Is incentive salience dynamically influenced by satiation state?

by

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Abstract

Pavlovian-conditioned cue-attributed incentive salience is directly related to both the current physiological state of an individual and the intensity of dopaminergic signaling within that individual's mesolimbic salience-attributing circuits (Zhang, Berridge, Tindell, Smith & Aldridge, 2009). The present study seeks to expand our understanding of these relationships by addressing three primary questions. First, is the incentive motivation or "wanting" of a conditioned reward cue influenced by a subject's satiation state during training? Second, if incentive salience is influenced by satiation state, can it be dynamically shifted when an individual transitions from one satiation state to another? Finally, does satiation state influence the dopamine-dependency of an individual's conditioned response to reward cues? Our results strongly indicate that hungry rats attribute more incentive salience to their prepotent Pavlovian-conditioned reward cues than sated rats. When the feeding schedules of sated and hungry rats were reversed prior to the last day of auto-shaping, rats shifted hungry→sated showed a marked decrease in cue-directed appetitive and consummatory behaviors, suggesting a corresponding decrease in cue-attributed incentive salience. Flupenthixol-mediated dopamine suppression led to increased cue biting among sated sign-trackers, but few other remarkable behavioral changes.

Keywords: hunger, motivation, satiation, flupinthixol, incentive salience

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Motivation has received widespread attention throughout the history of modern psychological research, with strong focus upon the development of addictive behaviors (Chambers, Taylor, & Potenza, 2003; Kalivas & Volkow, 2005; Robinson & Berridge, 2008). Drug-associated addictions have been a central focus of motivation research, and in recent years, neural changes known to be associated with the formation of addictions have been observed in subjects suffering from obesity, strongly indicating the possibility of food-centered addictions (Corsica & Pelchat, 2010; Liu, von Deneen, Kobeissy & Gold, 2010). Knowing that our motivations have the power to influence so many aspects of our lives, it is of great importance to move towards a more complete understanding of how motivational information is processed within the brain, and how this information is translated into behavior. The present study seeks to contribute to this understanding by investigating the effects of hunger and satiety on motivated behavioral responses to food reward cues.

Between the 1960s and 1970s, a new conception of motivation was developed and strengthened – one that suggested a division between "stimulus properties" and "organismic-state variables" (Bindra, 1974). The role of physiological states in the mediation of motivational drives has since been extensively deliberated (Houston, 1983; Toates, 1981), and continues to be an important subject in the discussion of motivation today. Recent empirical studies have formed the basis for increasingly advanced, "neurocomputational" theories of motivation, in which the "organismic-state variables" alluded to by Bindra and others have been assigned specific, computational roles in the attribution of incentive salience towards reward cues (Tindell, Smith, Berridge & Aldridge, 2009; Zhang et al., 2009).

Feeding behaviors directly control satiety, a critical organismic state, placing food-centered motivations among the most primitive and foundational motivations. From both social and medical perspectives alike, it is critically important to understand the ways in which hunger and satiety influence behavior. Obesity, a pervasive social and medical problem, has been studied extensively in the context of motivation and the "wanting" of food (Mirza et al., 2011; Rodriguez et al., 2012; Wang, Volkow & Fowler, 2002). As noted above, recent evidence suggests that many obese individuals experience "food addictions," motivational disorders mediated by the same neurochemicals and pathways currently understood to control drug addictions (Corsica & Pelchat, 2010; Liu et al., 2010).

The incentive sensitization theory of addiction proposes that neural circuits responsible for attributing incentive properties to reward cues become sensitized in addicted individuals (Robinson & Berridge, 2008). Auto-shaping paradigms have been used to assess behavioral responses to such reward cues, notably among Sprague Dawley rats (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009). These techniques of behavioral auto-shaping find their roots in the observations of Breland & Breland, who noted that cue-directed feeding behaviors can be elicited from animals who are trained to associate cues with food rewards (1961). In 1973, Jenkins & Moore published a study in which pigeons were trained to associate conditioned cues with either food or water rewards. These pigeons subsequently began to interact with their respective conditioned reward cues with characteristic eating/drinking motions (respective of the predicted reward) (Jenkins & Moore, 1973).

The studies of Breland, Breland, Jenkins, and Moore have set the stage for an important line of "auto-shaping" research in which subjects' cue-directed appetitive and consummatory behaviors are used to operationalize and assess motivational states. When placed in appropriate

auto-shaping paradigms, rats learn to associate Pavlovian-conditioned cues with the delivery of sucrose rewards, and in turn begin to exhibit appetitive and consummatory behaviors towards these conditioned cues (Mahler & Berridge, 2009). Furthermore, if two reward cues are present (one proximal and one distal to the location of delivery of the reward) most subjects will develop individual preferences for either one reward cue or the other (Flagel, Akil & Robinson, 2009; Mahler & Berridge, 2009).

The auto-shaping paradigm used in this study, adapted from Mahler & Berridge (2009), contains two potent reward cues: a lighted metal lever, and a steel "food cup" into which sucrose rewards are dispensed (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009). This paradigm has faithfully led to the development of two behavioral phenotypes among subjects, with "sign-trackers" attending to the distal cue (the "CS+" lighted lever) and "goal-trackers" attending to the proximal cue (the steel food cup) (DiFeliceantonio & Berridge, 2012; Flagel et al., 2009; Mahler & Berridge, 2009).

Mathematical models have been used to operationalize the level of incentive salience that individuals attribute to reward cues. The neurocomputational model of Zhang et al. (2009) employs the variable "κ" to represent physiological drives. Levels of cue-attributed incentive salience are computed by multiplying this factor by the learned values of specific reward cues (Zhang et al., 2009). In the present study, we were guided by this model to hypothesize that incentive motivation towards conditioned food-reward cues is influenced by an individual's satiation state, and that consequentially the shifting of these satiation states can effectively lead to a dynamic shift in the cue-directed attribution of incentive salience (by altering the value of "κ").

In addition to representing physiological drives, "κ" also serves as a mathematical representation of mesolimbic neuronal sensitization (Zhang et al., 2009). Accounting for this representation, and noting that the attribution of incentive salience has been determined to be a dopamine-dependent process (Flagel et al., 2009; Flagel et al., 2011; Robinson & Berridge, 2008), we hypothesized that the disruption of natural dopaminergic activity should decrease the value of κ, resulting in a corresponding decrease in the computed value of cue-attributed incentive salience. Furthermore, we hypothesized that this change should be accompanied by a reduction in cue-directed motivated behaviors (a result previously observed in Flagel et al., 2011). We sought to test this hypothesis by injecting previously-trained sated and hungry rats with flupenthixol, a potent dopamine antagonist (Ellingsen & Agmo, 2004; Flagel et al., 2011; García & Paredes, 2004). Following our hypothesis that that the κ values of hungry subjects would already be elevated by their hunger state, we hypothesized that the behaviors of hungry subjects would be most intensely altered by the flupenthixol treatment.

In the present study, Sprague Dawley rats were trained to associate Pavlovian-conditioned cues with the delivery of sucrose-pellet rewards. Rats were trained under either "sated" or "hungry" feeding schedules, and their food cup/CS+ lever-directed behaviors were recorded and analyzed in order to assess the effects of satiation state and cue-tracking phenotype on incentive salience-driven behaviors. A number of subjects had their feeding schedules reversed prior to the last day of operant training, and their subsequent behavior was used to assess whether cue-directed incentive salience can be dynamically altered by rapid changes in satiation state.

