Supplementary Information

Antagonism of Sigma-1 receptors blocks compulsive-like eating

Pietro Cottone, Ph.D.[1*]; Xiaofan Wang, M.D., Ph.D.[1*]; Jin Won Park, M.A.[1]; Marta Valenza, M.S.[1,2]; Angelo Blasio, Ph.D.[1,3]; Jina Kwak[1]; Malliga R Iyer, Ph.D.[4]; Luca Steardo, M.D.[3]; Kenner C Rice, Ph.D.[4]; Teruo Hayashi, M.D., Ph.D.[5]; Valentina Sabino, Ph.D.[1]

[1]Laboratory of Addictive Disorders, Departments of Pharmacology and Psychiatry, Boston University School of Medicine, Boston, MA, USA; [2]Department of Pharmacology and Human Physiology, School of Medicine, University of Bari, Bari, Italy; [3]Department of Human Physiology and Pharmacology, University of Rome La Sapienza, Rome, Italy; [4]Chemical Biology Research Branch, National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism, Rockville, MD, USA. [5]Cellular Stress Signaling Unit, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, USA.

*These authors contributed equally to this work

Correspondence and requests for materials should be addressed to:
Pietro Cottone (Email: cottone@bu.edu) or
Valentina Sabino (Email: vsabino@bu.edu)
Laboratory of Addictive Disorders, Departments of Pharmacology and Psychiatry
Boston University School of Medicine
72 E Concord St, R-618
Boston, MA 02118 USA
Phone: 617-638-5662 / Fax: 617-638-5668

Supplementary Material and Methods

Subjects

Male Wistar rats (*n*=121), weighing 180-230 g and 41-47 days old (Charles River, Wilmington, MA), were housed in wire-topped, plastic cages (27×48×20 cm) in a 12:12 h reverse light cycle (lights off at 10:00 AM), in a humidity (60%) and temperature-controlled (22°C) vivarium. Upon arrival, rats had access to corn-based chow (Harlan Teklad LM-485 Diet 7012 (65% [kcal] carbohydrate, 13% fat, 21% protein, metabolizable energy 341 cal/100 g); Harlan, Indianapolis, IN) and water *ad libitum* at all times. Procedures adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publication number 85-23, revised 1996) and the *Principles of Laboratory Animal Care* (http://www.nap.edu/readingroom/bookslabrats) and were approved by Boston University Institutional Animal Care and Use Committee (IACUC). All the experimental procedures used rats which were never food or water restricted/deprived, unless otherwise specified.

Drugs

BD-1063x2HBr salt (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrobromide]) was synthesized in the Drug Design and Synthesis Section Laboratory (M. R. Iyer, K. C. Rice) of the NIDA, Intramural Research Program, according to the previously reported procedure (de Costa *et al*, 1993). The sample used was analytically pure by C, H, N elemental analysis (values within 0.4% of the theoretical value for the compound). Doses of BD-1063 were calculated based on the base weight. BD-1063 was dissolved in isotonic saline and injected subcutaneously (*s.c.*, 2 ml/kg) 15 minutes before testing. DTG (1,3-di-(2-tolyl)guanidine) was purchased from Tocris Bioscience (Minneapolis, ME). DTG was suspended

in isotonic saline with a few drops of Tween-20 and injected subcutaneously (*s.c.*, 2 ml/kg) 15 minutes before testing. These pretreatment intervals were chosen to ensure full compound activities throughout the entirety of the behavioral testing (Hiranita *et al*, 2010, 2011; Rawls *et al*, 2002; Sabino *et al*, 2011; Sabino *et al*, 2009).

Development of an operant model of binge-like eating in rats

Baseline. After arrival, rats (*n*=42) were left to acclimate to the vivarium and were fed the standard Harlan Teklad diet in the home cage (see Subjects paragraph) for at least 1 week, at the end of which the regular Harlan Teklad chow diet was replaced with an AIN-76A-based diet, hereafter referred to as "Chow A/I." (5TUM diet formulated as 4-5g extruded pellets, 65.5% [kcal] carbohydrate, 10.4% fat, 24.1% protein, metabolizable energy 330 cal/100 g; TestDiet, Richmond, IN). After one week of maintenance on the Chow A/I diet, rats were trained to acquire operant self-administration for food and water in individual test cages (30×24×29 cm) in which they could obtain nosepoke-contingent food and water on a fixed ratio 1 (FR1) continuous schedule of reinforcement, as previously described (Cottone *et al*, 2009). The operant boxes had a grid floor and were located in ventilated, sound-attenuating enclosures (66×56×36 cm) (Blasio *et al*, 2011). Food reinforcers were delivered by a pellet dispenser (Med Associates Inc., St. Albans, VT). During the operant training, food pellets were 45-mg precision pellets (Chow A/I), identical in composition to the diet that rats received in the home cage as ~5 g extruded pellets. Therefore, in the operant chambers, rats were provided with a diet identical to the one received in the home cage to ensure that *Chow* rats' food intake during operant sessions was not influenced by any hedonic factor, but only by energy homeostatic needs (Cottone *et al*, 2009; Cottone *et al*, 2008a). Pellet delivery was paired with a light-cue (0.3 sec) located above the nosepoke hole.

