



Research report

Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues

Lindsay M. Yager, Terry E. Robinson*

Department of Psychology (Biopsychology Program), The University of Michigan, East Hall, 530 Church St, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:

Received 11 February 2010
 Received in revised form 11 April 2010
 Accepted 13 April 2010
 Available online 21 April 2010

Keywords:

Food cue
 Feeding
 Goal-tracking
 Sign-tracking
 Incentive salience
 Reinstatement
 Incentive motivation

ABSTRACT

Cues associated with food availability and consumption can evoke desire for food, sometimes leading to excessive intake. We have found, however, that food cues acquire incentive motivational properties (the ability to attract and to serve as conditional reinforcers) in some individuals (sign-trackers), but not others (goal-trackers). We asked, therefore, whether rats that are attracted (attribute incentive salience) to a food cue are the same individuals in which a food cue reinstates food seeking behavior, and whether this is modulated by hunger. We report that a food cue produced more robust reinstatement in individuals prone to attribute incentive salience to reward cues (sign-trackers), than in those that do not (goal-trackers). Furthermore, hunger significantly facilitated reinstatement in sign-trackers, but not goal-trackers. In conclusion, individual variation in the propensity to attribute incentive salience to food cues may contribute to susceptibility to eating disorders, and therefore, studies on the psychological and neurobiological basis of this variation may provide new insights into such disorders.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Cues associated with rewards can acquire such powerful control over behavior that individuals sometimes have difficulty resisting them. Indeed, cues that have acquired incentive motivational properties: (a) are attractive, eliciting approach toward them; (b) are “wanted”, in that individuals will work to get access to them; and (c) spur pursuit of their associated reward [1,2,10]. The incentive properties of food cues have been especially well characterized [1,14,18], and in both humans and non-human animals food cues can increase desire for food and evoke feeding behavior. For example, if moderately sated humans, who minutes earlier expressed no more desire to eat, are simply presented with a highly palatable food the desire to eat is reinstated [3]. Similarly, when presented with an auditory conditional stimulus (CS) or a context that had previously been associated with food availability otherwise sated rats reinstate feeding [13,19,12 for review].

There is, however, considerable individual variation in the ability of food-associated cues to motivate behavior to earn a food reward [9] and to elicit eating [5], which may be related to variation in the degree to which reward-related cues are attributed with incentive salience [6,14]. For example, if a localizable CS is

repeatedly paired with delivery of a food reward (the unconditional stimulus, [US]) the food cue itself becomes attractive eliciting approach and engagement with it, but only in some rats – these are called sign-trackers (STs) [14]. In other rats the food cue itself does not attract, but instead, upon CS presentation these animals learn to approach the location where food will be delivered – these animals are called goal-trackers (GTs) [14]. Furthermore, a localizable food cue is a more effective conditional reinforcer, reinforcing the learning of a new instrumental response, in STs than GTs [14]. Thus, the cue serves as an equally effective predictive CS in both STs and GTs, as it comes to evoke a conditional response (CR) in both, but it serves as a more attractive and “wanted” incentive stimulus in STs [14]. Although we have previously shown the ability of a food-associated cue to attract and to serve as a conditional reinforcer differs in STs and GTs [14], we have not shown whether the ability of a food cue to reinstate food-seeking behavior differs in STs and GTs. Therefore, the purpose of the present experiment was to study the ability of a food cue to reinstate food seeking behavior following extinction of an instrumental response required to obtain food, in rats characterized as STs or GTs. Furthermore, physiological states, such as hunger, potentially modulate the incentive value of food and food-associated cues for most individuals [1,18], and therefore, we also studied animals that were either sated or fasted.

2. Methods

Ninety-six male Sprague–Dawley rats (Harlan, Indianapolis, Indiana) weighing 300–350 g upon arrival were housed individually in a colony room on a

* Corresponding author at: Department of Psychology (Biopsychology Program), University of Michigan, 525 East University-East Hall, Ann Arbor, MI 48109, USA. Tel.: +1 734 763 4361.

