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

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implications for optimizing plant breeding and cultivation strategies.

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

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The sigma-1 receptor is widely distributed in the central nervous system and periphery. Originally mischaracterized as an opioid receptor, the sigma-1 receptor binds a vast number of synthetic compounds but does not bind opioid peptides; it is currently considered an orphan receptor. The sigma-1 receptor pharmacophore includes an alkylamine core, also found in the endogenous compound *N,N*-dimethyltryptamine (DMT). DMT acts as a hallucinogen, but its receptor target has been unclear. DMT bound to sigma-1 receptors and inhibited voltage-gated sodium ion (Na^+) channels in both native cardiac myocytes and heterologous cells that express sigma-1 receptors. DMT induced hypermobility in wild-type mice but not in sigma-1 receptor knockout mice. These biochemical, physiological, and behavioral experiments indicate that DMT is an endogenous agonist for the sigma-1 receptor.

The sigma-1 receptor binds a broad range of synthetic compounds (*1*). It has long been suspected that the sigma-1 receptor is targeted by endogenous ligands, and several candidates have been proposed (*2*, *3*). Although progesterone and other neuroactive steroids are known to bind sigma-1 receptors and regulate some of their functions (*1*, *4*), they do not exhibit agonist properties on sigma-1–regulated ion channels in electrophysiological experiments (*5*).

Our search for a sigma receptor endogenous ligand (or ligands) was based on a variant of the canonical sigma-1 receptor ligand pharmacophore (*6*), but with a more basic structure (Fig. 1A). Otherwise dissimilar sigma-1 receptor ligands possess a common *N*-substituted pharmacophore (Fig. 1A): an *N,N*-dialkyl or *N*-alkyl-*N*-aralkyl product, most easily recognized in the high-affinity sigma-1 receptor ligand, fenpropimorph (*7*). Similar chemical backbones can be derived from other sigma-1 receptor ligands such as haloperidol and cocaine (Fig. 1A). *N*-substituted trace amines harbor this sigma-1 receptor ligand pharmacophore, but their interactions with sigma receptors have not been determined. Of particular interest is the only known endogenous mammalian *N,N*-dimethylated trace amine, *N,N*-dimethyltryptamine (DMT) (*8*–*10*). In addition to being one of the active compounds in psychoactive snuffs (*yopo, epená*) and sacramental teas (*ayahuasca, yagé*) used in native shamanic rituals in South America, DMT can be produced by enzymes in mammalian lung (*11*) and in rodent brain (*12*). DMT has been found in human urine, blood, and cerebrospinal fluid (*9*, *13*). Although there are no conclusive quantitative studies measuring the abundance of endogenous DMT because of its rapid metabolism (*14*), DMT concentrations can be localized and elevated in certain instances. Evidence suggests that DMT can be locally sequestered into brain neurotransmitter storage vesicles and that DMT production increases in rodent brain under environmental stress (*8*). Although a family of heterotrimeric GTP-binding protein (G protein)–coupled receptors (GPCRs) known as the trace amine receptors (TARs) was discovered in 2001 (*15*), only two members of this family respond to trace amines and have been renamed trace amine-associated receptors (TAARs) (*16*). Because other binding targets for trace amines and DMT are likely (*8*), we first examined the sigma-1 receptor binding affinities of the trace amines and their *N*-methylated and *N,N*-dimethylated counterparts.

Competition assays against the sigma-1 receptor–specific ligand, (+)-[^{3}H]-pentazocine (10 nM), determined that the nonmethylated trace amines tryptamine, phenethylamine, and tyramine bound the sigma-1 receptor poorly (Fig. 1C), with dissociation constant (K_d) values of 431, 97.4, and >30,000 μM, respectively. By contrast, the *N*-methylated and *N,N*-dimethylated derivatives of these compounds bound sigma-1 receptors more tightly, with a clear increase in affinity as the ligands approached the sigma-1 receptor ligand pharmacophore (Fig. 1, A and B). With the exception of the *N*-methylated tyramines, this trend did not apply to the sigma-2 receptor, which differs pharmacologically and functionally from the sigma-1 receptor (Fig. 1C). Tryptamine, phenethylamine, and *N*-methyltyramine had the highest sigma-2 receptor affinities, with K_d values of 4.91, 7.31, and 6.61 μM, respectively. In contrast to sigma-1 receptors, *N*-methylation and *N,N*-

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dimethylation of tryptamine and phenethylamine decreased sigma-2 receptor affinity (Fig. 1C).