Water reinforcers were 100 µl in volume, delivered by a solenoid into a liquid cup nosepoke receptacle. The sessions were performed daily after dark cycle onset and were 1 hr in duration.

Testing. After attaining stable baseline performances in the 1-hr self-administration sessions, the testing procedure was initiated. Rats, matched for body weight, daily food intake, feed efficiency, and water and food responding in self-administration, were assigned to either a "*Chow*" control group, which, in the operant boxes, received the same 45-mg chow pellets offered in the training phase, or a "*Palatable*" group, which instead received a nutritionally complete, chocolate-flavored, high sucrose (50% kcal), AIN-76A-based diet, comparable in macronutrient composition and energy density to the chow diet (chocolate-flavored Formula 5TUL: 66.7% [kcal] carbohydrate, 12.7% fat, 20.6% protein, metabolizable energy 344 cal/100 g; formulated as 45 mg precision food pellets; TestDiet, Richmond, IN). This chocolate-flavored diet is strongly preferred by all rats (Cottone *et al*, 2008a, b). Subjects were tested daily.

Rate and regularity of sustained eating: inter food interval analysis

To identify differences between Chow vs. Palatable rats in the *rate* and *regularity* of sustained (not interrupted by drinking) eating, analysis of the ln-transformed duration of consecutive (uninterrupted by drinking) inter food intervals was performed (Cottone *et al*, 2007a; Cottone *et al*, 2007b). Inter food interval is a variable inversely correlated to eating rate. The mean, standard deviation, skewness, kurtosis and histogram entropy of the ln-transformed duration of each subject's consecutive inter food intervals was individually determined and then averaged across subjects. The normalized frequency histogram entropy (H) is a measure of categorical variability in the rate of ingestion (Shannon and Weaver, 1949), and was computed as follows:

$$H = -\frac{\sum_{i}^{n} p_i \log_2(p_i)}{\log_2(n)}$$

H is scaled between 0 and 1, with the denominator determined by the number of possible bins in the histogram (*n*) and the numerator a function of the proportion of observations that fall within a given histogram bin (p_i). Minimal (*H*=0) entropy occurs when all observations occur within a single histogram bin, whereas maximal entropy (*H*=1) occurs when each histogram bin has an equal probability, or a flat uniform joint density distribution. For entropy analysis, histograms were constructed from ln-transformed inter food intervals that fell from e^{-1} to e^{3} sec (~0.34-20.1 sec), with a bin width of $e^{0.2}$.

Significant decreases in the mean indicate an increased eating rate. Significant increases in the histogram entropy (a measure of categorical variability, reflected in an increasing number of populated histogram bins, each with more similar event frequencies), indicate a decreased regularity of intake. Conversely, a *decrease* in the kurtosis of the inter food interval distribution (a measure of the distribution's 'peakedness', reflected in a flatter top and taller tails of the distribution), is consistent with a decreased regularity of pellet-to-pellet feeding. Finally, a significant increase in the skewness (a measure of the distribution's symmetry, reflected in a selective increase of the frequency of the inter food interval falling to the left of the histogram) is consistent with a selective increase of the fast pellet-to-pellet responding.

Effects of the selective Sig-1R antagonist BD-1063 on operant binge-like eating

A different cohort of rats (*n*=16) was trained for the binge-like eating procedure and, following stabilization of intake, was pretreated with BD-1063 (0, 3.75, 7.5, 15, 30 mg/kg, subcutaneously (*s.c.*)) using a within-subject Latin square design. Rats were injected 15 min

prior to their operant binge-like eating session. This pretreatment interval was chosen to ensure full antagonist activity throughout the entirety of the food self-administration session (Sabino *et al*, 2011; Sabino *et al*, 2009). Rats had access to food and water *ad libitum* at all times.

Effects of the selective Sig-1R antagonist BD-1063 on high rate of responding for Chow A/I induced by food restriction

A different cohort of rats (n=7) was trained to acquire operant self-administration for Chow A/I diet (see "*Baseline*" in "Development of an operant model of binge-like eating in rats" paragraph). To increase the rate of responding for Chow A/I during the operant self-administration sessions, rats were food restricted in their home cages. For this purpose, 7 grams of Chow A/I food was provided in the home cages at the end of the operant self-administration sessions so that the total daily intake, including the food consumed during the self-administration session, equaled M±SEM: 20.7±0.8 grams (70% of a rat daily intake). This expedient was used *i*) to ensure that the rats consumed the entire home cage food intake before the beginning of the following self-administration session, *ii*) to induce an energy homeostatic overeating in the operant self-administration sessions and, therefore, *iii*) to increase the rate of responding for the Chow A/I diet in the operant self-administration sessions. Under these experimental conditions, the rate of responding for the Chow A/I diet of food-restricted rats was comparable to the rate of responding for the highly palatable sugary diet of *ad libitum*-fed *Palatable* rats. To assess the effects of BD-1063 (0, 30 mg/kg, *s.c.*) on high rate of responding for the Chow A/I diet in food-restricted rats, subjects were injected 15 min prior to their operant Chow A/I session, using a within-subject Latin square design.

