Published in final edited form as:

Psychopharmacology (Berl). 2013 August ; 228(3): 499–507. doi:10.1007/s00213-013-3051-7.

Attenuation of saccharin-seeking in rats by orexin/hypocretin receptor 1 antagonist

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Abstract

Rationale—The orexin/hypocretin system has been implicated in reward-seeking, especially for highly salient food and drug rewards. We recently demonstrated that signaling at the OxR1 receptor is involved in sucrose reinforcement and reinstatement of sucrose-seeking elicited by sucrose-paired cues in food-restricted rats. Because sucrose reinforcement has both a hedonic and caloric component, it remains unknown what aspect of this reward drives its reinforcing value.

Objectives—The present study examined the involvement of the orexin (Orx) system in operant responding for saccharin, a non-caloric, hedonic (sweet) reward, and in cue-induced reinstatement of extinguished saccharin-seeking, in ad libitum fed vs. food-restricted male subjects.

Methods—Male Sprague Dawley rats were fed ad libitum or food-restricted and trained to self-administer saccharin. We determined the effects of pretreatment with the OxR1 receptor antagonist SB 334867 (SB; 10–30 mg/kg) on fixed ratio (FR) saccharin self-administration, and on cue-induced reinstatement of extinguished saccharin-seeking.

Results—SB decreased responding and number of reinforcers earned during FR responding for saccharin, and decreased cue-induced reinstatement of extinguished saccharin-seeking. All of these effects were obtained similarly in food-restricted and ad libitum-fed rats.

Conclusions—These results indicate that signaling at the OxR1 receptor is involved in saccharin reinforcement, and reinstatement of saccharin-seeking elicited by saccharin-paired cues regardless of food restriction. These findings lead us to conclude that the Orx system contributes to the motivational effects of hedonic food rewards, independently of caloric value and homeostatic needs.

Keywords

orexin; hypocretin; SB 334867; addiction; obesity; reward-based feeding; saccharin; palatable food; conditioned stimuli

Introduction

Recent studies implicate Orx in food-reinforced behaviors including operant responding for high-fat and sweet food (Borgland et al. 2009; Cason et al. 2010; Cason and Aston-Jones 2012; Choi et al. 2010; Thorpe et al. 2005). Operant responding for food measures consummatory behavior associated with the reinforcement value of the food reward. In addition to its effects on consummatory behavior, our recent findings indicate that OxR1

signaling is involved in appetitive behavior, specifically cue-induced reinstatement to extinguished sucrose-seeking (Cason et al. 2010; Cason and Aston-Jones 2012). Cue-induced reinstatement of extinguished food-seeking measures conditioned behavior driven by cues previously associated with food rewards. This paradigm allows measurement of the motivational value of such cues to drive food seeking in the absence of actual food consumption. Importantly, increased consummatory or appetitive drives for food lead to excessive food intake and desire to consume food even in the absence of caloric need. Notably, the effects of Orx signaling on motivated behavior for food seem to be preferentially engaged for palatable foods with high reinforcing value (Borgland et al. 2009). However, a decrease in variable ratio and progressive ratio responding for regular chow food pellets following OxR1 antagonism or RNAi knockdown of Orx has been reported in mice (Sharf et al. 2010).

The ability to detect and procure food is an important survival mechanism, and cues associated with food are essential in this process. These cues interact with the brain's reward system to stimulate motivated behavior to seek and obtain food (Castellanos et al. 2009; Nijs et al. 2010). Such cues activate similar brain nuclei as do conditioned cues that drive seeking for drugs of abuse (Avena et al. 2008; Huang et al. 2005; Kelley et al. 2000; Rada et al. 2005; Spangler et al. 2004). Recent findings link the orexin (Orx) system with preference for stimuli associated with food or drug rewards, as well as with cue-driven reward-seeking behaviors (Borgland et al. 2009; Cason et al. 2010; Cason and Aston-Jones 2012; Choi et al. 2010; Smith et al. 2009; Smith and Aston-Jones 2012). Additionally, Orx neurons send dense projections to several nuclei within the mesolimbic dopamine and opioid systems that are activated by cues associated with food rewards ((Peyron et al. 1998; Sutcliffe and de Lecea 2002; Avena et al. 2008; Huang et al. 2005; Kelley et al. 2000; Rada et al. 2005; Spangler et al. 2004).

