Figure S1, Related to Figure 1

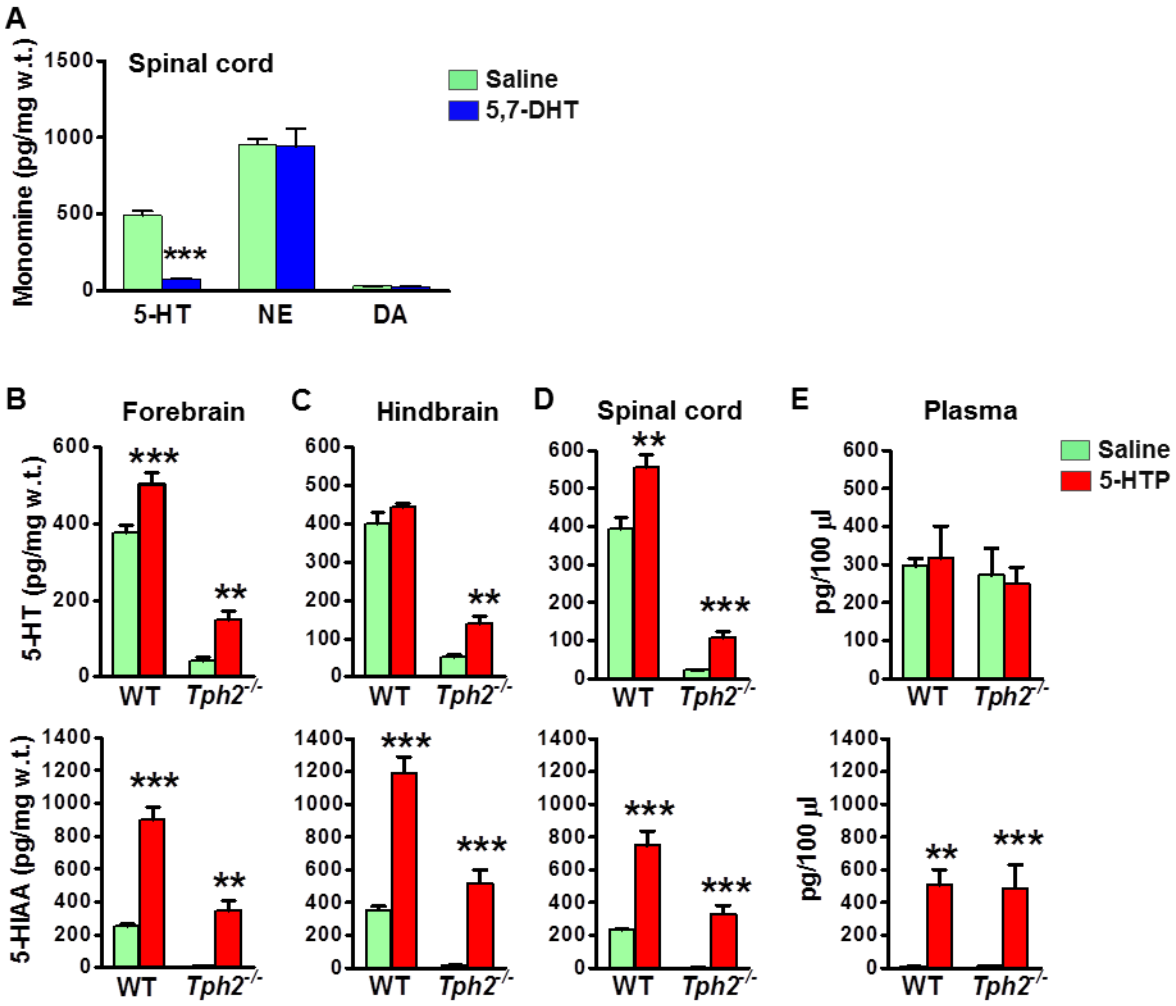

Figure S2, Related to Figure 3

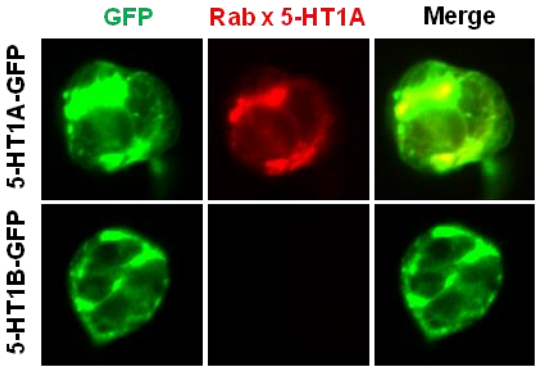

Figure S3, Related to Figure 3

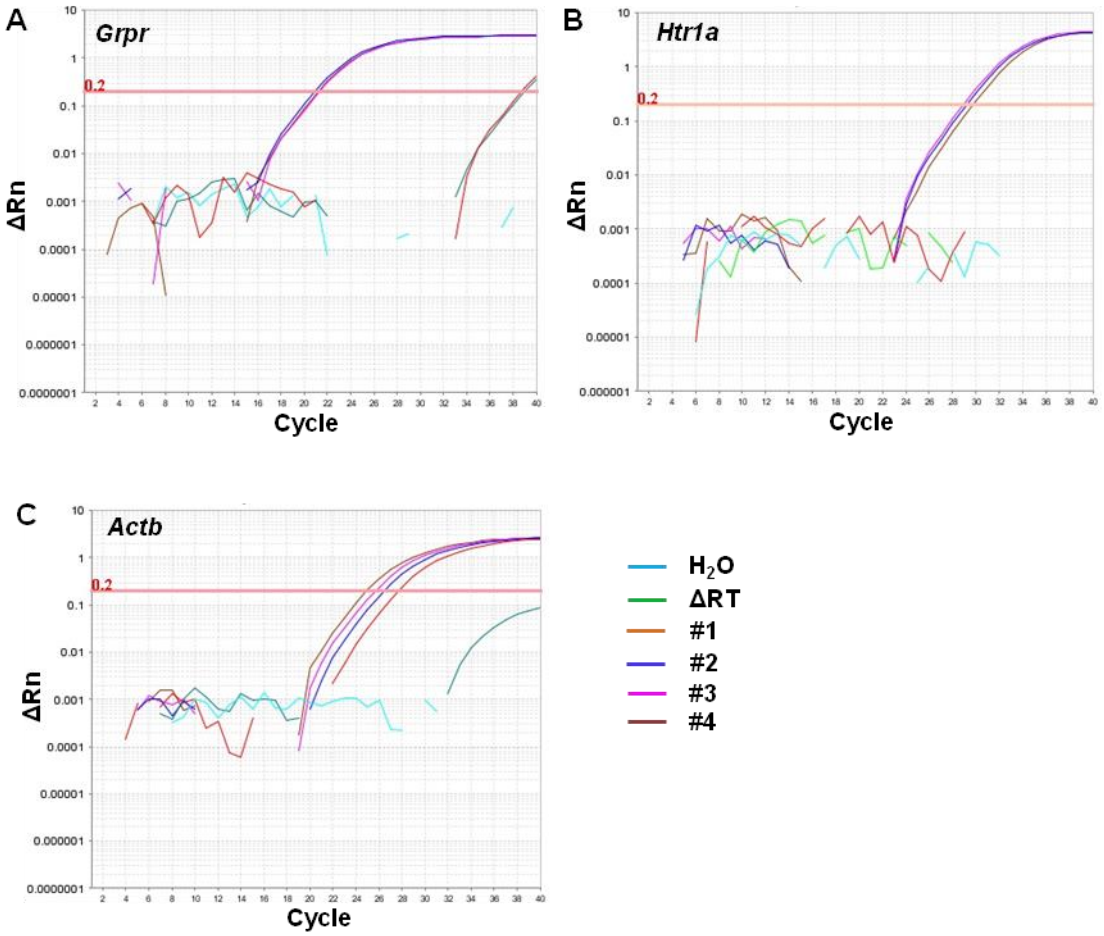

Figure S4, Related to Figure 4

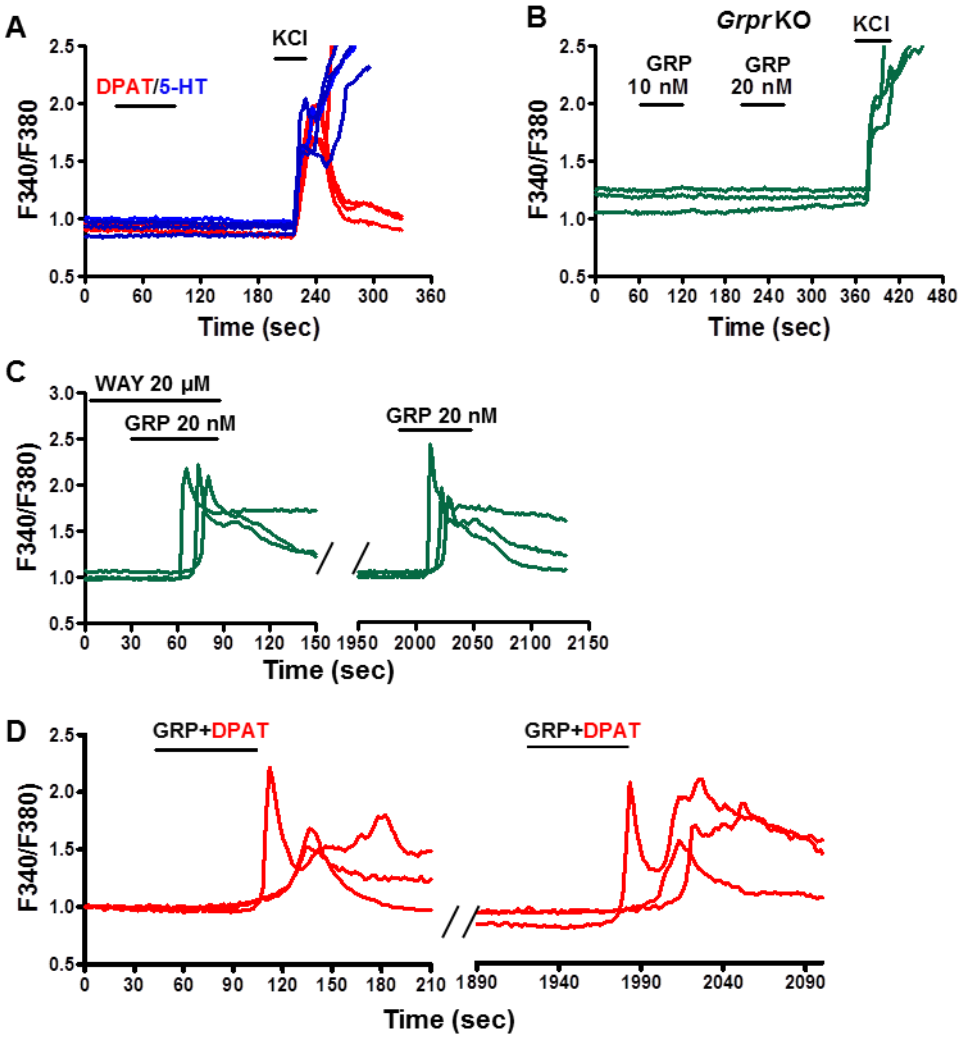

Figure S5, Related to Figure 5

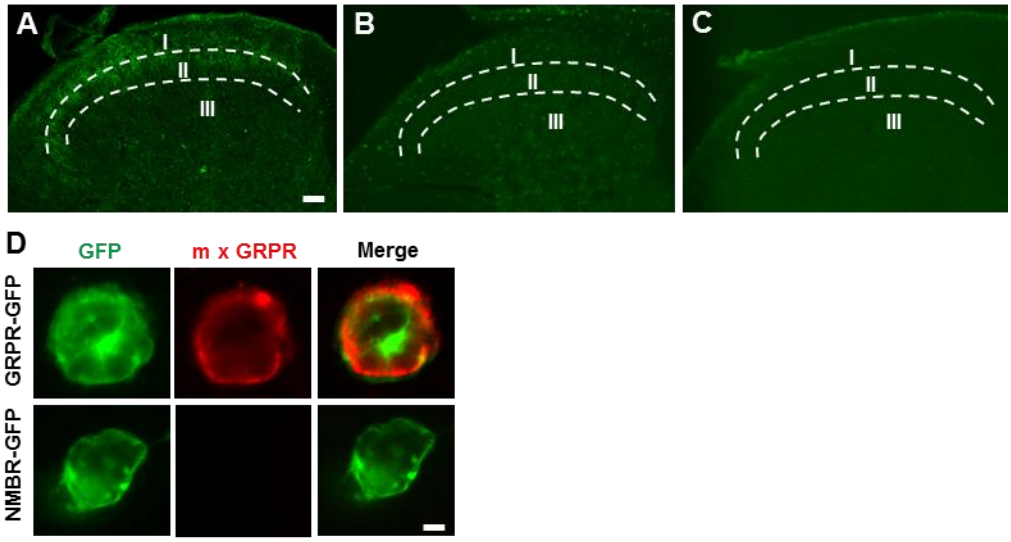

Figure S6, Related to Figure 5

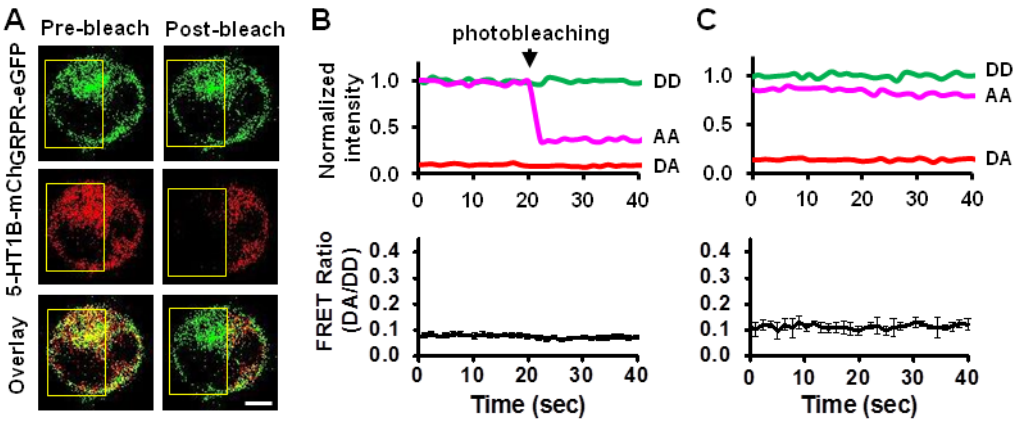

Figure S7, Related to Figure 7

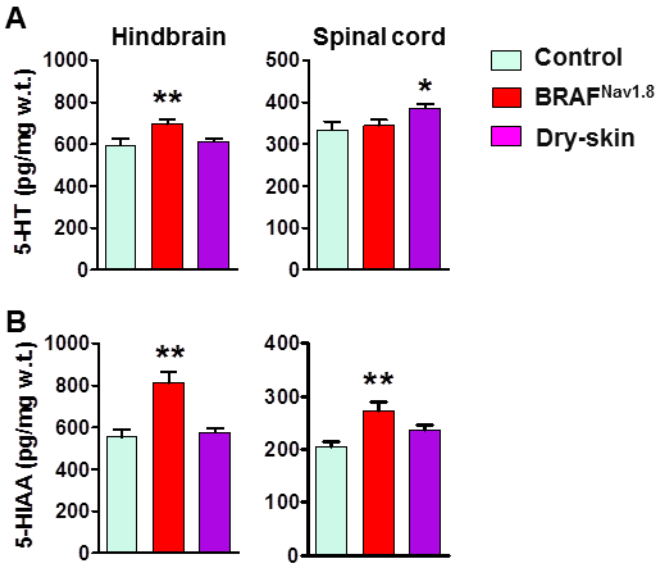

Table S1, Related to Figure 3

Cell #	ΔRT	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Actb	-	+	+	+	+	+	+	+	+	+	+
Grpr	-	-	+	+	+	+	+	+	+	+	+
Htr1a	-	-	+	+	+	-	+	+	+	-	+

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Reduced Spinal 5-HT Level after 5,7-DHT Injection and Enhanced Central 5-HT Concentrations by Systemic 5-HTP Administration, Related to Figure 1.

(A) Spinal 5-HT level was significantly reduced after 5,7-DHT treatment as detected by HPLC, while the levels of noradrenaline (NE) and dopamine (DA) were not affected.

