Supporting Information

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SI Materials and Methods

Construction of vMo, vMo-CT$_{5\text{-}HT2C}$, and 5-HT$_{2C}$-eGFP. Human melanopsin (vMo; cDNA clone BC113558.1) was obtained from Source BioScience and cloned into NheI and SacII restriction sites of the pmCherry-N1 vector using fusion PCR. The forward primer comprising the NheI restriction site and Kozak sequence, 5′-GCT AGC ACC ATG ATG AAC CCT CCT TCG GGG CCA AGA GTC CTG-3′, and the reverse primer, 5′-CCG CGG CAT CCT GGG GTC CTG GCT GGG GAT CAG CCC-3′, were created against the last coding nucleotids of vMo.

To construct adeno-associated virus (AAV) expression vectors, we modified pAAV-MCS vector (Stratagene) using the gateway vector conversion system (Invitrogen). In brief, cassette A (Invitrogen) was inserted into the HincII restriction site via blunt-end ligation to create a gateway destination vector. Entry clones were generated by cloning the gene of interest into a pENTR/D-TOPO shuttle vector according to the manufacturer's protocol (forward primer for directional cloning into pENTR/D-TOPO, 5′-C ACC ATG ATG AAC CCT CCT TCG GGG CCA AGA GTC CTG-3′). The reverse primer was created against the last coding nucleotide of mCherry with a multiple cloning site for the appendix of 5-HT$_{2c}$ C terminus and a stop codon (reverse primer, 5′-CTA GAT ATC GGT ACC ACT AGT CTT GTA CAG CTC GTC CAT GCC GCC-3′). LR recombination was performed to create final AAV expression clones. The C terminus of the human 5-HT$_{2c}$ receptor was fused to the expression clone using SpeI and KpnI restriction sites at the 3′ end of mCherry (forward primer, 5′-ACT AGT AAC AAA ATT TAC CGA AGG GCA TTC -3′; reverse primer, 5′-GAT ATC GGT ACC TCA CAC ACT GCT AAT CCT TTC GCT-3′). 5-HT$_{2c}$ receptor cDNA (Missouri S&T cDNA Resource Center) was cloned into NheI and SacII restriction sites of the peGFP-N1 vector (forward primer with Kozak sequence, 5′-GCT AGC ACC ATG GTG AAC CTG AGG AAT GCG GTG CAT TCA-3′; reverse primer, 5′- CCG CGG CAC ACT GCT AAT CCT TTC GCT AAC CAC ACT GGA-3′). The primer for directional cloning into AAV expression vector was as follows: forward, 5′-C ACC ATG GTG AAC CTG AGG AAT GCG GTG CAT; reverse, 5′-CTA GAT ATC GGT ACC ACT AGT CAC CTC GTT CTC GTA CTC GCA GAA CTT GTA CAG CTC GTC CAT GCC GAG-3′.

Double-floxed inverted ORF (DIO) viral vectors were created for cell type-specific expression of vMo-CT$_{5\text{-}HT2c}$ (AAV-DIO-vMo-CT$_{5\text{-}HT2c}$) in Cre recombinase driver mouse lines. Cre-dependent viral vectors were constructed using LR recombination of entry clones with modified pAAV-MCS expression vectors, including an inverted cassette A flanked by two distinct lox sites (LoxP and Lox2272) under the control of an EF1α promoter.

Fluo-4 Calcium Assay. A human embryonic kidney (HEK tsA-201) cell line (a subclone of the human embryonic kidney cell line HEK-293 that expresses the temperature-sensitive simian virus 40 T antigen; American Type Culture Collection) was used for transient expression of vMo, vMo-CT$_{5\text{-}HT2c}$, and 5-HT$_{2c}$-eGFP. Cells were grown in DMEM supplemented with 10% FBS (Life Technologies) and 1000 U penicillin-streptomycin (Life Technologies) in a humidified incubator under 5% CO_2. At 1 d before transfection, HEK tsA-201 cells were seeded onto 24-well plates (CytoOne). For heterologous expression of receptor chimeras, 60–70% confluent cells were transfected using Lipofectamine 2000 Transfection Reagent (Life Technologies) with 1 μg of DNA per plate. At 24 h after transfection, cells were seeded into poly D-lysine–coated, 96-well, black-walled, clear-bottomed microplates (BD Biosciences) and used the next day.