We tested the ability of tryptamine, *N*-methyltryptamine, and DMT to block sigma receptor photolabeling in rat liver homogenates by two radioactive photoaffinity labels, the sigma-1 receptor–specific cocaine derivative 3-[^{125}I]iodo-4-azidococaine ([^{125}I]-IACoc) (*17*) (Fig. 2A) and the sigma-1 and sigma-2 receptor fenpropimorph derivative 1-*N*-(2′,6′-dimethyl-morpholino)-3-(4-azido-3-[^{125}I]iodo-phenyl) propane ([^{125}I]IAF) (*18*) (Fig. 2B). Both of these compounds have been used to identify the drug binding region of the sigma-1 receptor (*18*, *19*). As anticipated, [^{125}I]-IACoc [sigma-1 K_d = 0.126 nM (*17*)] photolabeling of the 26-kD sigma-1 receptor (Fig. 2A) was protected best by DMT, with 61% protection by 50 μM DMT and almost 100% protection by 100 μM DMT. By contrast, tryptamine

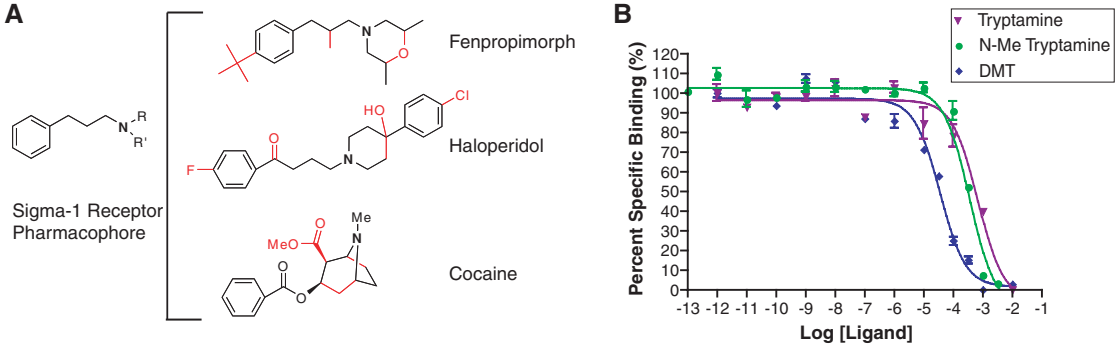

Fig. 1. Sigma-1 receptor ligand pharmacophore and binding affinities. (**A**) A basic sigma-1 receptor ligand pharmacophore variant of Glennon *et al*. (*6*) was derived by removal of the red bonds from the sigma-1 receptor ligands fenpropimorph, haloperidol, and cocaine. (**B**) Competitive binding curves of tryptamine, *N*-methyltryptamine, and DMT, against the radioactive sigma-1 receptor ligand [^{3}H]-(+)-pentazocine. Curves are shown as percent specific binding (5 μM haloperidol). (**C**) Sigma-1 and sigma-2 receptor K_d values of trace amines and their *N*-methylated and *N*,*N*-dimethylated derivatives (scheme S2). Included are SEM values (n = 3 binding experiments) and R^2 values for a nonlinear regression curve fit. Solid arrows denote the direction of increasing affinity.

C

R_1	R_2	Ligand	Sigma-1 K_D (μM) (± SEM, n=3), R^2	Sigma-2 K_D (μM) (± SEM, n=3), R^2	Ratio Sigma2/Sigma1
H—	H—	Tryptamine	431.55 (± 342.5, 0.87)	4.91 (± 5.2, 0.92)	0.011
H—	H_3C—	N-methyltryptamine	150 (± 61.6, 0.97)	12.82 (± 7.0, 0.94)	0.085
H_3C—	H_3C—	N,N'-dimethyltryptamine	14.75 (± 7.0, 0.96)	21.71 (± 10.8, 0.94)	1.472
H—	H—	Phenethylamine (PEA)	97.4 (± 23.3, 0.99)	7.31 (±5.2, 0.97)	0.075
H—	H_3C—	N-methylPEA	59.85 (± 22.3, 0.96)	21.93 (± 12.5, 0.94)	0.366
H_3C—	H_3C—	N,N'-dimethylPEA	12.89 (± 6.9, 0.92)	21.16 (± 11.8, 0.93)	1.642
H—	H—	Tyramine	>30,000	607 (± 422, 0.89)	>0.020
H—	H_3C—	N-methyltyramine	12.43 (± 2.7, 0.98)	6.61 (± 10.6, 0.91)	0.532
H_3C—	H_3C—	N,N'-dimethyltyramine	38.27 (± 10.3, 0.98)	64.29 (± 43.0, 0.94)	1.680
H—		N-ethyltyramine	1.62 (± 0.5 0.97)	N/A	N/A

