Supporting Online Material for

The Hallucinogen *N,N*-Dimethyltryptamine (DMT) Is an Endogenous Sigma-1 Receptor Regulator

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Supporting Online Material

Materials and Methods

Chemistry. Yields refer to isolated pure products after column chromatography. The products were characterized by comparison of their spectral (IR, ^{1}H and ^{13}C NMR, elemental and mass spectroscopy analyses) and physical data with those of authentic samples. All ^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$ relative to TMS (0.00 ppm). Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. The synthesis of compounds **1-5** is outlined in Scheme S2.

Preparation of 2-(1*H*-indol-3-yl)-*N,N*-dimethylethylamine (1): To 250 mg of 3-(2-bromoethyl)indole in a round-bottomed flask, a 10 M excess of dimethylamine (6 mL of 2M in MeOH) was added and the solution was stirred at r.t. overnight. After adding 5 mL sodium bicarbonate (2%), the reaction mixture was extracted with chloroform (3x5 ml) and back extracted with H_2O (1x5mL). The combined extracts were dried over $MgSO_4$ and evaporation of the solvent gave the product in crystalline form. Yield: 0.21 g, 84%, yellowish crystals. m.p.: 45-47 °C; TLC (EtAc:MeOH:acetic acid, 8:2:0.5 v/v/v): *R*F = 0.33. ^{1}H NMR, δ: 10.43 (s, 1 H), 7.42-7.06 (m, 5 H), 2.68 (m, 4H), 2.26 (S, 6 H). ^{13}C NMR ($CDCl_3$): δ 136.5, 124.3, 123.2, 121.2, 119.7, 117.6, 113.4, 111.1, 61.7, 41.2, 22.4. Anal. calcd for $C_{12}H_{16}N_2$: C, 76.55; H, 8.57; N, 14.88%. Found; C, 76.60; H, 8.70; N, 14.70%.

Preparation of *N,N*-dimethyl-2-phenylethylamine (2): To a stirring solution of phenylacetaldehyde (5 mM, 0.6 mL) and dimethylamine (5 mM, 3 mL of 2M in MeOH) in MeOH (1 mL), was added $NaCNBH_3$ (5 mM, 360 mg) in a round-bottomed flask. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and H_2O was added to the residue before extraction with CH_2Cl_2. The combined extracts were dried over $MgSO_4$, the solid was filtered off, and the solvent was evaporated under reduced pressure to give a yellow oil. The crude products were purified by column chromatography (silica gel, EtAc:n-hexane, 4:6) to afford pure product in quantitative yield, 0.52 g, 87%, yellow oil. TLC (EtAc:n-hex, 4:6 v/v): *R*F = 0.2. ^{1}H NMR, δ: 7.40-7.20 (m, 5 H), 3.20 (m, 4H), 2.20 (S, 6 H). ^{13}C NMR, δ: 141.5, 129.1, 128.6, 125.8, 60.9, 45.6, 32.2. Anal. calcd for $C_{10}H_{13}N$: C, 80.48; H, 10.13; N, 9.39%. Found; C, 90.60; H, 10.20; N, 9.40%.

Preparation of 4-(2-(methylamino)ethyl)phenol (3): To 250 mg of 4-hydroxyphenethyl bromide in a round-bottomed flask, a 10 M excess of methylamine (6.2 mL of 2M in MeOH) was added and the solution was stirred at r.t. overnight. After filtration, the solvent was removed to give a brown oil in 80% yield. TLC (EtAc:MeOH, 9:1 v/v): *R*F = 0.33. ^{1}H NMR, δ: 9.42 (s, 1 H), 7.10 (d, 2 H), 6.75 (d, 2 H), 3.60 (s, 3 H), 2.84 (t, 2 H),

2.60 (t, 2 H), 1.90 (s, 1 H). ^{13}C NMR, δ: 167.5, 131.2, 128.3, 112.4, 41.5, 36.4, 35.0. Anal. calcd for $C_9H_{13}NO$: C, 71.49; H, 8.67; N, 9.26%. Found; C, 71.50; H, 8.80; N, 9.20%.

