

SUPPLEMENTARY MATERIALS

I. Serotonin biosensors.

The cellular biosensor approach is effective for the on-line monitoring of secretion of signaling molecules from different cells, including taste cells. Central to this approach is a cell line that expresses appropriate surface receptors that are coupled to any intracellular signal, usually to Ca^{2+} mobilization, which could be easily visualized. To adapt this approach for exploring serotonin release from taste cells, we cloned and heterologously expressed a variety of 5-HT receptors, including the mouse 5-HT_{2C}, 5-HT_{1A}, 5-HT_{1B}, and 5-HT₄ receptors as well as the human 5-HT_{2B} receptor.

I.1. Molecular cloning of 5-HT receptors.

For cloning 5-HT receptors, RNA was isolated from the mouse brain or from mesenchymal stem cells (MSCs) derived from the human adipose tissue by using RNeasy mini kit (Qiagen). Next, isolated RNA was reverse-transcribed with SuperScript IV reverse transcriptase (Invitrogen) and random hexamer primers. For PCR amplification of coding sequences of 5-HT receptors, PhusionHot Start II DNA Polymerase (ThermoFisher Scientific) was used.

5-HT_{2C}. The coding sequence of 5-hydroxytryptamine receptor 2C (Htr2c, NM_008312.4) encompassing nucleotides 689-2065 was cloned from mouse brain by RT-PCR with the primers TAAGAATTCTGGCCACCATGGTGAACCTGGGCACT and TATGTCGACTGCACACTACTAATCCTCTCGCTG and subcloned into EcoRI and Sall sites of the pDsRed-Monomer-N1 mammalian expression vector in-frame with DsRed-Monomer sequence.

5-HT_{2B}. The coding sequence of 5-hydroxytryptamine receptor 2B (HTR2B, NM_000867.5) was amplified by RT-PCR from human MSCs with the primers GAGAA-GCTTCAGCAAATGGCTCTCTTACAGA and

GAGGTCGACTGTACATAACTAAGTCTTCA. The fragment encompassing nucleotides 432-1880 was cloned into HindIII and Sall sites of the pDsRed-Monomer-N1 vector.

5-HT_{1A}. The coding sequences of *Mus musculus* 5-hydroxytryptamine receptors 1A (Htr1a) were cloned by RT-PCR from mouse brain and inserted into the HindIII site of the pDsRed-Monomer-N1 vector by seamless cloning with In-Fusion® HD Cloning Kit according to the manufacturer (Takara). Primers for amplification of Htr1a sequence corresponding to nucleotides 556-1824 of NM_008308.4 were TCTCGAGCTCAAGCTT-GCAGGCATGGATATGTTTCAGTCT and GCAGAATTCGAAGCTTGCGGCAGAACTT-GCACTTGA.

5-HT_{1B}. The coding sequences of *Mus musculus* 5-hydroxytryptamine receptors 1B (Htr1b) were cloned by RT-PCR from mouse brain and inserted into the HindIII site of the pDsRed-Monomer-N1 vector by seamless cloning with In-Fusion® HD Cloning Kit according to the manufacturer (Takara). Primers for amplification of Htr1b sequence corresponding to nucleotides 461-1624 of NM_010482.2 were TCTCGAGCTCAAGCTT-GAGCTATGGAGGAGCAGGGT and GCAGAATTCGAAGCTTACCTGCGCAC-TTAAAGCGT.

5-HT₄. *Mus musculus* 5-hydroxytryptamine receptor 4 (Htr4) was cloned by RT-PCR from mouse brain with primers TATAAGCTTCCTGTAATGGACAACTTGATG and TATAAGCTTAGTATCACTGGGCTGAGC. The fragment encompassing nucleotides 207-1376 of NM_008313.4 was subcloned into the HindIII site of the pAcGFP1-Hyg-N1 vector in-frame with GFP sequence.

