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Leptin reverses sucrose-conditioned place preference in food-restricted rats

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Abstract

Previous studies have suggested that food restriction can modify performance in the conditioned place preference (CPP) paradigm. In the present study, we tested the hypotheses that food restriction would enhance the development of a CPP to low-calorie sucrose pellets and that peripheral leptin replacement in food-restricted animals would reverse this effect. Using a range of 45-mg sucrose pellets (0–15 pellets) as a reward, we observed that a significant place preference was conditioned in food-restricted, but not ad libitum-fed rats. This CPP was reversed either by treatment of food-restricted rats with the dopamine receptor antagonist α-flupenthixol (200 μg/kg ip) during the training protocol or by chronic subcutaneous replacement of leptin (125 μg/kg/day) that attenuated the food restriction-induced decrease of circulating leptin. We conclude that dopaminergic signaling and the fall of plasma leptin concentrations contribute to the CPP of food-restricted rats. This finding suggests that in addition to metabolic adaptations, hypoleptinemia results in behavioral adaptations during states of energy deprivation.

Keywords: Reward; Dopamine; Leptin; Place preference

1. Introduction

The conditioned place preference (CPP) is a behavioral paradigm that assesses the reward value of stimuli [1,2]. In this task, animals are trained to receive a rewarding stimulus on one side of a two-compartment chamber that has unique physical properties relative to the other side, where animals receive no stimulus or a control treatment. In a test situation, the animals are able to move freely between both compartments and will choose to spend more time on the side previously associated with the rewarding stimulus. Recently, it has been reported that food deprivation increased the CPP to cocaine in rats [3]. Although food has been shown previously to condition a place preference, and the food-deprivation state results in a more robust CPP [4–7], previous experimental paradigms have allowed rats ad libitum access to the conditioning food. This study design does not allow for evaluation of the mechanism(s) underlying the enhanced CPP of food restriction, since food-restricted rats will eat more during the training session than ad libitum-fed rats. This might result in differences in postingestive cues (e.g., hormonal responses) and, hence, the interoceptive states, of ad libitum vs. food-restricted rats. For example, Wilson et al. [8] have shown that the stimulation of DA release in response to ad libitum intake of the palatable liquid diet Sustacal was greater than DA release to a small amount of Sustacal in food-restricted rats. For many stimuli, including the stimulus of food, the conditioning of the place preference is dependent on intact DA transmission, as administration of a DA receptor antagonist during the training sessions blocks development of the CPP. Since CPP development to food reward is DA-dependent, it would seem important to keep the food stimulus quantitatively identical between ad libitum and food-restricted rats, and also to limit postingestional cues.

In the present study, we evaluated the ability of a small number of low-calorie sucrose reward pellets (0.15 kcal/pellet) to condition a place preference in free-feeding vs.

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food-restricted rats. We hypothesized that food restriction should shift the dose–response curve for sucrose-induced CPP to the left, in the food-restricted rats. Food restriction and deprivation are associated with the endocrine hallmark of low circulating insulin and leptin levels (e.g., Ref. [9]). Based on previous studies from our laboratory demonstrating that insulin can modulate the clearance of DA [10] and can interact with the postsynaptic DA receptor antagonist raclopride to decrease short-term intake of palatable sucrose solutions [11], we have suggested that insulin modulates performance in behavioral paradigms of reward and motivation (see Ref. [12] for discussion). However, the potential role of leptin in modulating performance in such paradigms has not been extensively evaluated. To date, only one observation has been made: that intraventricular leptin may reverse the decreased threshold frequency for self-stimulation in food restriction-sensitive LH sites [13]. Thus, in the present study, we further tested whether leptin replacement in the food-restricted animals results in a reversal of the CPP development of the food-restricted rats.

2. Methods

2.1. Subjects and experimental procedures

Experimental subjects were male albino rats (Simonsen, approximately 300–350 g), individually housed. After acclimation to the facility, rats received two habituation sessions with the sucrose pellets on consecutive days in their home cages (Days −3 and −2). They were weight-matched and assigned to specific experimental groups (vide infra). All procedures were approved by the Animal Studies Subcommittee of the VA Puget Sound Health Care System Research and Development Committee. See Table 1 for the experimental time line.

