Published in final edited form as:
Addict Biol. 2012 November ; 17(6): 956–963. doi:10.1111/j.1369-1600.2011.00339.x.

Attenuation of Cocaine Induced Locomotor Sensitization in Rats Sustaining Genetic or Pharmacologic Antagonism of Ghrelin Receptors

P. Shane Clifford,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Juan Rodriguez,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Destri Schul,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Samuel Hughes,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Tracey Kniffin,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Nigel Hart,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Shoshana Eitan,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Paul J. Wellman,
Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Luc Brunel,
Institut des Biomolécules Max Mousseron, UMR 5247, CNRS-UMI-UMII, Faculté de Pharmacie, 15 avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France

Jean-Alain Fehrentz, and
Institut des Biomolécules Max Mousseron, UMR 5247, CNRS-UMI-UMII, Faculté de Pharmacie, 15 avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France

Jean Martinez

Corresponding Author: Paul J. Wellman, Phone: 979-845-2557, FAX: 979-845-4727, PJW@PSYC.TAMU.EDU.

Authors contribution

PSC and PJW were responsible for the study concept and design. LB, J-AF and JM synthesized the JMV 2959 used in these studies. DS, SH, TK and PSC collected the locomotor data. SH collected the food intake data after ghrelin injection. PSC and PJW assisted with data analysis and interpretation of findings. PJW drafted the manuscript. SE, PSC, JR, NH, and JF provided critical revision of the manuscript. All authors critically reviewed content and approved the final version for publication.

Institut des Biomolécules Max Mousseron, UMR 5247, CNRS-UMI-UMII, Faculté de Pharmacie, 15 avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France

Abstract

Systemic infusions of the orexigenic peptide ghrelin (GHR) increase dopamine levels within the nucleus accumbens and augment cocaine stimulated locomotion and conditioned place preference in rats; observations that suggest an important role for ghrelin and GHR receptors (GHR-Rs) in drug reinforcement. In the present studies, we examined the development of cocaine locomotor sensitization in rats sustaining either pharmacologic antagonism or genetic ablation of GHR-Rs. In a pharmacologic study, adult male rats were injected (i.p.) with either 0, 3 or 6 mg/kg JMV 2959 (a GHR-R1 receptor antagonist) and 20 minutes later with either vehicle or 10 mg/kg cocaine HCl on each of 7 consecutive days. Rats pretreated with JMV 2959 showed significantly attenuated cocaine-induced hyperlocomotion. In a second study, adult wild type (WT) or mutant rats sustaining ENU-induced knockout of GHR-R (GHR-R ($^{-/-}$)) received daily injections (i.p) of vehicle (0.9% saline) or 10.0 mg/kg cocaine HCl for 14 successive days. GHR-R null rats treated repeatedly with cocaine showed diminished development of cocaine locomotor sensitization relative to WT rats treated with cocaine. To verify the lack of GHR-R function in the GHR-R ($^{-/-}$) rats, a separate feeding experiment was conducted in which WT rats, but not GHR-R ($^{-/-}$) rats, were noted to eat more after a systemic injection of 15 nmol ghrelin than after vehicle. These results suggest that GHR-R activity is required for the induction of locomotor sensitization to cocaine and complement an emerging literature implicating central GHR systems in drug reward. Ghrelin (GHR) is an orexigenic gut peptide that is transported across the blood brain barrier and interacts with GHR receptors (GHR-R) located on ventral tegmental dopamine neurons.

Key terms

ghrelin; ghrelin receptors; drug abuse; dopamine; locomotion; sensitization; ENU mutagenesis; GHR-R receptor antagonists

Introduction

Ghrelin (GHR) is a 28 amino acid peptide secreted peripherally from stomach and gut that functions as an orexigenic factor. Systemic or central administration of GHR enhances food intake (Kojima and Kangawa, 2005; Murakami et al., 2002; Shimbara et al., 2004) and augments feeding-associated behaviors such as hoarding and foraging (Keen-Rhinehart and Bartness, 2005). Systemic GHR is passively transported across the blood–brain barrier (Banks et al., 2008; Banks et al., 2002; Diano et al., 2006). GHR-receptors (GHR-Rs) are located on neurons located within the arcuate nucleus, the hippocampus and the ventral tegmental area (VTA) (Abizaid, 2009; Abizaid et al., 2006; Diano et al., 2006; Guan et al., 1997; Naleid et al., 2005; Zigman et al., 2006). These CNS sites are importantly linked to control of eating, modulation of memory and to reinforcement, respectively (Abizaid, 2009; Atcha et al., 2009; Carlini et al., 2010; Diano et al., 2006)

An emerging literature strongly suggests that GHR and GHR-Rs are positioned so as to modulate reinforcement to addictive drugs that act on brain dopamine. Changes in peripheral GHR levels can induce changes in dopamine signaling in brain reinforcement systems. GHR induces excitation of VTA neurons (Abizaid et al., 2006) and systemic GHR injection increases dopamine overflow within the nucleus accumbens (Abizaid et al., 2006; Jerlhag, 2008) and there is evidence that these changes may be specific to the shell region, but not the core region, of the nucleus accumbens (Quarta et al., 2009). Consistent with these effects are reports in which systemic administration of GHR enhanced cocaine-induced hyperlocomotion (Wellman et al., 2005) and chronic daily injection of GHR in rats induced

a degree of locomotor sensitization to a subsequent injection of cocaine (Wellman et al., 2008). More importantly, systemic and central administration of GHR can induce conditioned place preference (CPP) per se (Jerlhag, 2008), as well as enhance CPP induced by cocaine and by food (Davis et al., 2007; Egecioglu et al., 2010; Jerlhag et al., 2010; Perello et al., 2010). Tessari and colleagues (Tessari et al., 2007) reported that circulating GHR levels were positively related to the reinstatement of responding for intravenous cocaine. These studies indicate that augmenting GHR levels can facilitate the behavioral actions of cocaine and in particular may play a role in the sensitizing effects of drugs of abuse.