E-mail address: ter@umich.edu (T.E. Robinson).

12-h light/12-h dark cycle (lights on at 08:00 h). Water was available *ad libitum* throughout the experiment and food was available *ad libitum* through the end of Pavlovian training. After completion of Pavlovian training, all animals were restricted to approximately 16 g of rat chow per day, which maintained their weight at 85% of their free feeding weight. Their daily ration of food was given after each test session and animals were given one week to acclimate to the restricted diet before further testing. Following reliable instrumental responding, half the rats were again given *ad libitum* access to food and the remainder of the rats remained food restricted. All behavioral testing was conducted in sixteen standard (22 cm × 18 cm × 13 cm) test chambers (Med Associates Inc., St Albans, VT, USA) located in sound attenuating cabinets. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

2.1. Pavlovian training

To determine which rats were STs and which were GTs all animals were first trained using a Pavlovian procedure described previously [8]. Briefly, in five daily sessions consisting of 25 trials/session an illuminated retractable lever (the lever-CS) located either to the left or right of a centrally located food magazine was inserted into the chamber for 8 s on a random-interval 90-s schedule. Immediately following retraction of the illuminated lever a single 45-mg banana-flavored food pellet (the US) (BioServe, #F0059, Frenchtown, NJ, USA) was delivered into the food magazine. No instrumental response was required by the animal to initiate delivery of the food pellet. Following Pavlovian training animals were classified as sign-trackers or goal-trackers. Sign-trackers were defined as the 25% of animals who made the most lever presses during presentation of the CS and goal-trackers were defined as the 25% of animals who made the fewest number of lever presses. The remaining animals were not used further.

2.2. Instrumental training

One week after completion of Pavlovian conditioning, instrumental training began in chambers that contained a centrally located food magazine flanked on either side by a nose port. Animals were trained to make an instrumental response (a nose poke) for a single chocolate-flavored food pellet (BioServe, #F0299) and illumination of the active nose port (the light-CS) for 5 s on a fixed ratio (FR) 1/20 s time-out schedule of reinforcement. Responses into the active port during the time-out, or into the inactive port, had no programmed consequence. Rather than restricting the length of the session, animals were required to earn a fixed number of pellets each day, which increased across days. This procedure was used to ensure that all animals received exactly the same number of response-reward/light-CS pairings during instrumental training.

2.3. Extinction and reinstatement test

Once animals showed stable responding and were taking 80 pellets/session, extinction training began. The day prior to extinction training, a subset of STs and GTs were allowed *ad libitum* access to food (sated group) while the remaining animals remained food restricted (fasted group). Groups were matched based on the number of active nose pokes made during the final two days of training at the 80 pellet criterion. All animals underwent daily 40-min extinction sessions until they reached the criteria of two consecutive days with less than 15% of their active nose pokes during the final two days of instrumental training. During extinction sessions responses resulted in no programmed consequences (active nose pokes did not result in food delivery or illumination of the nose port). All animals reached the criterion for extinction in 4 days of extinction training. The day following extinction training, animals were given a single 40-min test for cue-induced reinstatement. During this test responses into the active nose port illuminated that port (i.e., produced the light-CS) for 2 s, but no food was delivered.

2.4. Statistics

Pavlovian conditioning, instrumental training, and extinction training were analyzed using linear mixed-effects models. To analyze data from the cue reinstatement test we addressed the following planned ("a priori") questions: (1) Did the cue reinstate responding? To test this we used paired *t*-tests to compare the number of active responses with the average number of responses during the last 2 days of extinction. (2) Were there group differences in the degree of reinstatement? To test this we analyzed data separately for sated and fasted animals using two-way repeated measures ANOVAs. Group differences in reinstatement were indicated by a significant phase-by-group interaction. (3) Did physiological state (fasted vs. sated) modulate the degree of reinstatement in STs or GTs? To test this we again used two-way repeated measures ANOVAs, and group differences were indicated by a significant phase-by-food group interaction. Significance was set at $p < 0.05$.