The constructs pDsRed-Monomer-N1/5-HT_{2C}, pDsRed-Monomer-N1/5-HT_{2B}, pDsRed-Monomer-N1/5-HT_{1A}, pDsRed-Monomer-N1/5-HT_{1B}, and pAcGFP1-Hyg-N1/5-HT₄ were confirmed by restriction enzyme digestion and sequencing.

I.1. Heterologous expression of 5-HT receptors.

The recombinant receptor 5-HT_{2C} was expressed in CHO cells, while 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2B}, and 5-HT₄ were expressed in HEK-293 cells. The cells of the HEK-293 and CHO lines were routinely cultured in the appropriate growth medium (1 ml) containing Dulbecco's modified Eagle's (DMEM) medium or the F12 medium (both from Invitrogen), respectively, 10%(v/v) fetalbovine serum (HyClone), glutamine (1 %), and the antibiotic gentamicin (100 µg/ml) (Invitrogen) in 12-well culture plates. Cells were grown in a humidified atmosphere (5 % CO₂ and 95 % O₂) at 37 °C. Before the day of transfection, cells were put in 12-well culture plates at the density of 3–5 × 10⁵ cells. The transient transfection was performed by adding 100 µl of OPTI-MEM (Gibco) containing plasmid DNA (1 µg) and FuGENE 6 (4 µl) (Promega) to cultured cells. After 12-h incubation, the transfection mixture was replaced with the growth culture medium. Basically, cells were assayed physiologically 24–72 h after transfection.

I.1.1. Monoclonal cells of 5-HT_{2C}-positive cells.

To generate monoclonal cells of 5-HT_{2C}-positive cells, CHO cells transfected with the pDsRed-Monomer/5-HT_{2C} construct were maintained in the F12 growth medium supplemented with 0.6 mg/ml G-418 for the following 3 weeks. The antibiotic-resistant cells were separated by their DsRed fluorescence at 590 nm using a FACS Aria SORP sorter (Beckton Dickinson), and those exhibiting most intensive red fluorescence were collected individually for further culturing in a 96-well plate. Overall, 53 monoclonal cells were obtained from individual 5-HT_{2C}-positive cells, and those were further maintained in the presence of 0.3 mg/ml G-418. Each monoclonal cell was screened for functionality with Ca²⁺ imaging, and 5 monoclonal cells of CHO/5-HT_{2C} cells were selected for further experimentation based on their responsiveness to nanomolar serotonin.

I.1.1. Monoclonal cells of 5-HT₄/PF cells.

Prior to the generation of a cell line stably expressing the 5-HT₄ receptor, we produced cells stably expressing the genetically encoded cAMP sensor Pink Flamindo (Harada et al., 2017). HEK-293 cells were transfected with the Pink Flamindo plasmid (a gift from Tetsuya Kitaguchi, Addgene plasmid # 102356; <http://n2t.net/addgene:102356>; RRID:Addgene_102356) by replacing the growth medium with the transfection mixture, containing plasmid DNA (1.6 µg) and Lipofectamine 3000 (4 µl) per 1 ml of serum-free DMEM. After 24-h incubation, the transfection mixture was replaced with the normal culture medium, and transfected cells were cultured in the presence of 0.6 mg/ml G-418 (InvivoGen) for 3 weeks. The antibiotic-resistant cells were analyzed using the FACS Aria SORP sorter (Beckton Dickinson), and those exhibiting most intensive Pink Flamindo fluorescence at 590 nm were collected individually for further culturing in a 96-well plate. Overall, individual Pink Flamindo (PF)-positive cells originated 63 cellular monoclonal cells, which were further maintained in the presence of 0.3 mg/ml G-418. Each monoclonal cell was screened for functionality with cAMP-imaging, and 7 monoclonal cells of HEK-293/PF cells were selected as generating sufficiently high cAMP responses to 10 µM forskolin.