(B-E) The concentrations of 5-HT (upper row) and 5-HIAA (lower row) in the forebrain (B), hindbrain (C), spinal cord (D) and plasma (E) were detected by HPLC with electrochemical detection. n = 4-5.

Error bars represent SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, versus saline.

Figure S2. 5-HT1A Antibody Is Specific, Related to Figure 3.

Rabbit anti-5-HT1A antibody (Rab x 5-HT1A) specifically labeled HEK 293 cell expressing 5-HT1A-GFP (red, upper row), but not 5-HT1B-GFP (lower row). Successful expression of 5-HT1A-GFP and 5-HT1B-GFP was confirmed by GFP fluorescence (green).

Figure S3. Co-Expression of *Grpr* and *Htr1a* in GRPR-eGFP Neurons, Related to Figure 3.

(A and B) qRT-PCR traces showing that *Grpr* (A) and *Htr1a* (B) were detected in 3 spinal GRPR-eGFP neurons (#2 - #4), but not in 1 spinal NMBR-eGFP neuron (#1).

(C) *Actb* mRNA was detectable in all 4 neurons.

Specificity of the PCR reactions were verified using two negative controls, in which cDNA was substituted with H_2O or no RT product (ΔRT).

Figure S4. GRP, 8-OH-DPAT and WAY100635 Are Specific, Related to Figure 4.

