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Effects of insulin and leptin in the ventral tegmental area and arcuate hypothalamic nucleus on food intake and brain reward function in female rats

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Abstract

There is evidence for a role of insulin and leptin in food intake, but the effects of these adiposity signals on the brain reward system are not well understood. Furthermore, the effects of insulin and leptin on food intake in females are underinvestigated. These studies investigated the role of insulin and leptin in the ventral tegmental area (VTA) and the arcuate hypothalamic nucleus (Arc) on food intake and brain reward function in female rats. The intracranial self-stimulation procedure was used to assess the effects of insulin and leptin on the reward system. Elevations in brain reward thresholds are indicative of a decrease in brain reward function. The bilateral administration of leptin into the VTA (15–500 ng/side) or Arc (15–150 ng/side) decreased food intake for 72 h. The infusion of leptin into the VTA or Arc resulted in weight loss during the first 48 (VTA) or 24 h (Arc) after the infusions. The administration of insulin (0.005–5 mU/side) into the VTA or Arc decreased food intake for 24 h but did not affect body weights. The bilateral administration of low, but not high, doses of leptin (15 ng/side) or insulin (0.005 mU/side) into the VTA elevated brain reward thresholds. Neither insulin nor leptin in the Arc affected brain reward thresholds. These studies suggest that a small increase in leptin or insulin levels in the VTA leads to a decrease in brain reward function. A relatively large increase in insulin or leptin levels in the VTA or Arc decreases food intake.

Keywords

leptin; insulin; food; reward; arcuate nucleus; ventral tegmental area

1. Introduction

During the last decades, there has been a gradual worldwide increase in the prevalence of overweight and obesity in humans [1]. In 2007–2008, the prevalence of obesity was 33.8% among adults in the United States. The prevalence of obesity was 32.2% among males and 35.5% among females [2]. The obesity rate is higher among females than among males but the differences in obesity rates between males and females vary somewhat between racial, ethnic, and age groups [2].

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Adiposity signals play a pivotal role in the regulation of food intake. Adiposity signals have been defined as compounds that are secreted in proportion to body fat mass, can cross the blood brain barrier, and have predictable effects on food intake and body weight [3,4]. The pancreatic hormone insulin and the fat-derived hormone leptin fulfill the criteria for adiposity signal [4]. Leptin receptors have been detected in hypothalamic nuclei that are involved in food intake, including the arcuate hypothalamic nucleus (Arc) [5,6]. Leptin receptors are also located on dopaminergic neurons in the ventral tegmental area (VTA)[7]. Insulin receptors have been detected in a wide range of brain areas including the Arc and VTA [7,8,9]. Extensive evidence points toward an important role for insulin and leptin in the regulation of food intake. Insulin decreases food intake and low insulin levels lead to an increase in food intake [10,11,12]. Strong evidence for a role of leptin in food intake has been provided by the observation that leptin deficient mice (ob/ob mice) or rats with a dysfunctional leptin receptor (fa/fa, Zucker rat) are hyperphagic and obese [13,14]. In addition, leptin decreases food intake in rats [15,16].

Several studies suggest that insulin in the Arc and VTA affect food intake. Food deprivation leads to increased neuropeptide Y (NPY) mRNA levels in the Arc and this is prevented by the administration of insulin [17,18]. NPY projections from the Arc to the paraventricular hypothalamic nucleus play an important role in stimulating food intake [19,20]. Blockade of the production of insulin receptors in the Arc with antisense oligodeoxynucleotides causes hyperphagia and increases fat mass in rats [21]. Figlewicz and colleagues showed that the administration of insulin into the Arc decreases the self-administration of a sucrose solution [22]. The same study also provided evidence for a role of insulin receptors in the VTA in the intake of palatable foods. It was shown that the stimulation of mu-opioid receptors in the VTA stimulates sucrose self-administration and this was blocked by the co-administration of insulin [22]. Some studies suggest that leptin in the Arc and the VTA play a role in food intake in rats. The administration of leptin into the Arc and VTA decreases food intake in rats [23,24]. Furthermore, knockdown of the leptin receptor in the VTA increases food intake [24] and increases responding for sucrose pellets under a progressive ratio schedule of reinforcement [25].