Furthermore, previous evidence indicates that the Orx system may regulate food-seeking behavior associated with food restriction. Food restriction produces changes in hormones that regulate adiposity signals and energy expenditure, and the action of these hormones is mediated at least in part through the Orx system (Lawrence et al. 2003; Lopez et al. 2000). Orx signaling mediates restriction-induced increases in operant responding (Vialou et al. 2011), and Orx is elevated in calorically restricted rats re-fed high-fat diet instead of regular chow (Pankevich et al. 2010). These studies highlight the potential of Orx signaling to modulate food-seeking behavior in the context of caloric restriction.

In support of previous findings, we recently demonstrated that signaling at the OxR1 is involved in sucrose reinforcement and in reinstatement of sucrose-seeking elicited by sucrose-paired cues, especially in food restricted animals (Cason and Aston-Jones 2012). Sucrose reinforcement has both hedonic and caloric components. Each component independently or synergistically can influence the reinforcing value of food, and both are increased during periods of food restriction (Levine et al. 2003a; Levine et al. 2003b). To determine if OxR1 signaling regulates the reinforcing effects of hedonic food rewards independently of their caloric value, the present study investigated the role of the OxR1 in operant responding for saccharin (an artificial sweetener whose reinforcing property is due strictly to its hedonic sweet taste as it lacks calories) and in cue-induced reinstatement of extinguished saccharin-seeking. We tested ad libitum-fed and food-restricted rats to compare to our previous findings using sucrose, and to determine if the ability of Orx signaling to modulate food-seeking behavior in the context of caloric restriction involves hedonic or caloric rewards. We hypothesized that OxR1 signaling regulates the motivation to obtain both caloric and non-caloric, palatable reinforcers.

Methods and Materials

Subjects

Male Sprague Dawley rats (Charles River, Wilmington, MA, USA) were singly housed under a reversed 12h/12h light/dark cycle (lights off 0600 h). Rats were divided into two groups: ad libitum fed ($n = 19$) and food-restricted ($n = 21$). Ad libitum rats had non-restricted access to food and water; food-restricted rats had free access to water and were food-restricted to 85% of the ad libitum rats' body weight. Food-restricted rats were given their daily food ration at 1500 h. Rats were housed in the animal facility at the Medical University of South Carolina (AAALAC-accredited). All experiments were approved by the Institutional Animal Care and Use Committee and conducted in accordance to the National Institutes of Health specifications outlined in their Guide for the Care and Use of Laboratory Animals.

Experiment 1: Fixed ratio responding for saccharin

Self-administration sessions were conducted as described previously (Cason and Aston-Jones, 2012). Briefly, rats were trained to lever press for saccharin reward (45 mg, 1% saccharin pellets, Bio-Serv, Frenchtown, NJ USA) on a fixed ratio 1 (FR1) schedule of reinforcement during daily 1 h sessions. Presses on the inactive lever had no programmed consequences. Pellet delivery was accompanied by a discrete tone + light cue. Rats were given 10 self-administration sessions in which they earned 10 saccharin pellets in each session. Once stable responding was established, rats were given injections (vehicle or SB) 30 min prior to a self-administration session. Each rat received two injections (ip) of SB on different sessions (10 or 30 mg/kg, 10 or 20 mg/kg, or 20 or 30 mg/kg). The order of injections was counterbalanced such that some rats received the higher dose of SB first while others received the lower dose first. Two or more days separated SB injections to allow responding to return to baseline.

Experiment 2: Cue-induced reinstatement of extinguished saccharin-seeking

Following self-administration sessions, rats from Experiment 1 underwent daily extinction sessions during which saccharin reward and cues were withheld as described previously (Cason and Aston-Jones, 2012). Then, rats ($n=14-15$ /group) were tested for cue-induced reinstatement of saccharin-seeking by delivering the tone + light cues (previously associated with saccharin administration) for active lever presses; no saccharin reward was delivered.