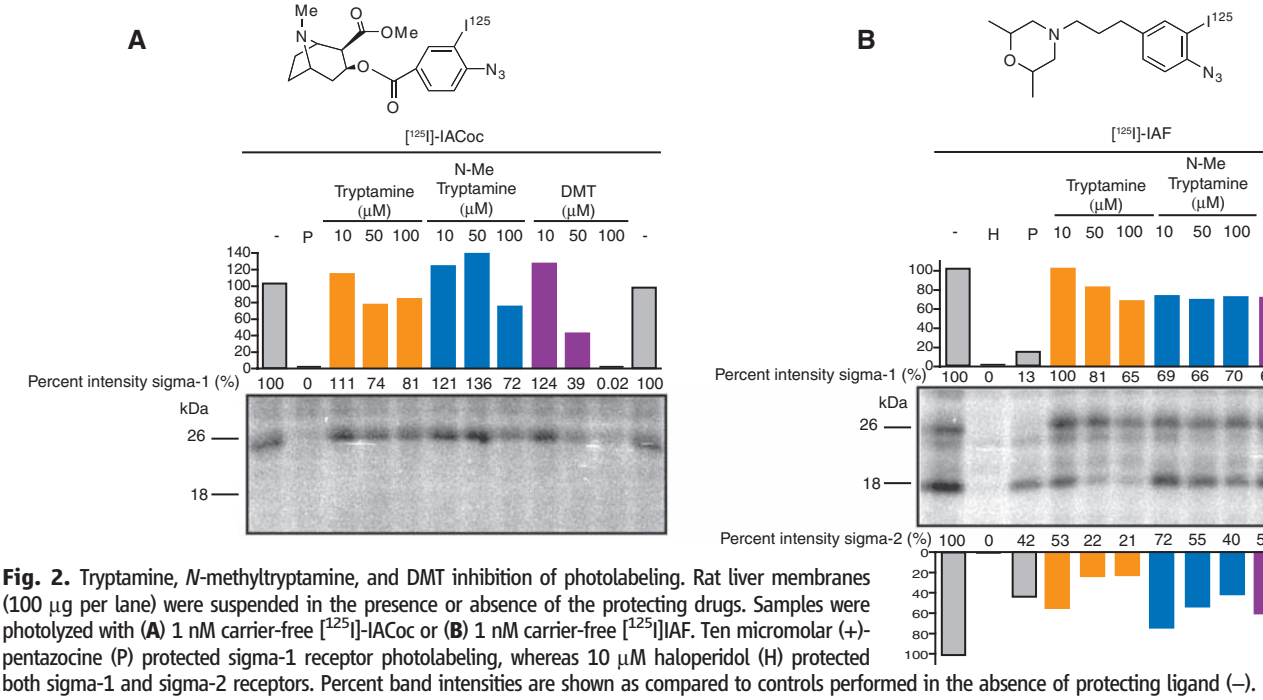

Fig. 2. Tryptamine, *N*-methyltryptamine, and DMT inhibition of photolabeling. Rat liver membranes (100 μg per lane) were suspended in the presence or absence of the protecting drugs. Samples were photolyzed with (**A**) 1 nM carrier-free [^{125}I]-IACoc or (**B**) 1 nM carrier-free [^{125}I]IAF. Ten micromolar (+)-pentazocine (P) protected sigma-1 receptor photolabeling, whereas 10 μM haloperidol (H) protected both sigma-1 and sigma-2 receptors. Percent band intensities are shown as compared to controls performed in the absence of protecting ligand (–).