3. Results

3.1. Pavlovian training

As expected from previous studies [14], during Pavlovian training animals designated STs and GTs acquired very different CRs (Fig. 1). STs learned to reliably and rapidly approach the lever-CS (Fig. 1A and C) and they vigorously engaged it (Fig. 1B). In contrast, GTs rarely approached the lever-CS, but upon its presentation they instead learned to reliably and rapidly approach the food cup (Fig. 1D and F), which they engaged vigorously (Fig. 1E). Thus, the lever-CS evoked a CR in both STs and GTs, but the CS itself became attractive only in STs, suggesting the lever-CS was attributed with incentive salience in STs but not GTs (see [6,14] for further discussion).

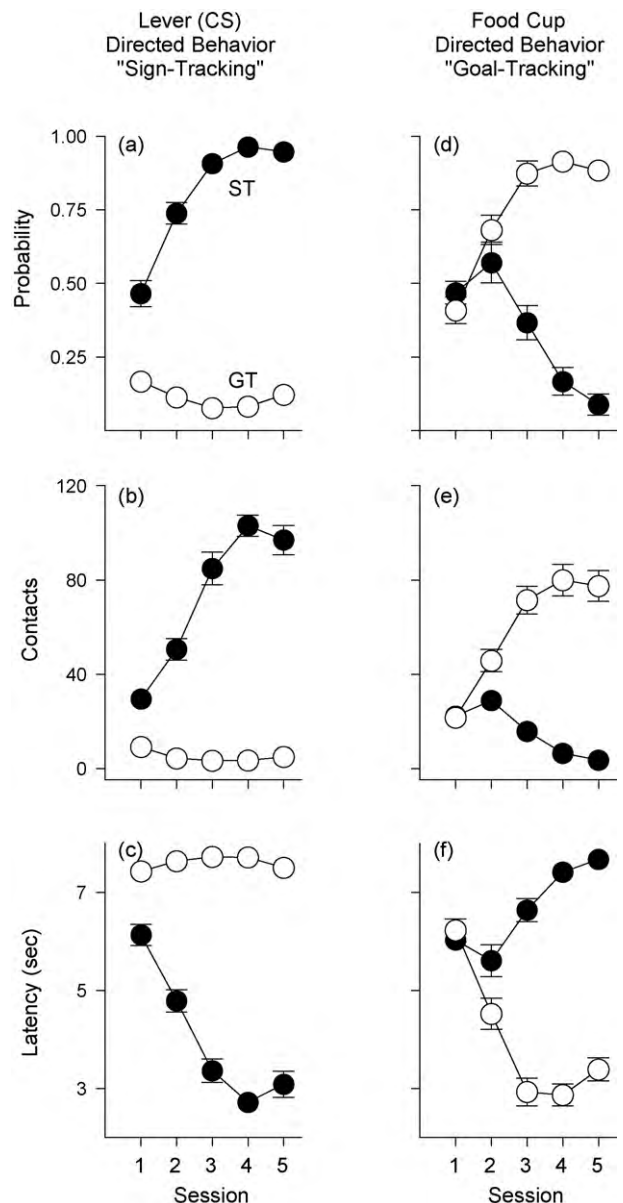


Fig. 1. Behavior directed towards the lever-CS vs. the location of food delivery (the food cup) during Pavlovian training in rats designated sign-trackers (STs; $n = 24$) or goal-trackers (GTs; $n = 24$). The mean \pm SEM for: (A) probability of approaching the lever-CS during the 8-s CS period, (B) number of lever contacts, (C) latency to first lever contact after CS presentation, (D) probability of approaching the food cup during the 8-s CS period, (E) number of food cup entries during the 8-s CS period, and (F) latency to the first food cup entry after CS presentation.