Preparation of 4-(2-(dimethylamino)ethyl)phenol (4): To 250 mg of 4-hydroxyphenethyl bromide in a round-bottomed flask, a 10 M excess of methylamine (6.22 mL of 2M in MeOH) was added and the solution was stirred at r.t. overnight. After filtration, the solvent was evaporated and gave the product in crystalline form in 92% yield, 0.23 g, light yellow. m.p.: 73-76 °C. TLC (EtAc:MeOH, 9:1 v/v): *R*F = 0.1. ^{1}H NMR, δ: 9.43 (s, 1 H), 7.12 (d, 2H), 6.70 (d, 2 H), 2.74 (m, 4 H), 2.30 (s, 6 H). ^{13}C NMR, δ: 167.5, 134.3, 130.2, 115.6, 60.6, 45.9, 32.4. Anal. calcd for $C_{10}H_{15}NO$: C, 72.69; H, 9.15; N, 8.48%. Found; C, 72.60; H, 9.30; N, 8.40%.

Preparation of 4-(2-(ethylamino)ethyl)phenol (5): To a stirring solution of tyramine (1 mM, 137 mg) and MeOH (5 mL) was added acetaldehyde in excess. Then excess $NaBH_3CN$ (1 mM, 62.8 mg) was added and the mixture stirred for 2 hours at r.t. After evaporating the solvent, methylene chloride (5 mL) was added and mixture was washed with H_2O (3x5 mL). The mixture was dried over $MgSO_4$, filtered, and evaporated to give the product in 72% yield as an orange oil. TLC (toluene:diethylamine, 4:1 v/v): *R*F = 0.5. ^{1}H NMR, δ: 9.48 (s, 1 H), 7.14 (d, 2H), 6.70 (d, 2 H), 2.85 (m, 2 H), 2.60 (t, 2 H), 250 (q, 2 H), 2.1 (s, 1 H), 1.1 (t, 3 H). ^{13}C NMR, δ: 167.5, 134.3, 130.2, 115.6, 48.2, 44.4, 35.6, 15.6. Anal. calcd for $C_{10}H_{15}NO$: C, 72.69; H, 9.15; N, 8.48%. Found; C, 72.50; H, 9.20; N, 8.50%.

Radiochemistry. Radiosynthesis of 3-[^{125}I]iodo-4-azidococaine ([^{125}I]-IACoc) (*S1*) and 1-*N*-(2',6'dimethyl-morpholino)-3-(4-azido-3-[^{125}I]iodo-phenyl) propane ([^{125}I]IAF) (*S2*) was performed as described.

Cell Culture and Transfection. HEK293 cells stably expressing hNav1.5 were provided by Dr. J.C. Makielski at the University of Wisconsin-Madison (*S3*). COS-7 cells were transiently transfected with recombinant cDNA encoding hNav1.5 using Lipofectamine. Both cell types were cultured on glass coverslips at 37ºC in 5% CO_2/air atmosphere, and used for electrophysiological recordings within 3-5 days.

Neonatal Myocyte Isolation and Culture. Neonatal mouse cardiac myocytes from 129/SvEvBrd x C57BL6/J sigma-1 receptor knock out (*S4*) and wild-type mice were enzymatically isolated and cultured on laminin-coated glass coverslips as previously described (*S5, S6*).

Preparation of Rat/Guinea Pig/Mouse Liver/Cell membranes. Preparation of rat, guinea pig, and mouse liver membrane homogenates was performed as previously

described (*S2, S7*) with the exception that mouse livers were obtained from age-matched adult 129/SvEvBrd x C57BL6/J sigma-1 receptor knock out (*S4*) and wild-type mice. HEK293 and COS-7 cell homogenates were prepared by passing cells through a custom-built cell cracker as previously described (*S8*).

Sigma Receptor Binding Assays. Competitive binding assays were performed as previously described (*S2*) with the exception of testing new ligands shown in Fig. 1C.