We seek in the present study to contribute to the investigation of food-related motivation by addressing three primary questions. First, is the incentive motivation or "wanting" of a

conditioned reward cue influenced by a subject's satiation state during training? Second, if incentive salience is influenced by satiation state, can it be dynamically altered via the transition from one satiation state to another? Finally, does satiation state during the acquisition of Pavolovian associations influence the dopamine-dependency of an individual's conditioned response to conditioned reward cues? We hope that our findings contribute positively to the growing body of empirical research currently underlying our understanding of motivation, and that the strengths and potential weaknesses of this study serve to motivate and inspire future investigations of the relationship between physiological variables, dopaminergic activity, and motivational processes.

Method

Apparatus

The operant chambers used for Pavlovian training in the present study were 30.5 cm wide × 24.1 cm deep × 21 cm tall. The floor, front and back walls, and ceiling were composed of clear plexiglass, and the side walls were composed of interchangeable steel plates. During training, 45 mg sucrose reward pellets were dropped into a stainless steel cup (referred to here as the “food cup”) imbedded in one of the steel side walls. This cup was flanked on either side by two retractable levers – one that remained extended throughout training sessions (labeled here as “CS-” according to the nomenclature of Mahler & Berridge, 2009), and one that extended only during the 8-seconds prior to sucrose pellet delivery (“CS+”). A small LED light was attached to the underside of the CS+ lever. Water was provided at all times within the operant chambers via a drinking tube, and a red house light was kept on in order to facilitate video recording and behavioral videoscoring.

The two retractable levers (CS+ and CS-) were each 4.5 cm long and extended 2 cm into the chamber. The food cup consisted of an opening in the chamber wall, 3 cm in diameter and elevated 0.7 cm from the chamber floor, containing a metal dish into which sucrose reward pellets were dispensed.

Procedure

Subjects. Thirty-two female Sprague Dawley rats from our own breeding colony (aged >56 days and weighing 200-260 grams at the time of operant testing) were used in the study. Subjects were housed in groups of 1, 2, and 3, in a reverse 12 hour light, 12 hour dark cycle, at approximately 21 degrees Celsius. Prior to their participation in this study, rats were provided with *ad libitum* standard rat chow and water. Dietary restrictions (described below) were put in place for a subset of subjects during habituation and testing. Our laboratory protocol has been approved by the University Committee on the Use and Care of Animals (approval number: 09574).

Four rats received a diet consisting partly of M&M ® candies during training (given *ad libitum* for one hour prior to auto-shaping sessions). This diet was prescribed in order to assess the effects of "super satiation" on cue-directed incentive salience attribution. The activity of these rats following their consumption of M&Ms ® was observed to be noticeably elevated with respect to the activity of those receiving the standard diet of rat chow (subjects tended to move rapidly and chase one another around their home cages following the consumption of M&Ms ®). This behavioral manifestation led us to omit the data collected from these rats from our general discussion below, as it appeared that their behavior was being uniquely affected by the nutritional qualities of their diet, and not solely by the quantity of food that they consumed.

The remaining rats (n=28) were assigned to either “sated” (n=16) or “hungry” (n=12) feeding groups. Subjects in the “sated” group were given *ad libitum* standard Purina ® rat chow at all times in their home cages. Rats in the “hungry” group were given *ad libitum* standard chow for six hours each day (this feeding period directly followed operant sessions on training days). All rats were provided with *ad libitum* water at all times. Rats were habituated to their respective feeding schedules for one week prior to training, and individual rat weights were monitored daily to ensure that each rat retained at least 85% of its respective free-feeding weight.

Preparation. In order to minimize subjects’ anxiety during training, rats were handled for ten minutes on each of four separate days prior to training in the operant chambers. To allow subjects to become familiar with the operant chamber sucrose pellet delivery system, rats were put into the operant chambers for approximately twenty minutes on the day before the first day of training. During this time, rats were presented with twenty sucrose pellets delivered variably on a schedule of approximately 1 pellet every 60 seconds. The CS- lever was extended throughout this session, but the CS+ lever remained retracted.

Experimental groups. Experimental groups were composed to address the study’s three primary questions: 1) whether the incentive motivation or "wanting" of a conditioned reward cue is influenced by a subject's satiation state during training, 2) if incentive salience can be dynamically shifted when an individual transitions from one satiation state to another, and 3) if satiation state influences the dopamine-dependency of an individual's conditioned response to reward cues.

Data collected from twenty rats (referred to below as “Group 1”) were used to address the question of whether the incentive “wanting” of conditioned reward cues is influenced by a subject's satiation state. Behavioral videoscoring of the recordings taken from the third day of

auto-shaping was performed (after rats had formed and enhanced their cue preferences under their respective feeding schedules). Behavioral data were compared for rats based upon their respective feeding schedules and cue preferences.

Data collected from twelve Group 1 rats (also designated here as "Group 2") were used to address the question of whether cue-attributed incentive salience is dynamically shifted following a transition between different satiation states. Following training on the third day, rats trained under the "sated" feeding schedule were provided with *ad libitum* chow for six hours, and were then deprived of food until training the following morning (to mimic a shift to the "hungry" feeding schedule). Conversely, rats trained under the "hungry" feeding schedule were allowed full access to *ad libitum* chow until operant training the following morning (to mimic a shift to the "sated" feeding schedule). These feeding-schedule modifications were implemented to impose a shift in satiation state, and thus a shift in the calculated incentive salience attributed to each individual's respective prepotent reward cue (Zhang et al., 2009).

Data collected from eight rats (Group 3) were used to address the question of whether satiation state influences the dopamine-dependency of an individual's conditioned cue-directed response. Half of the Group 3 rats (n=4) were maintained under the "sated" feeding schedule, and half (n=4) were maintained under the "hungry" feeding schedule. These rats were trained for eight days in the operant chambers. Upon the second- and third-to-last days of training, rats were given mock injections (one microliter for every gram of bodyweight) of physiological saline thirty minutes before their auto-shaping sessions. On the final (eighth) testing day, flupenthixol injections (of the same respective volumes) were given thirty minutes before the operant chamber session. Rats were left in their home cages in a darkened room during the thirty-minute period between injection and operant testing.

Training. Group 1 and 2 rats were placed in individual cages (identical to their home cages) for one hour prior to operant training, during which "sated" rats continued to receive *ad libitum* rat chow, and "hungry" rats continued to be food-deprived. During this time, all rats had access to *ad libitum* water. Group 3 rats were transferred directly from their home cages to the operant chambers (thirty minutes after the time of saline and flupenthixol injections, as noted above).

The duration of each full training session (given on Days 1-3 for Groups 1&2, and all training/testing days for Group 3) was approximately 43 minutes. During these sessions, rats were presented with twenty-five discrete "cues". Each cue period lasted for a duration of eight seconds, during which a 2.9 kHz tone was played within the operant chamber, and the CS+ lever was lit from beneath and extended. Each cue period terminated with the retraction of the CS+ lever, the ending of the auditory cue, and the delivery of a sucrose pellet. Following training, rats were weighed, fed, and returned to their home cages.

