A role for neuropeptide Y Y5 but not the Y1-receptor subtype in food-deprivation-induced reinstatement of heroin seeking in the rat

Tia Maric, Firas Sedki, Danielle Chafetz, Nicholas Schoela, Uri Shalev

Department of Psychology, Center for Studies in Behavioral Neurobiology/Groupe de Recherche en Neurobiologie Comportementale, Concordia University, Montreal, Quebec, Canada

Final Version: **http://dx.doi.org/ 10.1007/s00213-011-2362-9**

Correspondence:

Dr. Uri Shalev

Dept. of Psychology/CSBN

Concordia University

7141 Sherbrooke St. W

Montreal H4B 1R6

Quebec, Canada

E-mail: uri.shalev@concordia.ca

Acknowledgements:

This work was supported by the Natural Sciences & Engineering Council Discovery Program (US: 298915), the Fonds de la recherché en santé Quebec (CSBN), and the Canada Research Chairs program (US). We gratefully acknowledge the generous contribution of Lu AA33810 by Lundbeck Research USA, and the helpful comments made by Dr. Mary W. Walker (Lundbeck Research USA) on a previous version of this manuscript.

Abstract *Rational and objectives:* Neuropeptide-Y (NPY), an orexigenic peptide that is released during periods of food restriction, has been shown to have a significant modulatory impact on drug-related behaviors. We have previously reported that both acute food deprivation (FD) and NPY injections can reinstate extinguished drug-seeking behavior, a **proposed** animal model of relapse to drug abuse. However, it is not clear whether the FD effect on drug seeking is dependent on NPY transmission. Here we used the reinstatement model to assess the role of NPY Y1 and Y5-receptor-mediated transmission in FD-induced reinstatement of heroin seeking. *Methods:* Rats were trained to self-administer heroin for 10-12 days (0.1 mg/kg/infusion/IV). Animals then underwent extinction training followed by drug-seeking reinstatement tests under 21 hr of food deprivation and sated conditions. *Results:* Injections of a novel NPY Y5-receptor antagonist, Lu AA33810 (0.0, 1.0, or 30.0 mg/kg/IP), resulted in a significant attenuation of food deprivation-induced reinstatement of extinguished heroin seeking. However, no significant effects on reinstatement were found for the Y1-receptor antagonist, BIBO 3304 (0.0, 5.0, or 10.0 nmol/ICV). *Conclusions:* These results suggest that while signals mediated through NPY Y1 receptors play a modest role in reinstatement, activation of Y5 receptors has a critical function in FD-induced reinstatement of heroin seeking behavior.

Keywords: Neuropeptide Y (NPY), Reinstatement, Drug seeking, Heroin, Y1 receptors, Y5 receptors, Food deprivation

Introduction

Dietary manipulations, such as food deprivation and food restriction, can modulate the rewarding, reinforcing, conditioned, and locomotor-activating effects of drugs of abuse. Both acute food deprivation (FD) and prolonged food restriction (FR) can facilitate drug-related behaviors across drugs of abuse (Bell et al. 1997; Campbell and Fibiger 1971; Deroche et al. 1993), routes of administration (Cabeza de Vaca and Carr 1998; Carroll et al. 1979), and species (Comer et al. 1995; Rodefer and Carroll 1996). In addition, using the reinstatement procedure, a proposed animal model for relapse (Stewart and de Wit 1987), acute (24 hr) FD was shown to induce reinstatement of previously extinguished heroin and cocaine seeking under drug-free states in rats (Shalev et al. 2000; Shalev et al. 2003). Although advances have recently been made in the understanding of the brain mechanisms involved in the enhancement of drug reward by chronic FR (Carr 2007), the exact neuronal mechanisms underlying the effects of acute FD on drug seeking are not known.

We, and others, have suggested that neuropeptidergic and/or hormonal signals that change in response to energy deficits, and which have been shown to modulate the reward pathway (Fulton et al. 2000), might underlie the effects of dietary manipulations on drug seeking (Carr et al. 2000; Shalev et al. 2001). Neuropeptide Y (NPY) is a 36 amino acid orexigenic peptide, which is a widely distributed peptide found throughout the CNS (Allen et al. 1983). NPY is released in response to periods of food scarcity (Bi and Moran 2003), and drives the initiation of food seeking and consummation of food. NPY has also been implicated in increasing the motivation to obtain food (Jewett et al. 1995), which could suggest that NPY signaling plays a role in appetitive reinforcement. We have recently shown that acute intracerebroventricular

(ICV) injections of NPY can reinstate extinguished heroin-seeking behavior, mimicking the effects of acute FD, and can also increase on-going cocaine self-administration and cocaine-induced hyperactivity (Maric et al. 2008; Maric et al. 2009).

Six NPY receptor subtypes (Y1-Y6) have been identified in the mammalian brain. Of these, the Y1 and Y5 receptor subtypes have been most frequently associated with ingestive behavior in mammals (Kalra et al. 1999). For example, ICV administration of NPY Y1-receptor antagonists decreases food deprivation induced-feeding (Kask et al. 1998) and blockade of NPY Y5-receptor reduces food intake and body weight in diet-induced obese mice (Ishihara et al. 2006). Interestingly, these receptors have also been implicated in mediating psychoactive effects of drugs. For example, NPY Y1-receptor antagonists inhibit the locomotor enhancing effects of amphetamine (Kask and Harro 2000), and activation of NPY Y5-receptors has been shown to reduce morphine withdrawal precipitated by the opioid antagonist, naloxone (Woldbye et al. 1998).