On the third day, release of intracellular Ca^{2+} was measured using the Ca^{2+} indicator dye Fluo-4 (Fluo-4 Direct Calcium Assay Kit; Life Technologies). The Fluo-4 Ca^{2+} assay was performed as described in the manufacturer's manual. Cells were loaded with 50 μL of Fluo-4 calcium indicator dye and incubated for 1 h at 37 °C in 5% CO_2 while protected from light. In certain cases, all-*trans* or 9-*cis* retinal was supplemented to a final concentration of 10 μM. Then the Fluo-4 calcium assay was performed at room temperature using a multilabel plate reader (Victor X3; Perkin-Elmer). Ca^{2+} efflux was indicated by an increase of relative fluorescence. Excitation at 495 nm was achieved using a 485-nm excitation filter.

In each experiment, cells were stimulated with 2-s light pulses at 1-s intervals. Relative fluorescence increase was monitored over 3 min with a 510-nm emission filter using Victor 2030 Workstation software. HEK tsA-201 cells expressing 5-HT$_{2c}$ WT receptor (C-terminally tagged with eGFP) were stimulated with the potent 5-HT$_{2c}$ receptor agonist RO 60-0175 (10 μM) after 10 baseline measurements. Untransfected HEK cells and HEK cells expressing mCherry served as negative controls.

For each condition, fluorescence counts were normalized to the minimum fluorescence signal and averaged over six wells. The relative fluorescence increase was plotted as a function of time. An increase in fluorescence counts corresponds to an increase in intracellular calcium.

Production and Purification of AAVs. AAV1.flex.CBA.Arch-GFP.WPRE.SV40 (Addgene; 22222) and AAV 2/1.CAG.FLEX.tdTomato.WPRE.bGH (Allen Institute; 864) recombinant AAVs were purchased from Penn Vector Core. Viral titers were 1×10^{13} particles/mL. For construction of AAV-vMo-CT$_{5\text{-}HT2c}$ and AAV-DIO-vMo-CT$_{5\text{-}HT2c}$, recombinant AAV stock serotype 2 was produced according to the protocol outlined in the AAV Helper-Free System manual (Stratagene).

Cells. Low-passage AAV-293 HEK cells were maintained in DMEM (4.5 g/L glucose, 110 mg/L sodium pyruvate, and 2 mM L-glutamine) supplemented with 10% (vol/vol) heat-inactivated FBS in a 37 °C incubator at 5% CO_2 in 75-cm^2 tissue culture flasks. At 48 h before transfection, 50% confluent AAV-293 cells were transferred onto 15 100-mm tissue culture plates (1×10^6 cells) in 10 mL of DMEM growth medium.

Transfection. Cells were cotransfected at 70% confluence with equimolar amounts of vMo-CT$_{5\text{-}HT2c}$, pAAV-RC, and pHelper using a calcium phosphate-based protocol. The plasmid DNA was adjusted to a final concentration of 1 μg/μL in TE buffer (10 mM Tris·HCl and 1 mM EDTA; pH 7.5). Then 10 μg of each plasmid was transferred into a 15-mL conical tube, and 15 mL of 0.3 M $CaCl_2$ was added to the DNA solution. The DNA-$CaCl_2$ mixture was transferred dropwise to 15 mL of 2× Hank's balanced salt solution (HBSS) (280 mM NaCl, 1.5 mM Na_2HPO_4, and 50 mM Hepes; pH 7.1) under incidental agitation, followed by immediate application of 2 mL of the DNA-$CaCl_2$-HBSS suspension to each plate of cells under gentle swirling. At 24 h after transfection, the medium was replaced with fresh DMEM growth medium, and cells were returned to the 37 °C incubator for another 48 h.

Harvesting. At 3 d after transfection, cells were removed from the dishes, pelleted (centrifugation at 3,000 × *g* for 10 min at room temperature), resuspended in 7.5 mL lysis buffer (150 mN NaCl, 50 mM Tris·HCl pH 8.5) and lysed via three freeze/thaw cycles in

dry ice/ethanol and a 37 °C water bath (each for 10 min). To eliminate free DNA, the cell suspension was treated with 50 U Benzonase endonuclease (Sigma-Aldrich) for 2 h at 37 °C. The cell debris was spun down at 2,240 × *g* for 15 min at 4 °C. The supernatant was collected in a syringe and filtered into a 15-mL Falcon tube through a 0.45-μm filter unit to obtain the crude lysate, which was stored at 4 °C until purification.