To test the effects of the OxR1 antagonist SB on cue-induced reinstatement of saccharin-seeking, rats were given four test sessions in a within-subjects design: two late extinction sessions with no cues (vehicle or 30 mg/kg SB pretreatment) and two cue-induced reinstatement sessions (vehicle or 30 mg/kg SB pretreatment). The order of sessions was counterbalanced so that the test sessions were presented in different orders to different rats.

Drugs

SB 334867 [1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl urea hydrochloride] (SB) was generously donated by the National Institute of Drug Abuse, (Research Triangle Park, NC, USA), and suspended in 2% dimethylsulfoxide and 10% 2-hydroxypropyl- β -cyclodextrin (Sigma) in sterile water; SB was given in a volume of 4 ml / kg (ip) 30 min prior to self-administration or cue-induced reinstatement sessions.

Data Analysis

Mixed-model, factorial and one-way ANOVAs were utilized for analyses, with test day as a repeated measure when appropriate. Post hoc analyses were computed with the Tukey-Kramer test. All data are presented as mean $\pm$ SEM.

Results

Experiment 1: Fixed ratio responding

The mean number of lever presses and saccharin pellets during self-administration are shown in Figure 1. Repeated measures ANOVA revealed no significant group (ad libitum v. food-restricted) $\times$ test day interaction nor significant main effect of group or test day for active lever presses, inactive lever presses or saccharin reinforcements.

Acquisition—The number of days to meet the acquisition criteria for self-administration was similar across groups, regardless of food restriction. Ad libitum fed rats acquired saccharin self-administration within 12.84 ± 0.61 days and food-restricted rats acquired saccharin self-administration within 12.24 ± 0.71 days.

Maintenance—Ad libitum fed and food-restricted rats exhibited similar numbers of active presses for saccharin (100.63 ± 5.07 ; 115.40 ± 4.14 , respectively) and earned similar numbers of saccharin reinforcers (56.63 ± 2.73 ; 63.53 ± 1.89 respectively) during the last 10 days of self-administration.

Effect of OxR1 antagonist on maintenance of saccharin self-administration—

Figure 2 shows the effects of pretreatment with SB on established saccharin self-administration. Separate one-way ANOVAs were used in ad libitum fed and food-restricted rats to evaluate dose effects on numbers of presses and saccharin reinforcers earned. Pretreatment with 20 or 30 mg/kg SB significantly decreased the number of active presses (Fig 2a) during self-administration in ad libitum fed [$F(3,49) = 3.71$, $p < 0.001$] and food-restricted rats [$F(3,52) = 6.45$, $p < 0.001$]. The same doses decreased the number of saccharin reinforcers (Fig 2b) obtained compared to vehicle pretreatment in ad libitum fed [$F(3,49) = 7.24$, $p < 0.001$] and food-restricted rats [$F(3,52) = 7.76$, $p < 0.001$]. Responding on the inactive lever was minimal regardless of group, and there was not a significant effect of dose for inactive presses in ad libitum rats; however, there was a significant effect in food-restricted rats [$F(3,52) = 3.26$, $p < 0.05$ (Fig 2c)]. Post hoc analysis showed that food-restricted rats showed a small but significant increase in inactive presses following pretreatment with SB 10 mg/kg (due apparently to unusually high responding in one animal in the SB test session).

Experiment 2: Cue-induced reinstatement of extinguished saccharin-seeking

Both ad libitum fed and food-restricted rats extinguished within five extinction sessions. Figure 3 shows the mean number of active lever presses during late extinction sessions vs. cue-induced reinstatement of saccharin-seeking following pretreatment with SB 30 mg/kg or vehicle. During extinction, there was not a significant group (ad libitum vs food-restricted) $\times$ treatment (vehicle vs SB) interaction nor significant main effect of group or treatment. During reinstatement tests, there was not a significant group (ad libitum vs restricted) $\times$ treatment (vehicle vs SB) interaction nor a significant main effect of group. There was a significant main effect of treatment on active presses [$F(1,50) = 12.22$, $p < 0.001$]. Post hoc analysis of the main effect of treatment showed that rats given SB 30 mg/kg, regardless of food-restriction, had fewer active presses during cue-induced reinstatement than rats pretreated with vehicle ($p < 0.05$). There was not a significant group $\times$ treatment interaction or main effect of group or treatment on inactive presses.