Our previous findings, and the studies described above, suggest that NPY might be an important mediator of the FD effect on reinstatement of drug seeking. Here we tested the effects of NPY transmission blockade on FD-induced reinstatement of heroin seeking, using the specific, high affinity NPY Y1-receptor antagonist, BIBO 3304 (Wieland et al. 1998), and the novel NPY Y5-receptor antagonist, Lu AA33810. Lu AA33810 was recently demonstrated to have very high affinity for the Y5 receptor as well as *in vivo* efficacy after systemic administration (Walker et al. 2009), and is thus considered a superior candidate for use in our procedure.

Materials and methods

Subjects

Eighty-eight Long-Evans male rats (Charles River, St. Constant, QC; 300-350 g) were used. Following recovery from intravenous (IV) and intracerebroventricular (ICV) guide cannulation surgery, rats were housed in operant conditioning chambers under a reversed 12 hr light/dark cycle (lights off at 9:30 a.m.), throughout the experiment. *Ad libitum* access to water and food was available, except for the 21 hr food deprivation test condition. All rats were treated in accordance with the Canadian Council on Animal Care and approval was granted by the Concordia University Animal Research Ethics Committee.

Surgery

Rats were implanted with silastic catheters (Dow Corning, Midland, MI, USA) under anesthesia (xylazine and ketamine (10 + 100 mg/kg, intraperitoneal (IP)). Briefly, a small incision was made on the jugular vein's surface allowing for the insertion of a catheter, which was then secured to the vein. The remaining segment of the catheter was directed subcutaneously to the top of the skull where it was attached to a modified 22-gauge cannula (Plastics One Industries, Roanoke, VA). During the same surgery, animals from Experiment 1 were implanted with a 22-gauge cannula aimed 2 mm above the right lateral ventricle (AP -0.5, ML +1.4, DV -3.0, relative to bregma). Both cannulae were subsequently anchored to the skull with dental cement and jeweler screws. Following recovery from surgery, guide cannula placement was verified by demonstrating a short latency (<60 s) drinking response to angiotensin II (100 nmol, ICV). Throughout the experiment, the catheters were flushed daily with heparin and the antibiotic drug gentamicin in saline solution (7.5 IU + 12.0 μg/rat).

Apparatus

Operant conditioning chambers: Rats were individually housed in standard operant conditioning chambers (Med Associates, St. Albans, VT, or Coulbourn Instruments,

Allentown, PA, USA; 32.0 cm x 24.0 cm x 25.0 cm; 29.0 cm x 29.0 cm x 25.5 cm, respectively), which were kept in ventilated, sound-attenuating compartments. Each chamber contained two levers, which were placed 5 cm above the grid floor, and approximately 15 cm apart, on the right wall. The lever designated as 'inactive' remained extended, and presses on this lever did not result in any programmable consequences. The lever designated as 'active', extended at the beginning of each session and responses on that lever activated the infusion pump (Med Associates). Rats were attached to the infusion pump via a liquid swivel (Instech, Boulder, CO, USA) and a polyethylene-50 tubing shielded with a metal spring.

Elevated plus maze: Located in a dimly lit room, the maze was made out of wood and consisted of four arms (11.5 x 55.0 cm) positioned at right angles 50 cm above the floor. The maze comprised of two arms considered as "Closed" arms, which had 40 cm high walls and two "Open" arms, which had 1.0 cm high ledges.

ICV injections: The NPY Y1-receptor antagonist was injected using a microinfusion pump (Harvard Apparatus *11 plus*, Holliston, MA) fitted with a 10 μl Hamilton syringe (Hamilton Company, Reno, NV).

Drugs

Heroin HCL (National Institute for Drug Abuse, Baltimore, MD) was dissolved in sterile water (5 mg/ml), and then further diluted individually for each rat with 0.9% saline solution to a dose of 0.1 mg/kg/infusion. For experiment 1, BIBO 3304 ((R)-N-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N2-(diphen ylacetyl)-argininamide trifluoroacetate; TOCRIS, MO) was dissolved in 30% dimethyl sulfoxide (DMSO) and distilled water to a concentration of either 2.5 nmol/ul or 5.0 nmol/ul. For experiment 2, Lu AA33810 ((N-[[trans-4-[(4,5-dihydro[1]benzothiepino[5,4-d]thiazol-2-

yl)amino]cyclohexyl]methyl]-methanesulfonamide; Lundbeck Research USA, NJ) was dissolved in 5% DMSO, 1% methylcellulose and sterile saline. NPY receptors antagonists' doses were selected based on existing literature (Kask 2000; Walker et al. 2009). In addition, we verified in our laboratory that treatment with the high doses of the antagonists reduced free-feeding (BIBO 3304) and Y5-receptor-agonist-induced feeding (Lu AA33810).

