Supporting Information

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SI Materials and Methods

Chemicals and Specimen Collection. Chemicals were purchased from Sigma/Aldrich unless otherwise stated. Amines were purchased as free bases rather than hydrochloride salts. C57BL/6 mouse and Brown Norway rat urines were collected by using a metabolic cage, nonidentifiable human urine was purchased (Bioreclamation), and other urine samples were obtained from zoos or commercial sources as described in Fig. S5. All animal procedures were in compliance with institutional animal care and use committee guidelines.

TAAR Functional Assays. Reporter gene assays were performed as described (1) with the following minor modifications. Test urines were diluted in serum-free media containing penicillin G (100 units/mL; Invitrogen) and streptomycin sulfate (100 μg/mL; Invitrogen). SEAP activity was measured as fluorescence resulting from dephosphorylation of a substrate, 4-methylumbelliferyl phosphate. Fluorescence values were obtained by using an EnVision plate reader (Perkin-Elmer) and are reported directly without normalization. All TAARs, except mouse TAAR3, were expressed as fusion proteins with an N-terminal sequence of bovine rhodopsin (2).

Preparation of Urine Extracts. For Fig. 1*A*, urines (850 μL) were basified by addition of sodium hydroxide (150 μL, 1 M), and extracted with dichloromethane (2 × 480 μL). Twenty microliters of 1:1 PBS:dimethyl sulfoxide (DMSO) was added to pooled dichloromethane extracts and dichloromethane removed by mild heat (65 °C). Extracts were diluted in cell culture media for TAAR functional assays relative to the original urine volume. For Fig. 1*B*, mouse, rat, and human urines (425 μL) were basified by the addition of sodium hydroxide (75 μL, 1 M) and extracted with dichloromethane (6 × 800 μL). Twenty microliters of 0.1% formic acid/water was added to pooled dichloromethane extracts, and dichloromethane was removed by mild heat (65 °C).

Fractionation and Analysis of Bobcat Urine. Bobcat urine (5 mL) was basified by the addition of sodium hydroxide (1 mL, 1 M), and extracted with dichloromethane (3 × 2 mL). Dichloromethane extracts were pooled and concentrated to ≈500 μL by mild heat (65 °C). Concentrated bobcat extracts were separated by silica gel chromatography using a mobile solvent phase of increasing polarity. Thirty 1-mL fractions were collected using elution mixtures of solvent A (dichloromethane) and solvent B (methanol, 4% NH_4OH), at the following ratios (A:B): 100:0, 95:5, 90:10, 80:20, 70:30, and 50:50. Aliquots (100 μL) of each chromatography fraction were prepared for TAAR4 functional analysis by the addition of 1:1 PBS: dimethyl sulfoxide (10 μL), removal of organic solvent with mild heat, and dilution in cell culture media (1 mL) for direct testing in the reporter gene assay. Identified fractions with TAAR4 activator were then diluted 1:1 by the addition of 5% formic acid/methanol and analyzed by electrospray mass spectrometry using a hybrid linear quadrupole ion trap/FTICR mass spectrometer (LTQ FT; Thermo Fisher Scientific).

Quantitative LC/MS Analysis. Urines (350 μL for 1× analysis or 600 μL for 20× analysis) were basified to pH 12.0 by the addition of 10 M sodium hydroxide and extracted with dichloromethane (4 × 600 μL). Dichloromethane was partially removed by mild heat (55 °C). When sample volumes decreased ≈75%, 0.1% formic acid/water was added to extracts (350 μL for 1× analysis or 30 μL for 20× analysis). The remainder of the dichloromethane was then removed by returning samples to mild heat (55 °C). Extracts or 20× concentrated extracts were analyzed by LC/MS using a Hypercarb column (Thermo Scientific; 4.6 × 100 mm) on an Agilent 1200 HPLC instrument (Agilent Technologies). Samples were eluted (12-min run, flow rate 0.7 mL/min) using a linear gradient (0–60%) of solvent A (acetonitrile plus 0.1% formic acid) in solvent B (water plus 0.1% formic acid). The samples were analyzed in tandem by mass spectroscopy on an Agilent 6130 Quadrupole LC/MS system (Agilent Technologies). The number of ion counts with m/z =122 (the mass of ionized 2-phenylethylamine) was graphed over time, with a lower detection limit of 1 μM, and an integrated peak size linearly correlated with concentration up to 40 μM. Specimens indicating >40 μM 2-phenylethylamine were subsequently analyzed after dilution to measure in this linear range. For each sample, a control extraction of urine spiked with 14 μM 2-phenylethylamine was run in parallel to quantify recovery during extraction, inferred by difference measurement, and verify that observed peaks in the test specimen had the same retention time as 2-phenylethylamine. Calculations of 2-phenylethylamine concentration in original specimens were based on the observed recovery rate of 2-phenylethylamine in control extractions (average of 55%). Urine extracts were used because they enabled concentration of 2-phenylethylamine for analysis, and because direct quantification of 2-phenylethylamine in urine, without extraction, resulted in an underestimation of 2-phenylethylamine levels, as assessed in spiked specimens.