An alternative strategy to the aforementioned involves the assessment of cocaine's behavioral effects in animals sustaining inactivation of either GHR or GHR-Rs. GHR/GHR-R inactivation strategies include immunosuppression (Lu et al., 2009), RNA silencing (Shrestha et al., 2009) GHR-R antagonists (Abizaid et al., 2006; Halem et al., 2004; Moulin et al., 2007), and gene knockout strategies, primarily in mice (Abizaid et al., 2006; Sun et al., 2008). In the present study, we assessed the development of locomotor sensitization to daily administration of cocaine (10 mg/kg, i.p.) using two complementary approaches to antagonize GHR-Rs. In the first approach, we considered the impact on cocaine sensitization in rats sustaining pharmacologic inactivation of GHR-Rs using daily administration of the receptor antagonist JMV 2959 at doses of either 3 or 6 mg/kg for 7 consecutive days. JMV 2959 pretreatment was noted to block the feeding elicited in rats by the ghrelin agonist hexarelin (Moulin et al., 2007), whereas icv administration of JMV 2959 blocked feeding induced by icv administration of ghrelin (Salome et al., 2009). Systemic injection of 6 mg/kg JMV 2959 was noted to block the capacity of alcohol to induce CPP in mice without altering basal locomotion (Jerlhag et al., 2009). In the second approach, WT and GHR-R ($^{-/-}$) rats were administered vehicle or 10 mg/kg cocaine for 14 consecutive days. N-ethyl-N-nitrosourea (ENU)-driven target-selected mutagenesis has been used to ablate the GHR-R in rats (Till et al., 2007; Zan et al., 2003).

Methods

Animals

Animal research was conducted in accordance with the guidelines provided by the Texas A&M University Laboratory Animal Care Committee. Two different strains of rats were used in the present study. Adult male wild-type Sprague-Dawley rats (SD rats) obtained from Harlan (Houston, Texas) were used for the pharmacologic study. For the genetic ablation study, a FHH-Ghsr$^{m1/Mcwi}$ [GHR-R ($^{-/-}$)] strain was generated by the PhysGen Program in Genomic Applications (http://pga.mcw.edu) by N-ethyl-N-nitrosourea (ENU) mutagenesis of Fawn Hooded Hypertensive (FHH) strain animals. Briefly, ENU-treated males were backcrossed and offspring were screened using a Targeting Induced Local Lesions in Genomes (TILLing) approach (Till et al., 2007). GHR-R-specific primers GHR-R_F: 5′-GTTTGTCAGTAGGCATGCAG-3′ and GHR-R_R: 5′-GAAAGGCCATGTCTTAAGTTG-3′ were used to screen for mutations in exon 2 of GHR-R (GenBank accession number NM_032075). The GHR-R$^{m1/Mcwi}$ mutation was evidenced by a C>T transition of base pair of nucleotide 1027 of this sequence by Sanger sequencing, creating glutamine (CAG) to stop (TAG) codon change. Using *in silico* analysis, this mutation prematurely truncates the GHR-R protein by 21 amino acids. This mutant animal was backcrossed and then intercrossed for more than 15 generations. Sanger sequencing was used to confirm the animals are homozygous. PhysGen maintains the FHH-Ghsr$^{m1/Mcwi}$ colony as a homozygous breeding colony from which males were provided for phenotyping. Age-matched parental FHH/EurMcwi strain males were provided as controls in our experiments.

The Sprague-Dawley rats were acclimated to the laboratory for a week prior to the start of the pharmacologic study. WT and GHR-R ($^{-/-}$) rats were held in quarantine for 30 days after arrival at TAMU and were acclimated to the Psychology vivarium for 1 week prior to the start of the experiment. All animals were maintained on a 12-hour light/dark cycle. Testing commenced at approximately 09:00 hrs.

Drugs

A vehicle saline solution was prepared as 0.9% sodium chloride in distilled water. A solution of cocaine HCL was prepared by dissolving cocaine HCL into vehicle at a concentration of 10 mg/ml. The cocaine was provided by Dr. Kevin Gormley of the Basic Research Division of NIDA. JMV 2959 was a kind gift from Jean-Alain Fehrentz of the Institut des Biomolécules Max Mousseron, Faculté de Pharmacie. JMV 2959 HCL was dissolved into saline at a concentration of either 3 or 6 mg/ml. Cocaine and JMV 2959 solutions were administered i.p. in a volume of 1 ml/kg.

Apparatus

The assessment of locomotion was made in a set of 8 automated optical beam activity monitors (Model RXYZCM-16; Accuscan Instruments, Columbus, OH, USA). Each monitor is housed within a 40 × 40 × 30.5 cm acrylic cage. Activity monitors and cages were located in a sound-proof room with a 40 dB [SPL] white noise generator operating continuously. A multiplexor-analyzer monitored beam breaks from the optical beam activity monitors and tracked the simultaneous interruption of beams. The multiplexor-analyzer updated the animal's position in the acrylic cage every 10 msec using a 100% real-time conversion system. Computerized integration of the data obtained from the monitor afforded the recording of general activity using total distance (in cm) as the primary dependent measure (Sanberg et al., 1987).