Procedure

Self-administration training. Following a 24 hr habituation period in the operant conditioning chamber, all rats were trained to self-administer heroin with three 3 hr self-administration sessions per day under a fixed ratio 1 (FR-1), 20 s timeout schedule of reinforcement for a total of 10-12 days. The first daily session began shortly after the onset of the dark phase of the light/dark cycle. Each session began with the insertion of the levers into the chamber, the illumination of a houselight, and the activation of a cuelight/tone (2.9 Khz; 20 dB above background level) complex for 30 s. Subsequently, each response on the active lever resulted in the delivery of 0.1 mg/kg of heroin over 5 s, and the initiation of a 20 s timeout period. During the timeout period, lever presses were not reinforced, the houselight was turned off, and the cue-light/tone complex was turned on. At the end of each 3 hr session the active lever was retracted but the inactive lever remained in the chamber.

Extinction. Following 10-12 days of self-administration training, rats were submitted to extinction training that followed the same procedure as the self-administration days, but consisted of only one 3 hr session/day. During extinction, heroin syringes were removed but the rats continued to be exposed to all the contextual and response-contingent cues present during the self-administration training phase. Beginning on the second day of extinction training, rats in experiment 1 received sham ICV injections. Rats in experiment 2 received

injections of saline (0.5 ml, IP). Extinction training continued for a minimum of 4 days and until animals reached an extinction criterion of 15 or less active lever presses per 3 hr session before conducting reinstatement tests.

Reinstatement Test. Once rats met extinction criterion, they were exposed to two 3 hr reinstatement test sessions, which were preceded by either 21 hr of food-deprivation (food hoppers removed) or 21 hr of unlimited access to food, in a counterbalanced order. In experiment 1, rats were injected with the NPY Y1-receptor antagonist BIBO 3304 (0.0, 5.0 or 10.0 nmol in 2 μl, ICV), 15 min before each reinstatement test. BIBO 3304 was injected over a period of 4 min using a 28-gauge injector that extends 3 mm below the tip of the guide cannula. The injector was retained in place for an additional 60 s after the injection to ensure the dispersion of the drug. In experiment 2, 30 min before each reinstatement test, rats were injected with the NPY Y5-antagonist Lu AA33810 (0.0, 1.0, or 30 mg/kg, IP). Test sessions were separated by a minimum of two baseline days.

Elevated plus maze. Rats were handled daily for 1 week prior to the elevated plus maze experiment, and habituated to the maze apparatus (10 min exposure) and ICV or IP injection procedures over 2 days. Immediately following the last habituation day, all rats were food-deprived for 21 hr, by removing all food from the cages, with water available *ad libitum.* For experiment 1B, rats were randomly assigned to either the BIBO 3304 (10.0 nmol/rat/ICV, n=5) or vehicle (0.0 nmol/rat/ICV, n=5) group. Ten minutes following the injection, rats were brought from the animal facility to the testing room and were placed on the maze for 10 min. For experiment 2B, rats were randomly assigned to either the Lu AA33810 (30.0 mg/kg, IP, n=5) or vehicle (0.0 mg/kg, IP, n=5) group. Thirty minutes following the injection rats were brought from the animal facility to the testing room and were placed on the maze for 10 min.

For both experiments, following the maze test fecal boli was collected and counted, and the maze was wiped down with 70% ethanol to ensure environmental neutrality. Behavior on the plus maze was video-recorded and then scored by an observer who was unaware of treatment assignment. Two variables were observed and scored: 1. Time spent on closed arms, defined as time spent with all paws in the closed arm, or two hind paws in the closed arm and two front paws in middle or open arm. 2. Number of closed arm entries, defined as the number of closed arm entries from an open arm, or crossings from one closed arm to the other.

Statistical Analyses

Reinstatement data were analyzed with separate mixed design ANOVAs, which were conducted for each dependent variable (active and inactive lever responses) with a within-subjects factor of *condition* (baseline, sated, and 21 hr food deprivation (FD)), and a between-subjects factor of *BIBO 3304 dose* (0.0, 5.0, or 10.0 nmol/rat/ICV) for Experiment 1, or *Lu AA33810 dose* (0.0, 1.0 or 30.0 mg/kg, IP) for Experiment 2. Baseline condition was calculated by averaging the number of lever presses made during the last of day of extinction before each reinstatement test. Significant main effects were further analyzed using Fisher's LSD post-hoc tests. Elevated plus maze data were analyzed using separate One-Way ANOVAs with the between-subjects factor *of BIBO 3304 dose* (0.0 or 10.0 nmol/rat/ICV; experiment 1B), or *Lu AA33810 dose* (0.0 or 30.0 mg/kg, IP; experiment 2B) for the two dependent variables described above. Time spent in closed arms was analyzed as percent time in closed arms (out of total session time). The critical cut-off point for significant results in all analyses was set at $p \leq 0.05$.