Purification. Recombinant virus particles were purified from crude lysate using heparin-agarose columns. Chromatograph columns (732-1010; Bio-Rad) were filled with 3 mL of heparin-agarose type I (H6508; Sigma-Aldrich) and then incubated with the crude lysate for 2–4 h on a vertical shaker at 4 °C. Once the heparin-agarose had settled, the lysate was drained from the column. The column was washed with equilibration buffer [PBS (Life Technologies), 1 mM $MgCl_2$, 2.5 mM KCl; pH 7.2), and the virus particles were eluted with 15 mL of elution buffer (equilibration buffer with 500 mM NaCl; pH 7.2) into a filter unit (Amicon Ultra-15 100 kDa, UFC910024; Millipore). The virus solution was concentrated with a centrifugation step (2,240 × *g*) until <500 μL remained. To exchange the high-salt concentration buffer, the filter unit was refilled with PBS (Life Technologies; pH 7.4), and centrifugation was repeated twice. The final centrifugation step was run until 200 μL of the virus solution remained. This virus solution was collected in a 1-mL syringe, and aliquots were stored at 4 °C until further use.

Animals, Surgery, Virus Injection, and Implantation. Mice were housed on a 12 h light/dark cycle with food and water available ad libitum. Expression of double-floxed constructs in GABAergic and serotonergic neurons was done using Gad65-Ires-Cre mice [$Gad2^{tm2(cre)Zjh}$/J, stock no. 010802; Jackson Laboratory (1)] and ePet1-Cre mice [B6.Cg-Tg(Fev-cre)1Esd/J, stock no. 012712; Jackson Laboratory (2)].

For optical stimulation of GABAergic and serotonergic neurons, AAVs expressing double-floxed vMo-$CT_{5\text{-}HT2c}$ or Arch-GFP (Penn Vector Core) were injected into the DRN of GAD65-Ires-Cre homozygous or ePet1-Cre mice at age 2–3 mo. Rodents were deeply anesthetized with an i.p. injection of ketamine 10 mg/kg and xylazine 20 mg/kg and placed into a stereotactic frame (Stoelting). Body temperature was controlled with a heating pad. If necessary, additional doses of ketamine were applied to maintain anesthesia. To prevent corneal drying during surgery, the eyes were coated with a moisturizing balm.

The skin was opened with a sagittal incision along the midline, and a small craniotomy was performed for implantation using a microrodrill. The DRN was localized with the aid of stereotactic coordinates from the mouse brain atlas (3). A customized glass pipette attached to a 10-mL syringe was used for virus delivery at −4.5 mm posterior and 0 mm lateral to bregma at a depth of 2 mm. A micromanipulator was used to lower the pipette into the brain. The initial pressure injection was made at a depth of 2 mm, and additional injections were made at 100-μm steps up to 1.5 mm. The pipette was left in place after each injection for approximately 1 min, to ensure virus diffusion into the brain tissue. After the injection, a customized cannula guide (Plastics One) was lowered into the brain and secured to the skull with dental cement and a mounting screw. Dummy cannulae (Plastics One) were inserted to prevent contamination of cannula guides.

After implantation, the skin was sutured with surgical yarn, and carprofen (2 mg/kg) was injected s.c. for analgesia. Animals were placed individually into their home cages and allowed to recover for at least 14 d. Control animals were matched for genetic background and age, but received either saline injections or the same viral vector carrying a fluorophore alone (AAV-CMV-eGFP).

In Vivo Extracellular Recordings and Optical Stimulation. For extracellular in vivo recordings, anesthetized mice were placed into a stereotactic frame. Optrodes consisted of an optical fiber with 200 μm diameter (Thorlabs) fused to a glass-coated tungsten recording electrode (2 MΩ) with a tip-to-tip distance of ∼500 μm. Optrodes were coupled to a blue diode-pumped laser (CrystaLaser CL 2000; λ = 473 nm, 20 mW) for light delivery. Single-unit and multiunit potentials were amplified and filtered (model 1800 microelectrode AC amplifier; gain, 10 kHz; 300 Hz–10 kHz bandpass; A-M Systems). After noise elimination (50/60-Hz Hum Bug noise eliminator; Quest Scientific), potentials were stored with a sampling rate of 20 kHz using an A/D converter (CED Power 1401 mk, Cambridge Electronic Design, Cambridge, UK) and then analyzed offline using Spike2, version 7 (Cambridge Electronic Design). Blue light pulses of 10 s each were triggered with a 5-V TTL pulse. One trial lasted 70 s, including 30 s of baseline recording, 10 s of blue light, and then another 30 s of baseline recording. Ten trials were recorded for each cell. After the experiments, mice were perfused with 4% paraformaldehyde for histological analysis.