2.1.1. Experiment 1

The study design was based upon that of Lepore et al. [7]. Rats were divided into an ad libitum-fed group and a group restricted to 15 g chow/day; this represents approximately 50% of the normal chow intake for this age and strain of rats in our laboratory. This food restriction paradigm was begun the day following the sucrose habituation, continued for 2 days prior to CPP training (Days −1 and 0) and then was maintained throughout the remainder of the experiment (Days 1 through 7). The first experiment was run on three cohorts of rats, with a '0 sucrose pellet' condition being included in each separate run-through, along with pellet 'doses' of 5, 10, or 15. Final *n*s were: 0/ad libitum, $n=17$; 0/restricted, $n=15$; 5/ad libitum, $n=8$; 5/restricted, $n=7$; 10/ad libitum, $n=17$; 10/restricted, $n=18$; 15/ad libitum, $n=7$; and 15/restricted, $n=7$.

2.1.2. Experiment 2

All rats were food-restricted the day following sucrose habituation for 2 days (Days −1 and 0) prior to CPP training. On Day 0, the animals were divided into two weight-matched groups and then implanted with subcutaneous 7-day Alzet osmotic minipumps (Model 1007 D, Palo Alto, CA) for infusion of either 0.9% saline (Abbott Labs, North Chicago, IL) ($n=15$) or recombinant rat leptin (Sigma/RBI, St. Louis, MO) ($n=15$) in sterile saline at a dose estimated to provide replacement (125 μg/kg/day) [14].

2.1.3. Experiment 3

To confirm the role of dopaminergic activation in the CPP development of food-restricted rats, a third group of food-restricted rats received intraperitoneal injections of the general DA receptor antagonist, α-flupenthixol (Sigma/RBI), 200 μg/kg, 30 min prior to training sessions with sucrose ($n=8$) or intraperitoneal saline prior to no-reward sessions ($n=7$). This drug and dose were selected based on previous demonstrations of its efficacy to block CPP development and operant responding for sucrose [4,15].

2.2. CPP training paradigm

45-mg Noyes (PJ Noyes, Lancaster, NH) sucrose 'reward' pellets were used during both the sucrose habitu-

Table 1
General experimental timeline

Day −3	Day −2	Day −1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Sucrose delivery system habituation (home cage)	Sucrose delivery system habituation (home cage)	Experiment 1: off or first day of food restriction	Experiment 1: off or second day of food restriction	CPP training day 1	CPP training day 2	CPP training day 3	CPP training day 4	CPP training day 5	CPP training day 6	test day
		Experiment 2: first day of food restriction	Experiment 2: minipumps implanted second day of food restriction							Experiment 2: blood collected
		Experiment 3: first day of food restriction	Experiment 3: second day of food restriction							

ation and CPP training days. On alternating days of the 6-day training period, rats were placed on alternating sides of a two-compartment CPP apparatus (35 cm/side with a removable 10-cm divider between the sides) for a 30-min training session. The two sides were distinguished by white vs. black walls and by flooring texture. On one side, rats received a specific dose of the sucrose pellets and on the other side rats received no treatment. Both the side of the apparatus (white or black) and the days of conditioning (Days 1, 3, 5 vs. 2, 4, 6) for the sucrose pellets were counterbalanced.

2.3. Test

On the test day (Day 7), rats were placed in the apparatus for 15 min with free access to both compartments. Animals were videotaped and scored (by an observer blind to the experimental procedure) for the time spent in the sucrose-paired and the non-sucrose-paired compartment. The location of the rat was determined by his scapulae. Time spent in the area between compartments did not count towards either side. A difference score was then calculated (time spent in sucrose-paired side minus time spent in the non-sucrose-paired side). A positive difference score is indicative of the conditioning of a place preference.

2.4. Plasma leptin measurement

At the end of Experiment 2, immediately following the test session (Day 7), rats were euthanized by guillotine and trunk blood was collected. Plasma leptin was measured with a radioimmunoassay for rat leptin with reagents from Linco Research as previously described [16]. The working range of the assay is 0.5–50 ng/ml. Intra- and interassay coefficients of variation for the assay average 2.4% and 4.8%, respectively, at a leptin concentration of 1.6 ng/ml, and 4.1% and 3.0%, respectively, at a leptin concentration of 3.3 ng/ml. Plasma leptin concentrations in three ad libitum-fed albino rats averaged 2.1 ± 0.1 ng/ml. Plasma leptin concentrations in the food-restricted rats receiving leptin replacement infusion averaged 0.8 ± 0.1 ng/ml, suggesting that the leptin dose did not fully replace leptin to ad libitum-fed conditions. Nonetheless, leptin levels in the food-restricted rats that received leptin were substantially higher than those of food-restricted rats implanted with minipumps containing saline. Circulating plasma leptin concentrations were undetectable in the majority of those rats (< 0.1 ng/ml, $P < .0001$, unpaired t test).