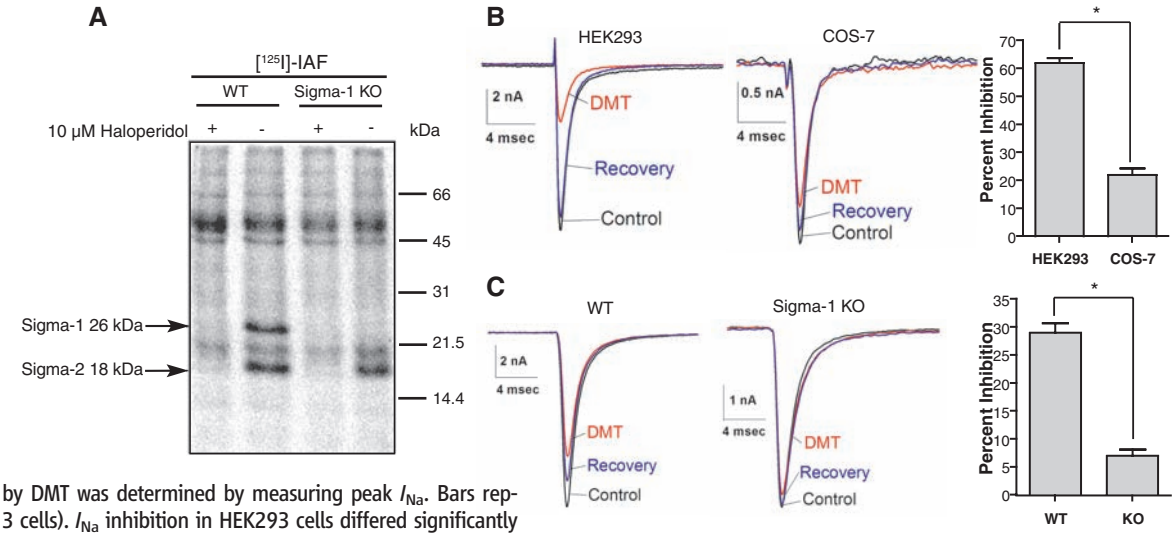

Fig. 3. Sodium channel inhibition by DMT. (**A**) In the presence or absence of 10 μM haloperidol, wild type (WT) or sigma-1 receptor knockout (KO) mouse liver homogenates (200 μg/lane) were photolabeled with 1 nM [^{125}I]IAF. (**B**) Examples of I_{Na} evoked by steps from –80 to –10 mV in HEK293 or COS-7 cells expressing hNav1.5 channel in the absence (control, black), presence (DMT, red), and after wash out (recovery, blue) of 100 μM DMT. Average inhibition by DMT was determined by measuring peak I_{Na}. Bars represent mean ± SEM (n = 3 cells). I_{Na} inhibition in HEK293 cells differed significantly from that in COS-7 cells (*P < 0.03). (**C**) Examples of I_{Na} evoked as described in (B) in neonatal cardiac myocytes from WT and KO mice in the absence (control, black), presence (DMT, red), and after wash out (recovery, blue) of 100 μM DMT. Current inhibition in WT was significantly different from that in KO (*P < 0.002, n = 7 neonatal cardiac myocytes).

and *N*-methyltryptamine protected minimally against sigma-1 receptor [^{125}I]-IACoc photolabeling, even at these high concentrations (Fig. 2A). Similarly, [^{125}I]IAF photolabeling of the sigma-1 [K_d = 194 nM (*18*)] receptor showed that DMT was the most potent protector. Ten micromolar DMT provided 31% protection, whereas 50 and 100 μM DMT provided 43 and 69% protection, respectively (Fig. 2B). With the exception of *N*-methyltryptamine, protection of [^{125}I]IAF sigma-2 [K_d = 2780 nM (*18*)] receptor photolabeling paralleled the sigma-2 binding data. Tryptamine afforded the greatest protection of sigma-2 receptor photolabeling, with values of 47, 78, and 79% for 10, 50, and 100 μM, respectively (Fig. 2B).