Procedures

Impact of JMV-2959 on the development of locomotor sensitization to cocaine in SD rats—The subjects of this experiment were 46 adult male Sprague-Dawley rats weighing 250–275 g at the start of the experiment. Our previous studies of cocaine sensitization employed this strain of rats (Miller et al., 1999; Nation et al., 2000). On two consecutive days, the rats were adapted to the locomotion chambers for 60 minutes per day. On the next three days, the rats were injected (i.p.) with 0.9% saline (1 ml/kg) 10 minutes before being placed into the activity chamber. The rats were placed into the locomotion chambers for 15 minutes, removed and then injected with 0.9% saline and then placed back into the chambers for 45 minutes. During the 7 day cocaine exposure period, a third of the rats in each cocaine injection condition were treated (i.p.) with either vehicle (0), 3 or 6 mg/kg JMV 2959 at 10 minutes before being placed into the locomotion chambers. After the 15 minute baseline period, the rats were injected (i.p.) with either saline or 10 mg/kg cocaine HCl and placed back into the locomotion chamber for 45 minutes with the room lights switched off. This pretreatment-treatment combination formed six test groups: vehicle-vehicle (n=6), vehicle-cocaine (n=8), 3 mg/kg JMV 2959-vehicle (n=8), 3 mg/kg JMV 2959-cocaine (n=8), 6 mg/kg JMV 2959-vehicle (n=8), and 6 mg/kg JMV 2959-cocaine (n=8). Food and water were not available in the locomotor chambers, but were freely available in the home cage between behavioral tests.

Development of locomotor sensitization to cocaine in GHR-R ($^{-/-}$) and WT rats—Animals were separated into WT and GHR-R ($^{-/-}$) groups based on genotype. At the start of behavioral testing, the rats weighed between 330 and 370 g and there were no significant differences in body weight by genotype. On two consecutive days, the rats were

adapted to the locomotion chambers for 45 minutes per day. On the next three days, the rats were placed in the chamber for 15 minutes, removed and injected (i.p.) with 0.9% saline and then placed back into the chambers for 45 minutes. Test animals within each group were then randomly assigned to receive (i.p) injections of either vehicle (0.9% saline) or 10.0 mg/kg cocaine HCl for 14 successive days, thus forming four test groups (WT/vehicle (n=4), WT/cocaine (n=7), GHR-R $^{(-/-)}$/vehicle (n=5), and GHR-R $^{(-/-)}$/cocaine (n=8). During sensitization testing, animals were placed in their respective test chambers for a 15 minute baseline-recording period prior to receiving either a vehicle or cocaine injection. Rats were then placed back in the chamber immediately following injection, at which time the room lights again were turned off and recording continued for another 45 minutes. Food and water were not available in the locomotor chambers, but were freely available in the home cage between behavioral tests.

Impact of 15 nmol ghrelin on food intake in GHR-R ($^{-/-}$) and WT rats—The present study is among the first to employ an ENU-based knockout of the GHR-R in the rat. To verify that this receptor was in fact non-functional in these rats, we examined the capacity of systemic ghrelin injection (15 nmol/rat) to stimulate acute food intake (Wren et al., 2000). Accordingly, a separate set of WT (n=5) and GHR-R ($^{-/-}$) (n=7) rats were tested for their feeding responses to systemic injection of ghrelin. Each rat underwent a series of five baseline ingestive trials. Each 60-min trial started at about 09:30 h and was conducted under full illumination in the home colony room. The start times for each rat were staggered in 1-min intervals to accommodate subsequent injection procedures. Rat body weights were recorded to the nearest g prior to each trial and each rat was then placed into a separate testing cage for 15 min with no access to food or water. Each rat was subsequently given access to a weighed amount of the pellet diet (approximately 20 g) and a drinking tube containing tap water. Food intakes were recorded to the nearest 0.1 g and were adjusted for any spillage collected on paper pads placed beneath the wire floor of each cage. Water intakes were recorded to the nearest 0.1 g, but were not corrected for spillage. On days 4 and 5, the rats were adapted to the injection protocol by daily sham injections (i.p.) of 0.9% saline (0.5 ml/rat) administered 15 min prior to the start of each ingestion trial. On the ghrelin test day, the rats were injected (i.p.) with 15 nmol/rat ghrelin (Pi Proteomics; Huntsville, Al) (in 0.5 ml vehicle), housed in the test cage and then given 60 min access to food and water fifteen min later. During the interval after each test trial, the rats had continuous access to food and water in the home cage.

Data Analyses

Because the treatment means and variances were proportional, the total distance traveled scores of each study were subjected to a square root transformation (Kirk, 1982). The overall design of the first study was a split-plot factorial consisting of the between-group factors of JMV 2959 treatment (0, 3 or 6 mg/kg) and cocaine dose (0 versus 10 mg/kg) and a within-group factor of days (1–7). In the second study, the overall design was a split-plot factorial design consisting of between-group factors of GHR receptor status (WT versus GHR-R $^{(-/-)}$) and cocaine exposure (vehicle versus 10 mg/kg cocaine) and a within-group factor of day (blocks 1–7 were formed using averages of 2 days total distance data). The changes in food intake in response to ghrelin were evaluated using split-plot analyses using the between-group factor of GHR receptor status (WT versus GHR-R $^{(-/-)}$) and the within-group factor of drug day (vehicle versus 15 nmol GHR). Statistical significance was deemed to be $p < 0.05$ and the Bonferroni procedure was used to examine mean group differences.

