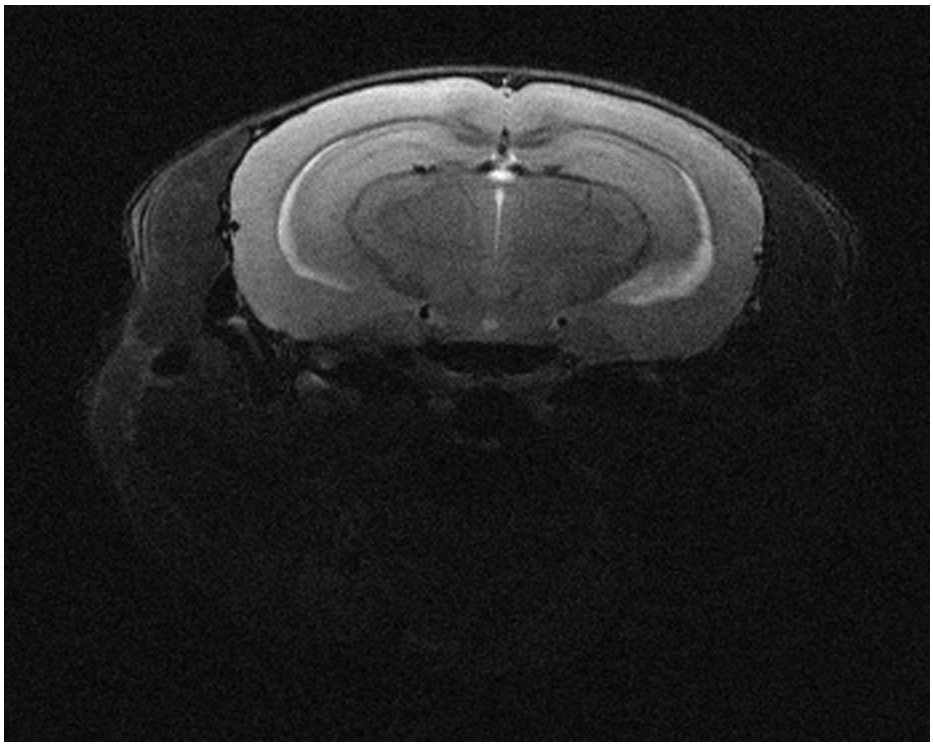


Fig. S3. Telemetric analysis of physiological parameters in *Tph2*-deficient mice. Alterations in sleep (*A*), mean arterial blood pressure (BP) (*B*), heart rate (HR) in beats per minute (bpm) (*C*), and respiratory rate in breaths per minute (Bpm) (*D*) in *Tph2*-deficient mice (6-month-old males, FVB/N-F7 genetic background). Parameters were averaged from 12 a.m. until 6 p.m. for 5 consecutive days. Filled bars, $Tph2^{-/-}$ ($n = 7$); open bars, control mice ($n = 10$). *, $P < 0.05$, ***, $P < 0.001$ (Student's *t* test) $Tph2^{-/-}$ vs. control mice.



Movie S1. Representative brain MRI (axial slices) of a 6-month-old control male mouse (C57BL/6-F6 genetic background).

Movie S1



Movie S2. Representative brain MRI (axial slices) of a 6-month-old *Tph2*-deficient male mouse (C57BL/6-F6 genetic background).

Movie S2

Movie S3. Pup-retrieval test (control mother, mixed background).

Movie S3

Movie S4. Pup-retrieval test (*Tph2*$^{-/-}$ mother, mixed background).

Movie S4

Table S1. Levels of 5-HT and its metabolite 5-HIAA in brain regions of *Tph2*-deficient mice

	Striatum		Frontal cortex		Hippocampus		Hypothalamus	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
$Tph2^{-/-}$	7.67 ± 2.56***	ND	10.78 ± 1.12***	ND	7.54 ± 1.95***	ND	7.13 ± 4.76***	ND
$Tph2^{+/-}$	718.98 ± 23.24	286.27 ± 9.36$^{\#\#}$	858.94 ± 38.09	159.18 ± 7.78	1152.21 ± 30.09	316.22 ± 10.98$^{\#}$	1738.09 ± 50.28	306.87 ± 8.26$^{\#\#\#}$
$Tph2^{+/+}$	623.68 ± 68.44	345.92 ± 17.31	840.75 ± 34.85	180.22 ± 10.85	1048.17 ± 68.55	379.69 ± 36.44	1932.18 ± 74.18	423.30 ± 23.56

Serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured by HPLC in lysates of brain regions from $Tph2^{-/-}$ ($n = 5$), $Tph2^{+/-}$ ($n = 11$), and $Tph2^{+/+}$ ($n = 6$) mice (mixed background). Data are presented in picograms per milligram of wet tissue as mean ± SEM; ND, not detectable. ***, $P < 0.001$ vs. $Tph2^{+/+}$ and $Tph2^{+/-}$ 1-way ANOVA, followed by Tukey's post hoc test; $^{\#}$, $^{\#\#}$, and $^{\#\#\#}$, $P < 0.05$, 0.01, and 0.001, respectively, vs. $Tph2^{+/+}$; Student's *t* test.

Table S2. Levels of 5-HT and its metabolite 5-HIAA in peripheral tissues and blood of *Tph2*-deficient mice

	Duodenum (pg)		Liver (pg)		Blood (ng/mL)		Spleen (pg)	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT,	5-HIAA
$Tph2^{-/-}$	7053.50 ± 701.78	2547.10 ± 349.66	70.58 ± 5.51	ND	4138.0 ± 426.4	ND	3208.03 ± 443.72	ND
$Tph2^{+/-}$	8609.05 ± 605.91	3166.91 ± 292.54	86.69 ± 14.60	ND	4161.3 ± 664.2	ND	4180.70 ± 300.04	ND
$Tph2^{+/+}$	6374.44 ± 483.94	2851.19 ± 546.21	95.81 ± 14.37	ND	3887.9 ± 444.8	ND	3033.81 ± 432.07	ND

Serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured by HPLC in tissue lysates and in whole blood of $Tph2^{-/-}$ ($n = 5$), $Tph2^{+/-}$ ($n = 4$) and $Tph2^{+/+}$ ($n = 6$) mice (FVB/N-F7 genetic background). For duodenum, liver, and spleen data are presented in picograms per milligram of wet tissue as mean ± SEM; ND, not detectable. No significant difference was observed between genotypes.