Confocal Calcium Imaging of Olfactory Sensory Neurons in Tissue Slices. Recordings were performed as described (3) with the following modifications. For calcium sensitive dye loading, slices of olfactory epithelium were incubated (30 min, 4 °C) in Hepes solution: 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes; pH = 7.3) containing Fluo-4/AM (2 μM; Molecular Probes). Slices were transferred to a recording chamber (Slice Mini Chamber; Luigs & Neumann) and visualized by using a Leica DM6000CFS confocal fixed stage upright microscope (Leica Microsystems) equipped with an apochromatic water immersion objective (HC X APO L20×/1.0 W) and infrared-optimized differential interference contrast (DIC) optics. Slices were anchored via stainless steel wires with 0.1-mm lycra threads and continuously superfused with Hepes-buffered solution. Changes in cytosolic calcium were monitored over time at 1.0 Hz frame rate. Stimulus application as well as solution exchange during interstimulus intervals was achieved by a custom-made, pressure-driven focal application device consisting of a software-controlled valve bank connected to a 7-in-1 "perfusion pencil." Rhodamine application controlled for uniform flow and even stimulus application throughout the epithelial sensory surface. Offline analysis of time-lapse experiments was performed by using LAS-AF software (Leica). All cells in a given field of view were marked as individual regions of interest (ROIs), and the relative fluorescence intensity for each ROI was calculated and processed as a function of time.

Modulation of 2-Phenylethylamine Levels in Lion Urine. PEA-depleted lion urine was prepared by the addition of 90 μL of Human MAO-B (BD Biosciences; 5 mg/mL) to 1 mL of 10% lion urine/PBS (Specimen 6, Fig. S5) and incubation (24 h, 37 °C). PEA-respiked lion urine was derived from PEA-depleted lion urine by incubation (2 h, 37 °C) with *R*-deprenyl hydrochloride (20 mM final concentration) followed by addition of 2-phenylethylamine to 31 μM, the original level in 10% lion urine. Quantitative

LC/MS analysis verified reduction of 2-phenylethylamine in PEA-depleted lion urine and recovery of 2-phenylethylamine in PEA-respiked lion urine (Fig. 6*C*). All behavior experiments involving PEA-respiked lion urine were done immediately after PEA readdition, because prolonged incubation of PEA-respiked lion urine (4 h, 37 °C) resulted in partial degradation of respiked 2-phenylethylamine because of residual MAO-B activity.

Open Field Behavioral Analysis. Rat behavioral responses to odors in the open field were measured as described (4) with the following modifications. Adult Sprague–Dawley rats (240–340 g; Janvier) were placed in the center of a 45 cm × 45 cm Plexiglass arena (TSE Systems) equipped with infrared sensors (distance 14 mm, illumination 80–120 lx). The arena contained glass dishes (36 mm) in each corner, with one dish containing test stimuli. Before testing, animals were habituated to the arena by introducing them for three consecutive days. Next, test stimuli (see below) were presented to each rat on subsequent days in a pseudorandomized order and pseudorandomized odor corner. Amines were applied as free bases rather than as hydrochloride salts because acidification decreases amine volatility. All tests were performed between 0800 and 1000 hours of a normal light cycle (lights on at 0500 hours). The arena was cleaned with soapy water between experimental sessions. Location of the rats was automatically recorded by using the infrared detectors and analyzed (TSE Systems software). Statistical significance was measured by using Wilcoxon Signed Test [$^{**}P < 0.01$; comparison with chance level (25%)].

Three different experiments were performed, each using 12 rats. In the first experiment (Fig. 5 *B* and *C*), each rat was exposed to 1 mL of water, 1 mL of lion urine (Specimen 1, Fig. S5), 1 mL of coyote urine, 5 μL of benzylamine, 5 μL of 2-phenylethylamine (PEA, free base, catalog no. 128945). After the experimental sequence, all animals were tested with water controls to verify the absence of residual effects. In the second experiment (Fig. S3), stimuli included PEA (0, 0.05, 0.5, or 5 μL) in 1 mL of water or 1 mL of giraffe urine, as well as 1 mL of 10% lion urine/water (Specimen 1, Fig. S5) as a control. In the third experiment (Fig. 6*C*), stimuli included 1 mL of water, 1 mL of 1% and 10% lion urine/PBS, 1 mL of 1% and 10% PEA-depleted lion urine/PBS, and 1 mL of 1 and 10% PEA-respiked lion urine/PBS. In experiment three, one animal was excluded from final analysis because this animal showed almost no exploratory behavior throughout the whole experiment leading to a presence of >90% in one quadrant.