Effects of the Sig-R agonist DTG on binge-like eating

A different cohort of rats (*n*=24) was trained for the binge-like eating procedure. Following stabilization of food intake, 16 randomly selected subjects of the 24 were pretreated with DTG (0, 15, 30 mg/kg, *s.c.*) using a within-subject Latin square design. Rats were injected 15 min prior to their operant binge-like eating session. This pretreatment interval was chosen to ensure full agonist? activity throughout the entirety of the food self-administration session (Hiranita *et al*, 2010, 2011; Rawls *et al*, 2002). Rats had access to food and water *ad libitum* at all times.

Effects of the selective Sig-1R antagonist BD-1063 on risk-taking behavior and compulsive-like eating

The same rats used for the development of the binge-like eating procedure (*n*=42) were tested in a light/dark rectangular box (50×100×35 cm) in which the aversive light compartment (50×70×35 cm) was illuminated by a 60 lux light. The dark side (50×30×35 cm) had an opaque cover and ~0 lux of light. A shallow, metal cup containing a pre-weighed amount of the same food received during self-administration (45-mg chow A/I pellets for *Chow* rats or 45-mg chocolate pellets for *Palatable* rats) was positioned in the center of the light compartment. The two compartments were connected by an open doorway which allowed the subjects to move freely between the two (Teegarden and Bale, 2007). Rats were habituated to the anteroom the day before testing and ~2 h before testing. White noise was present during both habituation and testing days. On the test day, following a 24-hr withdrawal period from the last access to the highly palatable food (withdrawal here and henceforth strictly meaning a period in which the palatable food was not provided), rats were pretreated with BD-1063 (0, 7.5, mg/kg, *s.c.*) 15 min

prior to being placed into the light compartment, facing both the food cup and the doorway (Teegarden *et al*, 2007). The time spent in the open compartment and the amount of food eaten during the test, were measured. The two dependent variables were then used to operationalize the constructs of "risk-taking behavior" and "compulsive-like eating". Due to rats' innate fear for bright, aversive environments, the time spent exploring the light compartment of the light/dark box under normal, control conditions is minimal. An increased time spent in this compartment, as compared to control conditions, resulting from the presence of the highly palatable diet, was operazionalized as "risk-taking behavior" (Colorado *et al*, 2006; Teegarden *et al*, 2007). Moreover, under normal, control conditions, eating behavior is typically suppressed when a rat faces adverse circumstances; a significant increase in food intake in spite of the adverse conditions, as compared to control conditions, was operazionalized as a construct of "compulsive-like eating" (Belin *et al*, 2008; Davis *et al*, 2010; Heyne *et al*, 2009; Hopf *et al*, 2010; Johnson and Kenny, 2010). The apparatus was cleaned with a water-dampened cloth after each subject. Rats had access to food *ad libitum* at all times; water was not available during the 10-min test.

Effects of the selective Sig-1R antagonist BD-1063 on motor activity

A different cohort of rats (n=20) underwent the same binge-like eating procedure and was used to test the effects of BD-1063 on motor activity. Motor activity of individually-housed rats was measured in Plexiglas chambers (27×48×20 cm) using an Opto-M3 activity system (Columbus Instruments, Columbus, OH). The Opto-M3 system consisted of a series of 16 sensor beams spaced 2.54 cm apart and able to measure horizontal activity. Sensor beams were located along the longest side of the horizontal plane of the cage. Each sensor beam provided both total

counts and ambulatory counts. A total count was accumulated every time a sensor beam was broken, while an ambulatory count was accumulated every time a new sensor beam was broken. The ambulatory count did not respond to the same sensor beam being broken and restored repeatedly, keeping the ambulatory counts from responding to rapid sensor beam interruptions. Therefore, two dependent variables were measured, total activity and ambulatory activity (Jensen *et al*, 2008; Kubera *et al*, 2011). Rats were previously acclimated to the testing room for 2 hr and testing was performed 24 hr after the last binge-like eating session. Total activity and ambulatory activity were recorded by a computer using the Multi Device Interface software over a 75-min period, which began right after rats were treated with BD-1063 (0, 7.5 mg/kg, *s.c.*) (within-subject Latin square design). To better control for potential motor activity effects of BD-1063, the first 15 minutes post-injection were excluded from the analysis, because it represented the pretreatment time used for the behavioral tests of this study.

Sig-1R gene expression in binge-like eating rats: quantitative Real-Time PCR

Two cohorts of *Chow* and *Palatable* rats were used for the quantification of the Sig-1R mRNA: a first cohort (*n*=12; randomly selected from the rats used in the DTG dose-response experiment, following a period of washout) was sacrificed 20-40 minutes after the end of the self-administration session. This time point was chosen to give enough time (80-100 minutes since the beginning of the session) for gene transcription changes to occur. A second cohort (*n*=16; randomly selected from the rats used in the BD-1063 dose-response experiment, following a period of washout) was sacrificed 24 hr following the last daily binge-like eating session. Rats were euthanized with isoflurane and decapitated; brains were quickly removed and sliced coronally in a brain matrix, with anterior cingulated cortex (ACC), prefrontal cortex