RESULTS

Impact of JMV 2959 on the development of cocaine-induced locomotor sensitization

On the last day of the baseline procedure (Day 0 in Figure 1), there were no significant differences in 45 minute locomotor scores ($p = 0.111$). Separate one-way ANOVAs were computed for the impact of 0, 3 or 6 mg/kg JMV 2959 on locomotion in rats treated with vehicle (0 mg/kg cocaine, Figure 1A). No significant changes from day 0 were noted in rats treated with either 0 or 3 mg/kg JMV 2959 ($ps \leq 0.446$). In contrast, 6 mg/kg JMV 2959 induced a significant suppression of locomotion on days 1–7 relative to baseline day 0 ($F(7,49) = 2.21$, $p < 0.05$). Accordingly, the subsequent analyses considered the impact of 0 versus 3 mg/kg JMV 2959 on the development of cocaine locomotor sensitization. A primary analysis considered the impact of JMV 2959 (0 versus 3 mg/kg) in rats treated with 10 mg/kg cocaine. These analyses revealed a significant overall effect of JMV 2959 dose ($F(1,14) = 7.359$, $p < 0.017$), of day ($F(1,14) = 24.41$, $p < 0.0001$) and a significant interaction between JMV 2959 treatment and day ($F(1,14) = 4.397$, $p < 0.05$). The latter interaction reflected the fact that the JMV 2959-cocaine and vehicle-cocaine groups exhibited similar increases in locomotion during days 1–4, but these groups diverged during days 5–7. In contrast, no such divergence was evident in the vehicle-vehicle and JMV 2959-vehicle groups. A two-way repeated measure ANOVA using the between-group factor of JMV 2959 dose (0 vs 3 mg/kg) and day (0–7) revealed a significant effect of JMV dose ($F(1,12) = 9.612$, $p < 0.01$) but no significant effect of day ($p < 0.309$) nor a significant interaction between JMV dose and day ($p < 0.609$).

Development of locomotor sensitization in WT and GHR-R $^{(-/-)}$ rats

The impact of ablation of the GHR-R in adult male rats on the development of cocaine-induced locomotor sensitization is displayed in Figure 2. A 2 way ANOVA revealed no significant ($p >$ at least 0.156) effect of GHR receptor status, cocaine exposure nor an interaction among these factors on locomotion scores after vehicle (block 0 in Figure 2). Although there were no initial differences between the WT and GHR-R ($^{-/-}$) groups treated with vehicle, these groups diverged over the 7 blocks such that by the last block, the GHR-R ($^{-/-}$) rats treated with vehicle showed significantly less locomotion than did the WT rats treated with vehicle. A multivariate analysis of variance computed using the difference between blocks 1–6 versus baseline revealed significant effects of GHR gene status on each of block 6 ($F(1,20) = 6.4$, $p < 0.02$) and block 7 ($F(1,20) = 15.6$, $p < 0.0001$) and a significant effect of cocaine exposure on each block ($F(1,20) =$ at least 20.65, $p < 0.0001$). The interaction between cocaine exposure and GHR gene status was not significant on block 6 ($F(1,20) = 2.25$, $p < 0.149$) whereas this interaction was significant on block 7 ($F(1,20) = 4.1$, $p < 0.05$). The latter interaction reflected the fact that the difference in locomotion scores between the WT and GHR-R ($^{-/-}$) rats during the last block relative to baseline was significantly larger in the cocaine exposure condition than in the vehicle treatment condition.

Attenuation of ghrelin-stimulated food intake in GHR-R $^{(-/-)}$ rats

Figure 3 depicts the changes in food intake induced by 15 nmol ghrelin in WT and GHR-R $^{(-/-)}$ rats. ANOVA of the food intake data revealed a significant effect of drug day ($F(1,10) = 6.812$, $p < 0.026$) and a significant interaction between GHR gene status and drug day ($F(1,10) = 10.779$, $p < 0.008$), but no significant effect of GHR gene status ($p < 0.173$). The interaction reflected a significant stimulation of food intake in WT rats but not in GHR-R $^{(-/-)}$ rats. Systemic injection of 15 nmol GHR did not significantly alter water intakes (data not depicted) in either WT or GHR-R $^{(-/-)}$ rats.

General Discussion

In the present studies, we considered the development of locomotor sensitization induced by repeated administration of 10 mg/kg cocaine in rats for which GHR receptors were subjected to pharmacological antagonism (0, 3 or 6 m/kg JMV 2959) or genetic ablation (GHR-R $^{(-/-)}$ null versus WT rats). In the first study, SD rats pre-treated with the GHR-R antagonist JMV 2959 showed significant attenuation of the development of hyperlocomotion to daily injections of 10 mg/kg cocaine. JMV 2959 alone produced some reduction of locomotion at 6 mg/kg but not at 3 mg/kg. Both doses, however, produced similar attenuation of cocaine-induced hyperlocomotion. This profile suggests that the attenuation of cocaine locomotor sensitization was not an artifact of the capacity of JMV 2959 to reduce baseline locomotion. The blunted development of cocaine locomotor sensitization reported herein parallels a recent study by Jerlhag (Jerlhag et al., 2010) in which administration of a GHR-R antagonist attenuated the acute hyperlocomotion induced by the psychostimulant cocaine as well as amphetamine; reduced the increase in accumbens dopamine produced by cocaine and most importantly, attenuated cocaine-induced CPP.