Spike sorting was performed using individual thresholds. Peristimulus time histograms (PSTHs) and action potential waveforms were generated using Spike2. Single-unit and multiunit recordings were exported as MATLAB files (MathWorks). Firing rates and interspike intervals were analyzed using MATLAB software. Baseline firing rate and interspike intervals were measured 30 s before light stimulation and 30 s after light stimulation and averaged over 10 trials for each cell. The dorsal raphe serotonergic neurons used for analysis met at least two of the following criteria characteristic of 5-HT–containing neurons (4, 5): slow firing rate (<3 Hz), regular firing pattern (coefficient of variation: SD of the interspike interval divided by the interspike interval mean <0.4), and triphasic extracellular waveform with a wide spike width (>1.5 ms).

Microiontophoresis. Microiontophoresis experiments were performed using three-barrel recording/iontophoresis carbon fiber electrodes (Carbostar-3; Kation Europe). All drugs (Sigma-Aldrich) were prepared in 0.9% NaCl (pH 4) and delivered with a constant-current Union-40 iontophoresis pump (Kation Scientific). Individual barrels contained 5 mM RO 60-0175 and 40 mM serotonin hydrochloride. Local drug administration was conducted using ejection currents of 60 nA, with a retention current of −10 nA to prevent leakage between ejection periods. Control experiments were conducted using four-barrel carbon fiber electrodes (Carbostar-4; Kation Europe), with the center barrel filled with 0.9% saline (pH 4). Each session comprised a 30-s baseline recording followed by a 30-s drug application and then a 30-s recovery period. Each cell was subjected to five stimulation periods. Serotonergic neurons were identified by reduced neuronal activity after iontophoretic application of 5-HT. Single-unit activity was analyzed by waveform sorting using Spike2.

Immunohistochemistry and Image Analysis. Immunohistochemical localization of the $5\text{-}HT_{2c}$ receptor in the DRN was performed using the following primary antibodies: polyclonal goat anti–$5\text{-}HT_{2c}$ receptor antibody (sc-15081, 1:100 dilution; Santa Cruz Biotechnology), monoclonal mouse anti–tryptophan-hydroxylase antibody (1:200 dilution; Sigma-Aldrich), and polyclonal rabbit anti-GAD65 antibody (ABN101, 1:600 dilution; Millipore). Immunohistochemistry was performed according to Bubar et al. (6). In brief, coronal sections (30 μM) were cut on a microtome-cryostat (HM-500; Microm) and collected in 24-well plates (CytoOne). Free-floating mouse brain sections were washed on an orbital shaker with PBS twice for 10 min each wash at room temperature, incubated in 20 mM sodium acetate, and then washed again with PBS twice for 10 min each. Sections were then incubated in 1.5% blocking serum [normal donkey serum (NDS); Millipore] in PBS for 2 h at room temperature to reduce nonspecific antibody binding. The blocking serum was aspirated, and sections were incubated with primary antibodies in 1.5% NDS in PBS for

48 h on an orbital shaker at 4 °C. After three 10-min washes with PBS, anti–species-specific secondary antibodies (donkey anti-mouse Alexa Fluor 568, donkey anti-goat Alexa Fluor 488, donkey anti-rabbit Alexa Fluor 568, and Alexa Fluor 488 donkey anti-rabbit; Life Technologies) diluted in 1.5% NDS (1:1,000) were incubated on the sections for 3 h at room temperature while protected from light, followed by three 10-min rinses with 1× PBS.

Sections were mounted onto Superfrost/Plus Microscope Slides (Thermo Scientific) and coverslipped using either Vectashield fluorescent mounting medium (Vector Laboratories) with or without DAPI (Biozol) or Roti-Mount FluorCare (Carl Roth). Control experiments were conducted to examine antibody specificity and tissue autofluorescence. For each combination of primary and secondary antibodies, no primary or no secondary antibody, as well as mismatched primary/secondary antibody combinations, were used. Control experiments revealed no cross-reactivity between antibodies of different species.