2.5. Statistics

Statistical analysis was performed using Statview (SAS Institute, Cary, NC) software. All data are presented as mean ± S.E.M. and $P < .05$ was considered statistically significant.

2.5.1. Experiment 1

A two-way ANOVA [(control vs. food-restricted) × dose of sucrose pellets] was performed to analyze differences between treatments over varied doses. Post hoc testing using Fisher's PLSD evaluated the differences both between and within treatment groups at specific doses.

2.5.2. Experiment 2

Unpaired t test (control vs. leptin) was performed to analyze differences between the treatment groups for difference scores.

2.5.3. Experiment 3

Unpaired t test (control vs. α-flupenthixol) was performed to analyze the difference between treatments.

3. Results

For all three experiments, all rats ate all the sucrose pellets put in the CPP chamber on their training days. Fig. 1 shows the CPP scores of ad libitum and food-deprived rats as a function of the 'dose' of sucrose pellets. Comparison between ad libitum-fed and food-restricted rats revealed a significant overall effect of sucrose 'dose' [$F(3,88) = 6.205$, $P = .0007$] and metabolic status [$F(1,88) = 5.941$, $P = .017$] and a borderline significance for interaction between 'dose' and metabolic status [$F(3,88) = 2.702$, $P = .0504$]. Post hoc testing revealed that none of the 'doses' of sucrose pellets (0, 5, 10, or 15) was able to condition a place preference in the ad libitum-fed rats. In contrast, food-restricted rats developed a significant CPP ($P < .001$ vs. '0' sucrose pellets) with 10 or 15 sucrose pellets (difference scores: 69 ± 11 and 59 ± 12, respectively). The difference score for ad libitum-fed rats at a 'dose' of 15 sucrose pellets (31 ± 9) did not differ from that of '0' sucrose pellets (-10 ± 16,

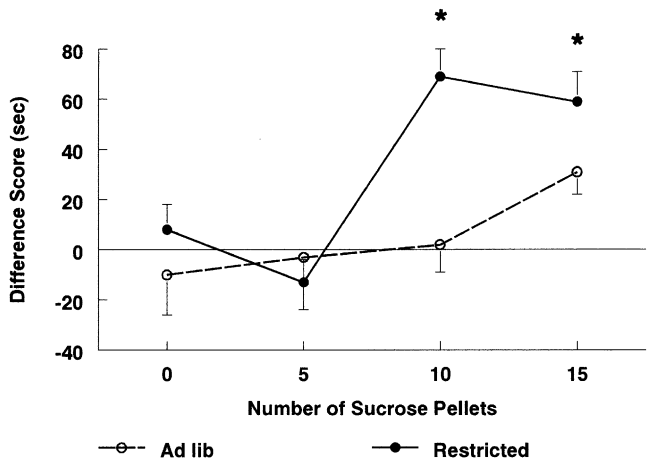

Fig. 1. Effect of food restriction and low-calorie sucrose pellets on development of CPP. See Methods for 'n's at each dose. * $P < .001$ for difference scores (vs. '0') at 10 and 15 sucrose pellets in the food-restricted rats.

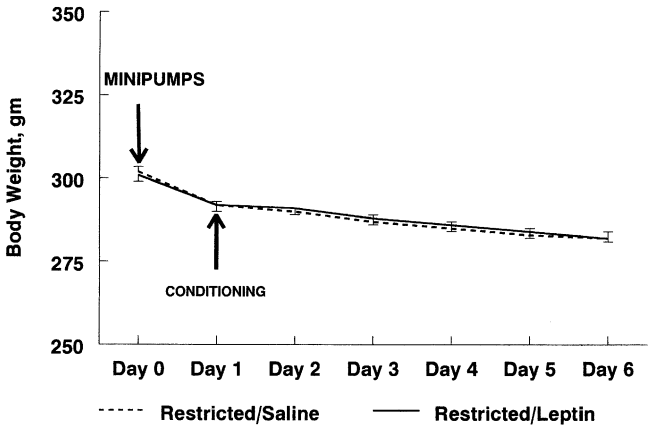

Fig. 2. Body weight in food-restricted rats receiving minipump infusions of saline ($n=15$) or 125 μg/kg/day recombinant rat leptin ($n=15$).