On feeding shift days (Day 4 for Group 2 rats), training consisted of three segments. During each of the first two segments, 10 unrewarded cues were presented (identical to the 8-second cue periods described above, but *not* followed by the delivery of a sucrose pellet). The third and final segment consisted of 10 *rewarded* cues (during which cues were followed by the delivery of sucrose pellets). These three segments were separated by 1-2 minutes, during which subjects remained in their respective operant chambers, and computer-scored data was recorded.

Rats belonging to Group 3 were trained under the full 43-minute schedule (consisting of 25 rewarded cues) for eight days. Systemic flupenthixol injections of 0.25 mg/kg were administered intraperitoneally to each of these rats on their last day of training, to assess the relationship between dopaminergic activity and satiety-related motivated behaviors.

Intraperitoneal injections of flupenthixol at this dose have been used in previous studies, leading to meaningful behavioral manifestations with minimal motor deficits (Ellingsen & Agmo, 2004; García & Paredes, 2004). Drug injections were prepared in physiological saline, and were administered using 27.5 gauge needles and 1 mL syringes. Mock injections of physiological saline were administered on each of the two testing days preceding the final testing day, to habituate subjects to the injection procedure.

Behavioral videoscoring

The behavioral videoscoring method of Mahler & Berridge (2009) and DiFeliceantonio & Berridge (2012) was adopted for data collection in this study. For each scored cue period, the frequency (number of events) and target (CS+ lever or steel food cup) of four distinct cue-directed behaviors was recorded for both the 8-seconds preceding the cue onset, and the 8-seconds during which the cue took place. The length of time between cue onset and the point of first cue contact was also recorded, resulting in two 0-8-second "latency" scores per cue period (one for the food cup, and one for the CS+ lever). Video recordings were made using two cameras – one directed upwards through the floor of the chamber (the "bottom" camera) and one directed through the rear plexiglass wall of the chamber towards the reward cues (the "side camera"). The precise time of cue onset was determined using the bottom camera, while most of the behaviors themselves were best observed using the side camera.

The four cue-directed behaviors that were recorded during scoring procedures were nibbles/sniffs, slow bites/dives, looks, and approaches. Nibbles and sniffs consisted of snout-to-cue contacts, sometimes accompanied by slight nibbling movements. Slow bites (CS+ lever) were scored when rats closed their jaws around the CS+ lever. Slow bites/dives (food cup) were scored when rats made forceful snout-cue contact with the top or bottom of the inside of the food

cup, turning their heads and forcing the sides of their heads/snouts into the food cup. “Looks” were scored each time a subject oriented her face towards one of the two reward cue. Food cup entries without contact were scored as “looking” behaviors. An “approach” was scored each time a subject oriented and moved towards one of the cues.

The first, fifth, tenth, fifteenth, twentieth, and twenty-fifth cues were scored for critical 25-cue training days (Day 3 for Groups 1 and 2, Days 6 and 7 for Group 3). On shift days (Day 4 for Group 2 rats), the second, fifth, eighth, and tenth cues of the second 10-cue (unrewarded) block were scored.

Analysis

Mixed ANOVAs were used to analyze the data collected. Data gathered on feeding shift days and flupenthixol injection days were compared using repeated measures ANOVAs. All statistical tests were carried out using IBM Statistics SPSS software, Version 19.

Results

Classification of Phenotype

Previous utilization of the auto-shaping paradigm described above has necessitated the assignment of individual subjects to separate groups based upon their respective conditioned reward cue preferences for either the food cup or the CS+ lever (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009). For the purposes of this study, subjects were classified as either “goal-trackers” (preferring the food cup) or “sign-trackers” (preferring the CS+ lever) based upon the frequency with which they contacted these two reward cues. Rats that on average bit, nibbled, and sniffed the food cup more than the CS+ lever during scored cues were classified as “goal-trackers.” Conversely, those that preferentially bit, nibbled, and sniffed the CS+ lever were

classified as "sign-trackers." For photographic representations of nibbling/sniffing and biting behaviors, please refer to Figure 1.

Individual Preferences of Group 1. Of the twenty rats belonging to Group 1, twelve were sated and eight were hungry during training. Of the twelve sated rats, nine were sign-trackers, and the remaining three were goal-trackers. Of the eight hungry rats, six were sign-trackers, and the remaining two were goal-trackers.

Individual Preferences of Group 2. Twelve Group 1 rats (eight sated, four hungry) were assigned to Group 2. Of these rats, only one (sated) rat developed goal-tracking tendencies. The remaining eleven were sign-trackers.

Individual Preferences of Group 3. Among Group 3 subjects, one (hungry) rat was a goal-tracker, and the remaining seven rats (four sated, three hungry) were sign-trackers.

Synopsis

Hungry individuals were substantially more attracted to their preptotent cues than sated individuals. This observation was confirmed by increases in both nibbling and sniffing behaviors, and total cue-directed behaviors. These data support our hypothesis that cue-directed motivated behaviors are elevated in individuals experiencing hunger during training.

Two physiological and psychological "shifts" were analyzed during this study – one induced by hunger, and one induced via the injection of a dopamine antagonist, flupenthixol. Group 2 hungry→sated rats exhibited a substantial increase in latency towards their preferred cues between their final training day and the day of their feeding shift, reflecting lowered incentive salience, as it took them longer to reach their preferred cue. Rats receiving flupenthixol exhibited mixed behavioral effects, with increased slow-biting activity among sated sign-trackers being the most pronounced behavior change observed among the groups tested.

Hunger elevated incentive motivation towards preferred reward cues. Hungry rats (n=8) nibbled and sniffed their respective prepotent cues an average of 40% more than sated rats (n=12) ($F(1,18) = 8.6$, $p < .01$). A corresponding increase in the total number of motivated behaviors directed toward subjects' preferred cues was also observed, with hungry rats engaging in approximately 22% more cue-directed behaviors overall ($F(1,18) = 3.820$, $p < .07$; Figure 2). Together, these results indicate that rats in a state of hunger attribute a greater degree of incentive salience toward their preferred reward cues than rats experiencing normal satiation.

We also observed that sated goal-trackers (n=3) directed fewer appetitive and consummatory behaviors towards their preferred reward cue (the food cup) than rats belonging to other feeding/cue-tracking phenotype groups. This difference was driven by decreases in both slow bites and nibbles/sniffs. These decreases (particularly the decrease in nibbles/sniffs) indicate that sated goal-trackers may attribute less incentive salience towards their preferred reward cue than other rats (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009), a phenotypic attribute that may potentially differentiate them from goal-trackers with respect to their pattern of attribution of cue-directed incentive salience.

Sated goal-trackers also took twice as much time (3.0 seconds) to contact their preferred reward cue as both sated sign-trackers (1.5 seconds) ($F(3,16) = 2.668$, $p < .10$), and hungry goal-trackers (also 1.5 seconds) ($F(3,16) = 2.668$, $p < .40$). This result again suggests that sated goal trackers attributed less incentive salience to their prepotent reward cue (the food cup).

Group 1 rats did not differ in their probability of contacting (nibbling, sniffing, and biting) their prepotent cues. However, hungry goal-trackers were on average the most likely to contact their non-prepotent cue (the CS+ lever), while sated goal-trackers were on average the *least* likely to contact their non-prepotent cue. This suggests that hungry goal-trackingers were

less focused in their cue-directed responses than sign-trackers in both feeding groups, attributing more incentive salience to their nonprepotent cue. However, in the case of the sated goal-trackers, it is possible that their decreased likelihood of contacting the non-prepotent CS+ lever largely influenced by an overall lack of activity.