Photoaffinity Labeling. Sigma-1 and sigma-2 receptors were photolabeled with 3-[^{125}I]iodo-4-azidococaine ([^{125}I]-IACoc) and 1-N-(2',6'-dimethyl-morpholino)-3-(4-azido-3-[^{125}I]iodo-phenyl) propane ([^{125}I]IAF) as described (*S2*) with the exception of the protecting ligands, tryptamine, *N*-methyltryptamine, and *N,N*-dimethyltryptamine. Following photolabeling of cell homogenates (100 μg) and prior to protein separation by SDS-PAGE (12%), the homogenates were solubilized with 1% Triton X-100 and centrifuged at 14000G for 30 minutes to separate the Triton extract. Sigma-1 receptor polyclonal antibody (*S9*) (3 μg) was added to the solubilized extract and incubated at 4 °C for 4 hr. Immune complexes were captured using protein A-sepharose (GE healthcare, Piscataway, NJ, USA).

Electrophysiology. I_{Na} was recorded from all cell types using whole-cell patch clamp at room temperature. External recording solutions were perfused by gravity feed at ~1-2 ml/min. Individual cells were located with an upright DIC microscope (Reichert Microscope Services, Depew, NY, USA) equipped with a Zeiss 40X water immersion objective (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA). Patch pipettes were fabricated from borosilicate or aluminosilicate glass (Garner Glass Co., Claremont, CA) and pipette shanks were coated with Sylgard to reduce electrode capacitance (*S10*). Prior to contact with the cell membrane, resistances ranged from 1-3 MΩ. Immediately after breaking in, cell capacitance and series resistance were determined by transient cancellation. Series resistance compensation was applied (85-95%), to reduce the effective series resistance below 2 MΩ. Recordings were made using an Axopatch-200B patch clamp amplifier (Axon Instruments/Molecular Devices, Foster City, CA, USA), interfaced to a PC. Data acquisition, voltage control, and analysis were carried out with PCLAMP7 software (Axon Instruments/Molecular Devices, Foster City, CA, USA).

External solution for recordings in HEK293 and COS-7 cells consisted of (mM): NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2 glucose 10, HEPES 10 (pH 7.4 NaOH). The pipette solution contained (mM): KCl 140, $MgCl_2$ 2, $CaCl_2$ 1, EGTA 5, glucose 10, HEPES 10 (pH 7.2 KOH). Extracellular solution for recordings from neonatal cardiac myocytes consisted of (mM): tetraetylammonium chloride (TEA-Cl) 100, NaCl 40, Glucose 10, $MgCl_2$ 1, CsCl 5, $CaCl_2$ 0.1, $NiCl_2$ 1, HEPES 10 (pH 7.3 CsOH). Intracellular solution contained (mM) CsCl 135, NaCl 5, EGTA 10, HEPES 10 (pH 7.3 CsOH). Sodium currents were typically elicited with pulses from -80 mV to -10 mV for 25 msec. Current-voltage (I-V) analysis was routinely used to determine healthy cells. Cells were held at –80 mV and depolarized with 25 msec pulses in 10 mV increments from -70 to +70 mV.

N,N-dimethyltryptamine (DMT) was first dissolved in DMSO, and then diluted in external solution to obtain the desired drug concentration. Final DMSO never exceeded 0.1% (by volume), and control experiments verified that this level of DMSO had no effect on sodium currents. DMT was applied in recording solution by gravity feed at ~1-2 ml/min. In general, currents were recorded at 15 sec intervals for ~5 minutes to obtain a stable baseline, after which the drug was applied. Drug effects typically appeared within 2-4 minutes of solution change and were recorded until a stable inhibition level was achieved.

Mouse Behavior. All mice were maintained on a normal light/dark cycle and handled in accordance with Animal Care and Use Guidelines of the University of Wisconsin, Madison. Hypermobility effects induced by DMT in age-matched adult 129/SvEvBrd xC57BL6/J wild type (WT) and sigma-1 receptor knockout (KO) mice were measured in an open-field assay. Mice were first acclimatized to the experimental room for one hour. WT and KO mice were injected with the monoamine oxidase inhibitor, pargyline (75 mg/kg, i.p.) two hours prior to DMT or vehicle injection (*S11*). The mice were observed in the open-field box for an hour and then injected with DMT (2 mg/kg, i.p.) (*S11*) or vehicle, and observed for 30 minutes. Each condition represents 8 to 14 mice (n = 8-14). For methamphetamine studies, WT and KO mice (n = 6) were placed in the open-field and observed for one hour prior to methamphetamine (3mg/kg, i.p.) injection (*S12*) and observation (1 hr.) A computer program was used to quantitate movement and after each experiment, 70% ethanol was used to remove odors from the open-field.