Next, HEK-293/PF cells were transfected with the plasmid vector pAcGFP1-Hyg-N1/5-HT₄ as described above, and then they were subjected to the antibiotic selection in the presence of G418 (0.3 mg/ml) and Hygromycin B Gold (0.1 mg/ml) (InvivoGen). Selected by Pink Flamindo and GFP fluorescence with the FACS Aria SORP sorter, 5-HT₄/PF-positive cells were cultured individually to originate monoclonal cells. Of 72 5-HT₄/PF monoclonal cells obtained, each was functionally assayed, and 4 monoclonal cells were selected as generating well-resolved intracellular cAMP signals in response to nanomolar serotonin (Fig. 3B in the main text).

I.1. Functional assay of 5-HT receptors coupled to Ca²⁺ mobilization

The serotonin responsiveness of cells expressing recombinant 5-HT_{2C}, 5-HT_{2B}, 5-HT_{1A}, or 5-HT_{1B} receptors was characterized with Ca²⁺ imaging. Assayed cells were plated onto a photometric chamber of nearly 150 μ l volume. The last was a disposable coverslip with attached ellipsoidal resin wall. The chamber bottom was coated with Cell-Tak (Corning) ensuring strong cell adhesion. Attached cells were loaded with the Ca²⁺ dye at room temperature (23–25 °C) by incubating them with Fluo-8AM (4 μ M) and Pluronic (0.02%) for 20 min followed by the 30-min rinse with the bath solution (mM): 140 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES-NaOH (pH 7.4), 10 glucose.

Individual CHO cells stably expressing 5-HT_{2C} responded to serotonin in an “all-or-nothing” manner: they either were irresponsive to the agonist at low doses or generated very similar Ca²⁺ signals to the agonist at different concentrations above the threshold (Fig.S1A, upperpanel). Note that sensitivity to serotonin, response lag and response magnitude, in terms of $\Delta F/F_0$, varied from cell to cell. As a result, when serotonin responses of a population of CHO/5-HT_{2C} cells were averaged by summarizing fluorescent signals of individual cells and representing the total fluorescence of a cell population as $\Delta F/F_0$ (Fig.S1A, bottom panel), the resultant dose- response curve became gradual (Fig.S1B).

