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# Ghrelin enhances cue-induced bar pressing for high fat food

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ABSTRACT

Ghrelin is an orexigenic hormone produced by the stomach that acts on growth hormone secretagogue receptors (GHSRs) both peripherally and centrally. The presence of GHSRs in the ventral tegmental area (VTA) suggests that ghrelin signaling at this level may increase the incentive value of palatable foods as well as other natural and artificial rewards. The present investigation sought to determine if ghrelin plays a role in relapse to such foods following a period of abstinence. To achieve this, thirty-six male Long Evans rats were trained to press a lever to obtain a high fat chocolate food reward on a fixed ratio schedule of 1. Following an extinction period during which lever presses were not reinforced, rats were implanted with a cannula connected to a minipump that continuously delivered ghrelin, a GHSR antagonist ([D-Lys-3]-GHRP-6), or saline in the VTA for 14 days. One week later, food reward-associated cues, food reward priming, and an overnight fast were used to induce reinstatement of the lever pressing response. Our results indicate that intra-VTA ghrelin enhances cue-induced reinstatement of responses for palatable food pellets. To the extent that the reinstatement paradigm is considered a valid model of relapse in humans, this suggests that ghrelin signaling facilitates relapse to preferred foods in response to food cues through GHSR signaling in the VTA.



## Introduction

Relapse to preferred foods and unhealthy eating habits is a primary concern for people undergoing weight reduction diets (Elfhag and Rossner, 2005; Kramer et al., 1989). This phenomenon is similar to drug relapse in that it can be triggered by the same environmental stimuli: re-exposure to the substance and/or cues associated with it, and exposure to stressful situations (Elfhag and Rossner, 2005; Grilo et al., 1989; Kayman et al., 1990; Gorin et al., 2004). Interestingly, some have demonstrated that cues associated with reinforcing stimuli can elicit feeding responses in satiated animals that similar to those seen when these test animals are hungry (Weingarten, 1983). Experimentally, relapse to food seeking is most frequently studied using an operant conditioning model that is also used in drug addiction research. The paradigm is characterized by 3 successive phases: self-administration training, extinction, and reinstatement (Epstein et al., 2006; Nair et al., 2009a). Over the last decade, the use of this model has facilitated the identification of a number of peptides and neurotransmitters that act centrally to modulate the reinstatement of food seeking, many of which also impact reinstatement of drug seeking (reviewed in Nair et al., 2009a). These include, but are not limited to, orexigenic peptides such as orexin and melanin-concentrating hormone (Cippitelli et al., 2010; Richards et al., 2008; James et al., 2011; Nair et al., 2008, Nair et al., 2009b; Boutrel et al., 2005; Wang et al., 2009). In this context, the role of ghrelin, the only circulating orexigenic hormone currently known, is relatively under-studied.

Ghrelin is a 28 amino acid-long peptide that is produced predominantly in the oxyntic glands of the stomach in times of negative energy balance (reviewed in Castaneda et al., 2010). It is passively transported across the blood brain barrier and acts on growth hormone secretagogue receptors (GHSRs) both inside and outside of the central nervous system (Banks et al., 2002; Diano et al., 2006; Ghigo et al., 2005). Peripheral, intra-cranial and hypothalamic ghrelin administration produces a robust dose-dependent feeding response that is not seen in animals lacking a functional GHSR gene (Lawrence et al., 2002; Wren et al., 2001; Tschop et al., 2000). Ghrelin administration also results in a number of outcomes that are typically observed in the presence of both natural (ex: food) and artificial (ex: drug) rewards, such as increased dopamine (DA) levels in the nucleus accumbens (NAc), increased locomotion and development of a conditioned place preference, suggesting a general role of ghrelin signaling in reward processing (Abizaid et al., 2006; Abizaid, 2009; Jerlhag et al., 2006a/b; Jerlhag, 2008). Likewise, disruption of ghrelin or ghrelin signaling via genetic or pharmacological manipulations can alter the behavioral and physiological response to reinforcers, including operant responding for palatable foods and drugs of abuse (Jerlhag and Engel, 2011; Landgren et al., 2011a; Skibicka et al., 2011a; Skibicka et al., 2011b; King et al., 2011; Landgren et al., 2011b; Clifford et al., 2011; Wellman et al., 2011; Perello et al., 2010; Jerlhag et al., 2010; Jerlhag et al., 2009; Egecioglu et al., 2010; Abizaid et al., 2011). Ghrelin's reward-related effects likely involve the dopaminergic mesolimbic system. Over 60% of DA cells within the ventral tegmental area (VTA) express the GHSR, and ghrelin binding in this area causes