For double-immunohistochemical localization of GAD65 and tryptophan hydroxylase, coronal sections were processed for heat-induced epitope retrieval using an unmasking buffer (10 mM sodium citrate buffer; pH 6) and a water bath. Sections were incubated for 15 min in boiling water. After 30 min of cooling, sections were rinsed twice in PBS and processed as described above.

Tracer studies were performed via pressure injection of double-floxed tdTomato virus (AAV2/1.CAG.FLEX.tdTomato.WPRE.bGH; Penn Vector Core) into the DRN of ePet1-Cre mice. Green fluorescent Nissl stain (NeuroTrace 500/525; Life Technologies) was used to provide an anatomic overview of amydaloid nuclear boundaries.

Digital images were acquired from brain sections using a Leica TCS SP5 confocal laser scanning microscope interfaced to a personal computer running Leica Application Suite AF 2.6 software. Objectives of 10×/0.3 NA, 20×/0.7 NA, and 40×/ 1.1 NA were used to capture all photomicrographs. Sequential Z-stacks were created for each section, and cross-talk among fluorophores was eliminated automatically with the AF 2.6 software. Captured images were transferred into ImageJ 1.45s (National Institutes of Health) for processing and image overlay.

Behavioral Assays. For the behavioral tests, male and female mice age 2–6 mo were injected stereotactically with virus solution and implanted as described above. Mice were caged individually and allowed to recover for at least 2 wk before behavioral testing. Animals were moved from the holding room to the behavior room at least 1 h before the test to allow for acclimatization.

Open-field test. The open-field test arena consisted of an acrylic chamber (30 × 30 × 30 cm; Noldus Information Technology), virtually subdivided into 16 equal squares (square size 7.5 × 7.5 cm) via a 4 × 4 grid and brightly illuminated by several 75W incandescent bulbs mounted above the arena. The inner four squares (14.5 × 14.5 cm) were defined as the center region; the remaining squares, as the border region.

Mice were anesthetized with isoflurane to facilitate withdrawal of the dummy cannula. After a ≥5-min recovery from isoflurane anesthesia, optical stimulation was applied through a fiberoptic cannula (200 μM; Doric Lenses) targeting the DRN. Fibers were attached to a patch cord coupled to a blue diode laser (CL 2000; CrystaLaser). Mice were placed into the center of the open field, and videos were analyzed for the time spent in the center, total distance traveled, and border-to-center transitions using automatic tracking software (EthoVision XT 8.5; Noldus Information Technology). Each individual animal was measured following the same protocol, comprising 3 min of constant light stimulation alternating with 3 min of no optical stimulation. One trial lasted 18 min, consisting of three periods without light stimulation (OFF) and three periods with light stimulation (ON). For each animal, data were averaged over three test periods.

Novelty-suppressed feeding test. Mice were deprived of food for 24–27 h before testing, with water available ad libitum. Individual mice were coupled to a fiber patch cord for constant light delivery under short isoflurane anesthesia and allowed to recover from handling for 5 min. A familiar food pellet (weighing ∼2 g) was placed on a filter paper (60 mm diameter) into the middle of a new aversive environment (arena: 30 × 30 × 30 cm), brightly illuminated with two 75W incandescent bulbs. The laser was switched on for 3 min, and the mice were placed into the corner of the arena covered with clean wood chips. The task ended when the mice first fed, defined as biting the food pellet with use of the forepaws. The latency to start feeding served as a measurement of anxious behavior. Subjects were recorded by a video camera for further analysis. After the experiment, the mice were removed from the experimental setup, released from the fiber patch cord, and placed in their home cages. Food consumption was measured for additional 5 min for potential feeding differences among test groups.

Statistics. Statistical analyses were performed using SigmaPlot 12.0 and IGOR Pro 6.03 (WaveMetrics) software. Data obtained from electrophysiological recordings and behavioral experiments were analyzed for normal distribution using the Shapiro–Wilk test. Within-subject comparisons for normally distributed data were analyzed using the paired t test. In all cases, the Wilcoxon signed-rank test was used to evaluate statistical significance. Differences between groups with normal distribution were determined using the Student t test. Non-normally distributed data were analyzed using the Mann–Whitney rank-sum test. For all results, the significance threshold was set at $P = 0.05$. Individual significant values in the figures are represented as follows: $^{*}P \leq 0.05$; $^{**}P < 0.01$; $^{***}P \leq 0.001$. All data are expressed as mean ± SEM.