An important biological activity of sigma receptor activation is the inhibition of ion channels, which operates through protein-protein interactions without mediation by G proteins and protein kinases (*20*–*22*). In addition to modulating various types of voltage-activated K^+ channels (*21*, *23*, *24*), the sigma-1 receptor associates with the Kv1.4 K^+ channel in posterior pituitary nerve terminals, as well as in *Xenopus* oocytes (*22*). Sigma receptor ligands also modulate N-, L-, P/Q-, and R-type Ca^{2+} channels in rat sympathetic and parasympathetic neurons (*25*). Sigma receptor ligands modulate cardiac voltage-gated Na^+ channels (hNav1.5) in human embryonic kidney 293 (HEK293) cells, COS-7 cells, and neonatal mouse cardiac myocytes (*26*). To evaluate the capacity of DMT to induce physiological responses by binding to sigma receptors, we examined the action of DMT on voltage-activated Na^+ current. Patch-clamp recordings from HEK293 cells stably expressing the human cardiac Na^+ channel hNav1.5 revealed voltage-activated Na^+ currents (I_{Na}) in response to voltage steps from –80 to –10 mV (Fig. 3B). Application of 100 μM DMT inhibited I_{Na} by 62 ± 3% (n = 3 HEK293 cells), which reversed upon DMT removal. With hNav1.5 transiently transfected into COS-7 cells, 100 μM DMT inhibited I_{Na} by only 22 ± 4% (n = 3 COS-7 cells), but photolabeling has shown that these cells have much lower concentrations of endogenous sigma-1 receptors compared to HEK293 cells (fig. S1 and Fig. 3B). The difference between DMT inhibition of I_{Na} in HEK293 and COS-7 cells (Fig. 3B, P < 0.03) thus demonstrates the dependence of I_{Na} inhibition on sigma-1 receptors. Experiments in cardiac myocytes demonstrated the same DMT action in a native preparation (Fig. 3C) and enabled further demonstration of sigma-1 receptor dependence by using a sigma-1 receptor

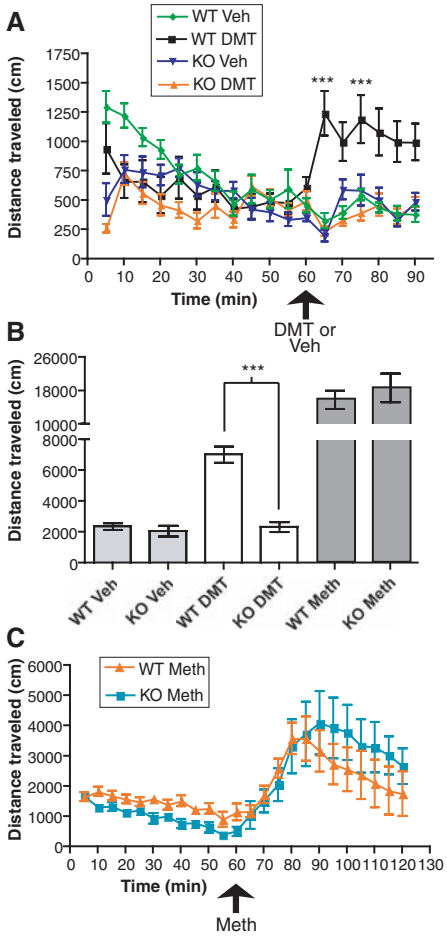

Fig. 4. DMT-induced hypermobility abrogated in the sigma-1 KO mouse. (**A**) Distances traveled by WT and KO mice were measured in an open-field assay in 5-min increments. Pargyline was injected 2 hours before DMT or vehicle (Veh) ip injection. Bars represent mean ± SEM (n = 8 to 14 mice). WT mice showed a significant (***P < 0.0001) increase in mobility in response to DMT as compared to KO mice. (**B**) Total distance traveled over 30 min after DMT, vehicle (Veh), or methamphetamine (Meth, n = 6 mice) injection in WT and KO mice. (**C**) Methamphetamine serves as a positive control for hypermobility in KO mice.

knockout mouse (*27*). [^{125}I]IAF photolabeling of liver homogenates from wild-type (WT) and sigma-1 receptor knockout (KO) mice indeed showed the absence of sigma-1 receptor (26 kD) in the KO samples (Fig. 3A). In WT neonatal cardiac myocytes, 100 μM DMT reversibly inhibited I_{Na} by 29 ± 3% (n = 7 WT myocytes), whereas I_{Na} was reduced by only 7 ± 2% (n = 7 KO myocytes) in KO myocytes (Fig. 3C, $P < 0.002$).