(A) 5-HT (blue traces) or 8-OH-DPAT (DPAT, red traces) was not able to induce Ca^{2+} response in dissociated WT spinal neurons.

(B) Up to 20 nM of GRP didn't evoke Ca^{2+} response in dissociated spinal neurons of *Grpr* KO mice.

(C) WAY100635 failed to block GRP-evoked Ca^{2+} spikes in dissociated WT spinal neurons.

(D) WT spinal neurons showed comparable Ca^{2+} response to two times of co-application of GRP (5 nM) and 8-OH-DPAT (10 μM) with 30 min wash in between.

Figure S5. The specificity Test of Mouse Anti-GRPR Monoclonal Antibody, Related to Figure 5.

(A) GRPR antibody staining on WT mouse spinal dorsal horn.

(B) GRPR antibody staining on bombesin-saporin treated mouse.

(C) GRPR staining after GRPR antigen adsorption.

(D) Mouse anti-GRPR antibody (mxGRPR) specifically labeled HEK 293 cells expressing GRPR-GFP (upper row) but not NMBR-GFP (lower row).

Scale bars, 100 μm in A, 20 μm in D.

Figure S6. No Detectable FRET Was Observed Between GRPR-eGFP and 5-HT1B-mCh, Related to Figure 5.

(A) Representative confocal images showing plasma membrane fluorescence intensities of HEK293 cells expressing 5HT-1B-mCherry (red) and GRPR-eGFP (green), before and after acceptor (mCh) photobleaching in the selected region of a cell (yellow box), Overlay images show typical example of a cell co-expressing 5HT-1B-mCh and GRPR-eGFP. Scale bar, 10 μm.

(B) Top: Plots showing background subtracted normalized fluorescence intensities of donor excitation/donor emission (green trace, 488 nm excitation, 515 nm emission: DD), donor excitation/acceptor emission (red trace, 488 nm excitation, 630 nm emission: DA) and acceptor excitation/acceptor emission (pink trace, 595 nm excitation, 630 nm emission: AA) from selected plasma membrane regions of the cells that were photobleached. Bottom: Averaged FRET ratio (DA/DD) in the photobleached regions. n = 9-11. Error bars represent SEM.

(C) Background subtracted normalized fluorescence intensities (top) and averaged FRET ratio (bottom) of non-photobleached regions in (A).

Figure S7. Enhanced 5-HT Level in Mice with Chronic Itch, Related to Figure 7.