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6. Bubar MJ, Seitz PK, Thomas ML, Cunningham KA (2005) Validation of a selective serotonin 5-HT2C receptor antibody for utilization in fluorescence immunohistochemistry studies. *Brain Research* 1063:105–113.

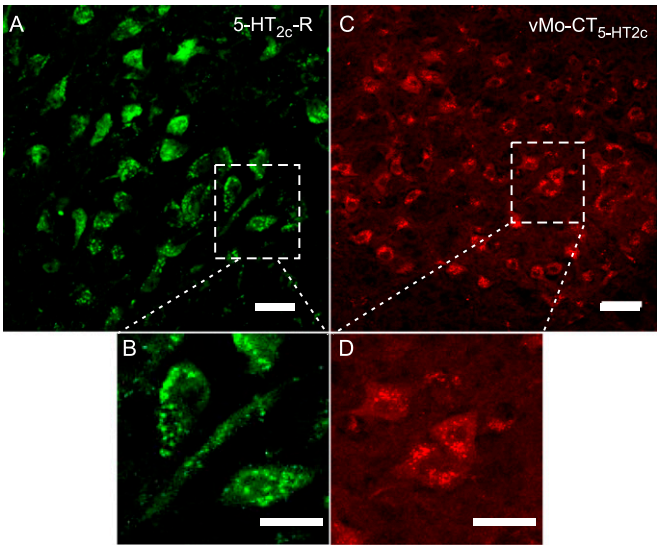

Fig. S1. Functional expression of vMo-$CT_{5\text{-}HT2c}$ in GABAergic neurons of the dorsal raphe. (*A*) Confocal images showing immunolabeling of endogenous 5-HT_{2c}-Rs (green) in the DRN. Boxed area is shown in higher magnification and in more detail in *B*. (*B*) Subcellular distribution of endogenous 5-HT_{2c}-Rs in vesicular structures of DR neurons. (*C*) Functional expression of light-activated vMo-$CT_{5\text{-}HT2c}$ in DR GABAergic neurons at 14 d after virus injection. (*D*) Higher-magnification image of the boxed region in *C* revealing punctate distribution of vMo-$CT_{5\text{-}HT2c}$ (red). Higher-magnification images indicate comparable subcellular distribution patterns in endogenous 5-HT_{2c}-Rs and light-activated vMo-$CT_{5\text{-}HT2c}$. (Scale bars: 50 μm in *A* and *C*; 30 μm in *B* and *D*.)