Both DMT and sigma receptor ligands influence animal behavior. DMT injection induces hypermobility in rodents concurrently treated with the monoamine oxidase inhibitor pargyline (*28*), and this action is not antagonized by blockers of dopamine or serotonin receptors, but is potently inhibited by haloperidol (*28*). Although haloperidol is thought to act in part through the dopamine D_2 receptor system, it is also a potent sigma-1 receptor agonist [sigma-1 inhibition constant (K_i) = 3 nM (*29*); sigma-2 K_i = 54 nM (*29*)] when inhibiting voltage-gated ion channels (*5*, *25*). Haloperidol reduces brain concentrations of DMT (*8*) and DMT inhibits haloperidol binding in brain tissue more robustly than the dopamine agonist apomorphine (*8*). On the basis of these findings, which were discovered before sigma receptor identification, DMT has been hypothesized to act through an unknown "hallucinogen" receptor (*8*). We confirmed results (*28*) that intraperitoneal (ip) administration of DMT (2 mg per kilogram of body weight) 2 hours after pargyline (75 mg/kg, ip) injection induced hypermobility in WT mice (7025 ± 524.1 cm, n = 12 WT mice) in an open-field assay. Identical drug treatments in sigma-1 receptor KO mice had no hypermobility action (2328 ± 322.9 cm, n = 12 KO mice, $P < 0.0001$; Fig. 4, A and B). This result is particularly important to our understanding of sigma-1 receptor biological function because the KO mice are viable and fertile (*27*). The sigma-1 receptor dependence of DMT-induced hypermobility parallels that induced by the sigma-1 receptor ligand (+)-SKF10047 in WT but not in KO mice (*27*). As a positive control, methamphetamine, which is thought to act through catecholaminergic systems, induced hypermobility in both WT and KO mice (3 mg/kg, ip, n = 6 mice; Fig. 4, B and C) with a reduced onset rate compared with that seen for DMT (Fig. 4, A and C). This indicates that behavioral actions of DMT depend on the sigma-1 receptor, which may provide an alternative research area for psychiatric disorders that have not been linked to dopamine or *N*-methyl-D-aspartate systems.

The binding, biochemical, physiological, and behavioral studies reported here all support the hypothesis that DMT acts as a ligand for the sigma-1 receptor. On the basis of our binding results and the sigma-1 receptor pharmacophore, endogenous trace amines and their *N*-methyl and *N,N*-dimethyl derivatives are likely to serve as endogenous sigma receptor regulators. Moreover, DMT, the only known mammalian *N,N*-dimethylated trace amine, can activate the sigma-1 receptor to modulate Na^+ channels. The recent discovery that the sigma-1 receptor functions as a molecular chaperone (*30*) may be relevant, because sigma-1 receptors, which are observed in the endoplasmic reticulum, associate with plasma membrane Kv 1.4 channels (*22*) and may serve as a molecular chaperone for ion channels. Furthermore, the behavioral effect of DMT may be due to activation or inhibition of sigma-1 receptor chaperone activity instead of, or in addition to, DMT/sigma-1 receptor modulation of ion channels. These studies thus suggest that this natural hallucinogen could exert its action by binding to sigma-1 receptors, which are abundant in the brain (*1*, *27*). This discovery may also extend to *N,N*-dimethylated neurotransmitters such as the psychoactive serotonin derivative *N,N*-dimethylserotonin (bufotenine), which has been found at elevated concentrations in the urine of schizophrenic patients (*10*). The finding that DMT and sigma-1 receptors act as a ligand-receptor pair provides a long-awaited connection that will enable researchers to elucidate the biological functions of both of these molecules.

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When Your Gain Is My Pain and Your Pain Is My Gain: Neural Correlates of Envy and Schadenfreude

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We often evaluate the self and others from social comparisons. We feel envy when the target person has superior and self-relevant characteristics. Schadenfreude occurs when envied persons fall from grace. To elucidate the neurocognitive mechanisms of envy and schadenfreude, we conducted two functional magnetic resonance imaging studies. In study one, the participants read information concerning target persons characterized by levels of possession and self-relevance of comparison domains. When the target person's possession was superior and self-relevant, stronger envy and stronger anterior cingulate cortex (ACC) activation were induced. In study two, stronger schadenfreude and stronger striatum activation were induced when misfortunes happened to envied persons. ACC activation in study one predicted ventral striatum activation in study two. Our findings document mechanisms of painful emotion, envy, and a rewarding reaction, schadenfreude.