In the present study, pharmacological inactivation of GHR-Rs using JMV 2959 induced a degree of suppression of locomotion with significant effects noted at 6 mg/kg, but not at 3 mg/kg. This is not unexpected given that GHR and GHR-Rs can modify locomotion. Acute administration of GHR (Jerlhag et al., 2006) as well as repeated systemic injection of GHR can facilitate locomotion (Wellman et al., 2005). Such an effect would not be unexpected given that GHR-Rs are located on neurons of the substantia nigra. GHR not only activates dopamine neurons within the VTA but also promotes dopamine function within the substantia nigra-striatal pathway (Andrews et al., 2009; Narayanan et al., 2010). In contrast, inactivation of GHR-Rs diminished substantia nigra dopamine function (Andrews et al., 2009). One consequence of such inactivation may be diminished locomotion. This is evident in food-associated locomotion (Abizaid et al., 2006; Blum et al., 2009; Jerlhag et al., 2006). In a recent study, Clifford et al. (2010) examined the interaction between food restriction and cocaine on locomotion in WT mice, GHR-null mice and GHR-R null mice. A key effect noted in that study is that GHR-R null mice failed to increase their baseline locomotion under conditions of food restriction. Pharmacological inactivation using JMV 2959 induced a degree of suppression of locomotion in the present study with significant effects evident at 6 mg/kg but not 3 mg/kg. Thus these results suggest that GHR-Rs can not only modulate dopamine reward related processes but can also modulate those related to locomotion.

In the second study, we examined the development of cocaine sensitization in GHR-R $^{(-/-)}$ null or WT rats. Acute administration of cocaine induced similar acute increases in hyper-locomotion in GHR-R $^{(-/-)}$ null and WT rats, but the GHR-R $^{(-/-)}$ null rats showed attenuated sensitization to repeated cocaine administration, relative to that noted in WT rats. To our knowledge, this is the first report of diminished drug sensitization and perhaps the first study of the behavioral characteristics in this ghrelin receptor deficient animal model. That the ENU technique produced a functional ablation of GHR-Rs in these rats is supported by the present finding that systemic injection of ghrelin induced feeding in WT, but not in GHR-R $^{(-/-)}$ null rats. While it should be noted that the ENU technique may produce mutations at loci other than that of the GHR-R, it is unlikely that the backcrossing method used to generate these rats produced rats bearing homozygous mutations at non-GHR-R sites. Additionally, the profile of results noted in the GHR-R $^{(-/-)}$ null rats is similar to that noted in SD rats treated with JMV 2959. The complementary outcomes noted in JMV 2959 and GHR-R $^{(-/-)}$ null rats supports the contention that the development of cocaine locomotor sensitization is partially dependent on functional GHR-Rs.

Given that GHR-Rs are critically involved in the induction of eating (Abizaid, 2009; Egecioglu et al., 2010; Tschop et al., 2000), antagonism of GHR-Rs have been a key focus of development for drug companies seeking to diminish appetite. Pharmacological antagonism of GHR-Rs can diminish baseline feeding and attenuate the rewarding action of food (Egecioglu et al., 2010; Perello et al., 2010). The present studies in which antagonism of GHR-R function reduced the development of cocaine sensitization may offer another modality of drug treatment for cocaine addiction. It has been suggested that GHR-R antagonists may represent a treatment modality for alcohol abuse (Jerlhag et al., 2009). Whether GHR-R antagonism alters sensitization induced by other drugs of abuse (i.e. morphine or nicotine) remains to be determined.

In contrast to the impact of inhibition of GHR signaling on cocaine behavioral function, our earlier laboratory studies showed that administration of GHR facilitates cocaine-induced hyperlocomotion and cocaine-induced CPP (Davis et al., 2007; Wellman et al., 2005; Wellman et al., 2008). It should also be noted that chronic activation of ghrelin receptors using daily administration of ghrelin can induce cross-sensitization to cocaine – suggesting an alteration of the coupling between GHR_Rs and dopamine neurons (Wellman et al., 2008). This effect may be related to an up-regulation of Dopamine1 receptors such that ghrelin can amplify dopamine signaling (Jiang et al., 2006). Such an outcome is the inverse of that noted in the present studies wherein antagonism of GHR-Rs diminished the development of sensitization to cocaine. A more general role for GHR-Rs in brain reinforcement is also indicated by recent studies in which pharmacological inactivation of GHR-Rs attenuates the CPP induced by ethanol (Jerlhag et al., 2009) and in which genetic ablation of GHR-Rs attenuates the CPP induced by ingestion of a high-fat diet (Perello et al., 2010). These converging outcomes strongly support the view that GHR receptors modulate reinforcement/reward function.

Acknowledgments

Portions of this report were supported by NIH R01-013188 to PJW. We thank Aron Geurts and Dr. Howard Jacob from the Medical College of Wisconsin for donating the *Ghr-r* knockout rat to the Knock Out Rat Consortium (KORC). The *Ghr-r* knockout rat is available to academic and non-profit researchers through the KORC (www.knockoutrat.org) and to for profit entities through Transposagen Biopharmaceuticals Inc (www.transposagenbio.com). JMV 2959 was a kind gift from Jean-Alain Fehrentz of the Institut des Biomolécules Max Mousseron, Faculté de Pharmacie.