Mouse Odor Responses in a Compartment Assay. Individual male mice (8 wk old) were placed in a test cage (17 × 28 cm) modified from previous designs (5). Aerosolized odors, dissolved in water or dipropylene glycol (DPG), were delivered through a gas port into a compartment of the arena such that 2/3 of the arena remained odor-free. Animals were subjected to 6-min trials consisting of 3 min of pure air delivery, followed by 3 min of odor delivery. The percentage change in odor compartment occupancy during stimulus application was calculated. Animals with <10% occupancy of the test compartment before odor exposure were excluded. Statistical significance was measured by comparison with wild-type water exposures by using a Student's *t* test.

Plasma Corticosterone Assay. Rats were exposed to aqueous odor-containing solutions (1 mL of water, 10% 2-phenylethylamine, 10% benzylamine, or 2% TMT, 30 min, $n = 16, 20, 8, 16$) in a small box (32 × 20 × 16 cm), and rapidly decapitated for plasma collection. Corticosterone levels were measured in duplicate by using a competitive radioactive binding assay as described (6).

1. Liberles SD, Buck LB (2006) A second class of chemosensory receptors in the olfactory epithelium. *Nature* 442:645–650.
2. Krautwurst D, Yau KW, Reed RR (1998) Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* 95:917–926.
3. Spehr M, et al. (2006) Essential role of the main olfactory system in social recognition of major histocompatibility complex peptide ligands. *J Neurosci* 26:1961–1970.
4. Fendt M (2006) Exposure to urine of canids and felids, but not of herbivores, induces defensive behavior in laboratory rats. *J Chem Ecol* 32:2617–2627.
5. Kobayakawa K, et al. (2007) Innate versus learned odour processing in the mouse olfactory bulb. *Nature* 450:503–508.
6. Pryce CR, Bettschen D, Bahr NI, Feldon J (2001) Comparison of the effects of infant handling, isolation, and nonhandling on acoustic startle, prepulse inhibition, locomotion, and HPA activity in the adult rat. *Behav Neurosci* 115:71–83.

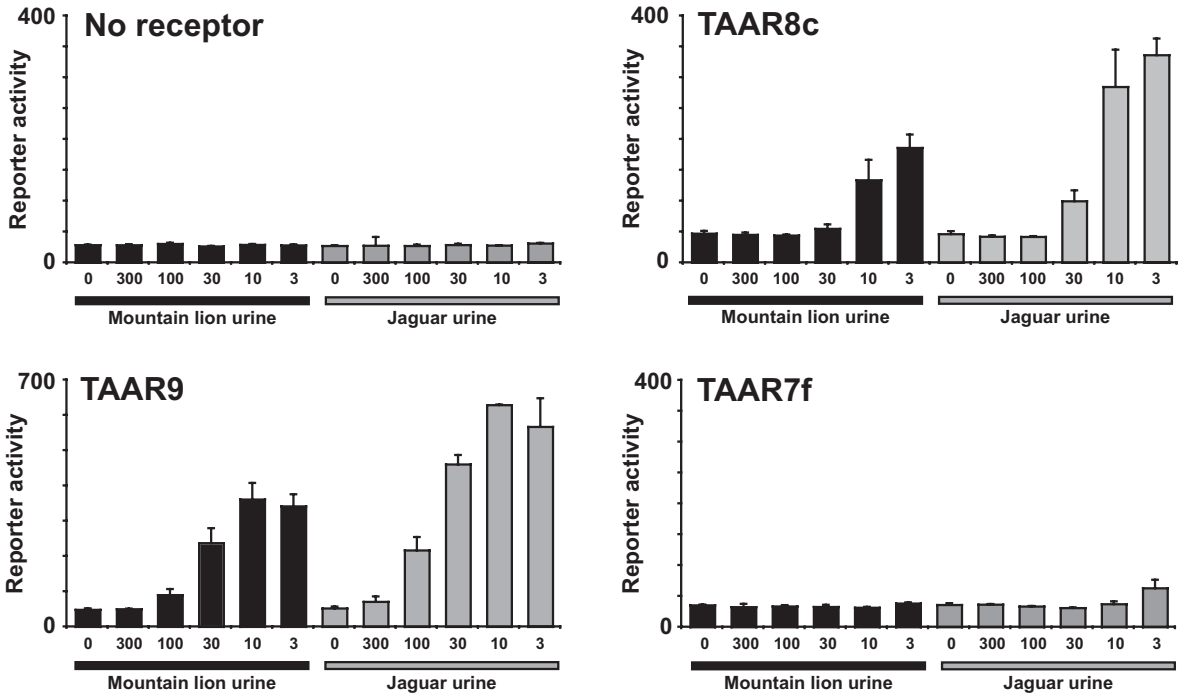


Fig. S1. Responses of TAAR9, TAAR8c, and TAAR7f to carnivore urines. HEK293 cells were transfected with TAAR and reporter plasmids, incubated with urine extracts indicated, and assayed for reporter activity (triplicates ± SD). Rat TAAR8c and rat TAAR9 detected urine extracts of carnivores (jaguar and mountain lion) and noncarnivores (Fig. 1: mouse, rat, human) with similar sensitivity. Mouse TAAR7f weakly detected jaguar urine. No responses were observed to animals odors in control cells transfected with reporter plasmid alone.