Results

Experiment 1A. Effects of treatment with the NPY Y1-receptor antagonist, BIBO 3304, on food-deprivation induced reinstatement of heroin seeking

Eight rats were removed due to catheter blockage (*n*= 5), illness (*n*= 1) or poor self-administration training (*n*= 2**).** One additional rat from the 5.0 nmol dose group was removed due to an extreme response rate on the active lever during the FD test (172 compared to a group average of 28.2). Final analyses included data from 29 rats in three *BIBO 3304 dose* groups, 0.0 nmol (*n*= 12), 5.0 nmol (*n*= 8) and 10.0 nmol (*n*= 9). On the last training day, the mean ± SEM number of infusions taken over the three 3 h sessions was 42.9 ± 3.6. The mean ± SEM numbers of active and inactive lever responses made during the last day of training were 159.8 ± 34.2 and 11.9 ± 2.3, respectively. The mean ± SEM number of extinction training days prior to the first test session was 8.1 ± 0.7.

Acute, 21 hr FD resulted in significant reinstatement of heroin seeking behavior, shown as an increase in the number of responses on the active lever, that was previously associated with drug delivery [*condition effect*; $F(2, 52)= 17.00$, $p< 0.001$; Figure 1a]. Post hoc comparisons found that the number of responses on the active lever during the FD condition was higher than in the baseline or sated conditions ($p< 0.05$). Pretreatment with 5.0 or 10.0 nmol of BIBO 3304 did not attenuate the FD effect on reinstatement of heroin seeking. In fact, responses on the active lever seemed to be increased during the FD test following treatment with the high dose of BIBO 3304 compared to vehicle (effect sizes: Cohen's d = 0.7).

No significant effects of *condition* or *BIBO 3304 dose* were found for inactive lever responses (Figure 1b).

Experiment 1B. The effects of treatment with the NPY Y1-receptor antagonist, BIBO 3304 on performance in the elevated plus maze in food deprived rats.

Following experiment 1A, rats (*n*= 10) were housed in the animal facility on the same reversed light/dark cycle (lights off at 9:30 a.m., on at 9:30 p.m.) for a period of 2 weeks for drug washout and habituation.

Although number of fecal boli was recorded, no statistical analysis was conducted, as most rats did not produce any. However, it is interesting to note that animals that did display excretion (*n*=2) were rats treated with BIBO 3304 (10.0 nmol/rat/ICV). BIBO 3304 treated rats spent significantly more time in the closed arms compared to vehicle treated rats [$F(1,8)= 5.37$, $p= 0.04$; Figure 2a], and had more entries into closed arms, but this difference failed to reach statistical significance [$F(1,8)= 3.70$, $p= 0.09$; Figure 2b]. Nevertheless, the higher number of closed arms entries reflects a high number of crossings between the two closed arms in the BIBO 3304 treated group, and rules out attenuated general locomotion in this group as the reason for the longer time spent in the closed arms.

Experiment 2A: Effects of treatment with the NPY Y5-receptor antagonist, Lu AA33810, on food-deprivation induced reinstatement of heroin seeking

Three rats were removed due to illness (n= 2), and training failure (n= 1). Final analyses included data from 37 rats in three treatment groups of *Lu AA33810 dose*, 0.0 mg/kg (*n*=17), 1.0 mg/kg (*n*=10), and 30.0 mg/kg (*n*=10). On the last training day, the mean ± SEM number of infusions taken over the three 3 h sessions was 43.5 ± 3.9. The mean ± SEM numbers of active and inactive lever responses made during the last day of training were 126.7 ± 22.8 and 15.6 ± 3.3, respectively. The mean ± SEM number of extinction training days prior to the first test session was 6.9 ± 0.4.

A statistically significant higher number of active lever presses under the FD condition compared to the baseline and sated conditions was observed [*condition:* $F(2, 68)= 18.54$, $p<$

0.0001], regardless of treatment (Figure 3a). In addition, a statistically significant interaction between c*ondition* and *Lu AA33810 dose* for active lever presses was found [$F(4, 68)= 2.68$, $p= 0.04$], reflecting an attenuation of FD-induced reinstatement by pretreatment with Lu AA33810. Subsequent one-way ANOVAs, followed by post hoc tests, revealed a significant increase in active lever presses in rats treated with vehicle or the low dose of Lu AA33810 in the FD condition, compared to the baseline and sated conditions [$F(2, 32)= 10.94$, $p= 0.0002$; $F(2, 18)= 8.39$, $p= 0.003$, respectively], but not in rats treated with the high dose of Lu AA33810. Finally, within the FD condition, *Lu AA33810 dose* effect approached significance [$F(2, 34)=2.78$, $p=0.08$], and post hoc tests revealed a statistically significant difference in active lever responses between rats treated with the high dose Lu AA33810 and the low dose-treated rats ($p< 0.05$). No significant effects for inactive lever presses were found (Figure 3b).

Experiment 2B. The effects of treatment with the Y5 receptor antagonist, LU AA33810, on performance in the elevated plus maze in food deprived rats.

Ten rats with heroin self-administration experience were kept in the animal facility under reversed light/dark cycle, as above, for 10 days to allow complete drug washout. The procedure for the elevated plus maze test followed the one used in experiment 1B.

Since very few fecal boli were produced, these data were not recorded. No statistically significant differences in percent time spent in the closed arms, or the number of entries into the closed arms were found (data not shown).