3.2. Instrumental training

Following Pavlovian training all animals were food restricted and then trained to nose poke for delivery of a food pellet, which was paired with illumination of the nose port (the light-CS). Animals in each group received the same number of response-reinforcer (and light-CS) pairings by requiring them to take a fixed number of pellets each session. During instrumental training there were no group differences in active [$F(1,71) = .002, p = .963$] or inactive responses/session [$F(1,132) = .184, p = .668$], and both groups learned to discriminate between the active and inactive ports (Fig. 2A). However, at all pellet criteria STs responded significantly faster than GTs [effect of group, $F(1,46) = 19.337, p < .001$] (Fig. 2B). Importantly, the group difference in rate of responding was not due to a difference in learning the instrumental task, as indicated

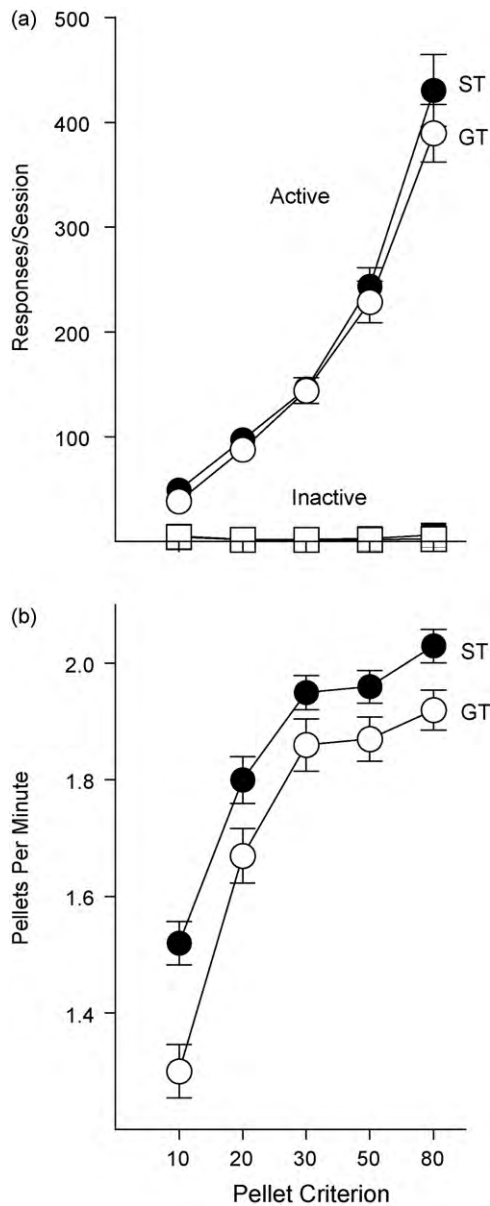


Fig. 2. Acquisition of instrumental responding (nose poke) for a food reward in sign-trackers ($n=24$) and goal-trackers ($n=24$) trained using a FR1 (20s time out) schedule of reinforcement. (A) Mean \pm SEM number of active and inactive responses/session across days of testing, in which the criterion number of pellets required was progressively increased, until animals were responding reliably for 80 pellets/day. (B) Mean \pm SEM number of pellets earned/minute.

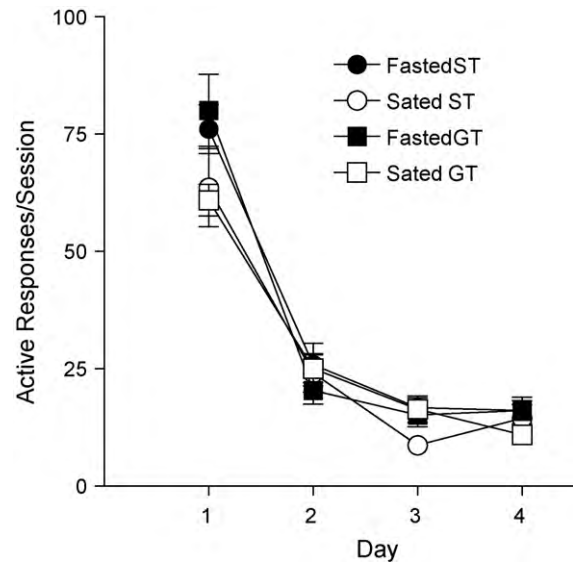


Fig. 3. Mean \pm SEM number of active responses/session during 4 days of extinction training in fasted sign-trackers ($n=14$) and goal-trackers ($n=13$) and sated sign-trackers ($n=10$) and goal-trackers ($n=11$).