Envy is one of the seven biblical sins, the Shakespearian "green-eyed monster," and what Bertrand Russell (*1*) called an unfortunate facet of human nature. It is an irrational, unpleasant feeling and a "painful emotion" (*2*) characterized by feelings of inferiority and resentment produced by an awareness of another's superior quality, achievement, or possessions (*3*). Understanding envy is important because of its broad implications, ranging from individual mat-

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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.

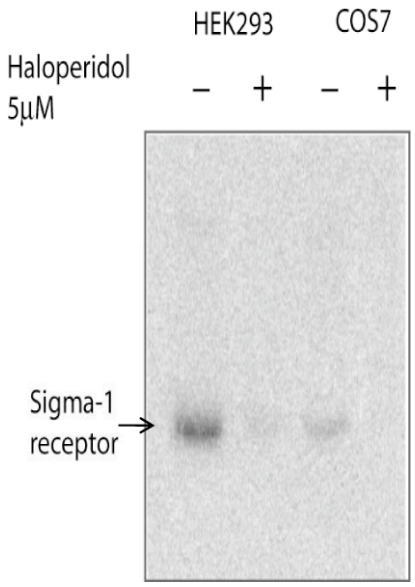
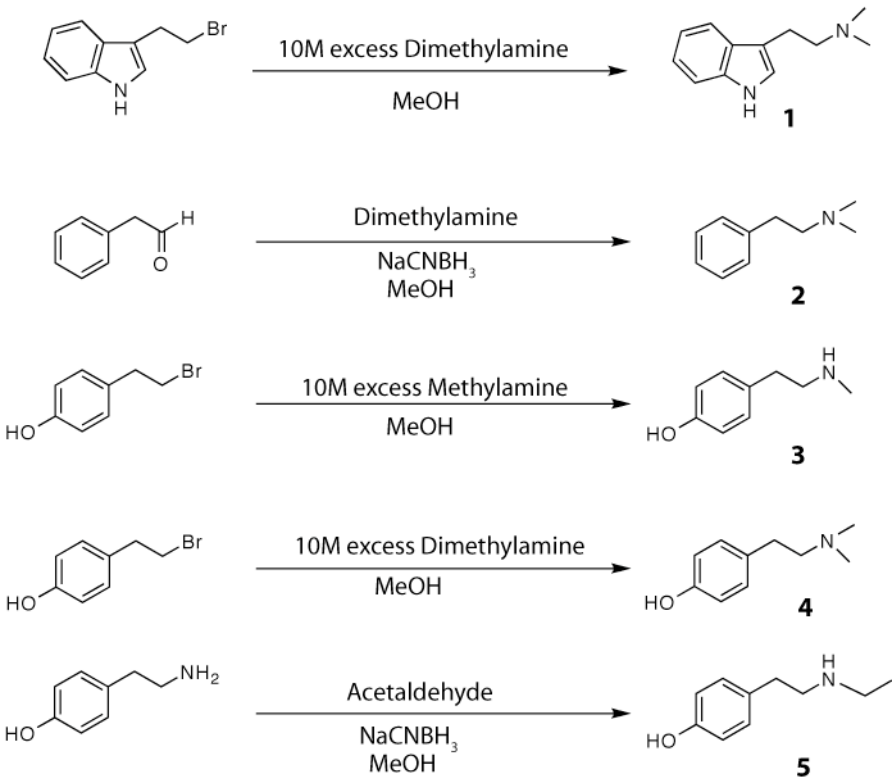


Fig. S1. Comparison of Sigma-1 Receptor Protein Levels in HEK293 and COS-7 cells. HEK293 and COS-7 cell homogenates (100 μg) were suspended in the presence or absence of 5 μM Haloperidol. Samples were photolyzed with 1 nM carrier-free 3-[^{125}I]iodo-4-azidococaine ([^{125}I]-IACoc) and immunoprecipitated 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

References

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