Discussion

The major finding in this report is that blockade of the NPY Y5-receptor with the novel antagonist, Lu AA33810, attenuated acute FD-induced reinstatement of heroin seeking. This result implies a critical role for Y5-receptor-mediated NPY transmission in FD-induced reinstatement. In contrast, pretreatment with BIBO 3304, an NPY Y1-receptor antagonist, did

not attenuate FD-induced reinstatement of heroin seeking, further emphasizing the specific role of the Y5-receptor in reinstatement of drug seeking. The attenuating effect of Lu AA33810 on drug seeking behavior was not due to general, non-specific effects of the antagonist, as inactive lever responding was not affected. Our results are consistent with studies that have shown that another Y5-antagonist, L-152,804, suppresses reward-related behavior. For example, it has been shown the oral delivery of L-152,804 attenuated alcohol-self-administration in alcohol preferring rats (Schroeder et al. 2005).

We have previously found that ICV injections of NPY, at doses known to stimulate feeding, increased cocaine self-administration and cocaine-induced locomotor activity, and induced reinstatement of previously extinguished heroin-seeking behavior in free-feeding animals (Maric et al. 2008; Maric et al. 2009). These findings imply that NPY mediates both the unconditioned and conditioned reinforcing effects of drugs of abuse and their associated cues. We have suggested that administration of NPY has the ability to activate or "mimic" a state of food-deprivation. Thus, it was concluded that NPY is involved in FD-induced reinstatement perhaps via a homeostatic mechanism expressed as a "hunger"-like state, or by direct augmentation of activity in the neuronal circuitry underlying the conditioned reinforcing properties of drug-associated cues. It was further speculated that NPY transmission might be necessary for the expression of FD-induced reinstatement. Our present findings support the latter speculation, albeit exclusively through the Y5-receptor.

NPY Y5-receptor is expressed in high numbers in the hypothalamic areas known to regulate food intake such as the paraventricular nucleaus (PVN), arcuate nucleus (ARC), and in limbic regions (Morin and Gehlert 2006; Wolak et al. 2003). Although the NPY Y1-receptor is critical in the regulation of food intake, activation of the Y5 receptors has also been shown to

stimulate feeding (Kalra et al. 1999; Parker et al. 2000). Activation of hunger pathways traditionally associated with the hypothalamus, could modulate the saliency of reward-associated cues for food or drug. Thus, Y5-receptor antagonism might have dampened hunger signals initiated by FD and thus inhibit FD-induced reinstatement of drug seeking. Indeed, treatment with the non-specific Y5-receptor antagonist, CGP71683A, significantly suppressed spontaneous nocturnal feeding and fasting-induced feeding (Kask et al. 2001). However, the role of Y5 receptor in the control of feeding has recently been questioned. Accumulating reports indicate that NPY Y5-receptor by itself does not have a major role in feeding behavior. For example, the Y5-receptor antagonist, NPY5RA-972, potently suppressed food intake induced by the Y5-receptor agonist cPP, but failed to attenuate NPY-induced feeding or spontaneous food intake (Turnbull et al. 2002). Similarly, Lu AA33810 was shown to have no effect on NPY-evoked food consumption (Walker et al. 2009). In addition, acute administration of a spironolactone Y5-receptor antagonist did not significantly reduce food intake or body weight in high-fat diet-fed mice, while treatment with a Y1-receptor antagonist resulted in a robust suppression of food intake and body weight (Mashiko et al. 2009). Finally, food deprivation seems to increase the expression of the Y1-receptor mRNA in the hypothalamus, while the expression of Y5 receptors is decreased (Widdowson et al. 1997; Xu et al. 1998).

In conclusion, although we cannot completely rule out hunger-suppression as the underlying mechanism for the effect of the Y5 receptor antagonist on FD-induced reinstatement of heroin seeking, the findings described above make this interpretation highly unlikely.

Another way for the Y5 receptor to modulate the reinstatement effect is through the mediation of anxiolytic responses. The present results, together with findings from previous studies,

suggest that Y5-receptor antagonism could be involved in *reducing* anxiety-producing states, an effect opposite to the one suggested for Y1 receptor antagonism described below. Systemic injections of Lu AA33810 at doses shown to decrease Y5 receptor agonist-induced feeding have been shown to produce anxiolytic and anti-depressant-like effects in rats (Walker et al. 2009). These results suggest that antagonism of the Y5-receptor could counteract the stress/anxiety condition associated with FD, which is one of the suggested mechanisms underlying FD-induced reinstatement (Shalev et al. 2006). However, this is probably not the case here, as we found no anxiolytic effect for the high dose of Lu AA33810 in the elevated plus maze test. Moreover, identifying the underlying pathway for an anxiolytic or anti-stress effect appears problematic. The amygdala has been identified as the critical area for the anxiolytic effects of NPY (Heilig 2004). As expected, the amygdala contains Y1 and Y5 receptors, with relatively higher Y5 receptor expression within basolateral nucleus (BLA) compared to the central amygdala (Wolak et al. 2003). Yet, it has been reported that the anxiolytic effects of NPY are specific to the BLA through exclusive *activation* of Y5 receptors (Sajdyk et al. 2002), thus challenging the notion of NPY Y5-receptors in the BLA as the critical target for Lu AA33810 in our study.