References

Abizaid A. Ghrelin and dopamine: new insights on the peripheral regulation of appetite. J Neuroendocrinol. 2009; 21:787–793. [PubMed: 19523165]

Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschop MH, Gao XB, Horvath TL. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J Clin Invest. 2006; 116:3229–3239. [PubMed: 17060947]

Andrews ZB, Erion D, Beiler R, Liu ZW, Abizaid A, Zigman J, Elsworth JD, Savitt JM, DiMarchi R, Tschoep M, Roth RH, Gao XB, Horvath TL. Ghrelin promotes and protects nigrostriatal dopamine function via a UCP2-dependent mitochondrial mechanism. J Neurosci. 2009; 29:14057–14065. [PubMed: 19906954]

Atcha Z, Chen WS, Ong AB, Wong FK, Neo A, Browne ER, Witherington J, Pemberton DJ. Cognitive enhancing effects of ghrelin receptor agonists. Psychopharmacology (Berl). 2009; 206:415–427. [PubMed: 19652956]

Banks WA, Burney BO, Robinson SM. Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. Peptides. 2008; 29:2061–2065. [PubMed: 18682266]

Banks WA, Tschop M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. J Pharmacol Exp Ther. 2002; 302:822–827. [PubMed: 12130749]

Blum ID, Patterson Z, Khazall R, Lamont EW, Sleeman MW, Horvath TL, Abizaid A. Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. Neuroscience. 2009

Carlini VP, Ghersi M, Schioth HB, de Barioglio SR. Ghrelin and memory: Differential effects on acquisition and retrieval. Peptides. 2010

Davis KW, Wellman PJ, Clifford PS. Augmented cocaine conditioned place preference in rats pretreated with systemic ghrelin. Regul Pept. 2007; 140:148–152. [PubMed: 17257691]

Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B, Gaskin FS, Nonaka N, Jaeger LB, Banks WA, Morley JE, Pinto S, Sherwin RS, Xu L, Yamada KA, Sleeman MW, Tschop MH, Horvath TL. Ghrelin controls hippocampal spine synapse density and memory performance. Nat Neurosci. 2006; 9:381–388. [PubMed: 16491079]

Egecioglu E, Jerlhag E, Salome N, Skibicka KP, Haage D, Bohlooly YM, Andersson D, Bjursell M, Perrissoud D, Engel JA, Dickson SL. Ghrelin increases intake of rewarding food in rodents. Addict Biol. 2010

Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van der Ploeg LH, Howard AD. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res Mol Brain Res. 1997; 48:23–29. [PubMed: 9379845]

Halem HA, Taylor JE, Dong JZ, Shen Y, Datta R, Abizaid A, Diano S, Horvath T, Zizzari P, Bluet-Pajot MT, Epelbaum J, Culler MD. Novel analogs of ghrelin: physiological and clinical implications. Eur J Endocrinol. 2004; 151(Suppl 1):S71–75. [PubMed: 15339248]

Jerlhag E. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. Addict Biol. 2008; 13:358–363. [PubMed: 18782383]

Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA. Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. Addict Biol. 2006; 11:45–54. [PubMed: 16759336]

Jerlhag E, Egecioglu E, Dickson SL, Engel JA. Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. Psychopharmacology (Berl). 2010

Jerlhag E, Egecioglu E, Landgren S, Salome N, Heilig M, Moechars D, Datta R, Perrissoud D, Dickson SL, Engel JA. Requirement of central ghrelin signaling for alcohol reward. Proc Natl Acad Sci U S A. 2009; 106:11318–11323. [PubMed: 19564604]

Jiang H, Betancourt L, Smith RG. Ghrelin amplifies dopamine signaling by cross talk involving formation of growth hormone secretagogue receptor/dopamine receptor subtype 1 heterodimers. Mol Endocrinol. 2006; 20:1772–1785. [PubMed: 16601073]

Keen-Rhinehart E, Bartness TJ. Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters. Am J Physiol Regul Integr Comp Physiol. 2005; 288:R716–722. [PubMed: 15576659]

Kirk, RE. Experimental Design. Wadsworth; Belmont, CA: 1982.

Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev. 2005; 85:495–522. [PubMed: 15788704]

Lu SC, Xu J, Chinookoswong N, Liu S, Steavenson S, Gegg C, Brankow D, Lindberg R, Veniant M, Gu W. An acyl-ghrelin-specific neutralizing antibody inhibits the acute ghrelin-mediated orexigenic effects in mice. Mol Pharmacol. 2009; 75:901–907. [PubMed: 19129426]

Miller DK, Nation JR, Wellman PJ. Sensitization of anorexia and locomotion induced by chronic administration of ephedrine in rats. Life Sci. 1999; 65:501–511. [PubMed: 10462077]

Moulin A, Demange L, Berge G, Gagne D, Ryan J, Mousseaux D, Heitz A, Perrissoud D, Locatelli V, Torsello A, Galleyrand JC, Fehrentz JA, Martinez J. Toward potent ghrelin receptor ligands based on trisubstituted 1,2,4-triazole structure. 2. Synthesis and pharmacological in vitro and in vivo evaluations. J Med Chem. 2007; 50:5790–5806. [PubMed: 17927165]