Cue-capture analysis was performed of all Group 1 rats (n=20). “Cue-capture” instances occurred when rats directed 100% of their contact behaviors (nibbling/sniffing and slow-biting) towards only one conditioned reward (either the CS+ lever or the food cup) throughout each of the scored cues during a single testing session. Of the eight hungry Group 1 rats assessed, two sign-tracking individuals demonstrated cue-capture (towards their prepotent cue) on their third day of training. Of the twelve sated rats assessed, two sign-tracking individuals demonstrated cue capture (again, towards their prepotent cue). No goal-tracking rats (n=5) were observed to exhibit cue-capture, suggesting that these rats were less focused in their cue-responses than sign-trackers.

Hungry goal-trackers exhibited elevations in motivated behavior towards their non-prepotent cue (Figure 3). Hungry goal-trackers (n=2) bit their non-preferred cue (the CS+ lever) more frequently than either sated goal-trackers (n=3) ($F(3,16) = 4.2$, $p < .05$) or hungry sign-trackers (n=6) ($F(3,16) = 4.2$, $p < .05$). This difference suggests that hungry goal-trackers may be less focused in their cue-directed biting responses than both sated goal-trackers and hungry sign-trackers.

This observed difference in cue-directed focus on the part of hungry goal-trackers was also detected in their latency to contact the CS+ lever, which was on average 75% lower than the latency of either sated goal-trackers ($F(3,16) = 7.3$, $p = .01$) or hungry sign-trackers ($F(3,16) = 7.3$, $p < .01$) to contact their respective non-prepotent cues. Hungry goal-trackers also showed an

increased probability of visiting (and contacting) their non-prepotent cue during the 8-second cue period. These results suggest that hungry goal-trackers may be less focused in their attribution of incentive salience towards potent conditioned reward cues.

Rats shifted hungry→sated attributed less incentive salience to their preferred reward cues. Hungry→sated sign-trackers (n=4) on average took over twice as much time (210%) to reach their preferred reward cue on the hungry day of their feeding shift than on the previous sated training day, whereas sated→hungry sign-trackers did not show any substantial change in their latency to contact their preferred cue under hungry conditions compared to their earlier sated performance. This strongly indicates that when rats trained under hungry conditions are suddenly sated, they attribute less incentive salience to their preferred reward cues after the feeding shift. Further analysis revealed that hungry→sated sign-trackers exhibited fewer cue-directed behaviors *overall* (towards both the food cup *and* the CS+ lever) on the sated shift day than the previous hungry training day. All of these findings suggest that cue-attributed incentive salience was dynamically decreased for these subjects when they transitioned from a state of hunger to a state of satiation.

Examined in isolation, the only Group 2 goal-tracker (sated→hungry) was 93% slower to contact the food cup on her hungry feeding-shift day than during the previous sated training day. However, she was nearly 310% faster to contact the CS+ lever after the sated→hungry transition. In addition, this rat performed 200% more CS+ lever-directed nibbles/sniffs and slow bites on the hungry feeding shift day than food cup-directed nibbles/sniffs and slow bites/dives, strongly indicating that she experienced a profound shift in her cue preference between the last sated day of training and the hungry shift day. No other rat was observed to undergo such a shift in cue preference following the feeding shift. This rat frequently oriented towards the the lever during

previous training sessions, so her sign-tracking behaviors may have been already in development prior to her last day of training. Overall, this rat was 24% faster to attend the *CS+ lever* on the hungry feeding shift day than the *food cup* on the previous sated training day. Thus, while she underwent a shift in cue preference, she was nonetheless faster to contact her *new* prepotent cue on the day of the feeding shift than her old prepotent cue during the previous training day. This observation appears to be consistent with our hypothesis that hunger increases cue-directed incentive salience.

Hungry→sated sign-trackers were less likely to visit and contact the (non-prepotent) food cup on their sated feeding-shift day than on the previous hungry training day. While this is consistent with our other observations indicating that satiety focuses cue-directed contacts, it should be noted that this difference may have been influenced by a general decrease in activity in hungry→sated individuals. Sated→hungry rats showed minimal change in their probability of approaching either cue between (sated) training and (hungry) shift days.

Of the seven sated→hungry sign-tracking subjects, one rat directed *all* of her contact behaviors towards the CS+ lever (during the 8-second cue period) on both the sated training day and the hungry shift day. One additional sated→hungry subject contacted both cues on her last sated training day, but directed all of her contact behaviors towards the CS+ lever on the hungry shift day. Thus, only one sated→hungry sign-tracker of the seven analyzed exhibited changes in cue-capture between training and shift days.

Cue-capture changes were much more pronounced among the four hungry→sated sign-trackers, two of which (half of the group) were not focused in their cue-directed contacts on the final hungry training day, but directed 100% of their contact behaviors towards the CS+ lever on

the sated shift day. This satiety-related increase in focus is consistent with our observations that hunger decreased focus among Group 1 subjects (see Figure 3).

Flupenthixol led to an elevation in slow-biting behaviors for rats trained under "sated" conditions (Figure 5). Hungry sign-trackers (n=3) showed only a slight decrease in their total number of CS+ lever-directed behaviors on the day of flupenthixol treatment with respect to the previous training day ($F = 0.571$, $p = .529$), exhibiting very little variation in their cue-directed nibbles/sniffs and slow bites between the two days (see Figure 5). However, sated sign-trackers (n=4) engaged in a greater total number of CS+ lever-directed behaviors on the day of flupenthixol treatment than on the previous training day ($F = 5.630$, $p = .098$). This change was greatly influenced by a substantial increase in CS+ lever-directed slow bites (by up to 2600% as many CS+ lever-bites as were observed on the previous day of training). Every sated rat (4 sign-trackers) showed an increase in slow-biting, and every hungry rat (3 sign-trackers and 1 goal-tracker) showed a decrease in slow bites/dives directed towards their respective preferred reward cues. CS+ lever-directed nibbles/sniffs were largely unchanged for sated sign-trackers between the mock-injection day and the day of flupenthixol treatment (Figure 5).

The frequency of non-prepotent cue-directed looks on the day of flupenthixol treatment was increased for hungry subjects (132% of previous training day), and decreased for sated subjects (49% of previous training day) ($F(1,5)$ for comparison of %training = 10.7, $p < .05$). This indicates that unimpeded dopaminergic activity may have acted in concert with hunger to focus subjects' incentive salience attribution towards their prepotent reward cues, an observation that is consistent with the neurocomputational theory of Zhang et al. (2009) and our expectations for the effect of systemic dopamine suppression in this experiment.

The risk of drug-induced motor impairment was a cause for concern during flupenthixol testing. While all rats appeared to be somewhat less mobile than usual when removed from the operant chambers following testing under flupenthixol, only one subject (a hungry goal-tracker) showed any signs of motor deficits. This subject was the only goal-tracker among the eight Group 3 rats tested with flupenthixol.

Examined alone, the Group 3hungry goal-tracker exhibited a general decrease in activity after being treated with flupenthixol, with the exception of a slight increase in her total CS+ lever-directed behaviors. She was slower to contact both the food cup and CS+ lever on the day of flupenthixol treatment (in contrast to the previous training day under saline).

