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Disruption of cue-potentiated feeding in mice with blocked ghrelin signaling

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HIGHLIGHTS

- A novel cue-potentiated feeding protocol has been adapted for use in mice.
- We examine the role of the peptide hormone ghrelin in cue-potentiated feeding.
- Ghrelin receptor antagonist blocks feeding potentiated by a positive conditioned stimulus.
- Ghrelin receptor-deficient mice eat in response to both negative and positive conditioned cues.
- A role for ghrelin in establishing a specific positive cue–food association has been established.

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ABSTRACT

The peptide hormone ghrelin regulates a variety of eating behaviors. Not only does it potently increase intake of freely-available food, but it also shifts food preference toward diets rich in fat, enhances operant responding for food rewards, and induces conditioned place preference for food rewards. Here, we postulated that ghrelin also enables cue-potentiated feeding, in which eating is enhanced upon presentation of a food-conditioned stimulus. To test this hypothesis, a novel cue-potentiated feeding protocol adapted for use in mice was designed and validated, and then the effects of pharmacologic ghrelin receptor (GHSR) antagonism and GHSR transcriptional blockade (as occurs in GHSR-null mice) were assessed. Sated C57BL/6J mice indeed demonstrated cue-potentiated intake of grain-based pellets specifically upon presentation of a positive conditioned stimulus (CS+) but not a negative conditioned stimulus (CS−). Treatment with a GHSR antagonist blocked potentiated feeding in sated C57BL/6J mice in response to the CS+. In contrast, while GHSR-null mice also lacked a potentiation of feeding specifically in response to the CS+, they displayed an enhanced intake of pellets in response to both the positive and negative conditioned stimuli. The pattern of immediate early gene expression within the basolateral amygdala – a brain region previously linked to cue-potentiated feeding – paralleled the observed behavior of these mice, suggesting uncharacteristic activation of the amygdala in response to negative conditioned stimuli in GHSR-null mice as compared to wild-type littermates. Thus, although the observed disruptions in cue-potentiated feeding are different depending upon whether GHSR activity or GHSR expression is blocked, a key role for GHSRs in establishing a specific positive cue–food association has now been established.
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1. Introduction

The rates of obesity have been steadily and dramatically increasing [1]. Understanding the pathways that regulate complex eating behaviors and ultimately disturb homeostatic control of food intake is crucial for the development of effective obesity treatments. While genetic factors undoubtedly contribute to obesity, an individual's environment and upbringing are also likely involved [2–4]. The human environment is replete with visual, auditory, and olfactory cues which, via associative learning and Pavlovian conditioning, can become intimately linked to food, resulting in the induction and maintenance of eating [5]. Prime examples include logos of commercial enterprises that sell food [6]. With continued exposure, these cues can form such a strong association with eating that they may override satiety signals that otherwise would normally lead to eating cessation [5]. Recurrent exposure to these cues potentially can lead to an overabundance of food intake resulting in an increased risk for obesity. Of note, the motivational salience of food cues as measured by visual attention is greater in obese individuals than in lean subjects, suggesting that higher sensitivity to cues associated with food may contribute to their lack of control over food intake [7].

The cue-potentiated feeding paradigm models habitual eating that occurs with strong cue associations linked to food. Several studies have found that food-sated rats increase food consumption after presentation of a conditioned stimulus previously paired with food

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during a period of caloric restriction [8,9]. These studies were performed with bland pellets similar to regular chow, signifying the strength of a conditioned cue's ability to enhance feeding behavior even without savory taste as a rewarding component. The amygdala and prefrontal cortex play a major role in this behavior as lesions of the basolateral amygdala (BLA) or medial prefrontal cortex (mPFC) in rats abolish the cue-induced potentiation of eating [9–12] and as connections from the BLA/basomedial amygdala and mPFC to the lateral hypothalamic area in rats are strongly activated by the positive conditioned stimulus [13]. While these studies using rats have determined some of the neural pathways and regional networks involved in cue-potentiated feeding, to our knowledge, this behavioral model has never been performed in mice using a non-savory food, which is an important distinction since a tasty or rewarding food adds another dimension to the learning aspect of conditioning. The use of mice in place of rats in this paradigm will facilitate studies that aim to identify the molecular mediators involved in shaping and activating these neural networks, as mice can be more easily genetically manipulated.

One potential mediator in the development of cue-potentiated feeding is the gastrointestinally-derived peptide hormone ghrelin [14]. Ghrelin potently induces intake of freely-available food upon binding to its receptor, the growth hormone secretagogue receptor (GHSR), in regions including the hypothalamus and brainstem, and it is through these pathways that endogenous ghrelin is thought to affect body weight homeostasis [15–17]. GHSR localization to the ventral tegmental area (VTA), hippocampus, and amygdala provides evidence that ghrelin also may mediate more complex eating behaviors that involve different aspects of learning, memory, and reward [18–20]. Indeed, several studies have investigated a role for ghrelin in complex eating behaviors. Ghrelin helps to define food preference — shifting consumption toward sweet diets and those high in fat, and ghrelin also enhances operant responding for sweet and fatty food rewards [21–26]. Furthermore, ghrelin enables acquisition of conditioned place preference for food rewards upon its pharmacologic administration or upon its natural elevation as induced by caloric restriction or psychosocial stress [21,27,28]. Several studies have indicated that blockade of ghrelin action, by pharmacologic blockade of or genetic deletion of GHSRs, blocks many of these same complex eating behaviors [21,22,28–30]. To our knowledge, only one study, using a Pavlovian-to-instrumental transfer protocol to study motivational incentive learning, has reported an enhancement in reward behavior upon blockade of ghrelin action [31]. The ability of ghrelin to enhance performance in tests of behavioral memory also may be relevant to the pathways required for cue-potentiated feeding [32,33]. Here, we test the hypothesis that in addition to its previously-reported effects on homeostatic eating, food preference, and reward-based eating, ghrelin also participates in the development and expression of cue-potentiated feeding as well as the regulation of BLA activity in response to conditioned cues.