Murakami N, Hayashida T, Kuroiwa T, Nakahara K, Ida T, Mondal MS, Nakazato M, Kojima M, Kangawa K. Role for central ghrelin in food intake and secretion profile of stomach ghrelin in rats. J Endocrinol. 2002; 174:283–288. [PubMed: 12176667]

Naleid AM, Grace MK, Cummings DE, Levine AS. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides. 2005; 26:2274–2279. [PubMed: 16137788]

Narayanan NS, Guarnieri DJ, DiLeone RJ. Metabolic hormones, dopamine circuits, and feeding. Front Neuroendocrinol. 2010; 31:104–112. [PubMed: 19836414]

Nation JR, Miller DK, Bratton GR. Developmental lead exposure alters the stimulatory properties of cocaine at PND 30 and PND 90 in the rat. Neuropsychopharmacology. 2000; 23:444–454. [PubMed: 10989272]

Perello M, Sakata I, Birnbaum S, Chuang JC, Osborne-Lawrence S, Rovinsky SA, Woloszyn J, Yanagisawa M, Lutter M, Zigman JM. Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. Biol Psychiatry. 2010; 67:880–886. [PubMed: 20034618]

Quarta D, Di Francesco C, Melotto S, Mangiarini L, Heidbreder C, Hedou G. Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. Neurochem Int. 2009; 54:89–94. [PubMed: 19118592]

Salome N, Hansson C, Taube M, Gustafsson-Ericson L, Egecioglu E, Karlsson-Lindahl L, Fehrentz JA, Martinez J, Perrissoud D, Dickson SL. On the central mechanism underlying ghrelin's chronic pro-obesity effects in rats: new insights from studies exploiting a potent ghrelin receptor antagonist. J Neuroendocrinol. 2009; 21:777–785. [PubMed: 19703102]

Sanberg PR, Zoloty SA, Willis R, Ticarich CD, Rhoads K, Nagy RP, Mitchell SG, Laforest AR, Jenks JA, Harkabus LJ, et al. Digiscan activity: automated measurement of thigmotactic and stereotypic behavior in rats. Pharmacol Biochem Behav. 1987; 27:569–572. [PubMed: 3659082]

Shimbara T, Mondal MS, Kawagoe T, Toshinai K, Koda S, Yamaguchi H, Date Y, Nakazato M. Central administration of ghrelin preferentially enhances fat ingestion. Neurosci Lett. 2004; 369:75–79. [PubMed: 15380311]

Shrestha YB, Wickwire K, Giraudo S. Effect of reducing hypothalamic ghrelin receptor gene expression on energy balance. Peptides. 2009; 30:1336–1341. [PubMed: 19540432]

Sun Y, Butte NF, Garcia JM, Smith RG. Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. Endocrinology. 2008; 149:843–850. [PubMed: 18006636]

Tessari M, Catalano A, Pellitteri M, Di Francesco C, Marini F, Gerrard PA, Heidbreder CA, Melotto S. Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats. Addiction Biology. 2007; 12:22. [PubMed: 17407494]

Till BJ, Cooper J, Tai TH, Colowit P, Greene EA, Henikoff S, Comai L. Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol. 2007; 7:19. [PubMed: 17428339]

Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature. 2000; 407:908–913. [PubMed: 11057670]

Wellman PJ, Davis KW, Nation JR. Augmentation of cocaine hyperactivity in rats by systemic ghrelin. Regul Pept. 2005; 125:151–154. [PubMed: 15582726]

Wellman PJ, Hollas CN, Elliott AE. Systemic ghrelin sensitizes cocaine-induced hyperlocomotion in rats. Regul Pept. 2008; 146:33–37. [PubMed: 17804092]

Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology. 2000; 141:4325–4328. [PubMed: 11089570]

Zan Y, Haag JD, Chen KS, Shepel LA, Wigington D, Wang YR, Hu R, Lopez-Guajardo CC, Brose HL, Porter KI, Leonard RA, Hitt AA, Schommer SL, Elegbede AF, Gould MN. Production of knockout rats using ENU mutagenesis and a yeast-based screening assay. Nat Biotechnol. 2003; 21:645–651. [PubMed: 12754522]

Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. J Comp Neurol. 2006; 494:528–548. [PubMed: 16320257]

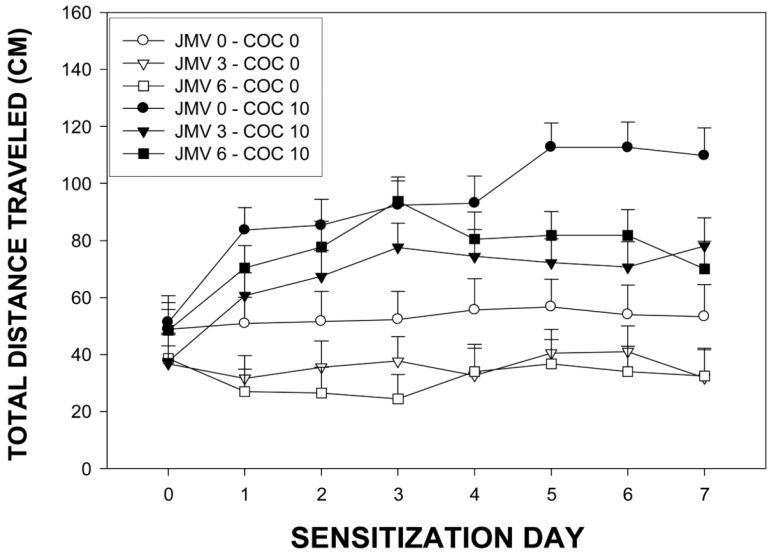

Fig.1.