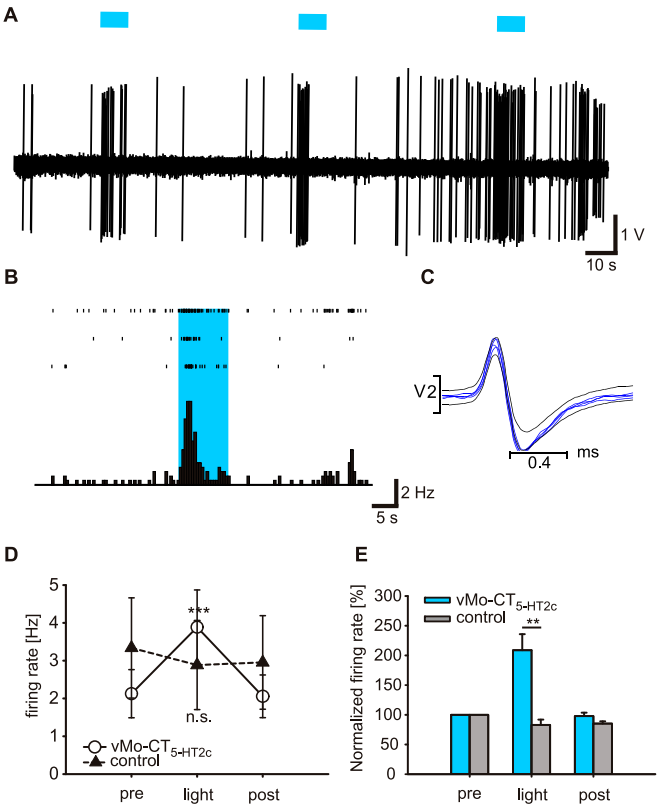

Fig. S2. Optical activation of DR GABAergic cells by vMo-$CT_{5\text{-}HT2c}$. Shown are optrode recordings from GAD65-Ires-Cre mice expressing vMo-$CT_{5\text{-}HT2c}$ in DR GABAergic cells. (*A*) Single trace representing repetitive light-induced activation of a GABAergic cell (blue bar, 10 s). (*B*) PSTH (bin size 500 ms) and raster plot of three sweeps. (*C*) Spike waveform of recorded trace (blue, average waveform). (*D*) Changes in firing rate for recorded cells before (pre), during (light), and after (post) light stimulation for vMo-$CT_{5\text{-}HT2c}$ (pre, 2.13 ± 0.64 Hz; light, 3.89 ± 0.99 Hz; post, 2.06 ± 0.56 Hz; $P < 0.001$; white circles, n = 11 cells from three animals) and control (pre, 3.34 ± 1.33 Hz; light, 2.88 ± 1.18 Hz; post, 2.95 ± 1.24 Hz; $P = 0.184$; black triangles, $n = 5$ cells from one animal). (*E*) Normalized increase in firing rate for recorded cells in *D* (control: pre, 100%; light, 82.93 ± 9.02%; post, 85.21 ± 3.86%; vMo-$CT_{5\text{-}HT2c}$: pre, 100%; light, 208.78 ± 27.01%; post, 97.84 ± 5.73%; $P < 0.002$).

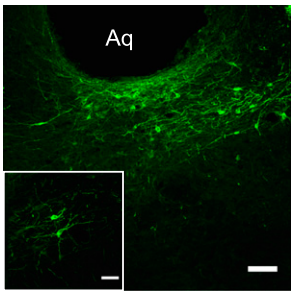

Fig. S3. Arch-GFP expression in the serotonergic dorsal raphe neurons. AAV-flex-Arch-GFP was expressed in ePet1-Cre mice. At 2–6 wk after virus injection, mice were analyzed for anxiety behavior (Fig. 4). After the behavioral experiments, mice were perfused for immunohistochemical experiments. GFP expression indicates that Arch-GFP was localized mainly in the dorsal part of the DRN. Aq, aqueduct. (Scale bars: 100 μm; box, 50 μm.)

Table S1. Numerical changes in firing rates for data presented in Fig. 2

	RO 60-0175			5-HT			Saline		
	Before	Drug	After	Before	Drug	After	Before	Drug	After
Fig. 2*E*, Hz									
Mean	3.20	7.70	3.57	3.64	10.7	3.81	4.05	3.76	4.14
SEM	0.45	0.81	0.49	0.49	1.5	0.44	0.85	0.67	1
P value vs. before		≤0.001			≤0.001			0.368	
Fig. 2*F*, %									
Mean	100	312.74	117.56	100	378.86	112.99	100	93.50	101.65
SEM	0	38.01	7.37	0	49.50	4.92	0	6.06	4.89
P value vs. saline		≤0.001			≤0.001				
Fig. 2*H*, Hz									
Mean	3.68	1.30	4.80	2.74	1.06	3.09	4.05	3.76	4.14
SEM	0.56	0.26	0.82	0.53	0.23	0.57	0.85	0.67	1
P value vs. before		0.002			≤0.001			0.368	
Fig. 2*I*, %									
Mean	100	36.52	151.67	100	38.78	129.97	100	93.50	101.65
SEM	0	8.98	29.91	0	7.19	24.12	0	6.06	4.89
P value vs. saline		≤0.001			≤0.001				

Table S2. Numerical values for data presented in Fig. 4 *E* and *F*

	vMo-$CT_{5\text{-}HT2c}$		Control		Arch-GFP-GAD65		Arch-GFP-ePet	
	OFF	ON	OFF	ON	OFF	ON	OFF	ON
Fig. 4*E*								
Mean	8.7	11.5	9	8.57	16.71	15.14	5.6	7.6
SEM	2.42	2.64	2.96	2.4	3.98	2.6	0.93	2.79
P value vs. OFF		0.284		0.874		0.637		0.449
P value vs. control		0.447				<0.01		0.798
Fig. 4*F*								
Mean	429.48	435.12	405.06	65.29	596.57	568.39	511.39	542.25
SEM	41.47	37.37	15.54	24.68	55.12	48.73	118.3	130.79
P value vs. OFF		0.772		0.331		0.557		0.5
P value vs. control		0.248						