Discussion

Our data show that blocking OxR1s significantly attenuates operant self-administration of saccharin in both calorically restricted and non-restricted rats. Similarly, pretreatment with an OxR1 antagonist decreases cue-induced reinstatement of extinguished saccharin-seeking in rats independent of food-restriction. These findings indicate that signaling at the OxR1 modulates responding for food rewards based at least in part on hedonic aspects of taste. We conclude that OxR1 signaling modulates motivated behavioral responses to non-caloric palatable foods and food cues associated with these rewards in addition to its roles in regulating caloric rewards. Collectively, these findings are consistent with the view that the Orx system is engaged in the motivation induced by highly salient rewards, including palatable caloric and non-caloric food rewards.

SB effects on responding for food or saccharin

In the present study, SB attenuated responding for saccharin in food-restricted and ad libitum fed rats. These results are consistent with previous reports that show SB attenuates FR responding in food-restricted rats for palatable food rewards including sucrose pellets (Cason et al. 2010; Cason and Aston-Jones 2012) and high fat food pellets (Nair et al. 2008). These findings are also consistent with other studies that show SB reduces PR responding in food-restricted rats for chocolate and sweets (Borgland et al. 2009; Choi et al. 2010), whereas it has no effect on PR responding for regular chow (Borgland et al. 2009).

Our findings are also supported by findings from Furudono et al (2006) that demonstrated Orx-A and other orexigenic peptides are involved in saccharin drinking (Furudono et al. 2006). In that study, intracerebroventricular administration of Orx-A increased ingestion of both water and saccharin solutions. Orx-A mRNA levels in the hypothalamus were increased following ingestion of the palatable saccharin solution, but not following water, indicating that Orx-A is particularly associated with ingestion of palatable food rewards (Furudono et al. 2006). Together with our results, these findings indicate that hedonic properties of non-caloric food rewards engage the Orx system to promote food-seeking and food-reinforced behaviors.

Notably, the present results contrast somewhat with our prior findings with sucrose (Cason and Aston-Jones 2012; see Table 1 for comparison). SB decreased active lever responding and the number of saccharin reinforcers earned in ad libitum and food-restricted rats equally (present results), but only decreased these measures for sucrose in food-restricted rats (Cason and Aston-Jones 2012). Additionally, results from recent studies demonstrated that the dual OxR1+OxR2 receptor antagonist, almorexant, attenuated self-administration of a sucrose solution in ad libitum fed rats (Srinivasan et al. 2012) whereas SB did not (Richards et al. 2008). Thus, the Orx system is differentially engaged by caloric vs non-caloric hedonic food rewards depending on the deprivation state during food consumption, and the OxR1 and OxR2 potentially play distinct roles in mediating these effects.

Other orexigenic peptide systems may also be involved in these behaviors. Interestingly, antagonism of the melanin-concentrating hormone (MCH) one receptor decreases operant responding for sucrose solution, but not saccharin solution, indicating that the MCH system in contrast to the orexin system may play a stronger role in homeostatic than non-homeostatic feeding (Karlsson et al. 2012). Central administration of the anorexigenic peptides insulin and leptin also decrease operant responding for palatable foods including sucrose (Figlewicz et al., 2006; Figlewicz and Benoit, 2009; Figlewicz et al., 2011). Interestingly, intraventricular injections of insulin attenuate sucrose self-administration but at doses (insulin 5 mU, or leptin 0.2 µg) that are sub-threshold for regulating homeostatic feeding behaviors [Figlewicz et al., 2006]. However, to our knowledge similar studies

examining effects of anorexigenic peptides on saccharin self-administration have not been conducted. In the case of sucrose self-administration, it is hypothesized that leptin and insulin act on midbrain dopaminergic regions, specifically within VTA and substantia nigra, to modulate reward driven feeding (Figlewicz and Benoit, 2009). It seems possible that central administration of these anorexigenic peptides may also attenuate saccharin self-administration in a similar manner to sucrose given that the taste of saccharin or sucrose alone produces an increase in plasma insulin levels (Just et al., 2008; Tonosaki et al., 2007; Ionescu et al., 1988).