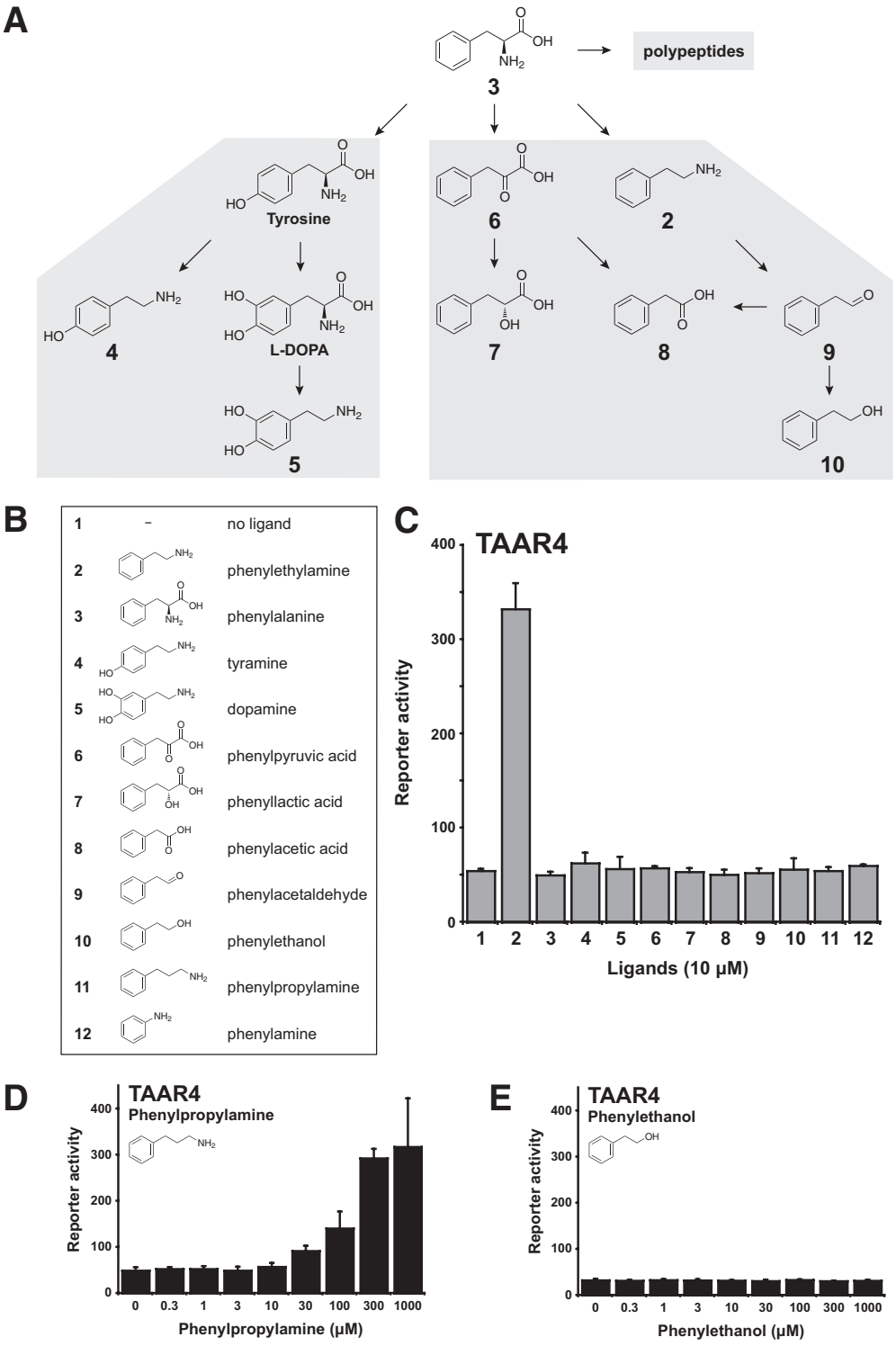


Fig. S2. TAAR4 has a narrow chemoreceptive field. (*A* and *B*) The names and structures of phenylalanine metabolites and other chemicals tested in TAAR4 functional assays. (*C*) TAAR4 detects 2-phenylethylamine but not related chemicals or other phenylalanine metabolites with similar sensitivity. HEK293 cells were transfected with TAAR4 plasmid and CRE-SEAP, incubated with ligands indicated (10 μM), and assayed for reporter activity (triplicates ± SD). TAAR4 was expressed as a fusion protein with an N-terminal sequence of bovine rhodopsin, which provided enhanced signal (2). (*D* and *E*) 3-phenylpropylamine activates TAAR4 at high concentrations, whereas 2-phenylethanol did not activate TAAR4 at any concentration tested.