(PFC), insular cortex (IC), dorsal striatum (DS), and nucleus accumbens (NAcc) punches collected on an ice-cold stage and stored at -80°C. ACC and PFC were chosen because of the role these areas play in executive function and in the integration of cognitive and motivational/emotional processes (Dalley *et al*, 2008; Kalivas and Volkow, 2005; Repunte-Canonigo *et al*, 2010); IC was chosen because of the role this area plays in encoding and storing information related to the valence (appetitive or noxious) and magnitude of the hedonic properties of stimuli (Kenny, 2011); DS was chosen because of the role this area plays in the acquisition and expression of instrumental actions (Yin *et al*, 2008); NAcc was chosen because of the role this area plays in mediating the rewarding or motivational characteristics of stimuli and in regulating effort-related functions (Salamone *et al*, 2007). These areas all express Sig-1Rs (Alonso *et al*, 2000; Bouchard and Quirion, 1997; Kawamura *et al*, 2000; Walker *et al*, 1992). Punches were taken using needles with a diameter of 2 mm (Fine Science Tools (USA), Inc., Foster City, CA) guided by an atlas (Palkovits, 1988), and stored at -80°C until processing. Total RNA was prepared from each punch using the RNeasy lipid mini kit (Qiagen, Valencia, CA) as recommended for animal tissue. Total RNA (1 μg), quantified by Nanodrop 1000 (Thermo Scientific, Wilmington, DE), was reverse transcribed with QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA), which includes a DNA removal step. For quantitative real-time PCR, Roche Light Cycler 480 Master-plus SYBR Green mix (Roche Applied Science, Indianapolis, IN) was used. Reactions (10 μl) were carried out in a 96-well plate Realplex2 machine (Eppendorf). The primers (0.5 μM final concentration, Sigma, St. Louis, MO), synthesized with a standard desalting purification, were the following: Cyclophilin A (Cyp), 5′-TAT CTG CAC TGC CAA GAC TGA GTG -3′ and 5′-CTT CTT GCT GGT CTT GCC ATT CC -3′; Sig-1R, 5′-GCT GCA GTG GGT GTT TGT GAA CG -3′ and 5′-GGT GGA AAG TGC CAG AGA TGA

TGG TA -3′. The Cyp sequence was amplified using a three-temperature protocol which included an initial 5 min at 94 °C to activate Taq polymerase, followed by 40 denaturation cycles at 95 °C for 20 sec, annealing at 58 °C for 15 sec, and extension at 72 °C for 10 sec. The Sig-1R sequence was amplified per a two-temperature protocol after an initial 5-min at 94 °C: 40 cycles at 94 °C for 15 sec and at 68 °C for 8 sec. The primers for Sig-1R hybridize to sequences within exons 3 and 4 of the Oprs-1 transcript and therefore amplify only the "long" rat isoform of the protein, corresponding to the characterized receptor (Ganapathy *et al*, 1999). Standard curves were constructed using sequenced PCR products. Results were analyzed by second derivative methods and expressed in arbitrary units, normalized to Cyp expression levels. Standards and samples were run in duplicate, and all reactions for a given brain region were performed concurrently. Gene-specific amplification was determined by melting curve analysis as one peak at the expected melting temperature and by agarose gel electrophoresis (Sabino *et al*, 2009).

Sig-1R protein levels in binge-like eating rats: western blotting

A different cohort of rats (n=12) underwent the binge-like eating procedure, and 24 hr following the last daily self-administration session, were sacrificed and brain areas were collected as described above. Punches were collected and homogenized in lysis buffer (20mM HEPES, pH=7.4, EDTA 2mM, 1% SDS, 10% sucrose) by sonication, and centrifuged at 10,000 g for 20 min at 4°C. The supernatant was transferred to a new tube, and protein concentration in lysates was determined by the BCA assay (Pierce) using BSA as standard. All samples were adjusted to an equal concentration. The lysates were then diluted with 4X LDS sample buffer (Invitrogen) and 10X reducing agent (Invitrogen), and heated for 10 min at 70°C, then put on ice for 3 min. 20-30ug of total protein aliquots were run on NuPAGE 4-12% Bis-Tris gradient gel.

Chow and *Palatable* samples of the same area were run in the same gel. After electrophoretic separation, the proteins were transferred to a PVDF membrane (Bio-Rad) at 30 V for 1 hr. Membranes were blocked in 5% nonfat dry milk in TBST buffer (10mM Tris-HCl, pH=7.5, 150mM NaCl, Tween-20 0.05%) for 1 hr. Membranes were then incubated with the following two primary antibodies overnight at 4°C: anti-Sig-1R rabbit polyclonal antibody (1:1,000 recognizing C-terminal 143-165aa Sig-1R (Hayashi and Su, 2007)) and anti-β-actin mouse monoclonal antibody, (1:5,000; sc-47778, Santa Cruz Biotechnology). After washing three times with TBST buffer, membranes were incubated with secondary antibodies goat anti-rabbit IgG-HRP (1:2,000; sc-2004, Santa Cruz Biotechnology) or goat anti-mouse IgG-HRP (1:2,000; sc-2005, Santa Cruz Biotechnology) for Sig-1R and β-actin, respectively, at room temperature for 2 hr. The blots were developed with an enhanced ECL chemiluminescent substrate according to the manufacturer's instructions (Thermo Scientific). Blots were exposed to autoradiography film (Denville Scientific, Inc) and band densities were quantified using the ImageJ software (NIH). Sig-1R expression levels were calculated as percentage relative to β-actin expression.