(A) The level of 5-HT was significantly upregulated in hindbrain of $BRAF^{Nav1.8}$ mice and in the spinal cord of dry-skin mice.

(B) $BRAF^{Nav1.8}$ mice showed enhanced level of 5-HIAA in both hindbrain and the spinal cord.

Table S1. Expression of *Grpr* and *Htr1a* in GRPR-eGFP$^+$ Spinal Neurons.

Grpr message was detected in all GRPR-eGFP$^+$ superficial dorsal horn neurons (#2-10) by single cell RT-PCR. *Htr1a* signal was detected in 7 out of 9 GRPR-eGFP$^+$ neurons (#2-4, 6, 7, 10). No *Grpr* or *Htr1a* expression was detected in ΔRT control or one NMBR-eGFP spinal neuron (#1).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mice

Adult male C57BL/6J mice, $Lmx1b^{f/f/p}$ mice (Ding et al., 2003), $Tph2^{-/-}$ mice (Kim et al., 2014), $Htr1a^{-/-}$ mice (Heisler et al., 1998), *Grpr* KO mice (Hampton et al., 1998) and $BRAF^{Nav1.8}$ mice (Zhao et al., 2013) were used for the study.

All mouse strains were maintained in a congenic C57BL/6J background and their wild-type littermates were used as controls for all experiments. C57BL/6J male mice (Jackson Laboratory) were also used for pharmacological studies. Mice were housed in clear plastic cages in a controlled environment at a constant temperature of 23°C and humidity of 50% ± 10% with food and water available *ad libitum*. The animal room was on a 12/12 h light/dark cycle with lights on at 0700. Male mice 7 to 12 weeks old were used to test for behavior and staining. Experimental procedures were conducted in accordance with policies of the National Institutes of Health and were approved by the Animal Studies Committee at Washington University School of Medicine.

Drugs and Chemicals

CQ, 5,7-DHT, 5-HTP, (R)-(+)-8-OH-DPAT (5-HT1A agonist), DOI (5-HT2A agonist), BW 723C86 (5-HT2B agonist), α-ME-5-HT (5-HT2 agonist), 1-(3-Chlorophenyl) biguanide hydrochloride (m-CPBG, 5-HT3 agonist) and WAY100635 were purchased from Sigma-Aldrich (St. Louis, MO). GRP_{18-27} was purchased from Bachem (King of Prussia, PA). CP 93129 (5-HT1B agonist), LY344864 (5-HT1F agonist), m-cpp (5-HT2C agonist), RS 67506 (5-HT4 agonist), EMD 386088 (5-HT6 agonist) and AS 19 (5-HT7 agonist) were purchased from R&D Systems.

All chemicals were dissolved in sterile saline. The volume of drug solutions was 5 μL for i.t. and i.c. injections and 50 μL for nape i.d. injections. The dose chosen was based on our previous work (Sun and Chen, 2007; Sun et al., 2009) or determined in pilot experiments on a small group of animals. The doses of drugs were as follows: CQ, 200 μg nape i.d; 5-HTP, 10 mg/kg, i.p.; 5-HT receptor agonists, 5 nmol, i.t.; Other detailed information for time and doses for their use was indicated in results or figure legends.

Acute Scratching Behavior

Scratching behaviors were performed as previously described (Sun and Chen, 2007; Sun et al., 2009). Briefly, the injection area was shaved two days before experiments. To avoid cross-tachyphylaxis, each mouse was used for just one test. Prior to the experiments, each mouse was placed in a plastic arena (10 × 11 × 15 cm) for 30 min to acclimate. Mice were briefly removed from the chamber for injection. Animal behaviors were videotaped (SONY HDR-CX190) from a side angle and played back on computer for assessments by observers blinded to the treatments and the genotypes of the animals. Hind limb scratching behavior towards the injected area was observed for 30 min with 5 min intervals. One bout of scratch was defined as a lifting of the hind limb to the injection site and then a replacing of the limb back to the floor or to the mouth, regardless of how many scratching strokes took place in between (Sun and Chen, 2007).

Intradermal (i.d.) Injection: The injections were performed as previously described (Shimada and LaMotte, 2008). Pruritogen of 50 μl was injected intradermally into the nape of neck.

Intracisternal (i.c.) Injection: The i.c. injection was performed as described earlier (Reijneveld et al., 1999). Briefly, the animal was placed in the prone position with their neck draped over a 15 mL cylinder. A volume of 5 µL solution was injected using a Hamilton syringe between the occiput and C_1 while mouse head was immobilized by the performer's thumb and midfinger.

Intrathecal (i.t.) Injection: Intrathecal injections into the lumbar region of unanaesthetized mice were performed as described previously (Hylden and Wilcox, 1980). Briefly, 30-gauge needle attached on 10 µL-Hamilton Syringe was inserted into the intervertebral space between L5 and L6. Drugs were injected in a volume of 10 µl.