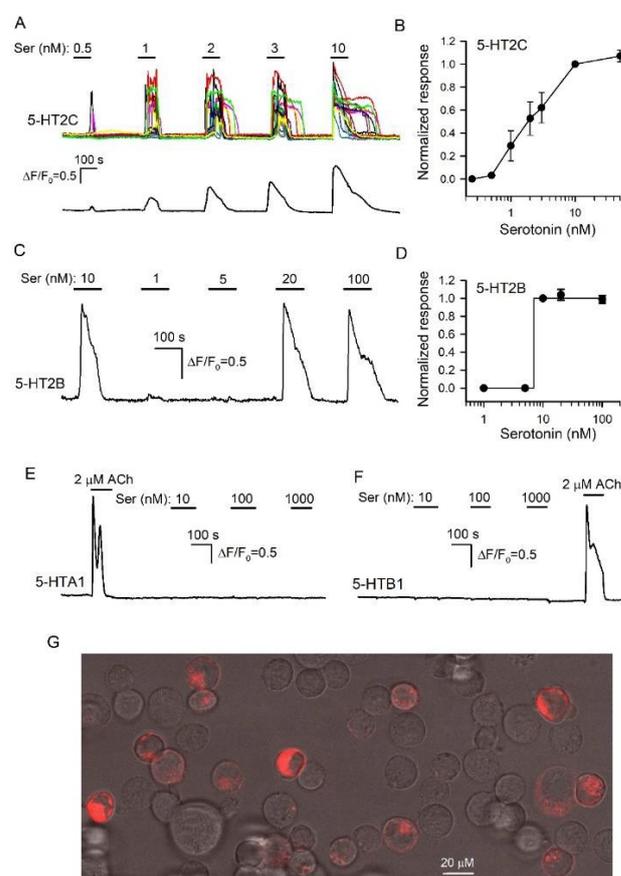


Figure S1. Responsiveness of cells transfected with different 5-HT receptors. (A) Upper panel, the concurrent monitoring of intracellular Ca²⁺ in 5-HT_{2C}-positive CHO cells loaded with Fluo-8; 20 out of 86 concurrently assayed cells were arbitrarily chosen. The cells were stimulated by serotonin at different doses as indicated. The deviation of cytosolic Ca²⁺ from a resting level in a particular cell was quantified by the ratio $\Delta F/F_0$, where $\Delta F = F - F_0$, F is the instant Fluo-8 fluorescence, F_0 is the averaged fluorescence in the very beginning of the recording. The bottom panel, the populational Ca²⁺ trace that was generated by averaging 86 individual fluorescence traces being similar to those shown in the upper panel. (B) Dose-response curve generated by averaging populational serotonin responses of 5-HT_{2C}-cells over 5 experiments, including one shown in (A). The cell response was calculated as $R = \Delta F/F_0$, where $\Delta F = F_{\max} - F_0$, F_0 is cell fluorescence measured immediately before seroto-

nin application, F_{\max} is the fluorescence peak characteristic of a given serotonin response. The averaged response to 10 nM serotonin was taken as a unit. The data are presented as a mean \pm S.D. ($n=5$). (C) Serotonin responses of 5-HT_{2B}-positive HEK-293 cells stimulated serially as indicated. (D) Dose response curve for the 5-HT_{2B}-biosensor. For each particular 5-HT_{2B}-cell, a Ca²⁺ transient ($\Delta F/F_0$) elicited by serotonin at a given dose was normalized to a response to 3 nM serotonin. The data (cycles) are presented as a mean \pm S.D. ($n=38$). The solid line represents the Heaviside step function $H(S-7)$ with S being serotonin concentration. (E, F) Serotonin never stimulated Ca²⁺ signaling in HEK-293 cells, which heterologously expressed the 5-HT_{1A} receptor (E) or 5-HT_{1B} receptor (F). (G) Images of HEK-293 cells transiently transfected with the 5-HT_{1A} receptor fused with the red fluorescent protein DsRed.

Being transiently transfected with the 5HT_{2B} receptor, HEK-293 cells also responded to serotonin with Ca²⁺ transients in the “all-or-nothing” manner (Fig.S1C, D). In contrast, we failed to observe serotonin-induced Ca²⁺ signaling in HEK-293 cells transiently transfected with 5HT_{1A}(Fig.S1E) or 5HT_{1B} (Fig.S1F). It is noteworthy that fluorescence of the DsRed marker fused with the receptor protein was apparently located in the plasmalemma in many cells (Fig.S1G). Thus, although recombinant 5HT_{1A} and 5HT_{1B} receptors were apparently transported on the cell surface, they did not endow transfected cells with responsiveness to serotonin, in terms of Ca²⁺ signaling, presumably because these recombinant serotonin GPCRs were not coupled to the phosphoinositide cascade in HEK-293 cells.

I. Assay of individual taste cells with the serotonin biosensor.

Basically, the biosensor approach allowed for detecting depolarization-induced serotonin release from individual cells of the type III from the CV papilla, if they were sufficiently robust. In the representative successful experiment ($n=7$) illustrated in Fig.S3, a taste cell, which was identified as type III by serotonin release, was stimulated by 70 mM KCl in series. Synchronously with depolarizing stimulation, the serotonin sensor generated pretty similar responses visualizing transient serotonin release. The control application of exogenous serotonin (10 nM) elicited the markedly higher response, showing that the biosensor was not saturated by released serotonin. This observation indicated that in robust cells of the type III, the KCl-induced serotonin release was reproducible in a series of 3 stimulation at least.