Statistical analysis

Food intake and inter food intervals during the first 15 days of self-administration were analyzed using two-way analyses of variance (ANOVAs) with Diet History as a between-subjects factor and Day as a within-subjects factor. Student's *t*-test was used to interpret significant group differences. To determine whether the "acquisition" of binge-like eating resembled an associative learning process, the following sigmoidal four-parameter logistic regression function was fit to intake (Hartz *et al*, 2001):

$$y = min + \frac{(max - min)}{1 + 10^{(logET50 - x)Hillslope}}$$

The *x* and *y* indicate the day and the food intake, respectively. The *min* and *max* parameters indicate the minimum and the maximum amount of food eaten, respectively, and model intake before and asymptotic intake after diet history-induced behavioral adaptation ("learning"). The *Hillslope* describes the rate and valence of intake adaptation. The ET_{50} (Effective $Time_{50}$) (Clark *et al*, 1991; Naidu *et al*, 2003) describes the number of sessions that passed until 50% of maximal behavioral adaptation occurred. A similar analysis was performed for the inter food interval, entropy, and the home cage food intake. The effects of BD-1063 or DTG on food intake, water intake, inter food interval mean, skewness, kurtosis, entropy and total activity and ambulatory activity were analyzed using two-way mixed design ANOVAs with Diet History and Treatment as between- and within-subjects factors, respectively. The effects of BD-1063 treatment on high rate of response for Chow A/I induced by food restriction were analyzed using a paired Student's *t*-test. For motor activity, three independent experiments were performed. Pair-wise effects were interpreted using within-subject Dunnett's tests (vs. vehicle condition). The effects of BD-1063 on the time spent in the open compartment and food eaten in the light/dark test were analyzed using two-way repeated measures ANOVAs with Diet History and Treatment as between-subjects factors. The effects of Diet History on Sig-1R mRNA and protein levels were analyzed using unpaired Student's *t*-tests. Variables which failed the test for normality were analyzed as ranked (Akritas, 1990). Pair-wise effects were interpreted using between-subjects Fisher LSD's tests. The statistical packages used were Instat 3.0 (GraphPad, San Diego, CA, USA), and Systat 11.0 (SPSS, Chicago, IL, USA).

Supplementary Figures and Tables

Figure S1

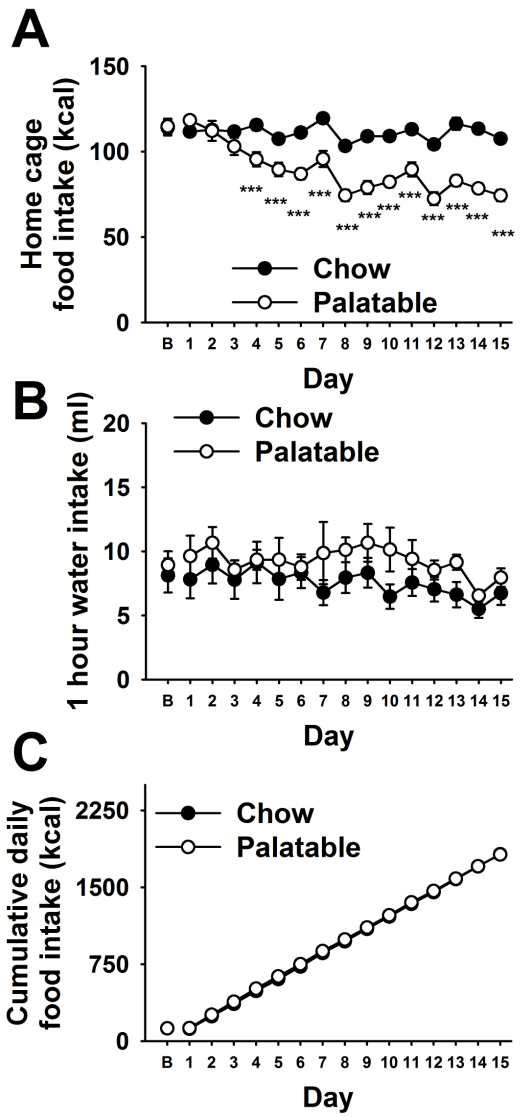

Figure S1. Effects of daily, 1-hour self-administration of a highly palatable diet on food and water intake in male Wistar rats (*n*=20-22/group). **A**) Home cage food intake. **B**) 1-hour water intake during self-administration sessions. **C**) Cumulative daily food intake. Panels represent *M*±SEM. *** Differs from *Chow* $p<0.001$ (unpaired Student's *t*-test).