Cue-induced reinstatement of extinguished food-seeking

The present study is the first to investigate a role of Orx in cue-induced reinstatement of extinguished saccharin-seeking. Our data demonstrate that signaling at the Ox1R regulates cued reinstatement of saccharin-seeking independent of caloric restriction, and furthermore that signaling at the OxR1 is involved in the motivation to seek palatable foods that lack caloric content. These findings are consistent with recent studies showing that SB decreases cue-induced reinstatement of extinguished sucrose-seeking (in food-restricted rats; see below and Cason et al. 2010; Cason and Aston-Jones 2012) as well as reinstatement of extinguished drug-seeking elicited by cues or contexts (Dayas et al. 2008; James et al. 2011; Lawrence et al. 2006; Smith et al. 2009; Smith et al. 2010; Smith and Aston-Jones 2012; Wang et al. 2009). Together these findings indicate that OxR1 signaling modulates appetitive behaviors such as cue-driven motivated behavior to seek food or drugs of abuse. In contrast, SB did not affect pellet-primed reinstatement of high-fat food-seeking (Nair et al. 2008), indicating that orexin's influence on reinstatement may be sensitive to the specific type of food reward or reinstatement modality. Collectively, these data support a role for Orx signaling in the anticipation of palatable food rewards.

Using our paradigm, rats showed similar levels of cue-induced reinstatement of extinguished seeking for saccharin and sucrose (active presses for saccharin: 34.36 ± 5.29 , ad libitum and 32.77 ± 6.37 , food restricted; for sucrose: 32.44 ± 5.30 , ad libitum and 33.43 ± 4.69 , food-restricted; sucrose data taken from Cason and Aston-Jones 2012). The effect of SB on reinstatement responding for saccharin was substantial: SB decreased the number of active presses approximately by half compared to vehicle pretreatment (Figure 3). A similar reduction in responding for sucrose was seen previously in food-restricted rats (Cason and Aston-Jones 2012). Taken together, our findings indicate that signaling at the OxR1 is strongly involved in appetitive behaviors such as cue-induced reinstatement of motivation for palatable food rewards including non-caloric food rewards. Furthermore, this increase in appetitive drive for non-caloric reinforcers is not affected by caloric restriction, suggesting that metabolic needs are not responsible for the increase in appetitive drive for cue-driven saccharin.

Notably, the present results also differ from our previous finding that SB was only effective in food-restricted rats at attenuating cued reinstatement of sucrose-seeking (Cason and Aston-Jones 2012). In the present study, SB was equally effective at attenuating cued reinstatement of extinguished saccharin-seeking in both ad libitum fed and food-restricted animals. Thus, as observed during self-administration of these sweet rewards, the Orx system is differentially engaged by cues associated with caloric vs non-caloric hedonic foods depending on the deprivation state.

It seems unlikely that the effects of SB in the present studies were due to a generalized locomotor effect. Although SB (10 mg/kg) produced a slight reduction in inactive lever responding, higher doses of SB (20–30 mg/kg) had no effect on inactive lever presses. Furthermore, our previous studies using sucrose reinforcement (Cason and Aston-Jones 2012) show that SB (10–30 mg/kg) had no effect on responding or cue-induced

reinstatement in ad libitum fed rats on either active or inactive levers. Similarly, other studies have shown that SB has no significant effect on established responding for cocaine rewards (Borgland et al. 2009; Smith et al. 2009). These findings indicate that SB does not substantially impair lever-pressing behavior.