Food intake is controlled by homeostatic and hedonic mechanisms [26]. The main function of the homeostatic mechanisms is to ensure that there is a balance between anabolic and catabolic influences [3]. Hedonic foods with a high fat content or high glycemic index can override the homeostatic mechanisms, which can lead to the consumption of excessive amounts of food and body weight gain [27,28]. Studies with animal models suggest that insulin and leptin affect the rewarding aspects of food. In animal studies, reward is often defined as an event (food, drugs of abuse, sex, etc.) that increases the probability of a given response [29,30]. Furthermore, pairing of the rewarding event with a specific environment leads to the development of a preference for that environment [30]. The administration of insulin and leptin prevents high-fat food-induced conditioned place preference and decreases operant responding for a sucrose solution [31,32]. The effects of food deprivation and the administration of insulin and leptin on the brain reward system have been investigated with a rate-frequency intracranial self-stimulation procedure (ICSS). A lowering of the rate-frequency reward threshold has been interpreted as potentiation of brain reward function and vice versa [33]. Chronic food deprivation lowers the rate-frequency reward threshold and this effect can be reversed by chronic icv administration of insulin [33]. The same chronic insulin treatment protocol also increases the rate-frequency reward thresholds of ad libitum fed control rats [33]. Leptin increases the rate-frequency reward thresholds in a subgroup of rats in which food deprivation decreases the rate-frequency reward thresholds [34]. Overall, these findings suggest that food deprivation potentiates brain reward function as assessed with the rate-frequency ICSS method and insulin and leptin decrease brain reward function.

These studies indicate that significant progress has been made towards understanding the role of adiposity signals in the regulation of food take. However, many questions remain unanswered. For example, despite the fact that obesity is more prevalent among females than among males, almost all obesity and food intake studies have been conducted with male animal models. It is critical to investigate the effects of leptin on food intake in female animal models because accumulating evidence suggests that there are differences in leptin levels and leptin sensitivity between males and females [35,36]. Furthermore, previous studies have not investigated whether the administration of insulin into the VTA or Arc affect the intake of regular lab chow. The aim of the present studies was to investigate the role of insulin and leptin in the VTA and Arc in food intake and brain reward function in female rats. The first set of experiments investigated the effects of leptin and insulin in the VTA and Arc on the consumption of regular lab chow in *ad libitum* fed female rats. The second set of experiments investigated the effects of leptin and insulin in the VTA and Arc on brain reward function in female rats. The state of the brain reward system was assessed with a rate-independent discrete-trial ICSS procedure. This ICSS method has been used extensively by our group and other groups to study the rewarding effects of drugs of abuse and drug withdrawal on the brain reward system [37,38,39].

2. Material and Methods

2.1. Animals

Female Sprague Dawley rats (Harlan labs, Prattville, AL) weighing 175–200 g at the beginning of the experiments were used. Animals were housed in a temperature- and humidity-controlled vivarium and maintained on a 12 h light-dark cycle (lights off at 9 AM). The animals were group housed (two per cage) for the ICSS studies and single housed for the studies in which food intake was recorded. All testing occurred at the beginning of the dark cycle. Food and water were available *ad libitum* in the home cages. All subjects were treated in accordance with the National Institutes of Health guidelines regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the University of Florida Institutional Animal Care and Use Committee.

2.2. Drugs

Leptin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and insulin, 100 U/ml, was purchased from Henry Schein (Melville, NY, USA). Insulin was diluted with distilled water. Leptin was dissolved in 10 mM NaOH and after the leptin was dissolved the pH was titrated from about 2 to 7.3 with 10 mM NaOH. Distilled water was used to obtain the final leptin concentrations.

2.3. Apparatus

The experimental apparatus consisted of twelve Plexiglas chambers (30.5 × 30 × 17 cm; Med Associates, Georgia, VT, USA), each housed in a sound-attenuating melamine chambers (Med Associates, Georgia, VT, USA). The operant chambers consisted of a metal grid floor and a metal wheel (5 cm wide) centered on a sidewall. A photobeam detector was attached next to the response wheel and recorded every 90 degrees of rotation. Brain stimulation was delivered using constant current stimulators (Model 1200C, Stimtek, Acton, MA, USA). Subjects were connected to the stimulation circuit through bipolar leads (Plastics One, Roanoke, VA, USA) attached to gold-contact swivel commutators (model SL2C Plastics One, Roanoke, VA, USA). A computer controlled the stimulation parameters, data collection, and all test session functions.