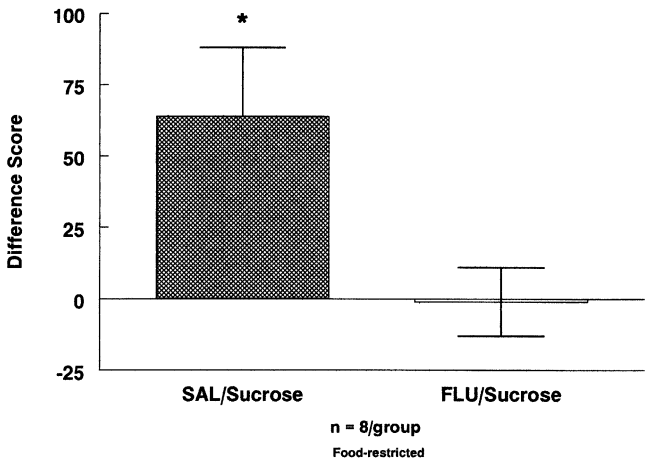

Fig. 4. Effect of α-flupenthixol (200 μg/kg ip) to inhibit place preference conditioned with 10 sucrose pellets in food-restricted rats. * $P=.034$ for difference score (vs. '0').

$P=.1009$); and this score was lower (approaching statistical significance, $P=.0685$) in comparison to the score of the food-restricted rats at the 'dose' of 15 sucrose pellets. Thus, consistent with our hypothesis, food restriction enhanced the ability of a low-calorie but highly palatable reward to condition a place preference, in a paradigm that was ineffective in ad libitum-fed rats.

In Experiment 2, food-restricted rats received subcutaneous minipump infusions of either sterile saline or recombinant rat leptin. Fig. 2 shows the trajectory of body weights in the two groups of rats: as would be expected, the leptin infusion had no suppressive effect on body weight in rats that were already 50–60% food-restricted. However, as shown in Fig. 3, the leptin replacement had a significant effect on the place preference conditioned by a dose of 10 sucrose pellets. Comparable to what was observed in Experiment 1, a significant CPP was observed in the saline-infused rats (difference score: 59 ± 17, $P=.0004$). Rats

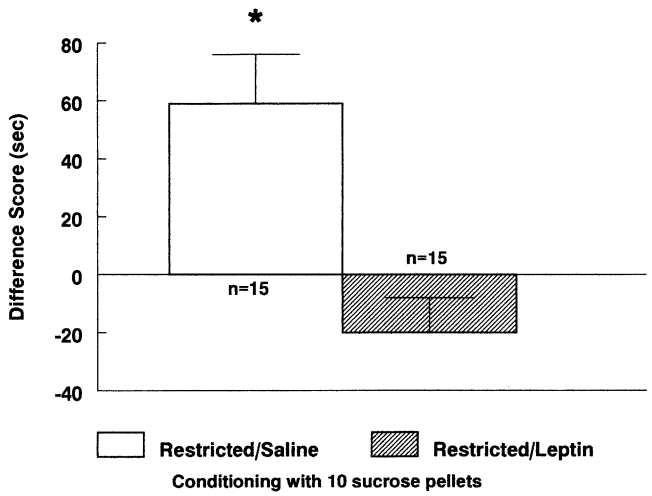

Fig. 3. Effect of peripheral leptin replacement to inhibit place preference conditioned with 10 sucrose pellets in food-restricted rats. * $P=.0004$ for difference score (vs. '0').

receiving leptin infusion did not demonstrate a CPP (difference score: -20 ± 12). This effect was highly significant vs. the saline-infused rats (unpaired t test, $P=.0009$). This result suggests that the low leptin level of food-restricted rats directly contributed to the place preference conditioned by the sucrose pellets.