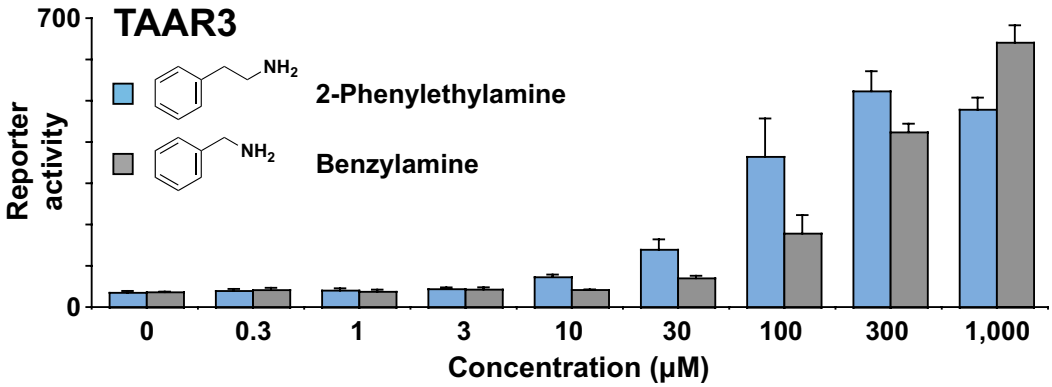


Fig. S3. TAAR3 detects both 2-phenylethylamine and benzylamine. Reporter gene assays were performed on HEK293 cells transfected with mouse TAAR3 and CRE-SEAP. TAAR3 detects numerous primary amines including isoamylamine as a preferred ligand (1), 2-phenylethylamine ($EC_{50} \approx 100$ μM), and benzylamine ($EC_{50} \approx 200$ μM). TAAR3 detects 2-phenylethylamine with 30-fold reduced sensitivity compared with TAAR4 and similarly detects benzylamine, which does not elicit avoidance behavior.