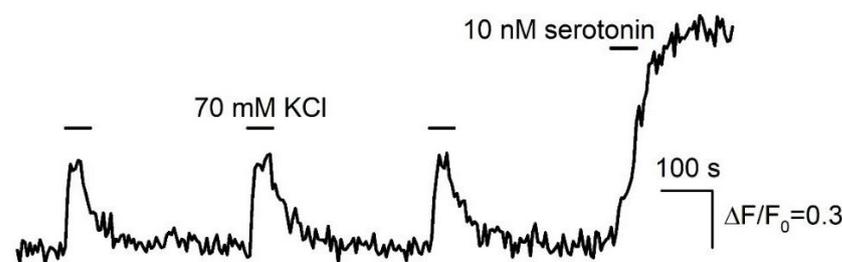


Figure S2. Release of serotonin from an individual CV cell of the type III assayed with the biosensor approach. The noisy experimental trace represents the cAMP signal in a nearby 5-HT₄/PF-positive HEK-293 cell. The sequential depolarization of the taste cell by 70 mM KCl, as indicated by the line segments above the cAMP trace, elicited rather reproducible responses of the serotonin biosensor. The deviations of cytosolic cAMP were quantified by the ratio $\Delta F/F_0$, where $F = F - F_0$, F is the instant fluorescence of the cAMP sensor Pink Flamindo, F_0 is the averaged fluorescence in the very beginning of the recording. The bath solution included (mM): 140 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES-NaOH (pH 7.4), 10 glucose. The taste cell was stimulated by the substitution of 140 mM NaCl for 70 mM NaCl+70 mM KCl.

Note however that the enzymatic dissociation of taste buds, which was necessary to obtain individual taste cells, compromised their functionality. Since taste cells remained much more robust being embedded in a taste bud, stimulated serotonin secretion from

taste buds was preferably studied at the level of individual taste buds, given that among taste bud cells, solely type III cells produced and released serotonin.

I. Effects of CASR ligands on ion currents in taste cells of the type II.

As a control for specificity, we studied effects of the CASR agonist NPS R-568 and antagonist NPS-2143 on VG currents in type II cells, which did not express CASR (Bystrova et al., 2010). Characteristic of these cells are VG Na⁺ currents and outward currents via non-selective CALHM channels. It turned out that NPS R-568 (0.5 μM) irreversibly diminished VG outward currents by 33±5% (8 cells) compared to control (Fig.S3A, B) but affected VG Na⁺ currents negligibly (Fig.S3, inset). Type II cells were weakly sensitive to 2 μM NPS-2143, which reversibly decreased VG Na⁺ and outward currents by 6-14% (n=6) (Fig.S3C,D).