Figure S2

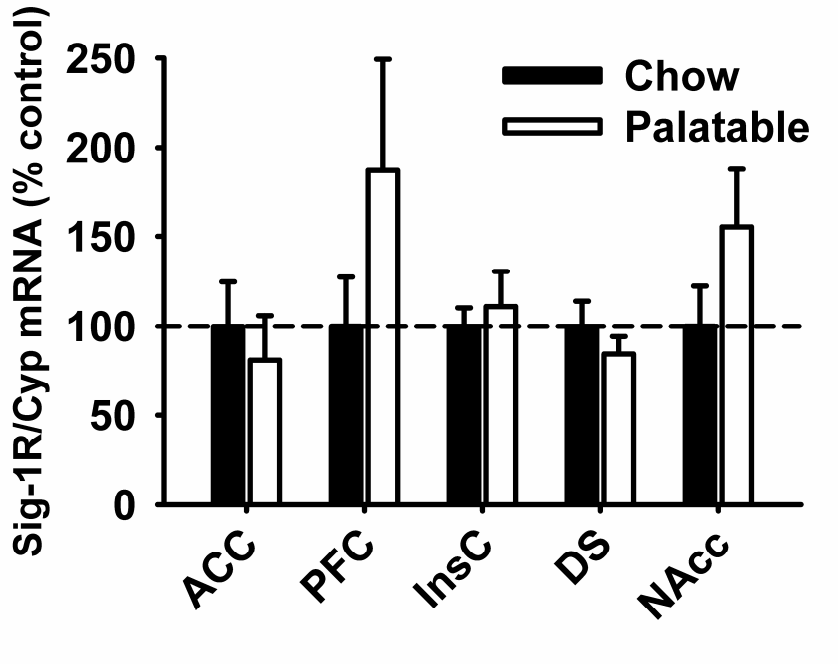

Figure S2. Effects of daily 1-hr self-administration of a highly palatable diet on Sig-1R mRNA expression in prefrontal cortex (PFC), anterior cingulated cortex (ACC), insular cortex (IC), nucleus accumbens (NAcc), and dorsal striatum (DS) in male Wistar rats (*n*=6/group). Rats were sacrificed 20-40 minutes after the last binge-like eating session. This time point was chosen to give enough time (80-100 minutes since the beginning of the session) for gene transcription changes to occur. Panels represent *M*±SEM expressed as percent of *Chow* group.

Figure S3

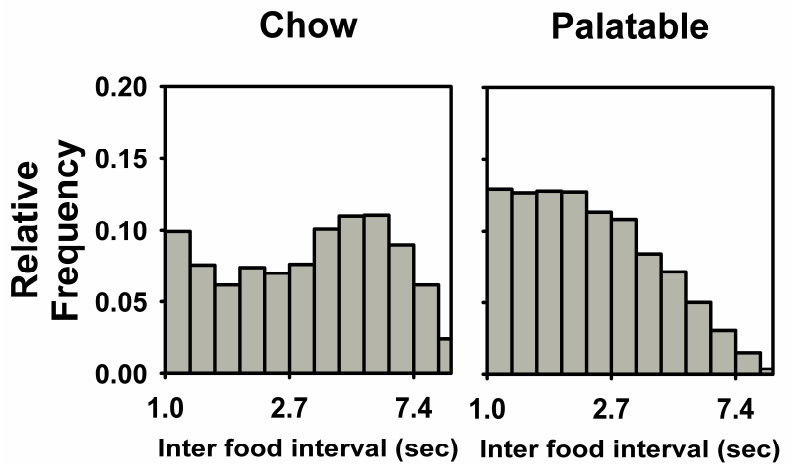

Figure S3. Effects of daily 1-hr self-administration of a highly palatable diet on relative frequency histograms of inter food intervals of *Chow* and *Palatable* rats during the 15th test day (*n*=20-22/group).

Figure S4

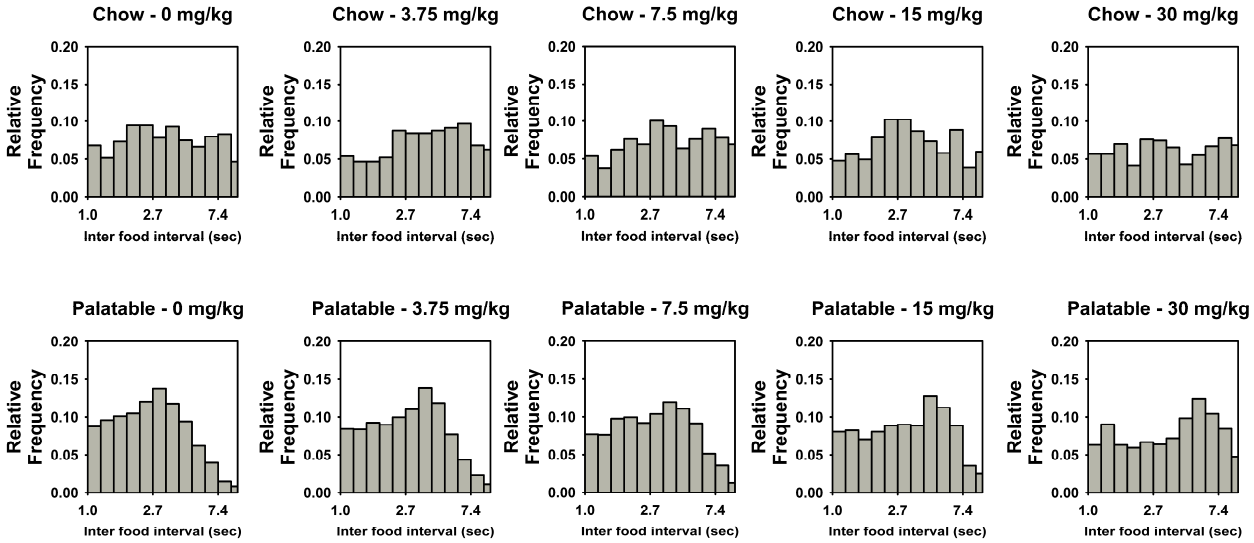

Figure S4. Effects of pretreatment (–15 min) with the selective Sig-1R antagonist BD-1063 (0, 3.75, 7.5, 15, 30 mg/kg, *s.c.*) on relative frequency histograms of inter food intervals of *Chow* and *Palatable* rats in male Wistar rats (*n*=8/group).