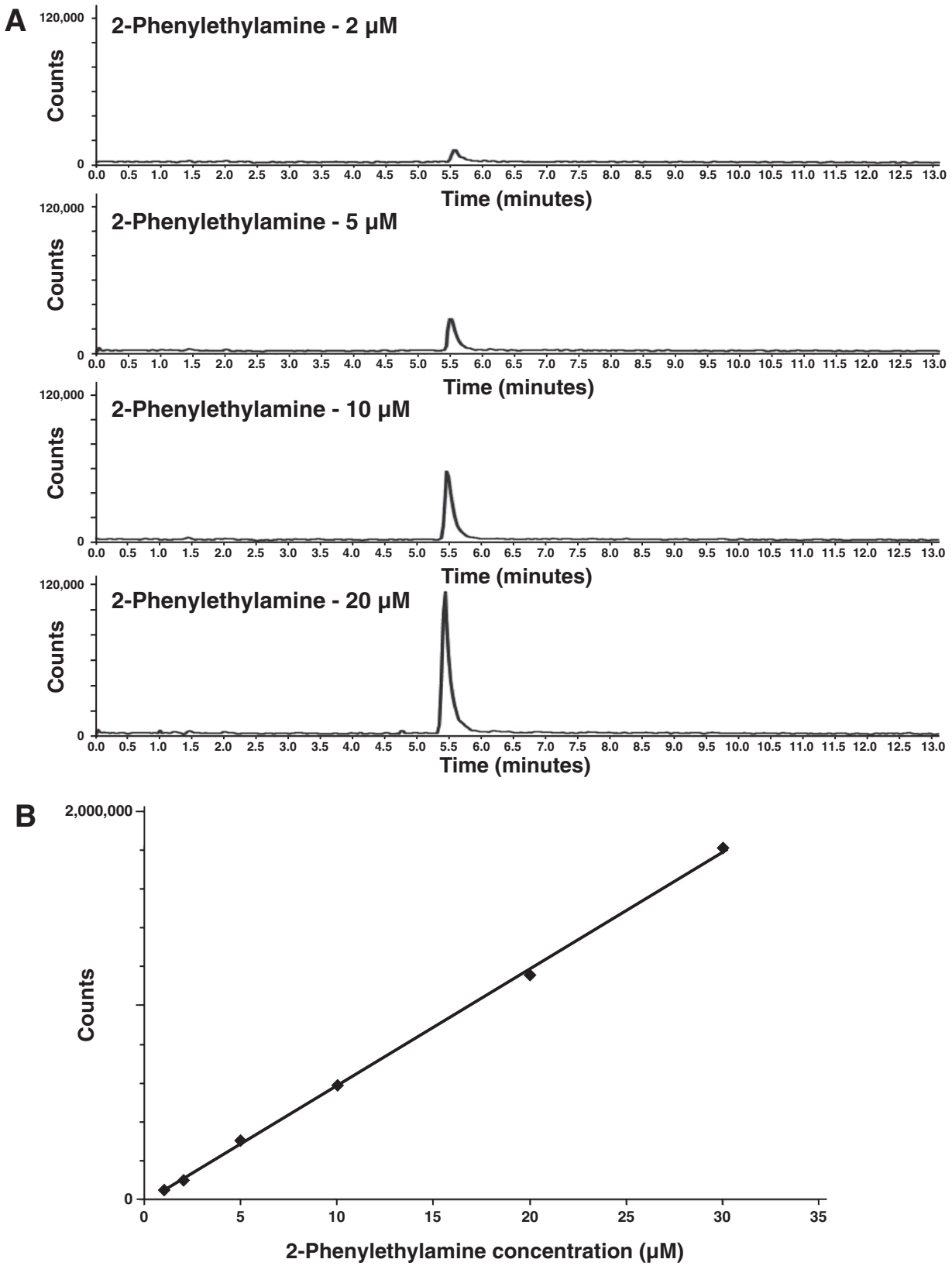
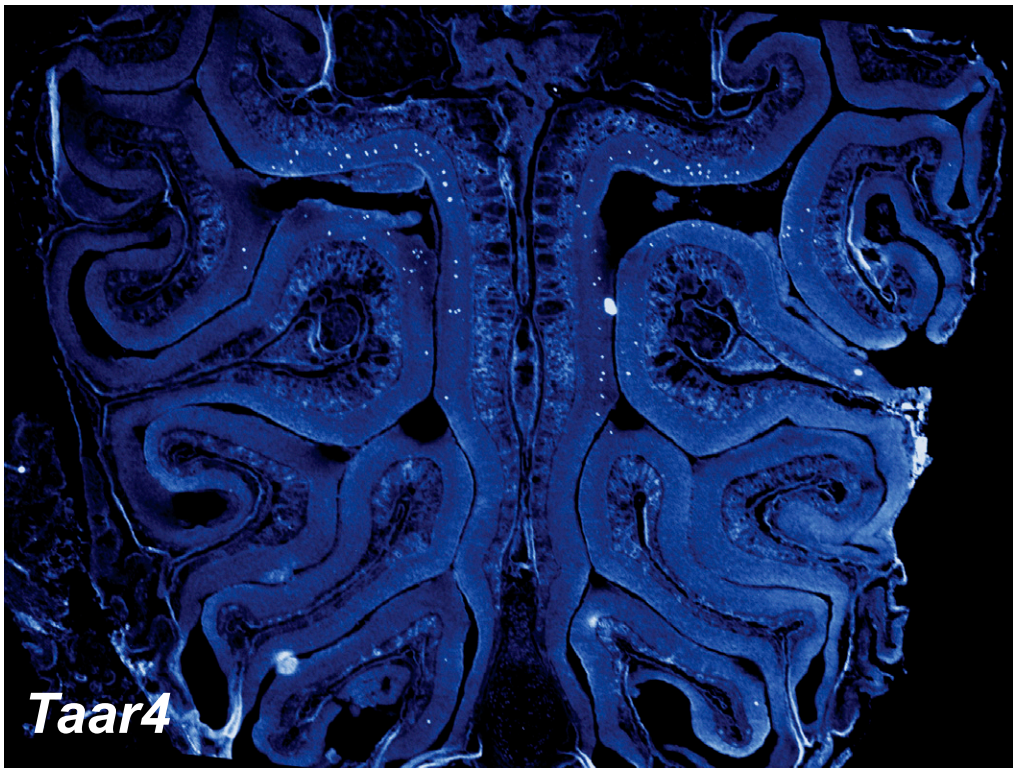


Fig. S4. Quantitative analysis of 2-phenylethylamine by LC/MS. (*A*) LC/MS was performed on solutions containing various concentrations of 2-phenyethylamine, and the number of ion counts with *m/z* = 122 were graphed versus retention time. Analysis of 2-phenylethylamine standards yielded single peaks of consistent retention time whose integrated areas were correlated with concentration. (*B*) Plotting integrated 2-phenylethylamine peak area versus 2-phenylethylamine concentration enabled calculation of 2-phenylethylamine concentration in unknown samples based on linear regression analysis of peak area by using the sum of least square method (Excel, **Microsoft**).