by a non-significant group-by-criteria interaction [$F(4,46) = 1.775, p = .150$]. Thus, STs and GTs learned the instrumental task at the same rate, but STs completed their sessions faster, suggesting that STs were more motivated to work for either the food reward, its associated cue (the nose port light-CS), or both

3.3. Extinction and reinstatement

Following instrumental training, the ST and GT groups were divided into fasted and sated sub-groups as described above, and then underwent extinction training. During extinction there were no group differences (ST, GT, fasted, sated), and all four groups extinguished to the same low level of responding [effect of group, $F(3,44) = 1.096, p = .361$; effect of session, $F(3,44) = 133.75, p < .001$; group-by-day interaction, $F(9,44) = 1.963, p = .067$] (Fig. 3). Following extinction all animals were tested for the ability of contingent presentation of the food cue (nose port light-CS) to reinstate responding. Sated STs significantly reinstated responding for the

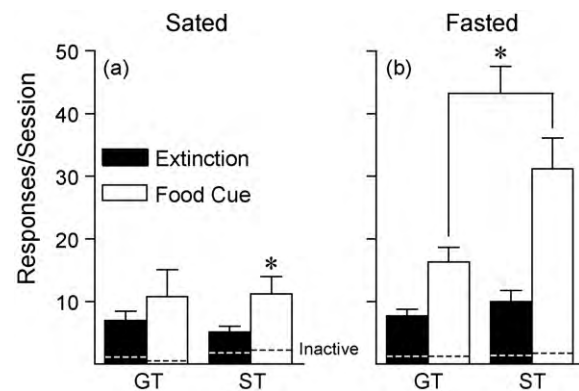


Fig. 4. Cue-induced reinstatement test. During the 40-min reinstatement test session responses (nose pokes) into the active port resulted in presentation of the cue previously paired with food delivery during instrumental training (the nose port light-CS), but no food reward. Mean \pm SEM number of active responses in (A) sated sign-trackers (ST) and goal-trackers (GT), and (B) fasted STs and GTs after extinction (black bars) and during the reinstatement test (white bars). The dashed lines indicate the mean number of responses in the inactive port. Significant difference, $*p < .05$.

cue alone, relative to extinction levels [paired $t(9) = .035$], whereas sated GTs did not [paired $t(10) = -.325$]. However, in sated animals, there were no significant differences between STs and GTs in the degree of reinstatement, as indicated by a non-significant phase-by-group interaction [$F(1,19) = .254, p = .620$] (Fig. 4A). Under fasted conditions both STs and GTs reinstated food-seeking behavior (Fig. 4B). However, fasted STs showed significantly more robust reinstatement than fasted GTs, as indicated by a significant group-by-phase interaction [$F(1,25) = 5.135, p = .032$; effect of group, $F(1,25) = 7.433, p = .012$; effect of phase, $F(1,25) = 28.784, p < .001$]. In addition, hunger did not significantly increase responding in GTs [interaction, $F(1,22) = 1.235, p = .279$], but did significantly increase responding in STs [interaction, $F(1,22) = 6.096, p = .022$].