Orexin and reward circuitry

It is unknown where Orx acts to influence saccharin- or sucrose-motivated behaviors (Cason et al. 2010; Cason and Aston-Jones 2012), but we speculate that the midbrain dopaminergic, basal forebrain cholinergic, and opioid systems seem plausible. Several studies have implicated Orx connections to VTA in reward-driven behaviors. For example, OxR1 activation in VTA is important for cue-induced reinstatement of cocaine-seeking (Aston-Jones et al. 2009; Harris et al. 2005; Mahler et al. 2012), and microinjection of orexin into VTA reinstates an extinguished morphine preference (Harris et al. 2005). Almorexant's ability to attenuate sucrose reinforcement in ad libitum fed rats, however, does not require Orx signaling in the VTA (Srinivasan et al. 2012); this may indicate a role for OxR2s elsewhere in sucrose reinforcement. Previous studies have also shown that non-VTA systems may be involved in orexin actions during feeding. Presentation of food or associated stimuli elicits cortical acetylcholine release (Fadel et al. 1996; Inglis et al. 2004; Moore et al. 1993) that requires signaling via the OxR1 (Frederick-Duus et al. 2007), and nucleus accumbens projections to hypothalamus stimulate Orx neurons that, in turn, increase ingestion of palatable food (Stratford and Kelley 1997; Stratford and Kelley 1999; Zhang and Kelley 2000; Zheng et al. 2007). More recently, OxR1 signaling in the paraventricular nucleus of the thalamus (PVT) has been found to increase reward-driven feeding as well as dopamine levels in nucleus accumbens (Choi et al. 2012). Additionally, given that we used systemic administration we cannot rule out peripheral effects of OxR1 signaling in our effects.

Sweets and related cues have been proposed to activate brain 'liking' and 'wanting' systems (Berridge and Robinson 1998; Berridge et al. 2010). 'Liking' mechanisms include hedonic circuits with hotspots in the NAc and ventral pallidum. Microinjection of orexin-A into the opioid hedonic hotspot in the ventral pallidum increased positive taste reactivity to sucrose in rats (Ho and Berridge 2009). Additionally, studies from our laboratory indicate that VTA-projecting neurons from the medial NAc shell and ventral pallidum, in the area of an hedonic hotspot, are involved in cue-induced reinstatement of extinguished cocaine-seeking (Mahler and Aston-Jones 2012). 'Wanting' mechanisms, on the other hand, include a much larger opioid network as well as mesolimbic dopamine systems and corticolimbic glutamate signals that interact with both systems. 'Wanting' can motivate increases in consumption even if hedonic 'liking' is not enhanced and occurs when the food cue is encountered in a mesolimbically primed state that can be influenced by hunger (Zhang et al. 2009). Furthermore, other studies demonstrate that sites within both 'liking' and 'wanting' systems are involved in cue- and context-induced reinstatement of food-seeking, but have not specifically investigated the role of Orx inputs to these areas (Floresco et al. 2008; Hamlin et al. 2006; Marchant et al. 2009; McLaughlin and Floresco 2007; Petrovich et al. 2002). Future studies involving central manipulation of OxR1s are needed to determine potential sites of action where Orx may act to influence saccharin-motivated behavior; however, the above findings suggest that targets in the midbrain dopaminergic, basal forebrain cholinergic or opioid system may be involved.

Differential involvement of Orx system in caloric vs non-caloric food reward: Effect of food restriction

The finding that SB decreased responding for saccharin reinforcement, and cue-induced reinstatement of saccharin seeking, in ad libitum fed rats is particularly interesting given that

SB does not attenuate sucrose-taking or -seeking in ad libitum fed rats (Cason and Aston-Jones 2012). Together, these findings indicate that the Orx system is differentially engaged by caloric vs non-caloric hedonic food rewards depending on the deprivation state during food consumption or food-seeking, and that the Orx system is responsive not only to states of negative energy balance but also to non-caloric, hedonic properties of food rewards. These findings show that the Orx system can be engaged by palatability of hedonic food rewards alone, independent of caloric value, and that conditioned responses of these cells may specifically activate brain reward circuits independent of metabolic circuits that regulate food intake. Interestingly, during food restriction the elevated motivation for caloric rewards becomes Orx-dependent. The mechanisms by which the Orx system is involved in consumption or seeking of caloric foods only during food restriction are unclear, but may involve the fact that Orx neurons are responsive to metabolically linked factors such as ghrelin, agouti related peptide and leptin, that also influence reward-driven behaviors (Davis et al. 2011; Perello et al. 2010, Shan and Yeo 2011; Dietrich et al., 2012). For example, disruption of ghrelin receptor signaling decreases operant responding for food reward and hedonic feeding, and this effect is correlated with decreased OxR1 expression in reward related areas (Davis et al., 2012). Further studies are needed to better understand the interaction between food restriction, caloric food consumption or seeking, and the orexin system. Furthermore, all of the neuroendocrine signals above act within lateral hypothalamic circuitry to modulate caloric intake and reward and could modulate orexin tone in the lateral hypothalamus differentially between caloric and non-caloric rewards and during periods of food restriction.