Finally, NPY has been shown to affect reward-related behaviors, as well as homeostatic feeding behavior (Brown et al. 2000). As previously mentioned, Y5 receptors have been shown to be involved in drug-reinforced behaviors (Schroeder et al. 2005), and thus it is possible that Y5 receptors mediate the effect of FD on drug-seeking through the modulation of reward pathways, and more specifically, the mesocorticolimbic dopaminergic (DA) pathway. DA projections from the ventral tegmental area (VTA), to nucleus accumbens (NAc) and to the prefrontal cortex (PFC) constitute this pathway, which is thought to

orchestrate motivational, affective and learning-based reward processing (Kelley and Berridge 2002; Wise 2002). DA transmission has also been shown to be critically involved in the reinstatement of drug seeking behavior (Bossert et al. 2005), and we have recently demonstrated that activation of the DA D1 receptors is critical for acute FD-induced reinstatement of heroin seeking (Tobin et al. 2009). Consistent with this suggestion, it was reported that NPY Y5-receptor protein and mRNA are strongly expressed in mesolimbic regions such as the NAc (Wolak et al. 2003) and VTA (Morin and Gehlert 2006; Parker and Herzog 1999). More recently, it was reported that extracellular DA levels were enhanced in mesocorticolimbic projecting areas, including NAc, following central administration of either NPY or a Y5-selective agonist [cPP1-7,NPY19-23,Ala31,Aib32,Gln34]hPP (Quarta et al. 2011). Thus, it is plausible that NPY mediates behaviors associated with dopamine release in the mesocorticolimbic pathway, including reinstatement of drug seeking, via activation of NPY Y5-receptors, and administration of Lu AA33810 might have interfered with this effect in our food deprived rats.

The lack of attenuation of FD-induced reinstatement by the NPY Y1-recpetor antagonist, BIBO 3304, was somewhat surprising. Hypothalamic NPY Y1-receptors are thought to mediate the orexigenic properties of NPY, and blockade of these receptors reduces feeding behavior in free-feeding and diet-induced obese animals (Kanatani et al. 2001; Kask et al. 1998). We have previously suggested that FD-induced reinstatement of drug seeking is mediated by activation of orexigenic pathways, and more specifically, NPY receptors, and reported that NPY administration results in reinstatement of heroin seeking (Maric et al. 2008). We therefore expected the activation of the Y1-receptor to be critically involved in this effect.

Instead, it appeared that NPY Y1-receptor antagonism enhanced, rather than attenuated, the reinstatement effect, exclusively in the food-deprivation condition. Although statistical significance was not established and thus these data should be reviewed with caution, an impressive effect size found within the food-deprivation condition suggests that a synergistic effect between the food deprivation condition and Y1-receptor antagonist treatment might be present and thus merits consideration.

It has been shown that NPY transmission reduces anxiety related phenomena (Heilig et al. 1989), an effect thought to be mediated by the Y1 receptor, as ICV injections of BIBO 3304 in a novel open field test, resulted in an increased defecation, a measure of anxiety, that occurred without affecting locomotion (Kask and Harro 2000). In addition, treatment with the Y1-receptor antagonist, BIBP 3226, induced conditioned place-avoidance (Kask et al. 1999). Moreover, blockade (experiment 1B) or antisense inhibition of NPY Y1-receptors (Wahlestedt et al. 1993), results in an increased anxiety as measured in the elevated plus-maze test. Pharmacologically-induced anxiety, for example, using yohimbine, is known to significantly reinstate extinguished drug seeking (Le et al. 2005; Shepard et al. 2004). We therefore suggest that the effects observed within the food deprivation condition in experiment 1A might have been a result of an increased anxiogenic experience associated with acute food deprivation, due to the treatment with the Y1-receptor antagonist.

In conclusion, our findings suggest that NPY transmission via specific NPY receptor activation, namely, the Y5 receptors, is critical for the expression of FD-induced reinstatement of heroin seeking. Although the effect would appear to be consistent with involvement of NPY Y5 receptors in mesolimbic projection areas, the precise anatomical locus and cellular mechanism remain to be defined.

References

Allen YS, Adrian TE, Allen JM, Tatemoto K, Crow TJ, Bloom SR et al. (1983) Neuropeptide Y distribution in the rat brain. Science 221:877–879

Bell SM, Stewart RB, Thompson SC, Meisch RA (1997) Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. Psychopharmacology 131:1–8

Bi S, Moran TH (2003) Response to acute food deprivation in OLETF rats lacking CCK-A receptors. Physiol Behav 79:655–661

Bossert JM, Ghitza UE, Lu L, Epstein DH, Shaham Y (2005) Neurobiology of relapse to heroin and cocaine seeking: An update and clinical implications. Eur J Pharmacol 526:36–50

Brown CM, Coscina DV, Fletcher PJ (2000) The rewarding properties of neuropeptide Y in perifornical hypothalamus vs. nucleus accumbens. Peptides 21:1279–1287

Cabeza de Vaca S, Carr KD (1998) Food restriction enhances the central rewarding effect of abused drugs. J Neurosci 18:7502–7510

Campbell BA, Fibiger HC (1971) Potentiation of amphetamine-induced arousal by starvation. Nature 233:424–425

Carr KD (2007) Chronic food restriction: enhancing effects on drug reward and striatal cell signaling. Physiol Behav 91:459–472