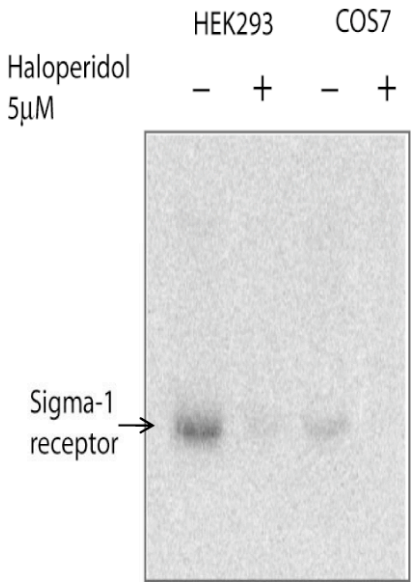
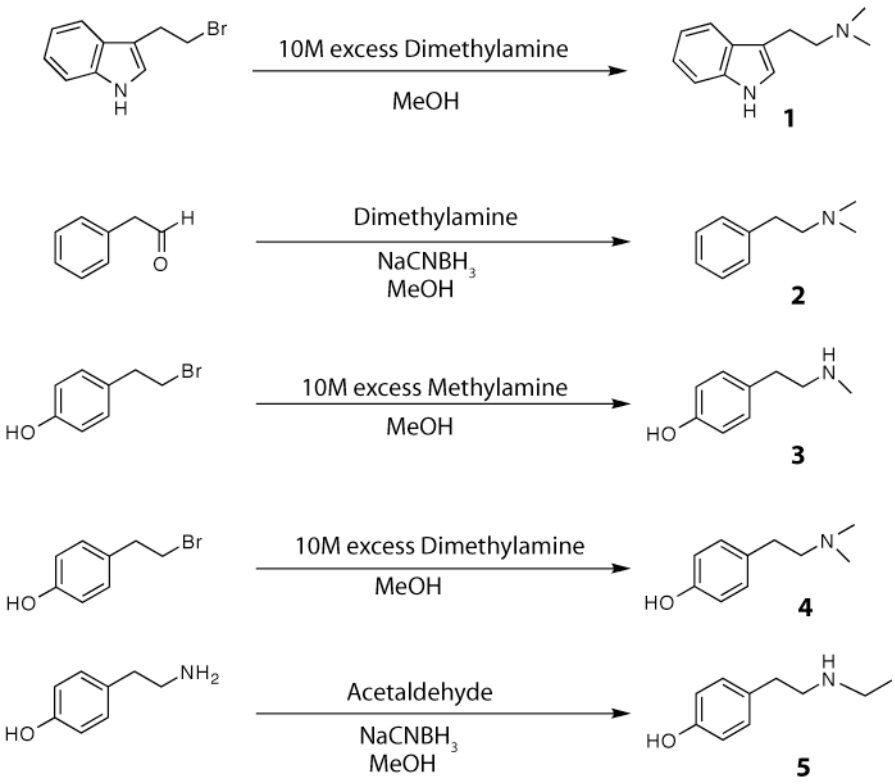


Fig. S1. Comparison of Sigma-1 Receptor Protein Levels in HEK293 and COS-7 cells. HEK293 and COS-7 cell homogenates (100 μg) were suspended in the presence or absence of 5 μM Haloperidol. Samples were photolyzed with 1 nM carrier-free 3-[^{125}I]iodo-4-azidococaine ([^{125}I]-IACoc) and immunoprecipitated with anti sigma-1 receptor antibody, revealing lower sigma-1 receptor (26 kD) expression in COS-7 cells as compared to HEK293 cells.



Scheme S2

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