4. Discussion

Cues associated with a primary reward can acquire the ability to act as conditional stimuli (CSs), evoking conditional responses (CRs), but they do not necessarily also acquire incentive motivational properties, and thus the ability to act as incentive stimuli [14]. In fact, there is considerable individual variation in the extent to which a CS acquires two properties of an incentive stimulus – the ability to attract and the ability to serve as a conditional reinforcer [6,14]. In the present study, we asked whether there is similar individual variation in the ability of a food cue to reinstate instrumental responding after extinction of the response, and whether this is modulated by physiological state (hunger). When they were sated a food cue was not very effective in reinstating responding in either animals previously shown to be attracted to a food cue (STs) or in animals not attracted to a food cue (GTs). When they were fasted, a food cue reinstated responding in both STs and GTs, however, it was significantly more effective in reinstating responding in STs than GTs, and hunger significantly increased reinstatement in STs but not GTs. Finally, during instrumental training STs appeared more motivated to obtain the food reward (and its associated cue), because they worked at a faster rate than GTs.

Traditionally, the initiation of appetite and feeding was considered a homeostatic response to energy depletion – internal feedback signals, such as decreases in glucose levels and fat stores drive an organism to seek out and consume food to reestablish energy balance [11,16,20]. Although physiological signals may play a direct role in initiating feeding, it is now well established that organisms eat not only in response to energy depletion but also in response to other stimuli, such as cues in the environment previously associated with food availability and/or consumption [13,16,19]. Indeed, cues associated with food delivery can even evoke conditioned physiological responses, such as anticipatory increases in blood insulin levels [21]. Furthermore, physiological state often modulates behavior not directly, but by altering the motivational properties of cues [1,18]. For example, the incentive value of a food cue changes depending on whether the animal is in a food deprived or sated state. The important finding here is that hunger amplified the incentive value of the food cue in STs to a greater extent than in GTs, as indicated by two observations: (1) fasted STs showed significantly more robust reinstatement than fasted GTs, and (2) hunger significantly increased reinstatement responding in STs but had no significant effect in GTs (i.e., there was no difference in active responses in fasted vs. sated GTs). Thus, there appears to be individual variation in the degree to which physiological state modulates the incentive motivational properties of a food cue.

In the present study the food cue did not produce robust reinstatement in sated animals (although there was a small effect in STs). There are, however, reports that a food cue can evoke eating in sated individuals [13,19]. The reason for this apparent discrepancy

may be because in this study animals were tested under extinction conditions, where no food was available, and in previous studies animals were allowed to consume food following cue presentation. Thus, properties of the food itself may be important for eliciting reinstatement in sated animals, as a small food prime itself is able to elicit robust reinstatement of food-seeking behavior in sated animals [4].

It is important to emphasize that the differences reported here between STs and GTs are not due to differences in the number of rewards earned or the number of response-reinforcer/light-CS pairings. We utilized a procedure in which these variables were held constant across groups by requiring all rats to earn a fixed number of pellets each day, ensuring that they received the same number of rewards and light-CS presentations. Indeed, there were no differences in the rate with which STs and GTs learned the instrumental response to obtain food, and thus group differences in cue reinstatement are not attributable to differences in the strength of the associations learned during instrumental training. However, it is interesting that STs and GTs did differ in how quickly they completed their sessions – their rate of responding. This may reflect an intrinsic difference in motivation for the food reward itself, or it could also be that during instrumental training the cue associated with food delivery facilitated responding to a greater degree in STs than GTs. We cannot differentiate these two possibilities in the present experiment.

In conclusion, we show that a food cue is more effective in reinstating instrumental responding following extinction in rats that are prone to attribute incentive salience to reward-related cues, and that hunger amplifies these differences. Variation in the propensity to attribute incentive salience to food cues may be important in studying individual variation in the propensity to develop eating disorders and obesity. For example, it has been shown that obese individuals are less sensitive to internal physiological cues that trigger eating [17] and more responsive to external environmental cues [15]. It is possible, therefore, that obese individuals attribute excessive incentive salience to food cues and these cues then come to control their behavior in maladaptive ways. Of course, studies in humans that have revealed differences in the ability of food cues to instigate desire and eating in obese vs. non-obese individuals [15] are all conducted “after the fact”, so to speak, and it would be desirable to be able to predict earlier which individuals may find it difficult to resist food cues, and why this might be. Thus, further studies on variation in the propensity to attribute incentive salience to food cues, and the neurobiological basis of this predisposition [7], may prove informative in this regard.