Carr KD, Kim G, Cabeza de Vaca S (2000) Hypoinsulinemia may mediate the lowering of self-stimulation thresholds by food restriction and streptozotocin-induced diabetes. Brain Res 863:160–168

Carroll ME, France CP, Meisch RA (1979) Food deprivation increases oral and intravenous drug intake in rats. Science 205:319–321

Comer SD, Lac ST, Wyvell CL, Curtis LK, Carroll ME (1995) Food deprivation affects extinction and reinstatement of responding in rats. Psychopharmacology Psychopharmacology 121:150–157

Deroche V, Piazza PV, Casolini P, Le Moal M, Simon H (1993) Sensitization to the psychomotor effects of amphetamine and morphine induced by food restriction depends on corticosterone secretion. Brain Res 611:352–356

Fulton S, Woodside B, Shizgal P (2000) Modulation of brain reward circuitry by leptin. Science 287:125–128

Heilig M (2004) The NPY system in stress, anxiety and depression. Neuropeptides 38:213–224

Heilig M, Soderpalm B, Engel JA, Widerlov E (1989) Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. Psychopharmacology 98:524–529

Ishihara A, Kanatani A, Mashiko S, Tanaka T, Hidaka M, Gomori A et al. (2006) A neuropeptide Y Y5 antagonist selectively ameliorates body weight gain and associated parameters in diet-induced obese mice. Proc Natl Acad Sci U S A 103:7154–7158

Jewett DC, Cleary J, Levine AS, Schaal DW, Thompson T (1995) Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior. Psychopharmacology 120:267–271

Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS (1999) Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev 20:68–100

Kanatani A, Hata M, Mashiko S, Ishihara A, Okamoto O, Haga Y et al. (2001) A typical Y1 receptor regulates feeding behaviors: effects of a potent and selective Y1 antagonist, J-115814. Mol Pharmacol 59:501–505

Kask A, Harro J, von Horsten S, Redrobe JP, Dumont Y, Quirion R (2002) The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neurosci Biobehav Rev 26:259–283

Kask A, Kivastik T, Rago L, Harro J (1999) Neuropeptide Y Y1 receptor antagonist BIBP3226 produces conditioned place aversion in rats. Prog Neuropsychopharmacol Biol Psychiatry 23:705–711

Kask A, Rago L, Harro J (1998) Evidence for involvement of neuropeptide Y receptors in the regulation of food intake: studies with Y1-selective antagonist BIBP3226. Br J Pharmacol 124:1507–1515

Kask A, Vasar E, Heidmets LT, Allikmets L, Wikberg JE (2001) Neuropeptide Y Y(5) receptor antagonist CGP71683A: the effects on food intake and anxiety-related behavior in the rat. Eur J Pharmacol 414:215–224

Kask A, Harro J (2000) Inhibition of amphetamine- and apomorphine-induced behavioural effects by neuropeptide Y Y(1) receptor antagonist BIBO 3304. Neuropharmacology 39:1292–1302

Kelley AE, Berridge KC (2002) The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 22:3306–3311

Le AD, Harding S, Juzytsch W, Funk D, Shaham Y (2005) Role of alpha-2 adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats. Psychopharmacology 179:366–373

Maric T, Cantor A, Cuccioletta H, Tobin S, Shalev U (2009) Neuropeptide Y augments cocaine self-administration and cocaine-induced hyperlocomotion in rats. Peptides 30:721–726

Maric T, Tobin S, Quinn T, Shalev U (2008) Food deprivation-like effects of neuropeptide Y on heroin self-administration and reinstatement of heroin seeking in rats. Behav Brain Res 194:39–43

Mashiko S, Moriya R, Ishihara A, Gomori A, Matsushita H, Egashira S et al. (2009) Synergistic interaction between neuropeptide Y1 and Y5 receptor pathways in regulation of energy homeostasis. Eur J Pharmacol 615:113–117

Morin SM, Gehlert DR (2006) Distribution of NPY Y5-like immunoreactivity in the rat brain. J Mol Neurosci 29:109–114

Parker EM, Balasubramaniam A, Guzzi M, Mullins DE, Salisbury BG, Sheriff S et al. (2000) [D-Trp(34)] neuropeptide Y is a potent and selective neuropeptide Y Y(5) receptor agonist with dramatic effects on food intake. Peptides 21:393–399

Parker RM, Herzog H (1999) Regional distribution of Y-receptor subtype mRNAs in rat brain. Eur J Neurosci 11:1431–1448

Quarta D, Leslie CP, Carletti R, Valerio E, Caberlotto L (2011) Central administration of NPY or an NPY-Y5 selective agonist increase in vivo extracellular monoamine levels in mesocorticolimbic projecting areas. Neuropharmacology 60:328–335

Rodefer JS, Carroll ME (1996) Progressive ratio and behavioral economic evaluation of the reinforcing efficacy of orally delivered phencyclidine and ethanol in monkeys: effects of feeding conditions. Psychopharmacology (Berl) 128:265–273

Sajdyk TJ, Schober DA, Gehlert DR (2002) Neuropeptide Y receptor subtypes in the basolateral nucleus of the amygdala modulate anxiogenic responses in rats. Neuropharmacology 43:1165–1172