Samples	Source	PEA concentration (μM)
Bear	Kishel's Scents & Lures, Butler, PA	2.7
Bobcat 1	Predator Pee, Lexington Outdoors, Robbinston, ME	24.5
Bobcat 2	Predator Pee, Lexington Outdoors, Robbinston, ME	35.3
Bobcat 3	Predator Pee, Lexington Outdoors, Robbinston, ME	11.1
Bobcat 4	Kishel's Scents & Lures, Butler, PA	5.3
Bobcat 5	Leg Up Enterprises, Lovell, ME	72.5
Bobcat 6	Harmon's Trophy Hunting Products, Ellijay, GA	6.7
Bobcat 7	Mark June's Lures, Calhoun, NE	21.6
Bobcat 8	Fox Hollow, Marble Hill, GA	12.2
Bobcat 9	Minnesota Trapline Products, Pennock, MN	21.3
Cat 1	Collected	3.6
Cat 2	Bioreclamation, Hicksville, NY	2.4
Cheetah 1	Great Plains Zoo, SD	5.2
Cheetah 2	Great Plains Zoo, SD	8.7
Coati	Stone Zoo, MA	2.5
Cougar 1	Stone Zoo, MA	3.4
Cougar 2	Stone Zoo, MA	2.9
Cougar 3	Stone Zoo, MA	6.8
Cougar 4	Stone Zoo, MA	3.6
Cow	Lexington Outdoors, Lincoln, ME	< 100 nM
Coyote 1	Predator Pee, Lexington Outdoors, Robbinston, ME	3.8
Coyote 2	Predator Pee, Lexington Outdoors, Robbinston, ME	0.9
Coyote 3	Leg Up Enterprises, Lovell, ME	5.3
Coyote 4	Harmon's Trophy Hunting Products, Ellijay, GA	17.5
Coyote 5	Wildlife Research Center, Ramsey, MN	23.6
Coyote 6	Mark June's Lures, Calhoun, NE	15.5
Coyote 7	Minnesota Trapline Products, Pennock, MN	3.8
Coyote 8	Fox Hollow, Marble Hill, GA	9.8
Deer 1	Kishel's Scents & Lures, Butler, PA	0.4
Deer 2	Kishel's Scents & Lures, Butler, PA	0.5
Deer 3	Harmon's Trophy Hunting Products, Ellijay, GA	0.6
Deer 4	In Heat Scents, Kinston, AL	< 100 nM
Elk 1	Pete Rickard, Cobleskill, NY	< 250 nM
Elk 2	Harmon's Trophy Hunting Products, Ellijay, GA	< 100 nM
Elk 3	Harmon's Trophy Hunting Products, Ellijay, GA	< 100 nM
Elk 4	Harmon's Trophy Hunting Products, Ellijay, GA	< 100 nM
Ferret	Bioreclamation, Hicksville, NY	0.3
Fisher	Kishel's Scents & Lures, Butler, PA	18.5
Fox 1	Predator Pee, Lexington Outdoors, Robbinston, ME	6.0
Fox 2	Predator Pee, Lexington Outdoors, Robbinston, ME	3.6
Fox 3	Predator Pee, Lexington Outdoors, Robbinston, ME	64.7
Fox 4	Harmon's Trophy Hunting Products, Ellijay, GA	5.8
Fox 5	Mark June's Lures, Calhoun, NE	1.5
Fox 6	Wildlife Research Center, Ramsey, MN	11.8
Fox 7	Minnesota Trapline Products, Pennock, MN	20.4
Fox 8	Minnesota Trapline Products, Pennock, MN	2.7
Fox 9	Fox Hollow, Marble Hill, GA	0.5
Gerbil	Bioreclamation, Hicksville, NY	0.9
Giraffe 1	Franklin Park Zoo, MA	< 100 nM
Giraffe 2	Franklin Park Zoo, MA	< 100 nM
Guinea pig	Bioreclamation, Hicksville, NY	< 100 nM
Hamster	Bioreclamation, Hicksville, NY	1.5
Horse	Capron Park Zoo, MA	< 100 nM
Human	Bioreclamation, Hicksville, NY	0.1
Jaguar 1	Stone Zoo, MA	129.1
Jaguar 2	Stone Zoo, MA	173.0
Jaguar 3	Stone Zoo, MA	86.0
Jaguar 4	Stone Zoo, MA	75.2
Jaguar 5	Stone Zoo, MA	79.6
Jaguar 6	Stone Zoo, MA	115.4
Jaguar 7	Stone Zoo, MA	161.7
Jaguar 8	Stone Zoo, MA	65.6
Jaguar 9	Stone Zoo, MA	59.1
Jaguar 10	Stone Zoo, MA	68.9
Jaguar 11	Stone Zoo, MA	57.8
Lion 1	Franklin Park Zoo, MA	522.9
Lion 2	Capron Park Zoo, MA	44.4
Lion 3	Capron Park Zoo, MA	645.3
Lion 4	Capron Park Zoo, MA	58.1
Lion 5	Franklin Park Zoo, MA (pool of 3 animals)	461.0
Lion 6	Franklin Park Zoo, MA (pool of 3 animals)	309.0
Llama 1	Capron Park Zoo, MA	0.3
Llama 2	Stone Zoo, MA	0.4
Lynx 1	Minnesota Trapline Products, Pennock, MN	6.5
Lynx 2	Kishel's Scents & Lures, Butler, PA	5.8