Previous work has strongly suggested that appetitive behaviors (nibbles and sniffs) are more representative of cue-directed incentive salience attribution than consummatory behaviors (slow bites) (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009). It is therefore possible that the changes in sign-tracking slow bites noted here do not represent a substantial change in cue-directed incentive salience attribution after flupenthixol treatment.

None of the hungry (n=4) and sated (n=4) rats receiving flupenthixol exhibited substantial changes in their probability of visiting either cue when compared to their previous mock injection (saline) day.

All instances of cue-capture among Group 3 subjects were observed in sign-trackers. One sign-tracking rat from each feeding group contacted the food cup at least once on the last day of saline injection, but subsequently directed 100% of their cue-contacts towards the CS+ lever on the day of flupenthixol treatment. In addition, one sign-tracking rat from each feeding group directed100% of their contact behaviors towards the CS+ lever on both the saline training day and flupenthixol testing day. While no differences were found between feeding groups, these

changes in cue capture suggest that rats receiving flupenthixol exhibited greater exclusivity in their cue-directed behaviors, regardless of satiety state.

Goal-trackers attended more to the food cup than sign-trackers prior to cue onset. Goal-trackers exhibited a greater tendency to attend to the food cup during the 8-seconds preceding cue onset than sign-trackers. This trend was observed for slow bites/dives, nibbles/sniffs, looks, probability of approach, total contact behaviors (nibbles/sniffs + slow bites/dives), total non-contact behaviors (looks + approaches), and total behaviors ($F(3,16) = 7.5$, $p < .01$, see Figure 6). Because the food cup was ever-present in the chamber, it was expected that pre-cue behaviors directed towards the food cup would occur with greater frequency than pre-cue behaviors directed towards the lever slot. It is also not surprising that these food-cup-directed behaviors were engaged in most frequently by goal-trackers, and not by sign-trackers, given that goal-trackers, by definition, made more frequent contact with the food cup during the cue period.

Discussion

Results and Conclusions

Our first question at the outset of this study was to assess whether incentive salience can be influenced by satiation state. Following the current neurocomputational model of incentive salience (Zhang et al., 2009), we hypothesized that satiation state, represented by the variable "κ," could directly modify cue-directed incentive salience. Among Group 1 rats, we observed that the total number of prepotent cue-directed behaviors (particularly nibbles/sniffs) exhibited by hungry subjects was substantially elevated relative to sated individuals. These results, indicating increased motivated behavior for both sign-tracking and goal-tracking subjects, strongly suggest that hunger is a potent mediator of cue-directed incentive salience.

In addition, we observed that hungry goal-trackers were less focused in their cue-directed responses than other subjects, contacting their non-prepotent cue faster, approaching it more often, and biting it with greater frequency (Figure 3). These subjects also contacted their non-prepotent cue with greater probability. These results suggest that hungry goal-trackers may attribute less incentive salience to their prepotent conditioned reward cue than rats experiencing other satiation states and cue-tracking phenotypes, and more incentive salience to their non-prepotent conditioned reward cue.

Next, we sought to address the question of whether rapid changes in satiation state can lead to the dynamic re-computation of cue-directed incentive salience. Following the same neurocomputational model of incentive salience adopted to answer Question 1 (Zhang et al, 2009), we hypothesized that subjects experiencing reversed satiation states would exhibit increased (or decreased) cue-directed appetitive and consummatory behaviors, respective of the feeding shift (sated→hungry or hungry→sated) that they experienced. Our data show that hungry→sated sign-trackers interacted less frequently with both reward cues after becoming sated, and exhibited a potent decrease in latency to contact their preferred reward cue. These findings together support our hypothesis that the alleviation of physiological drives (represented by "κ") results in calculated decreases in incentive salience attribution towards reward cues.

Finally, we sought to address the question of whether systemically-decreased dopaminergic activity leads to a corresponding decrease in incentive salience attribution to reward cues. Again, we referred to the neurocomputational model of incentive salience, which states that "κ" is dependent upon mesolimbic dopaminergic signaling, as well as current physiological states (Zhang et al., 2009). Prepotent cue-directed slow-biting behaviors were greatly elevated in sated sign-trackers following flupenthixol injection. However, nibbles, sniffs,

looks, and approaches were not profoundly changed. Observations made by Mahler & Berridge (2009) and DiFeliceantonio & Berridge (2012) indicate that nibbles and sniffs may be more representative of incentive salience than slow bites, which are consummatory in nature and are usually delayed until ingestion.

At the outset of the present study, we asked 1) whether incentive salience is influenced by hunger, and 2) whether this influence can be dynamically shifted by changes in satiation state. The results described here provide compelling evidence in support of our hypotheses: that cue-directed incentive salience is computed from one's current physiological state, and that this incentive salience can be dynamically re-computed in response to changes in physiological state. With regards to our third question, whether dopaminergic activity modulates hunger-driven incentive salience attribution, no strong evidence was found to support or confirm our hypothesis. Sated sign-trackers did show a marked increase in slow-biting behaviors towards their preferred cue (the CS+ lever), but this observation may not have strong implications for incentive salience (DiFeliceantonio & Berridge, 2012; Mahler & Berridge 2009).

Limitations

Size was the primary limitation of this study. While several differences between groups were observed, it is difficult to draw definite conclusions based upon the small number of subjects used. One of the most difficult challenges imposed by the size limitation of within this study was imposed upon our ability to study the effects of feeding and drug manipulations on a sufficient number of subjects from each cue-preference phenotype (sign-trackers as well as goal-trackers). Of the 28 rats analyzed, only 6 (21%) showed goal-tracking tendencies. Increasing the number of subjects in future studies should increase the number of goal-tracking subjects observed, thus allowing for a more complete study of this behavioral phenotype.

Our study of the dynamic computation of incentive salience has been made possible by the manipulation of each subject's motivational state. While we believe that the feeding schedule manipulation that was imposed upon Group 2 rats led to the shift in satiety that we desired, it must be noted that rats in the "hungry→sated" group gained a substantial amount of weight during the night preceding training on the fourth day, such that their weights during testing on the fourth day were between 102% and 113% of their weights taken on the previous day. This extra weight did not appear to adversely affect subjects' behavior, but it was a noticeable consequence of the feeding schedule manipulation.

Lastly, in our attempt to deliver a conservative, previously-tested dose of flupenthixol (to minimize confounding behavioral deficits) it is possible that we chose a dose that was not appropriate for the present study. Behavioral videoscoring revealed that only one rat (out of eight that received the injection) showed any significant signs of behavioral/motor deficits during testing. Therefore, it was possible that a higher dose could be used to achieve more significant results.

Future directions

The present study strongly supports the theory that cue-directed incentive motivation is correlated with satiation state. However, our study has only depicted behavioral correlations under two states of satiation: hungry and normally-sated. Our intention at the outset of the present study was to examine the motivated behaviors of rats experiencing three states of satiation: hunger, normal satiation, and "super" satiation. While hunger and satiation were operationalized in a straight-forward manner through the use of restricted feeding schedules, it was difficult to impose a state of "super" satiation, in which rats could be observed after having consumed *more* than a normally-sated rat. An attempt was made to create such an experience for

four fats by feeding them highly-palatable M&M ® candies one hour prior to training. While these rats were observed to eat substantial amounts of M&Ms ® during the pre-training session, they were noticeably more mobile and playful with one another after their exposure to the candies. Wary that such behavioral manifestations could be attributed to the unusual nature of these rats' diets, it was decided that they should not be considered as an appropriately "super" sated group. However, it would be useful to understand the effect of satiation-in-excess on motivated behavior, and the development of an appropriate super-sated model should be pursued.