Acknowledgements

This research was supported by a grant from the National Institute on Drug Abuse to TER (R37 DA04294). We thank Kristin Pickup for technical assistance and Dr. Kent Berridge for helpful comments on an earlier version of the manuscript. This paper is dedicated to Dr. Bryan Kolb, in celebration of his many contributions to the field of behavioral neuroscience.

References

- Berridge KC. Reward learning: reinforcement, incentives, and expectations. In: Medin DL, editor. Psychology of learning and motivation, vol. 40. New York: Academic Press; 2001. p. 223–78.
- Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev* 2002;26:321–52.
- Cornell CE, Rodin J, Weingarten H. Stimulus-induced eating when satiated. *Physiol Behav* 1989;45:695–704.
- Eiseler LA. Effects of food primes on operant-behavior of non-deprived rats. *Anim Learn Behav* 1978;6:308–12.
- Fedoroff IC, Polivy J, Herman CP. The effect of pre-exposure to food cues on the eating behavior of restrained and unrestrained eaters. *Appetite* 1997;28:33–47.

- [6] Fligel SB, Akil H, Robinson TE. Individual differences in the attribution of incentive salience to reward-related cues: implications for addiction. *Neuropharmacology* 2009;56:139–48.
- [7] Fligel SB, Robinson TE, Clark JJ, Clinton SM, Watson SJ, Seeman P, et al. An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: implications for addiction. *Neuropsychopharmacology* 2010;35:388–400.
- [8] Fligel SB, Watson SJ, Robinson TE, Akil H. Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology (Berl)* 2007;191:599–607.
- [9] Johnson WG. Effect of cue prominence and subject weight on human food-directed performance. *J Pers Soc Psychol* 1974;29:843–8.
- [10] Lovibond PF. Facilitation of instrumental behavior by a pavlovian appetitive conditioned-stimulus. *J Exp Psycho Anim Behav Process* 1983;9:225–47.
- [11] Mayer J. Glucostatic mechanism of regulation of food intake. *N Engl J Med* 1953;249:13–6.
- [12] Nair SG, Adams-Deutsch T, Epstein DH, Shaham Y. The neuropharmacology of relapse to food seeking: methodology, main findings, and comparison with relapse to drug seeking. *Prog Neurobiol* 2009;89:18–45.
- [13] Petrovich GD, Ross CA, Gallagher M, Holland PC. Learned contextual cue potentiates eating in rats. *Physiol Behav* 2007;90:362–7.
- [14] Robinson TE, Fligel SB. Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. *Biol Psychiatry* 2009;65:869–73.
- [15] Schachter S. Obesity and eating. Internal and external cues differentially affect eating behavior of obese and normal subjects. *Science* 1968;161:751–6.
- [16] Shin AC, Zheng HY, Berthoud HR. An expanded view of energy homeostasis: neural integration of metabolic, cognitive, and emotional drives to eat. *Physiol Behav* 2009;97:572–80.
- [17] Stunkard A, Koch C. Interpretation of gastric-motility. I. Apparent bias in reports of hunger by obese persons. *Arch Gen Psychiatry* 1964;11:74–82.
- [18] Toates FM. Motivational systems. New York: Cambridge University Press; 1986.
- [19] Weingarten HP. Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science* 1983;220:431–3.
- [20] Woods SC, Seeley RJ. Adiposity signals and the control of energy homeostasis. *Nutrition* 2000;16:894–902.
- [21] Woods SC, Vasselli JR, Kaestner E, Szakmary GA, Milburn P, Vitiello MV. Conditioned insulin-secretion and meal feeding in rats. *J Comp Physiol Psychol* 1977;91:128–33.