Mink	Minnesota Trapline Products, Pennock, MN	3.1
Moose 1	Harmon's Trophy Hunting Products, Ellijay, GA	0.2
Moose 2	Harmon's Trophy Hunting Products, Ellijay, GA	0.7
Moose 3	Harmon's Trophy Hunting Products, Ellijay, GA	0.7
Mountain lion 1	Predator Pee, Lexington Outdoors, Robbinston, ME	62.7
Mountain lion 2	Predator Pee, Lexington Outdoors, Robbinston, ME	25.3
Mountain lion 3	Harmon's Trophy Hunting Products, Ellijay, GA	51.6
Mouse 1	Collected (pool of 5 animals)	1.8
Mouse 2	Collected (pool of 5 animals)	1.4
Mouse 3	Collected (pool of 5 animals)	1.1
Mouse 4	Collected	1.2
Mouse 5	Collected	0.7
Ocelot 1	Capron Park Zoo, MA	3.6
Ocelot 2	Capron Park Zoo, MA	2.5
Pig	Harmon's Trophy Hunting Products, Ellijay, GA	< 100 nM
Porcupine	Stone Zoo, MA	< 100 nM
Rabbit 1	Kishel's Scents & Lures, Butler, PA	< 100 nM
Rabbit 2	In Heat Scents, Kinston, AL	< 100 nM
Rabbit 3	Harmon's Trophy Hunting Products, Ellijay, GA	< 100 nM
Raccoon 1	Minnesota Trapline Products, Pennock, MN	89.5
Raccoon 2	Kishel's Scents & Lures, Butler, PA	12.7
Rat 1	Collected	0.6
Rat 2	Collected	0.7
Rat 3	Collected	1.4
Rat 4	Collected	0.5
Rat 5	Collected	0.8
Rat 6	Collected	1.4
Serval 1	Capron Park Zoo, MA	385.9
Serval 2	Capron Park Zoo, MA	426.5
Serval 3	Capron Park Zoo, MA	220.0
Serval 4	Capron Park Zoo, MA	194.5
Snow leopard 1	Stone Zoo, MA	3.3
Snow leopard 2	Stone Zoo, MA	2.2
Snow leopard 3	Great Plains Zoo, SD	16.9
Snow leopard 4	Great Plains Zoo, SD	3.8
Squirrel	Kishel's Scents & Lures, Butler, PA	< 100 nM
Tiger 1	Great Plains Zoo, SD	129.1
Tiger 2	Great Plains Zoo, SD	98.2
Tiger 3	Great Plains Zoo, SD	112.7
Tiger 4	Great Plains Zoo, SD	320.0
Tiger 5	Great Plains Zoo, SD	49.1
Wolf 1	Predator Pee, Lexington Outdoors, Robbinston, ME	1.1
Wolf 2	Predator Pee, Lexington Outdoors, Robbinston, ME	36.7
Wolf 3	Predator Pee, Lexington Outdoors, Robbinston, ME	14.4
Wolf 4	Leg Up Enterprises, Lovell, ME	22.7
Wolf 5	Harmon s Trophy Hunting Products, Ellijay, GA	16.0
Woodchuck	Kishel s Scents & Lures, Butler, PA	< 100 nM
Zebra	Franklin Park Zoo, MA	< 100 nM

Fig. S5. 2-phenylethylamine (PEA) levels in each of 123 individual urine specimens from 38 mammalian species used for Fig. 3. The sources of samples are shown, and zoo specimens from the same species either originated from different animals, or in some cases from the same animals collected on different days. Purchased specimens from the same species and source originate from different lots. Mouse and rat samples were collected overnight by using a metabolic cage. One cat sample was collected overnight by using nonabsorbent litter (NoSorb Beads; Catco).

A



B

Receptor	Location in OE
TAAR1	not detected
TAAR2	dorsal
TAAR3	dorsal
TAAR4	dorsal
TAAR5	dorsal
TAAR6	ventral
TAAR7s	dorsal, ventral
TAAR8s	dorsal
TAAR9	dorsal

Fig. S6. Expression patterns of TAARs in olfactory epithelium. (*A*) Expression of *Taar4* in coronal sections of mouse olfactory epithelium is visualized by fluorescent in situ hybridization as described (1). Neurons expressing TAAR4 are dispersed in a dorsal zone of the olfactory epithelium. (*B*) The location of other TAAR-expressing neurons along the dorsal-ventral axis is summarized. All TAARs are expressed dorsally, except for TAAR6 and at least one TAAR7 subfamily member.