Relevance to dieting

Our findings may be relevant to the observation that obese patients display enhanced neuronal activation compared to lean controls in response to images of palatable foods (Rothmund et al. 2007; Stice et al. 2008). Similar to our findings with saccharin-seeking, this occurs in both fed and fasted conditions indicating that this enhanced activation of the reward circuitry in response to palatable foods is independent of caloric needs (Rothmund et al. 2007; Stice et al. 2008). Notably, increased palatability of food promotes overconsumption of food and diet-induced obesity (Lucas and Sclafani 1990; Sclafani 1987; Sclafani and Xenakis 1984). Thus, it is tempting to speculate that the difficulty humans have in dieting involves persistent conditioned responses of Orx neurons associated with hedonic properties of food that are relatively independent of caloric properties. Treatments that decrease such Orx signaling may provide novel therapies for psychological aspects of food cravings.

Acknowledgments

This research was supported by PHS grants DA 023354, DA 017289 and DA06214.

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Figure 1.

Operant responding for saccharin in ad libitum fed and food-restricted rats. There was no difference in the number of active presses ($p > 0.05$), inactive presses ($p > 0.05$) or saccharin pellets ($p > 0.05$) between ad libitum ($n=19$) and food-restricted ($n=21$) rats.

Figure 2. Attenuation of fixed ratio responding for saccharin by the OxR1 antagonist SB-334867 (SB). Rats were pretreated with SB or vehicle 30 min prior to the self-administration session. SB (20 or 30 mg/kg) reduced active lever presses and the number of saccharin pellets obtained in ad libitum ($p < 0.05$; $n = 10-13$, per dose) or food-restricted rats ($p < 0.05$; $n = 10-13$, per dose). SB had no significant effect on inactive lever presses in ad libitum fed rats; however, SB (10 mg/kg) produced a slight increase in inactive presses in food-restricted rats * $p < 0.05$ versus vehicle injection.

Figure 3.

Effects of the OxR1 antagonist, SB-334867 (SB), on lever pressing during extinction and cue-induced reinstatement of saccharin-seeking. Rats were pretreated with SB (30 mg/kg) or vehicle 30 min prior to (A) late extinction sessions (no cues or reward) or (B) cue-induced reinstatement of responding (tone + light cues only, no saccharin). Pretreatment with SB had no effect on active presses for saccharin during extinction (left panel), whereas SB significantly attenuated reinstatement of active lever pressing for saccharin in both ad libitum and food-restricted rats. * indicates $p < 0.01$ versus vehicle + cue.

Table 1

Comparison of operant responding for saccharin and sucrose reward and the effects of SB on operant responding in ad libitum and food-restricted rats. Data for sucrose experiments taken from (Cason and Aston-Jones 2012).

Self-Administration			
	Saccharin		Sucrose
	Ad Libitum v. Food-restricted		Ad Libitum v. Food-restricted
Active Presses	=		Food-restricted ↑
Pellets	=		Food-restricted ↑
Inactive Presses	=		=

SB Effects on Self-Administration				
	Dose of SB	Ad Libitum		Food-restricted
		Saccharin	Sucrose	Saccharin
Active Presses	10	=	=	=
	20	↓	=	↓
	30	↓	=	↓
Pellets	10	=	=	=
	20	↓	=	↓
	30	↓	=	↓
Inactive Presses	10	=	=	↑
	20	=	=	=
	30	=	=	=

SB Effects on Extinction and Cue-induced Reinstatement				
	Ad Libitum		Food-restricted	
	Saccharin	Sucrose	Saccharin	Sucrose
Active Presses	=	=	=	=
Extinction	↓	=	↓	↓
Reinstatement				

Symbols: “=” indicates no difference, “↓” indicates a decrease in behavior, “↑” indicates an increase in behavior.