Schroeder JP, Overstreet DH, Hodge CW (2005) The neuropeptide-Y Y5 receptor antagonist L-152,804 decreases alcohol self-administration in inbred alcohol-preferring (iP) rats. Alcohol 36:179–186

Shalev U, Finnie PS, Quinn T, Tobin S, Wahi P (2006) A role for corticotropin-releasing factor, but not corticosterone, in acute food-deprivation-induced reinstatement of heroin seeking in rats. Psychopharmacology 187:376–384

Shalev U, Highfield D, Yap J, Shaham Y (2000) Stress and relapse to drug seeking in rats: studies on the generality of the effect. Psychopharmacology 150:337–346

Shalev U, Marinelli M, Baumann MH, Piazza PV, Shaham Y (2003) The role of corticosterone in food deprivation-induced reinstatement of cocaine seeking in the rat. Psychopharmacology 168:170–176

Shalev U, Yap J, Shaham Y (2001) Leptin attenuates acute food deprivation-induced relapse to heroin seeking. J Neurosci 21:RC129

Shepard JD, Bossert JM, Liu SY, Shaham Y (2004) The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. Biol Psychiatry 55:1082–1089

Stewart J, de Wit H (1987) Reinstatement of drug-taking behavior as a method of assessing incentive motivational properties of drugs. In: Bozarth MA (ed) Methods of assessing the reinforcing properties of abused drugs, New York, pp 211–227.

Tobin S, Newman AH, Quinn T, Shalev U (2009) A role for dopamine D1-like receptors in acute food deprivation-induced reinstatement of heroin seeking in rats. Int J Neuropsychopharmacol 12:217–226

Turnbull AV, Ellershaw L, Masters DJ, Birtles S, Boyer S, Carroll D et al. (2002) Selective antagonism of the NPY Y5 receptor does not have a major effect on feeding in rats. Diabetes 51:2441–2449

Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M (1993) Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. Science 259:528–531

Walker MW, Wolinsky TD, Jubian V, Chandrasena G, Zhong H, Huang X et al. (2009) The novel neuropeptide Y Y5 receptor antagonist Lu AA33810 [N-[[trans-4-[(4,5-dihydro[1]benzothiepino[5,4-d]thiazol-2-yl)amino]cycloh exyl]methyl]-methanesulfonamide] exerts anxiolytic- and antidepressant-like effects in rat models of stress sensitivity. J Pharmacol Exp Ther 328:900–911

Widdowson PS, Upton R, Henderson L, Buckingham R, Wilson S, Williams G (1997) Reciprocal regional changes in brain NPY receptor density during dietary restriction and dietary-induced obesity in the rat. Brain Res 774:1–10

Wieland HA, Engel W, Eberlein W, Rudolf K, Doods HN (1998) Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO 3304 and its effect on feeding in rodents. Br J Pharmacol 125:549–555

Wise RA (2002) Brain reward circuitry: insights from unsensed incentives. Neuron 36:229–240

Wolak ML, DeJoseph MR, Cator AD, Mokashi AS, Brownfield MS, Urban JH (2003) Comparative distribution of neuropeptide Y Y1 and Y5 receptors in the rat brain by using immunohistochemistry. J Comp Neurol 464:285–311

Woldbye DP, Klemp K, Madsen TM (1998) Neuropeptide Y attenuates naloxone-precipitated morphine withdrawal via Y5-like receptors. J Pharmacol Exp Ther 284:633–636

Xu B, Kalra PS, Moldawer LL, Kalra SP (1998) Increased appetite augments hypothalamic NPY Y1 receptor gene expression: effects of anorexigenic ciliary neurotropic factor. Regul Pept 75-76:391–395

Figure legend

Fig. 1 The effects of treatment with the NPY Y1-receptor antagonist, BIBO 3304, on acute food deprivation (FD)-induced reinstatement of heroin seeking. Data shown are means (+SEM) of active (a) and inactive (b) lever responses during baseline and following pre-treatment with Vehicle, 5.0, or 10.0 nmol/rat BIBO 3304 (ICV), in the sated condition and following 21 hr FD. The baseline condition represents the average number of lever presses made, with no antagonist treatment, during the last extinction day prior to each test session. * $p<0.05$ compared to baseline and sated conditions

Fig. 2 The effects of treatment with the NPY Y1-receptor antagonist, BIBO 3304, on performance in the elevated plus maze. Data shown are mean (+SEM) time spent on the closed arms of the maze, as percent of total time (a), and mean (+SEM) number of entries into the closed arms (b), in the Vehicle and 10.0 nmol/rat (ICV) treatment groups. * $p<0.05$ compared to Vehicle treatment.

Fig. 3 The effects of treatment with the NPY Y5-receptor antagonist, Lu AA33810, on acute food deprivation (FD)-induced reinstatement of heroin seeking. Data shown are means (+SEM) of active (a) and inactive (b) lever responses during baseline and following pre-treatment with Vehicle, 1.0, or 30.0 mg/kg Lu AA33810 (IP), in the sated condition and following 21 hr FD. The baseline condition represents the average number of lever presses made, with no antagonist treatment, during the last extinction day prior to each test session. * $p<0.05$ compared to baseline and sated condition. # $p<0.05$ compared to the 30.0 mg/kg group within the FD condition.