Finally, in Experiment 3, we verified that the development of a CPP to the sucrose pellets in food-restricted rats was dependent upon dopaminergic activity. As mentioned above, rats in both groups of this experiment (saline- or α-flupenthixol-treated) ate all 10 of the sucrose pellets available to them on their three training days. Nonetheless, rats treated with the DA receptor antagonist α-flupenthixol did not develop a CPP (difference score: -1 ± 12), whereas the saline-treated rats developed a significant CPP (difference score: 64 ± 14, $P=.034$) that did not differ from the 10-pellet CPP score of the rats in Experiment 1 (Fig. 4). The effect of α-flupenthixol was statistically significant (saline- vs. α-flupenthixol-treated rats, unpaired t test, $P=.036$). A control group of food-restricted rats received α-flupenthixol but '0 sucrose pellets' during CPP training and did not develop a CPP or aversion (difference score: -23 ± 23, $n=8$).

4. Discussion

The main finding of the present study is that peripheral leptin replacement that attenuates the decline of leptin during a period of food restriction reverses the CPP to a limited amount of a highly palatable food. This response to peripheral leptin administration is presumably mediated by a central action of leptin since leptin gains access to its targets in the CNS across the blood–brain barrier [17,18]. Because in our specific paradigm — which was designed to differentiate between ad libitum-fed and food-restricted rats — the ad libitum-fed animals did not demonstrate a CPP, we could not test whether further elevation of leptin levels in

free-feeding rats could also constrain a CPP. Leptin levels in food-restricted rats were only replaced to approximately 40% of normal physiological concentrations, yet this level of leptin replacement was sufficient to prevent the CPP. This observation suggests that a critical component of the food-restricted condition that allows the manifestation of a CPP is a marked decrease of circulating leptin and therefore delivery of leptin to the CNS. Our study design does not allow us to differentiate between mechanistic contributions of altered learning during the training days, or altered performance motivation on the test day; it is possible that both may contribute. Increased performance in a runway task for food reward occurs with greater degrees of food restriction [19] and Spyraki et al. [20] demonstrated a place preference to food reward in food-deprived (but not nondeprived) rats that were only deprived during training, but not on the test day. The development of a CPP is a form of associative learning about environmental cues that signal the presence of a food reward and can be considered an adaptive behavioral response to food restriction. Thus, the results of this study demonstrate that the decrease of plasma leptin during chronic food restriction has an important role in behavioral adaptation to energy restriction as has previously been suggested for neuroendocrine and metabolic adaptations [9,21,22].

In contrast to previous studies in which a place preference was conditioned during ad libitum access to food, we chose sucrose pellets with low caloric content, 0.15 kcal/pellet. Consumption of 10 pellets would provide a total caloric value of 1.5 kcal. This represents a very minor proportion of energy intake in both the ad libitum-fed and food-restricted rats' daily caloric intake (about 1.25% and 2.5%, respectively). All of the food-restricted rats ate all of their chow every day, despite the fact that some of them had three training days with the sucrose pellets. Because a CPP could be induced with as few as 10 pellets in food-restricted, but not ad libitum-fed rats, these results suggest that the physicochemical attributes of the pellets per se are not responsible for the CPP, but that they interact with the rats' altered internal milieu to become effective in training the rats. Not surprisingly, our third experiment indicates that dopaminergic activity was critically required for the development of the CPP. The microdialysis study of Wilson et al. [8] demonstrated that striatal interstitial DA levels, in response to a food stimulus, are elevated in food-restricted (vs. nonrestricted) rats trained to expect a food reward. The present study suggests that if there are food restriction-related changes of dopaminergic signaling, they are substantive enough to have behavioral consequences.

The recent study of Fulton et al. [13] demonstrated that intraventricular leptin administration could reverse the leftward shift of the frequency/reward curves for self-stimulation in food restriction-sensitive lateral hypothalamic sites. Our study adds further support to the conclusion of Fulton et al., that one way in which leptin may act to decrease food intake is to decrease the reward value of food. Inasmuch as the CPP paradigm assesses reward, this appears to be true. Neither of these studies directly evaluates whether leptin can decrease activation of midbrain DA neurons (or DA signaling), perhaps specifically to food reward. It is possible, for instance, that the effects of hypoleptinemia and altered DA activity are convergent. The report of leptin receptor mRNA expression in the substantia nigra pars compacta [23] — a major nucleus of midbrain DA neurons — together with these behavioral studies provides a rationale for further investigation into the possible relationship between physiological concentrations of leptin and midbrain DA neuronal activity.

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