Another important consideration that should be made in future work stemming from the present study is that a large sample size may be needed to obtain sufficient numbers of individuals belonging to both cue-preference phenotypes. As noted above, this study yielded only a small amount of goal-tracking subjects. While the data produced by these goal-trackers provided several interesting results, these findings must be confirmed in a greater number of goal-trackers.

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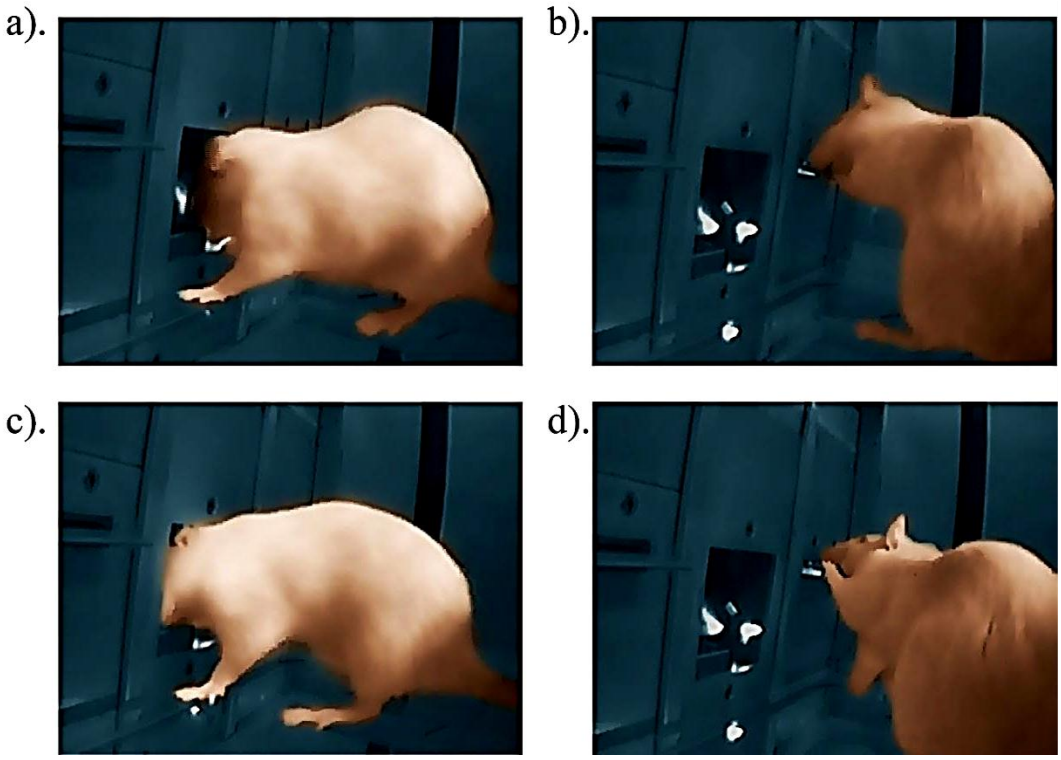

Figure 1. Contact behaviors scored. All behaviors are shown from the side camera angle (color and contrast added to highlight rat position). Behaviors shown: a). Food cup nibble/sniff. b). CS+ lever nibble/sniff. c). Food cup, slow bite/dive. d). CS+ lever slow bite.

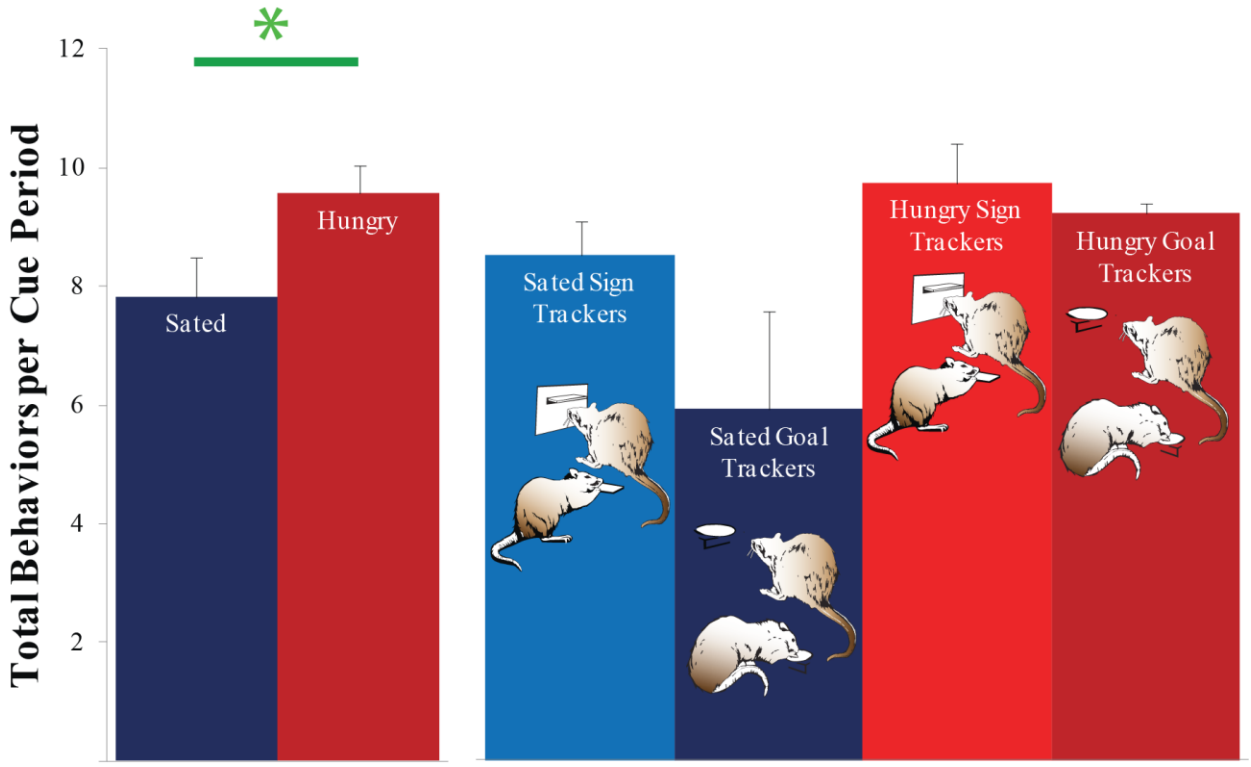

Figure 2. Hungry rats of both cue-tracking phenotypes were observed to exhibit more total behaviors towards their preferred reward cues than sated rats of both phenotypes. This difference was shown for both phenotypes combined (left) and for both phenotypes separated by satiation state (right). (" * " denotes *p*-value less than .07).