5,7-DHT Treatment

The protocol is similar to literatures (Oatway et al., 2004). Endogenous spinal 5-HT fibers were ablated in C57BL/6J mice using 5,7-dihydroxy-tryptamine (5,7-DHT)(Sigma-Aldrich). To prevent the uptake of 5,7-DHT into noradrenergic neurons, mice were pre-treated with desipramine hydrochloride (Sigma-Aldrich)(25 mg/kg, i.p.) for 45 min. Mice were then administrated with either 5,7-DHT (20 µg, i.t.) or vehicle (0.9% saline, 5 µl). Behavioral tests were performed 2 weeks after 5,7-DHT injection. The spinal cords were then dissected out for monoamines measurements using HPLC.

Immunhistochemistry and *in situ* Hybridization

Immunohistochemical staining and *in situ* hybridization were performed as previously described (Zhao et al., 2006). Briefly, mice were anesthetized with an overdose of ketamine, and fixed by intracardiac perfusion with cold PBS (0.01M, pH 7.4) followed by 4% paraformaldehyde. The brain, spinal cord and small intestine were immediately removed, postfixed in the same fixative overnight at 4 °C and cryoprotected in 30% sucrose solution. Frozen tissue was sectioned at 20~25 µm thickness using a cryostat. Free-floating sections were blocked in a solution containing 2% donkey serum and 0.3% Triton X-100 in PBS for 1 h at room temperature. The sections were incubated with primary antibodies overnight at 4 °C followed by the use of FITC or Cy3-conjugated secondary antibodies (Jackson

ImmunoResearch). The following primary antibodies were used: rabbit anti-5-HT (1:5,000, Immunostar), rabbit anti-5-HT1A (1:200, Santa Cruz) and chicken anti-GFP (polyclonal, 1:500, Aves Labs). For the *in situ* hybridization study, a digoxigenin-labeled cRNA probe was used as described earlier (Zhao et al., 2006). Images were taken using a Nikon Eclipse Ti-U microscope.

Small Interfering RNA Treatment

Negative control siRNA (SIC001) and selective siRNA duplex for mouse *Htr1a* mRNA knock-down (SASI_Mm01_00197594) were purchased from Sigma. RNA was dissolved in diethyl pyrocarbonate-treated PBS and prepared immediately prior to administration by mixing the RNA solution with a transfection reagent, *in vivo*-jet PEI® (Polyplus-transfection SA). The final concentration of RNA was 1.25 µg/10 µl. siRNA was delivered to the lumbar region of the spinal cord. Injection was given twice daily for 6 consecutive days as described previously with some modifications (Kawasaki et al., 2008; Liu et al., 2011; Luo et al., 2005; Tan et al., 2005). Behavior testing was carried out at 24 h after the last injection.

Immune-Electron Microscopy

Immune-electron microscopic studies were performed as previously described (Li et al., 1997; Pang et al., 2006). Briefly, three adult male GRPR-eGFP mice were perfused transcardially with 4% paraformaldehyde. Lumbar enlargement of spinal cord was cut serially into 50-µm thick cross sections on a vibratome (Microslicer DTM-1000; Dosaka EM, Kyoto, Japan). Subsequently, 5-HT and GFP were labeled by the immunogold–silver method and by the immunoperoxidase method, respectively. The sections were incubated with a mixture of rabbit anti-5-HT antibody (1:2,000; Incstar Corporation, Stillwater, MN) and guinea pig anti-GFP antibody (1.5 µg/ml) (Nakamura et al., 2008) at room temperature for 24 h followed by a mixture of 1.4-nm gold-particle-conjugated goat anti-rabbit IgG (1:100, Nanoprobes, 2003, Stony Brook, NY) and biotinylated donkey anti-guinea pig IgG (1:100, 706-065-148; Jackson Immunoresearch, West Grove, PA). The sections were then postfixed with 1% (w/v)

glutaraldehyde in 0.1 M PB (pH 7.4) for 10 min and washed in distilled water. Subsequently, silver enhancement was done in the dark with HQ Silver Kit (2012; Nanoprobes). Then the sections were incubated with avidin-biotin-peroxidase complex (1:50, Elite ABC Kit; Vector) for 6 hrs. Next, the sections were incubated in 0.05 M Tris-HCl (pH 7.6) containing 0.02% (w/v) DAB (Dojindo, Tokyo, Japan) and 0.003% (v/v) H_2O_2 for 20–30 min at room temperature. Then the sections were osmated, counterstained with 1% (w/v) uranyl acetate, dehydrated, embedded as described previously (Pang et al., 2006). Further, 50-nm-thick ultrathin sections were cut with a diamond knife mounted on an ultramicrotome and examined with a JEM-1400 electron microscope (JEM, Tokyo, Japan). The digital micrographs were captured by VELETA (Olympus,Tokyo, Japan).

HPLC

The concentrations of monoamines (5-HT, NE, DA and 5-HIAA) were measured using HPLC with electrochemical detection as previously described (Zhao et al., 2006). Briefly, mice were anesthetized with an overdose of ketamine, and their brains and spinal cords were immediately removed and frozen on dry ice. The brains were divided into the rostal and caudal halves at the juncture of the medulla/pons region. Blood was taken from the heart and centrifuged at 12,000 rpm for 10 min at 4°C to separate plasma from blood cells. The concentrations of the amines were calculated with respect to the mean peak height values obtained from standard runs set in the internal standard mode using CSW32 software (DataApex, Prague, Czech Republic). The resulting values were corrected for volume and expressed as pg of amine per mg of wet tissue or per 100 µL of plasma. For analyzing the effect of 5-HTP injections on indole amine concentrations, samples were collected one hour after 5-HTP or saline administration.