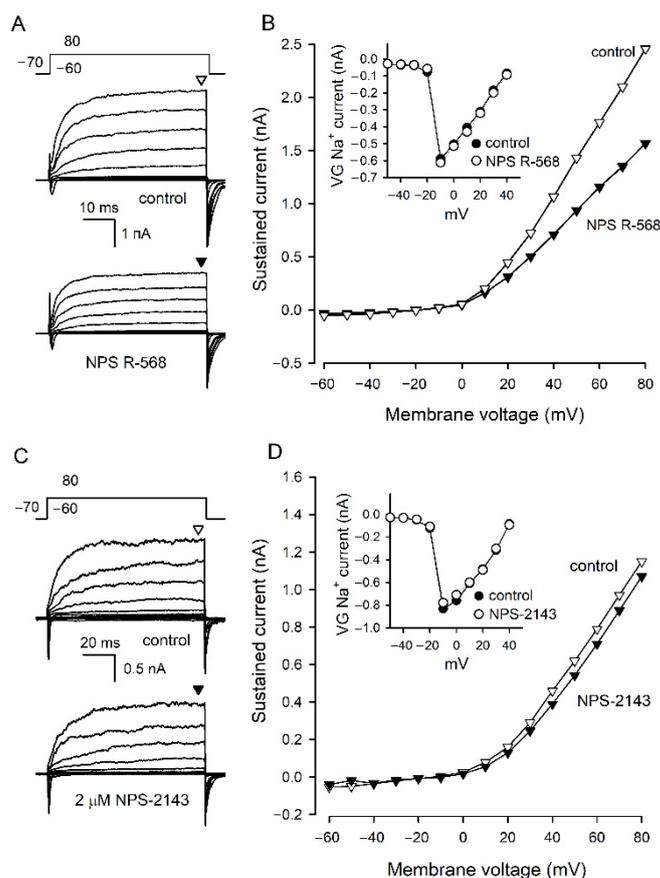


Figure S3. Effects of CASR ligands on VG currents in type II cells. (A) Representative (n=8) perforated patch recording of integral currents in control and in the presence of 0.5 μM NPS R-568. The cell was held at -70 mV and polarized from -60 to 80 mV by 50-ms voltage pulses with the 10-mV increment. The patch pipette contained 140 mM CsCl and Amphotericin B (400 μg/ml), the bath solution contained 140 mM NaCl. (B) Voltage dependence of the sustained current shown in (A) in control (▽) and in the presence of 0.5 μM NPS R-568 (▼). The sustained currents were evaluated at the moments indicated by the symbols above the current traces. Insert, the I-V curve of the VG Na⁺ currents shown in (A) (C, D) Representative (n=6) recordings of integral currents in control and in the presence of 2 μM NPS-2143 and their I-V curves (D). Recording conditions as in (A).

I. Assay of serotonin release from foliate taste buds.

The abundance and robustness of individual type III cells in a preparation of dissociated taste buds were critical for accomplishing this study. The CV papilla as a taste cell source was more preferable than the foliate papilla because the much larger number of

sufficiently robust cells of the type III could be isolated from it. Nevertheless, foliate cells of the type III were also assayed in the limited number of experiments. As was found, foliate cells of the type III also exhibited their invariance of depolarization-induced release of serotonin at varied bath Ca^{2+} (Fig.S4).

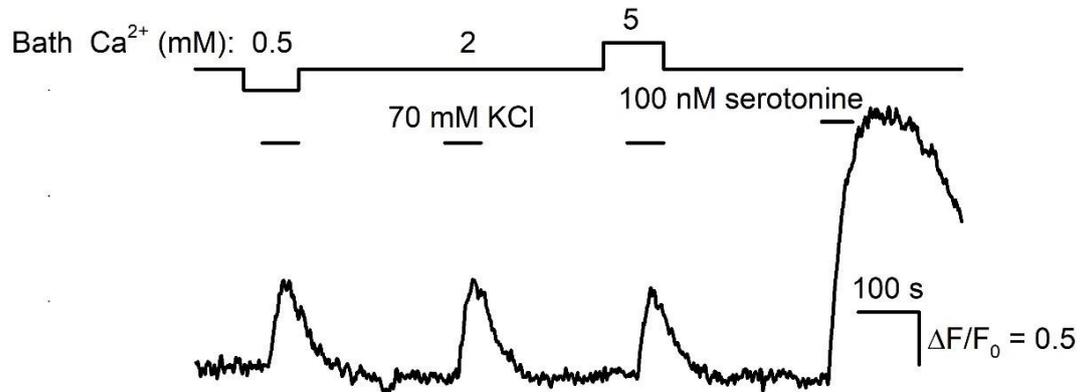


Figure S4. Assay of serotonin release from a foliate taste bud with the 5-HT₄/PF- biosensor at varied bath Ca^{2+} . The representative (n=5) bottom trace represents the evolution of the cAMP signal in the serotonin biosensor during the assay. The depolarization of taste bud cells by 70 mM KCl stimulated serotonin release that was apparently unaffected by a change in bath Ca^{2+} (solid line above the cAMP trace). The control application of exogenous serotonin (100 nM) elicited the much higher response, indicating that the biosensor was not saturated by released serotonin. The deviations of cytosolic cAMP were quantified by the ratio $\Delta F/F_0$, where $\Delta F = F - F_0$, F is the instant fluorescence of the cAMP sensor Pink Flamingo, F_0 is the averaged fluorescence in the very beginning of the recording.