Mean group total changes in total distance traveled scores during the test period (cm/45 min). On day 0, the rats were injected (i.p.) with vehicle at 10 min prior to the 15 min baseline period and then again with vehicle just prior to the 45 min test period. During days 1–7, the rats were injected with either vehicle (JMV 0), 3 mg/kg JMV 2959 (JMV 3) or 6 mg/kg JMV 2959 (JMV 6) at 10 min prior to the 15 min baseline period and then injected with either vehicle or 10 mg/kg cocaine (COC 0, COC 10) just prior to the 45 min test period on days 1–7. The lines above and below each symbol represent the S.E.M.

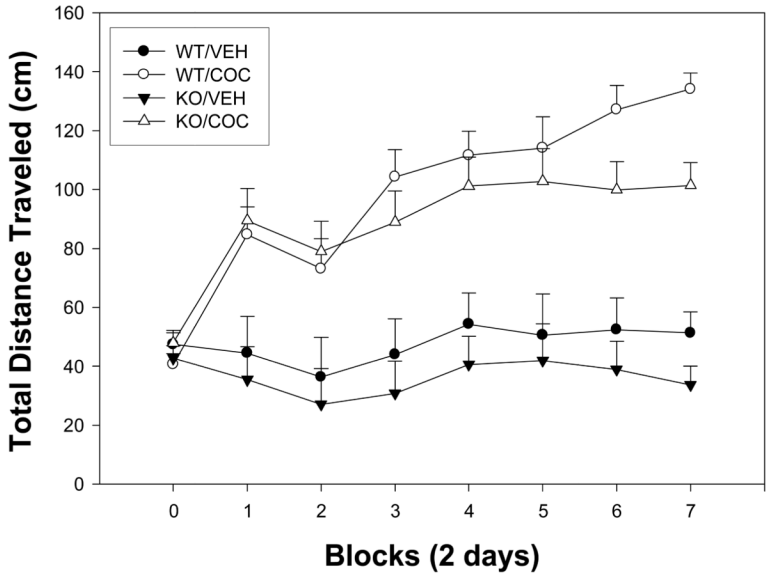

Fig.2.

Mean group total changes in total distance traveled scores during the test period (cm/45 min) for WT and GHR-R $^{(-/-)}$ (KO) rats injected with vehicle on Day 0 and then with either vehicle (VEH) or 10 mg/kg cocaine (COC) just prior to the 45 min test period on days 1–14 (shown in 2 day blocks). The lines above and below each symbol represent the S.E.M.

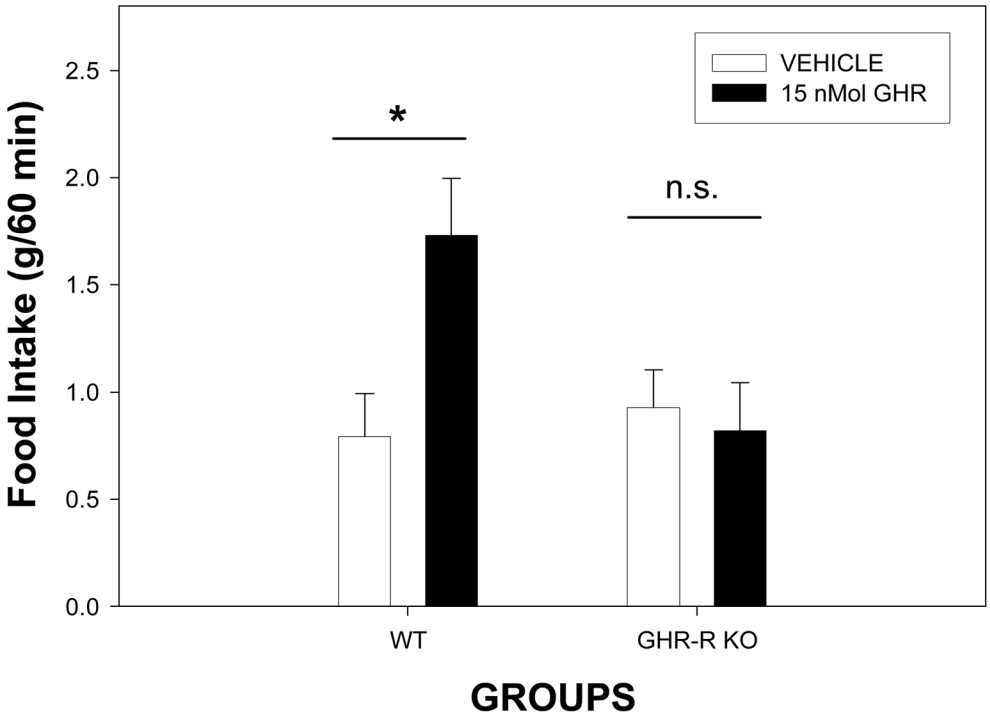

Fig 3.

Mean group 60 min food intake after vehicle or 15 nmol/rat (i.p.) ghrelin in WT (n=5) and GHR-R($^{-/-}$) (n=7) rats. The lines above and below each symbol represent the S.E.M. * denotes a significant difference between vehicle and ghrelin treatments at $p < 0.05$.