Single Cell qRT-PCR

Single-cell qRT-PCR was carried out using Ambion® Single Cell-to-CT™ Kit (Life technologies) in accordance with manufactures instructions. Briefly, single $eGFP^+$ neuron in lamina I of spinal cord

slices from GRPR-eGFP mouse or NMBR-eGFP mouse was identified by green fluorescence under microscope. Negative pressure was applied to the pipette to isolate cytosol of the cell, which was extruded into 10 µl cell lysis/Dnase I solution for RNA extraction and genomic DNA digestion. After reverse transcription (25°C,10 min/42°C, 60 min/85°C, 5 min) target cDNA was pre-amplified for 14 cycles (95°C, 15 sec/60°C, 4min) in the presence of 0.2x pooled TaqMan assays (Life technologies). One GRPR-eGFP neuron was used as ΔRT control for which reverse transcriptase as omitted at cDNA synthesis step. Diluted pre-amplification products (1:20 in 1x TE buffer) was used for final qPCR reaction (4 µl, 40 cycles of 95°C 5 sec/60°C 30 sec; StepOnePlus, Applied Biosystems) to examine target gene expression. TaqMan assays used are: *Grpr*, Mm01157247_m1; *Htr1a*, Mm00434106_s1; *Actb*, Mm01205647_g1. Data were analyzed using StepOne Software (v2.2.2.) with automatic baseline and threshold was set to 0.2.

Plasmid

Myc-GRPR, HA-5-HT1A, HA-5-HT1B, GRPR-GFP, 5-HT1A-mCherry, 5-HT1B-mCherry, NMBR-GFP, 5-HT1A-GFP and 5-HT1B-GFP were constructed using polymerase chain reaction and subcloned into a pcDNA3.1 (Life Technologies) using In-Fusion HD kit (Clontech Laboratories, Inc.).

Cell Culture and Transfections

HEK293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2. Stable HEK293 cell lines were made as described previously (Liu et al., 2011). Briefly, cells were first transfected with plasmid containing the neomycin resistance by electroporation (GenePulser Xcell, Bio-Rad). Stable transfectants were selected in the presence of 500 µg/ml G418 (Invitrogen). To generate lines co-expressing two distinct epitope-tagged receptors, the cells were subjected to a second round of transfection and selected in the presence of 500 µg/ml G418 and 100 µg/ml hygromycin (Roche). Clones expressing Myc-GRPR, HA-5-HT1A, HA-5-HT1A/Myc-GRPR, and HA-5-HT1B/Myc-GRPR were generated. For FRET

experiments and antibody verification, receptors were transiently transfected into HEK293 cells using Lipofectamine 2000^{TM} (Invitrogen) following the manufacturer's instruction. FRET was done at 37°C 24 h after transfection. GRPR and 5-HT1A antibody staining was carried out 24 h after transfection as described (Liu et al., 2011). Dilution of tested antibodies were 1:200 for rabbit anti-5-HT-1A and 1:1,000 for mouse anti-GRPR.

Dissociation of Spinal Neurons

Primary culture of spinal dorsal horn neurons was prepared from 5-7-days-old C57BL/6J mice (Zhao et al., 2013). After decapitation under deep anesthesia with diethylether, a laminectomy was performed and dorsal horn of spinal cord was dissected out with a razor blade and incubated in Neurobasal-A Medium (Gibco) containing 30 µl papain (Worthington) at 37 °C for 20 min. Enzymatic digestion was stopped by adding another 2 ml Neurobasal-A medium. After washing with the same medium for three times gentle trituration was performed using flame polished glass pipette until solution became cloudy. The homogenate was centrifuged at 1,500 rpm for 5 min and supernatant was discarded. Cell pellets were resuspended in culture medium composed of Neurobasal medium (Gibco, 92% vol/vol), fetal bovine serum (Invitrogen, 2% vol/vol), HI Horse Serum (Invitrogen, 2% vol/vol), GlutaMax (2 mM, Invitrogen, 1% vol/vol), B27 (Invitrogen, 2% vol/vol), Penicillin (100 µg/ml) and Streptomycin (100 µg/ml) and then plated onto 12-mm coverslips coated with poly-D-lysine. After three days neurons were used for calcium imaging studies.

Calcium Imaging

Calcium imaging experiments were performed as described previously (Liu et al., 2011). The cells were loaded with Fura 2-acetomethoxy ester (Molecular Probes) for 30 min at 37°C. After washing, cells were imaged at 340 and 380 nm excitation to detect intracellular free calcium. Calibration was performed using Fura-2 Calcium Imaging Calibration Kit (Invitrogen) following the manufacturer's

instructions. Each experiment was done at least three times and a minimum of 200 cells were analyzed each time.