Table S1

Table S1. Non-linear sigmoidal regression analysis of 1-hour food intake, inter food interval, entropy and home cage food intake in Palatable rats

	Variable			
Sigmoidal regression parameter	1 hour food intake	Inter response interval	Entropy	Home cage food intake
Min (kcal)	8.0 ± 2.1	2.4 ± 0.1	0.41 ± 0.01	76.9 ± 4.9
Max (kcal)	40.6 ± 2.0	3.7 ± 1.6	0.56 ± 0.02	117.3 ± 4.6
ET50 (days)	3.1 ± 0.4	1.9 ± 0.2	2.8 ± 0.7	3.9 ± 0.7
Hillslope (unitless)	2.0 ± 0.5	-9.0 ± 23.4	-2.4 ± 1.3	-2.4 ± 1.1
Goodness of fit (*r*)	0.98	0.92	0.91	0.94
p value	<0.0001	<0.0001	<0.0001	<0.0001

Table S1. Parameter values (*M*±SEM, where applicable) from non-linear four-parameter sigmoidal regression analysis of 1-hr food intake, inter food interval, entropy, and home cage food intake of *Palatable* male Wistar rats (*n*=20/group) across 15 days of receiving limited (1-hr) daily access to a highly palatable chocolate-flavored sugary diet. The parameters describe the associative acquisition curves of operant binge-like eating, inter food interval (a variable inversely correlated to eating rate), and home cage hypophagia that resulted from this diet history (Cottone *et al*, 2008b). The functions are graphically represented in Figure 1B in the main text (operant binge-like eating) and Supplementary Figure 2A and B (inter food interval and home cage hypophagia).

Supplementary References

Akritas MG (1990). The rank transform method in some two-factor designs. *Journal of the American Statistical Association* **85**(409): 73-78.

Alonso G, Phan V, Guillemain I, Saunier M, Legrand A, Anoal M*, et al* (2000). Immunocytochemical localization of the sigma(1) receptor in the adult rat central nervous system. *Neuroscience* **97**(1): 155-170.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008). High impulsivity predicts the switch to compulsive cocaine-taking. *Science* **320**(5881): 1352-1355.

Blasio A, Narayan AR, Kaminski BJ, Steardo L, Sabino V, Cottone P (2011). A modified adjusting delay task to assess impulsive choice between isocaloric reinforcers in non-deprived male rats: effects of 5-HT(2A/C) and 5-HT (1A) receptor agonists. *Psychopharmacology (Berl)*.

Bouchard P, Quirion R (1997). [3H]1,3-di(2-tolyl)guanidine and [3H](+)pentazocine binding sites in the rat brain: autoradiographic visualization of the putative sigma1 and sigma2 receptor subtypes. *Neuroscience* **76**(2): 467-477.

Clark WM, Madden KP, Rothlein R, Zivin JA (1991). Reduction of central nervous system ischemic injury by monoclonal antibody to intercellular adhesion molecule. *Journal of neurosurgery* **75**(4): 623-627.

Colorado RA, Shumake J, Conejo NM, Gonzalez-Pardo H, Gonzalez-Lima F (2006). Effects of maternal separation, early handling, and standard facility rearing on orienting and impulsive behavior of adolescent rats. *Behavioural processes* **71**(1): 51-58.

Cottone P, Sabino V, Nagy TR, Coscina DV, Zorrilla EP (2007a). Feeding microstructure in diet-induced obesity susceptible versus resistant rats: central effects of urocortin 2. *The Journal of physiology* **583**(Pt 2): 487-504.

Cottone P, Sabino V, Roberto M, Bajo M, Pockros L, Frihauf JB*, et al* (2009). CRF system recruitment mediates dark side of compulsive eating. *Proc Natl Acad Sci U S A* **106**(47): 20016-20020.

Cottone P, Sabino V, Steardo L, Zorrilla EP (2007b). FG 7142 specifically reduces meal size and the rate and regularity of sustained feeding in female rats: evidence that benzodiazepine inverse agonists reduce food palatability. *Neuropsychopharmacology* **32**(5): 1069-1081.

Cottone P, Sabino V, Steardo L, Zorrilla EP (2008a). Intermittent access to preferred food reduces the reinforcing efficacy of chow in rats. *American journal of physiology* **295**(4): R1066-1076.

Cottone P, Sabino V, Steardo L, Zorrilla EP (2008b). Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food. *Neuropsychopharmacology* **33**(3): 524-535.

Dalley JW, Mar AC, Economidou D, Robbins TW (2008). Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. *Pharmacol Biochem Behav* **90**(2): 250-260.

Davis JF, Loos M, Di Sebastiano AR, Brown JL, Lehman MN, Coolen LM (2010). Lesions of the medial prefrontal cortex cause maladaptive sexual behavior in male rats. *Biol Psychiatry* **67**(12): 1199-1204.

de Costa BR, He XS, Linders JT, Dominguez C, Gu ZQ, Williams W*, et al* (1993). Synthesis and evaluation of conformationally restricted N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamines at sigma receptors. 2. Piperazines, bicyclic amines, bridged bicyclic amines, and miscellaneous compounds. *Journal of medicinal chemistry* **36**(16): 2311-2320.