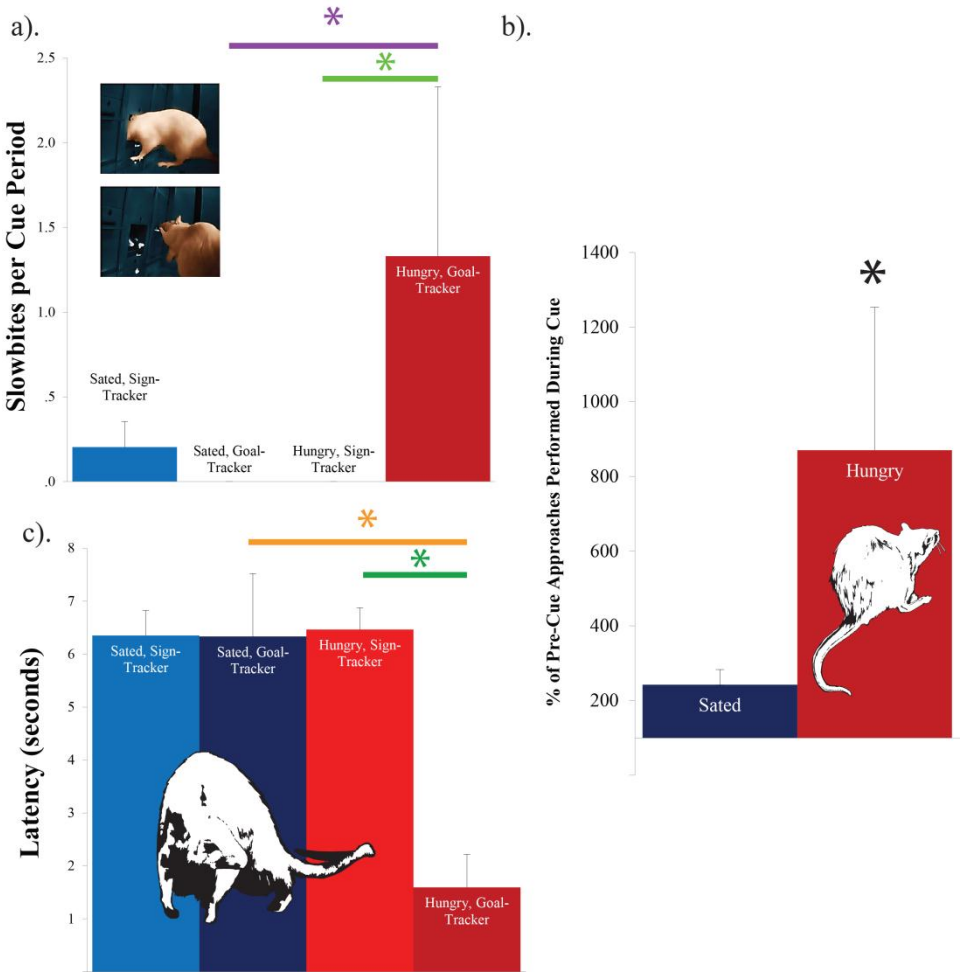

Figure 3. Hungry subjects, especially hungry goal-trackers, showed increased attention to their respective non-prepotent cues. a). Hungry goal-trackers performed more slow bites on their non-prepotent cue during the cue period than hungry sign-trackers or sated goal-trackers. b). Hungry and sated rats approached their respective non-prepotent cues with greater frequency during the cue period than during the pre-cue period (indicated here by the percentage of approaches made during the cue period with respect to the pre-cue period) However, hungry rats approached their nonprepotent cue with far greater frequency. c). Hungry goal-trackers approached their non-prepotent cue with greater latency than either hungry sign-trackers or sated sign-trackers (during the cue period). (“ * ” denotes difference with *p*-value less than .05).

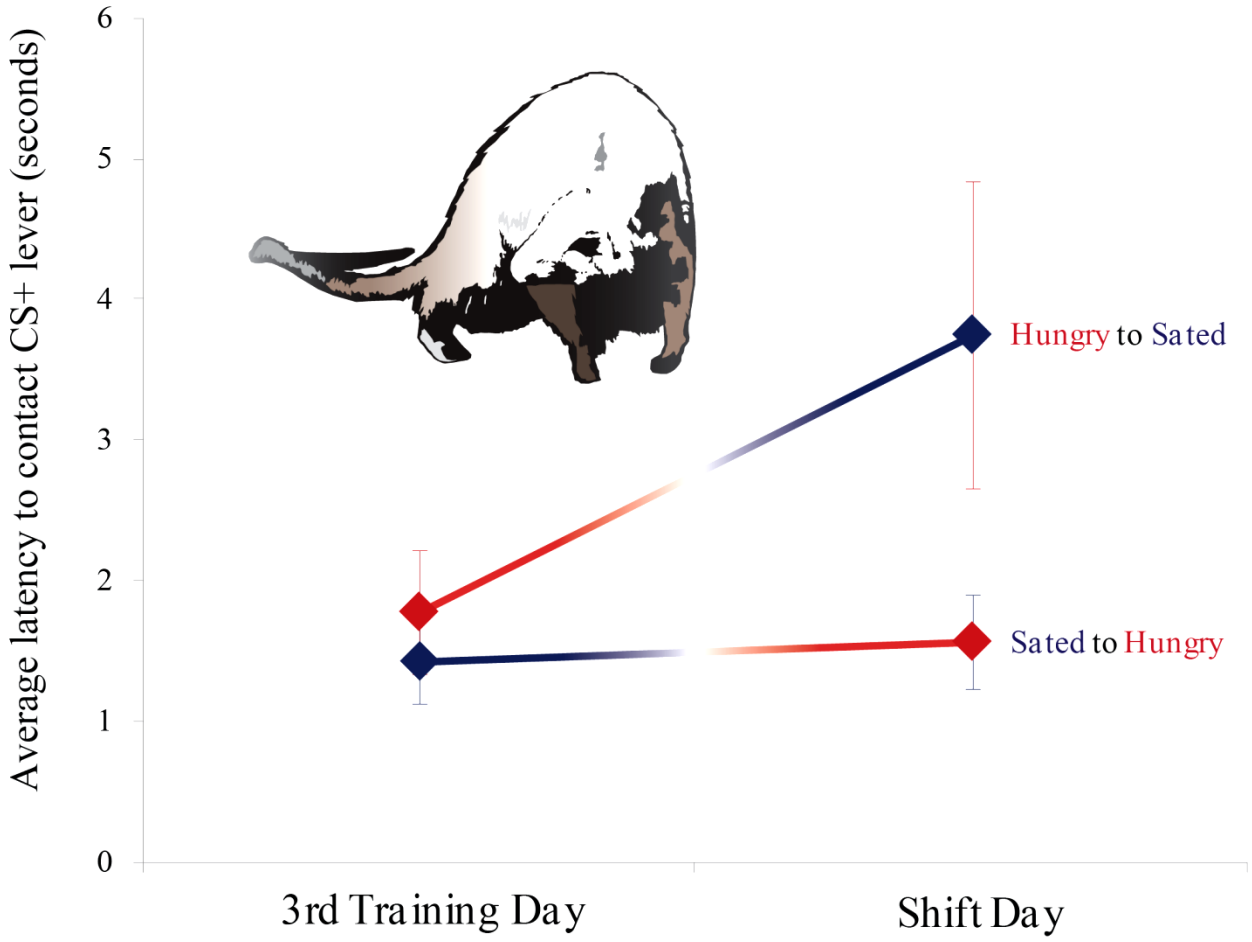

Figure 4. Hungry→sated sign-trackers were observed to take 110% longer to contact the CS+ lever after being sated. Sated→hungry rats showed no respective change in their respective latency.