Co-Immunoprecipitation and Western Blot Analysis

Tissues or cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and proteinase inhibitors) and membrane proteins were prepared as described earlier (Liu et al., 2011). Solubilized samples (200 µg) were incubated with either rabbit antibody against HA (BD bioscience), mouse antibody against c-Myc (Covance) or GRPR (Abmart) overnight at 4 °C. The complex was precipitated with 50% TrueBlot™ anti-rabbit or anti-mouse IgG bead slurry (eBioscience). After washing, the beads were boiled in LDS sample buffer (Invitrogen) with 50 mM dithiothreitol for 10 min. Western blot was carried out with mouse anti Myc (1:1,000), rabbit anti HA (1:1,000), mouse anti-GRPR (1:5,000) or rabbit anti-5-HT1A (1:5,000, Abcam). The GRPR mouse monoclonal antibody was custom-made via Abmart. Specificity of the antibody was validated using different approaches. First, GRPR staining signals were markedly ablated in the superficial dorsal horn of bombesin-saporin-treated mice (Sun et al., 2009; Zhao et al., 2014) (Figure S5B). Second, antigen adsorption completely blocked staining in control spinal sections (Figure S5C). Finally, HEK 293 cells expressing GRPR but not NMBR were specifically labeled by GRPR antibodies (Figure S5D).

Confocal Subcellular FRET Imaging

HEK 293 cells transiently expressing fluorescently tagged receptors were seeded in 29 mm glass bottom dishes. The FRET imaging and calculations were performed as described previously (Karunarathne et al., 2013). Basal FRET between eGFP (donor) and mCherry (acceptor) was measured by rapid photobleaching of the acceptor in a defined region of a single cell (ROI) which expresses both the donor and the acceptor (Figure 5E, yellow box). ROIs were photobleached using Andor FRAP-PA unit. The unbleached region of the same cell was used as control. Laser intensities of

the photobleaching lasers were adjusted using an acoustic tunable optical filter (AOTF) to prevent photobleaching of the donor. Before and after photobleaching, a series of time lapse images were captured with donor excitation-donor emission (DD) and donor excitation-acceptor emission (DA).

Electrophysiological Recording

Spinal cord slices were obtained from GRPR-eGFP mice 3-4 weeks of age. Laminectomies were performed in cold sucrose based solution (in mM): sucrose 300, KCl 2, NaH_2PO_4 1.25, $CaCl_2$ 1, $MgCl_2$ 5, $NaHCO_3$ 26 and glucose 11. Transverse sections (400 µm) were taken with a vibratome 3000 tissue slicer and transferred to a recovery chamber containing standard artificial cerebrospinal fluid (in mM): NaCl 140, KCl 2.5, NaH_2PO_4 1.4, $CaCl_2$ 2, $MgCl_2$ 2, $NaHCO_3$ 25 and glucose 11. For recording, slices were transferred to a recording chamber (Warner RC-21G) and continuously perfused (2 ml/min) with ACSF. Drugs were applied via the perfusion system. For Giga-seal whole cell recording, thick wall borosilicate pipettes were pulled (Sutter P-97) to a diameter of 3-5 MΩ and filled with an intracellular solution consisting of (in mM); K gluconate (130), NaCl (10), $MgCl_2$ (1), EGTA (0.2), HEPES (10), MgATP (1), NaGTP (5). GRPR-eGFP neurons were visualized with an Olympus BX-51 upright microscope and FITC fluorescent filter. Current clamp signals were control and acquired with a multiclamp 700B amplifier, digidata 1440 and pClamp 10 software. Signals were low pass filtered at 2 kHz and digitized at 5 kHz. Firing patterns were examined by injection of steps of positive current for 500 ms. Input resistance was tested every 20 s for drug induced changes by injection of negative (20 pA) current. Membrane depolarizations were measured by subtracting steady state value (mV) under control conditions from the peak value (mV) observed during agonist application conditions. For neurons that fired action potentials in response to agonist application the peak membrane potential value was defined as the AP threshold of the first AP where the slope became greater than 5mV/ms. Series resistance was monitored in voltage clamp mode by measuring the instantaneous current in response to small voltage steps; recordings having greater than 20% change in series resistance were discarded. Data were analyzed offline (clampfit 10) and plotted in Origin 8 graphing software.

Chronic Itch Models

Dry Skin (Xerosis): The dry skin model was set up as described (Akiyama et al., 2010; Miyamoto et al., 2002). Briefly, the nape of mice at 8-12 weeks age was shaved and a mixture of acetone and diethylether (1:1) was painted on the neck skin for 15 sec, followed by 30 sec of distilled water application (AEW). This regiment was administrated twice daily for 10 days. Littermate control mice received water only for 45 sec on the same schedule. Spontaneous scratches were examined on the morning following the last AEW treatment. Baseline scratching behaviors were recorded for 60 min. Mice were returned to home cages for 20 min followed by drug injection. Ten minutes later scratching behaviors were recorded for another 60 min.

Statistical Analysis

All values are expressed as the means ± standard error of the mean (SEM). Statistical analyses were performed using Prism 5 (version 5.03, GraphPad, San Diego, CA). One-Way Analysis of Variance was used to test the equality of three or more means at one time. For comparison of the mean of two groups a Student's *t* test was used. $p < 0.05$ was considered statistically significant.

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