Ganapathy ME, Prasad PD, Huang W, Seth P, Leibach FH, Ganapathy V (1999). Molecular and ligand-binding characterization of the sigma-receptor in the Jurkat human T lymphocyte cell line. *J Pharmacol Exp Ther* **289**(1): 251-260.

Hartz SM, Ben-Shahar Y, Tyler M (2001). Logistic growth curve analysis in associative learning data. *Animal cognition* **3**: 185–189.

Hayashi T, Su TP (2007). Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* **131**(3): 596-610.

Heyne A, Kiesselbach C, Sahun I, McDonald J, Gaiffi M, Dierssen M*, et al* (2009). An animal model of compulsive food-taking behaviour. *Addiction biology* **14**(4): 373-383.

Hiranita T, Soto PL, Tanda G, Katz JL (2010). Reinforcing effects of sigma-receptor agonists in rats trained to self-administer cocaine. *J Pharmacol Exp Ther* **332**(2): 515-524.

Hiranita T, Soto PL, Tanda G, Katz JL (2011). Lack of cocaine-like discriminative-stimulus effects of sigma-receptor agonists in rats. *Behav Pharmacol* **22**(5-6): 525-530.

Hopf FW, Chang SJ, Sparta DR, Bowers MS, Bonci A (2010). Motivation for alcohol becomes resistant to quinine adulteration after 3 to 4 months of intermittent alcohol self-administration. *Alcoholism, clinical and experimental research* **34**(9): 1565-1573.

Jensen DR, Knaub LA, Konhilas JP, Leinwand LA, MacLean PS, Eckel RH (2008). Increased thermoregulation in cold-exposed transgenic mice overexpressing lipoprotein lipase in skeletal muscle: an avian phenotype? *Journal of lipid research* **49**(4): 870-879.

Johnson PM, Kenny PJ (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nature neuroscience* **13**(5): 635-641.

Kalivas PW, Volkow ND (2005). The neural basis of addiction: a pathology of motivation and choice. *The American journal of psychiatry* **162**(8): 1403-1413.

Kawamura K, Ishiwata K, Tajima H, Ishii S, Matsuno K, Homma Y*, et al* (2000). In vivo evaluation of [(11)C]SA4503 as a PET ligand for mapping CNS sigma(1) receptors. *Nuclear medicine and biology* **27**(3): 255-261.

Kenny PJ (2011). Common cellular and molecular mechanisms in obesity and drug addiction. *Nature reviews Neuroscience* **12**(11): 638-651.

Kubera M, Grygier B, Wrona D, Rogoz Z, Roman A, Basta-Kaim A*, et al* (2011). Stimulatory effect of antidepressant drug pretreatment on progression of B16F10 melanoma in high-active male and female C57BL/6J mice. *Journal of neuroimmunology* **240-241**: 34-44.

Naidu KA, Fu ES, Sutton ET, Prockop LD, Cantor A (2003). The therapeutic effects of epidural intercellular adhesion molecule-1 monoclonal antibody in a rabbit model: involvement of the intercellular adhesion molecule-1 pathway in spinal cord ischemia. *Anesthesia and analgesia* **97**(3): 857-862.

Rawls SM, Baron DA, Geller EB, Adler MW (2002). Sigma sites mediate DTG-evoked hypothermia in rats. *Pharmacol Biochem Behav* **73**(4): 779-786.

Repunte-Canonigo V, Berton F, Cottone P, Reifel-Miller A, Roberts AJ, Morales M*, et al* (2010). A potential role for adiponectin receptor 2 (AdipoR2) in the regulation of alcohol intake. *Brain research* **1339**: 11-17.

Sabino V, Cottone P, Blasio A, Iyer MR, Steardo L, Rice KC*, et al* (2011). Activation of sigma-Receptors Induces Binge-like Drinking in Sardinian Alcohol-Preferring Rats. *Neuropsychopharmacology* **36**(6): 1207-1218.

Sabino V, Cottone P, Zhao Y, Iyer MR, Steardo L, Jr., Steardo L*, et al* (2009). The sigma-receptor antagonist BD-1063 decreases ethanol intake and reinforcement in animal models of excessive drinking. *Neuropsychopharmacology* **34**(6): 1482-1493.

Salamone JD, Correa M, Farrar A, Mingote SM (2007). Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology* **191**(3): 461-482.

Shannon CE, Weaver W (1949). *The Mathematical Theory of Communication* Urbana: University of Illinois Press.

Teegarden SL, Bale TL (2007). Decreases in Dietary Preference Produce Increased Emotionality and Risk for Dietary Relapse. *Biol Psychiatry*.

Walker JM, Bowen WD, Goldstein SR, Roberts AH, Patrick SL, Hohmann AG*, et al* (1992). Autoradiographic distribution of [3H](+)-pentazocine and [3H]1,3-di-o-tolylguanidine (DTG) binding sites in guinea pig brain: a comparative study. *Brain research* **581**(1): 33-38.

Yin HH, Ostlund SB, Balleine BW (2008). Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. *The European journal of neuroscience* **28**(8): 1437-1448.