2. Materials and methods

2.1. Animals

C57BL/6J mice (Charles River, Wilmington, MA) were used in Experiments 1 and 2. GHSR-null and wild-type littermates, used in Experiments 3 and 4, were generated by breeding mice heterozygous for the GHSR-null allele, obtained after more than 10 generation backcrossing onto a C57BL/6J genetic background [34]. All studies were approved by the UTSW Institutional Animal Care and Use Committee.

2.2. Primary cue-potentiated feeding paradigm

2.2.1. Conditioning

This protocol (Fig. 1A, used for Experiments 1, 3, and 4) was modeled after reported cue-potentiated protocols designed for use in rats with standard chow [9,11–13]. Two-month-old mice, housed 2–3 per cage, were placed on a restricted feeding schedule which provided access to standard chow (Teklad Global Diet #2016 Madison, WI, which provides 3.0 kcal/g of energy and contains 16.4 g % protein, 4.0 g % fat , and 48.5 g % carbohydrates) for 3 1/2 h per day. Such was maintained during a run-in period (Days 1–5) and throughout a "simple" conditioning phase (Days 6–12) and a subsequent "discrimination" conditioning phase (Days 13–26).

Conditioning sessions were performed by placing individual mice into conditioning chambers (Model ENV307A, Med Associates, Inc., St. Albans, VT) just before the 3 1/2 h period of food availability. During the first, "simple" conditioning phase (Days 6–12), daily conditioning sessions were performed by pairing a light cue, which would become the conditioned positive stimulus (CS+), with delivery of a single 14-mg grain-based Dustless Precision Pellet (BioServ, Frenchtown, NJ, which provides 3.6 kcal/g of energy and contains 18.7% protein, 5.6% fat, and 59.1% carbohydrates). The CS+ was assigned to each mouse in a counterbalanced fashion as either the main "house" light (affixed near the ceiling) of the chamber or its central "nose-poke" light (affixed to the lower wall area of the chamber). Cues lasting 2 s in duration were given at random intervals every 30–90 s. A single food pellet was dispensed immediately after each CS+ into a food hopper using a programmed automatic pellet dispenser. Thirty cues were delivered per 30 min-long simple conditioning session. During the second, "discrimination" conditioning phase (Days 13–26), daily conditioning sessions were performed using both positive light cues [conditioned positive stimuli (CS+)] and negative light cues [conditioned negative stimuli (CS−)]. A single food pellet was dispensed upon presentation of the CS+; no food pellet was dispensed upon presentation of the CS−. The CS− was assigned to be whichever light cue was not used as the CS+. Twenty CS+ and 20 CS− cues of 2 s duration each were delivered in random order and at random intervals every 30–90 s during each 40 min-long discrimination conditioning session. When not in the conditioning chambers, mice were housed in their home cages.

2.2.2. Test sessions

During the first three days following completion of the conditioning (Days 27–29), mice were kept in their home cages with ad lib-access to standard chow. For Experiment 1, acquisition of cue-potentiated feeding was assessed on Day 30 by placing mice in the conditioning chambers for three 10-min test sessions: a baseline session where no cue was presented (Session 1), a session where only the CS+ was presented (Session 2 or 3), and a session where only the CS− was presented (Session 2 or 3). Ten cues of 2 s duration were delivered at random intervals every 30–90 s during Sessions 2 and 3. The orders of the CS+ test session and CS− test session were counterbalanced between animals. During these three test sessions, mice had free access to 30 food pellets within the food hopper. Between sessions, mice were placed into their home cages briefly, while the pellets remaining were counted.

2.3. Cue-potentiated feeding with ghrelin receptor antagonist

For Experiment 2, the above protocol was modified slightly to allow more time for the mice to adapt to receiving an oral gavage of either a ghrelin receptor antagonist or its vehicle prior to each conditioning session (Fig. 2). As such, the "simple" conditioning phase was extended to two weeks rather than one (Days 6–19), while the "discrimination" conditioning remained two weeks in length (Days 20–33). Also, for this modified protocol, mice were allowed free access to 20-mg grain-based Dustless Precision Pellets (BioServ, which provides 3.35 kcal/g of energy and contains 21.3 g % protein, 2.8 g % fat, and 54 g % carbohydrates) instead of standard chow during the 3 1/2 h-long daily feeding periods provided in the home cages after each conditioning session. These grain-based pellets were provided ad lib in home cages in the days

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