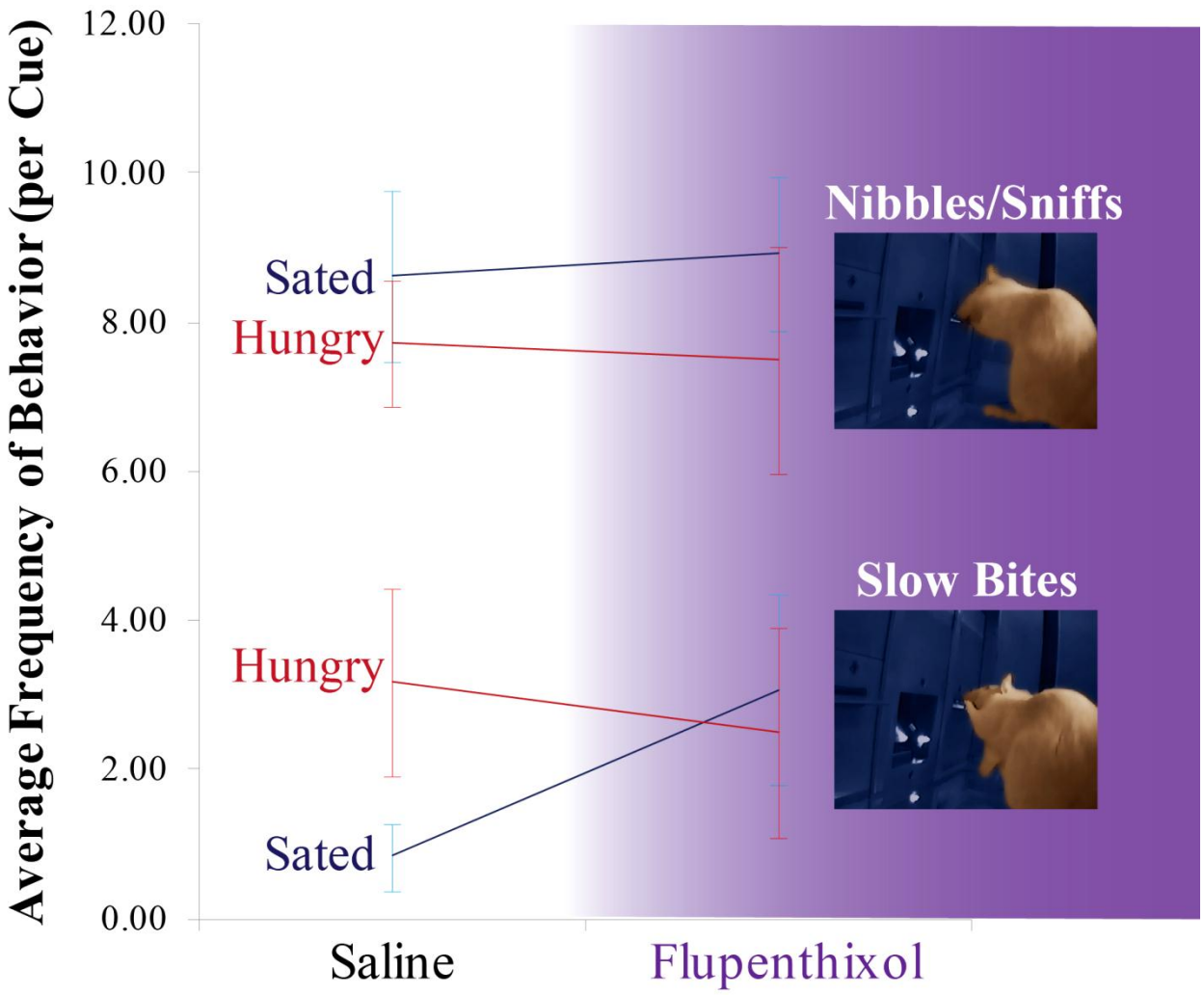

Figure 5. Sated sign-trackers showed more slow-biting behaviors towards the CS+ lever during the cue period on the day of flupenthixol treatment than on the previous training day, but no significant changes in nibbling/sniffing behaviors. Hungry sign-trackers showed little behavioral change towards the CS+ lever with regards to either slow-biting or nibbling/sniffing.

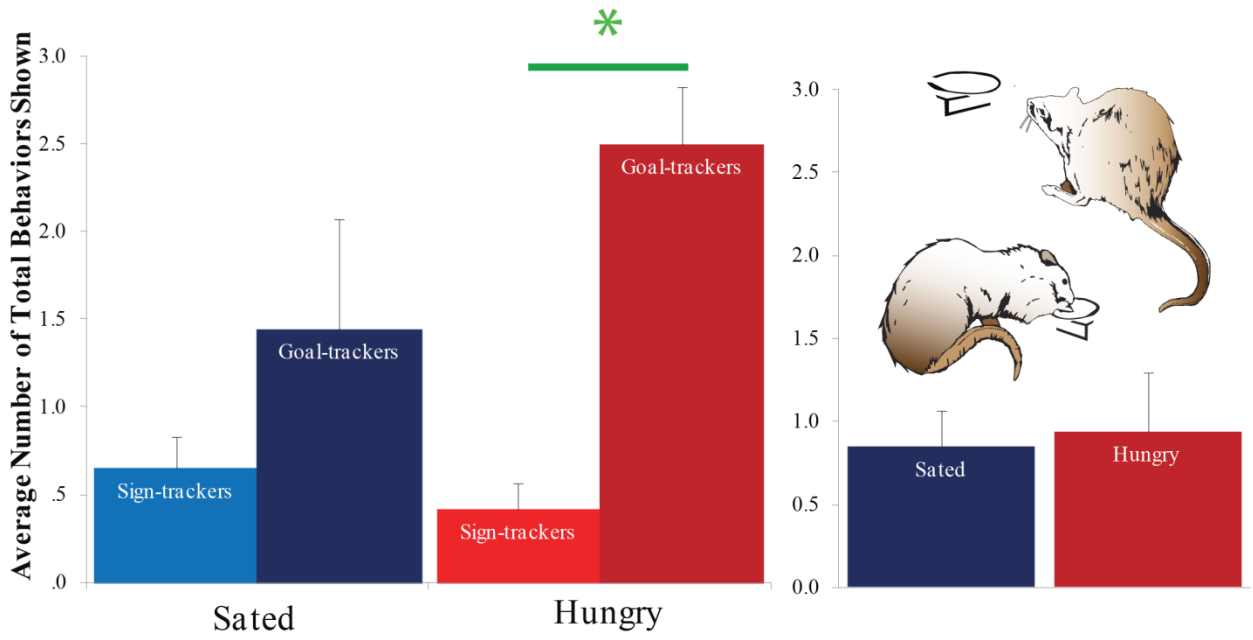

Figure 6. Goal-centered behaviors were elevated in goal-trackers relative to sign-trackers during the 8-second pre-cue period (left). This difference was not dependent upon feeding schedule (right). (" * " denotes difference with *p*-value less than .01).