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synaptic re-organization of local DA cells, ultimately increasing the ratio of excitatory (glutaminergic) to inhibitory (gabaergic) afferents to these cells (Abizaid et al., 2006). The resulting reduction in DA neurons' firing threshold and increased accumbal DA turnover might underlie ghrelin's orexigenic effects at this level (Abizaid et al., 2006). Ghrelin's action in the mesolimbic system may be specifically related to adding incentive value to food stimuli (Skibicka et al., 2011a; Egecioglu et al., 2010).

A role for ghrelin in the reinstatement of food seeking is suggested by the fact that ghrelin activates the HPA stress response system, and that stress is intimately linked with food seeking relapse in dieting individuals and rats tested in the reinstatement model (Ghitza et al., 2006; Asakawa et al., 2001; Greeno and Wing, 1994; Grilo et al., 1989; Tassone et al., 2003). Indeed, mice with targeted deletion of the GHSR gene or mice treated with a ghrelin receptor antagonists show lower reinstatement of operant responses to cues previously associated with food (Walker et al., 2012). In addition, caloric restriction, which increases plasma ghrelin levels, facilitates reinstatement of both food and drug seeking (Nair et al., 2009a). Elevated ghrelin levels are also associated with alcohol cravings in people who try to abstain from drinking, and such cravings often precede relapse episodes (Koopmann et al., 2012; Koob and Volkow, 2010). Finally, endogenous ghrelin levels are positively correlated to the reinstatement of cocaine seeking in rats (Tessari et al., 2007).

In the current study, we sought to determine if ghrelin administration within the VTA could restore previously extinguished bar pressing for pellets, a rodent model of reinstatement or relapse in rats. This was examined in response to stimuli that have been associated with the reinstatement of reward seeking behaviors in the past. Specifically, we evaluated the reinstatement of these responses after exposure to a cue previously associated with the reward, the reward itself (i.e. re-exposure to the high fat pellet), or an overnight fast. We chose chocolate-flavored high-fat food pellets as the reward because preferred foods are often sweet and rich in fat and ghrelin preferentially increases intake of sweet and high-fat foods (Shimbara et al., 2004, Disse et al., 2010). The choice of the administration route was based on the presence of GHSRs within this node of the reward system and evidence suggesting the VTA plays a role in the reinstatement of both cocaine and heroin seeking (Abizaid et al., 2006; Guan et al., 1997; Zigman et al., 2006; Wang et al., 2009, Wang et al., 2007; Bossert et al., 2004; Stewart, 1984). In order to limit the effects of endogenous ghrelin levels, which naturally rise in times of negative energy balance at GHSRs located outside of the mesolimbic system, reinstatement was tested in animals that were sated (i.e. not chronically food restricted) and therefore not in negative energy state.

## Methods

### *Subjects & apparatus*

Male Long Evans rats (216–375 g, average = 283 g, n = 39) were obtained from Charles River and singled-housed in a room under a reverse dark/light cycle (lights off at 8:00 AM). After a 1-week acclimation period, baseline food intake was measured daily for 5 days. The rats were subsequently food restricted as to maintain 85% of their baseline body weight. All testing was performed during the dark phase, in operant chambers (Coulbourn Instruments®) containing a grid floor, house light, pellet delivery magazine, and two levers 2.5 cm off the floor — one selected as "active" and the other as "inactive". Active and inactive lever presses were recorded by GraphicState software.

### *Procedure*

#### *Autoshaping, training & extinction*

Testing began 5 days after the start of food restriction. All animals were subjected to a single 3-hour long autoshaping session during which high-fat chocolate pellets (Bioserv, F05879 — 45 mg, 35% fat) were delivered every 5 min. Each pellet delivery was accompanied by a 2 s light/tone stimulus. Starting on the next day, rats were trained for 3 h every second day, for 24 days (12 training sessions). During training, pressing on the active lever resulted in the delivery of a pellet and exposure to the food cue (light/tone stimulus). Each pellet delivery was followed by a 20 s timeout period, during which pressing on the lever had no consequence. Similarly, pressing on the inactive lever at any point had no programmed consequence. Training sessions were conducted during the morning (9:00 AM–12:00 PM) or afternoon (1:00 PM–4:00 PM), which alternated for each rat. At the start of the first 5 sessions, 3 pellets were placed on the active lever in order to facilitate acquisition. On training days, rats were fed their maintenance food ration (75% of their baseline food intake or about 18–20 g of food) 1 h after the conditioning session. On "off" days, rats were fed 1 h after the start of the dark phase.

One day after the completion of the training phase, rats were exposed to a series of 3-hour extinction sessions, during which lever pressing (active or inactive) had no programmed consequence. Rats received daily extinction sessions until they reached the extinction criterion, defined as pressing on the active lever less than 30 times over 3 h on 3 consecutive days. Rats were then returned to an ad lib feeding schedule until the day of surgery.

#### *Surgery and recovery*

Stereotaxic surgery was performed 1 to 6 days after the end of the extinction period. Isoflurane and oxygen were used to anesthetize rats for surgery. A cannula (Plastics One, 3300P-SPC), connected to a minipump (Alzet, model 2002) by a 4 cm long vinyl tube (Plastics One, C312-VT) was then implanted into the VTA. Coordinates for implantation were 5.3 mm posterior to bregma, 2 mm lateral to the midline, and 7.6 mm below the skull surface (Paxinos and Watson, 1986). The cannula was anchored into position by dental acrylic placed around 3 stainless steel screws embedded within the skull. The minipumps, implanted subcutaneously between the shoulder blades, delivered saline, ghrelin (10 nmol; Pi Protemics, PI-G-01), or a ghrelin receptor antagonist, [D-Lys-3]-GHRP-6 (200 nmol; Peptides International, OGH-3656-PI) into the VTA at a rate of 0.5 μL/h for 14 days. Following surgery, rats received a subcutaneous injection of meloxicam (Metacam®, 0.1 mL) to minimize pain and discomfort and were placed in a recovery cage until normal behavioral activity was observed.

Recovering rats were fed mashed food (chow and water mixture) for 1 day following the surgery, and then returned to an ad lib feeding schedule for a period of 4 days, during which food intake was recorded daily.

#### *Reinstatement*

Rats were tested for reinstatement of food seeking after a short (two days) and very mild (about 85% of their regular intake) food restriction conditions that were not sufficient to make them lose weight. In fact, rats were about 50 g heavier in average than they were during the training and extinction period of the experiment. Food seeking (i.e. active lever pressing) was reinstated by 1) pre-exposure to the food pellets (food priming), 2) contingent presentations of the light-tone food cue upon lever pressing, or 3) an overnight fast. Rats also received an additional session during which no reinstatement stimulus was used. All rats were exposed to the 4 conditions in a counterbalanced order. In the food priming condition, 5 pellets were delivered in a non-contingent fashion and I second apart and after each rat was placed in the box at the start of the session. In the food cue condition, rats were presented with the tone/light cue 3 times at the start of the session, and upon all active lever presses during the session. In the overnight fast condition, food was removed from the home cage approximately 18 h before the session. Forty-eight hours separated each reinstatement session. With the exception of the tone/light stimulus presentations in the food cue condition, the reinstatement sessions themselves were identical to extinction sessions. Locomotion during these sessions was recorded using an