2.4. Surgical Procedures

Cannulae and electrode implantations: At the beginning of all the intracranial surgeries, the rats were anesthetized with an isoflurane/oxygen vapor mixture (1–3% isoflurane) and placed in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set 3.3 mm below the interaural line (flat skull). The rats were prepared with 11 mm stainless steel 23 gauge cannulae above the VTA or Arc using flat skull coordinates according to Paxinos and Watson, 1998. Bilateral cannulae were implanted 2.5 mm above the VTA (anterior-posterior [AP] -5.3 , medial lateral [ML] ± 1.0 mm, dorsal-ventral [DV] -5.2 from dura) or 2.5 mm above the Arc (AP -3.8 mm, ML ± 0.5 , DV -6.8 from dura). At the end of the surgery, 11 mm removable 30 gauge wire stylets were inserted in the cannulae to maintain patency. For the electrode implantations, the incisor bar was set 5 mm above the interaural line. The rats were prepared with stainless steel bipolar electrodes (model MS303/2 Plastics One, Roanoke, VA) 11 mm in length in the medial forebrain bundle at the level of the posterior lateral hypothalamus (AP -0.5 mm; ML ± 1.7 mm; DV -8.3 mm from dura). The electrodes and cannulae were permanently secured to the skull using dental cement anchored with four skull screws.

2.5. Intracranial self-stimulation procedure

Rats were trained on a modified discrete-trial ICSS procedure [40], as described previously [39,41]. The subjects were initially trained to turn the wheel on a fixed ratio 1 (FR1) schedule of reinforcement. Each quarter turn of the wheel resulted in the delivery of a 0.5 sec train of 0.1 msec cathodal square-wave pulses at a frequency of 100 Hz. After the successful acquisition of responding for stimulation on this FR1 schedule, defined as 100 reinforcements within 10 minutes, the rats were trained gradually on a discrete-trial current-threshold procedure. Each trial began with the delivery of a non-contingent electrical stimulus, followed by a 7.5 sec response window within which the animal can respond to receive a second contingent stimulus identical in all parameters to the initial non-contingent stimulus. A response during this 7.5 sec response window was labeled a positive response, while the lack of a response was labeled a negative response. During a 2 sec period immediately after a positive response, additional responses had no consequences. The inter-trial interval (ITI) that followed either a positive response or the end of the response window (in the case of a negative response), had an average duration of 10 sec (ranging from 7.5 sec to 12.5 sec). Responses that occurred during the ITI resulted in a further 12.5 sec delay of the onset of the next trial. During training on the discrete-trial procedure, the duration of the ITI and delay periods induced by time-out responses were gradually increased until animals performed consistently at standard test parameters. The subjects were then tested on a current-threshold procedure in which stimulation intensities varied according to the classical psychophysical method of limits. A test session consisted of four alternating series of descending and ascending current intensities starting with a descending series. Blocks of three trials were presented to the subject at a given stimulation intensity, and the intensity was altered systematically between blocks of trials by 5 μ A steps. The initial stimulus intensity was set 30 μ A above the baseline current-threshold for each animal. Each test session typically lasted 30–40 minutes and provided two dependent variables for behavioral assessment: brain reward thresholds and response latencies. *Brain reward thresholds*: The current threshold for a descending series was defined as the midpoint between stimulation intensities that supported responding (i.e., positive responses on at least two of the three trials), and current intensities that failed to support responding (i.e., positive responses on fewer than two of the three trials for two consecutive blocks of trials). The threshold for an ascending series was defined as the midpoint between stimulation intensities that did not support responding and current intensities that supported responding for two consecutive blocks of trials. Thus, four threshold estimates were recorded, and the mean of these values was taken as the threshold for each subject on each test session. In this ICSS procedure,

elevations in brain reward thresholds are indicative of a decrease in brain reward function [38,42]. *Response Latencies*: The time interval between the beginning of the non-contingent stimulus and a positive response was recorded as the response latency. The response latency for each test session was defined as the mean response latency on all trials during which a positive response occurred.

2.6. Intracranial microinjections

Drugs were administered bilaterally into the VTA and Arc by using 30 gauge stainless steel injectors that extended 2.5 mm (length of injector tip was 13.5 mm) beyond the guide cannulae. The injection volume was 0.5 μ l/side and the drug was infused over a 66 second period as described previously [43,44]. The rats were gently retrained by hand during the infusions. The infusion speed was regulated by a Harvard Apparatus syringe pump (model 975) and the pump was equipped with 10 μ l syringes (Model 901 RN; Hamilton, Rena, NE, USA). The syringes were connected to the injectors with Tygon microbore PVC tubing (0.25 mm ID $\times$ 0.76 mm OD). The injectors were left in place for 30 seconds post-injection to allow diffusion from the injector tip. The dummy stylets, 11 mm, were re-inserted immediately after the injectors were removed.

2.7. Histology

The brains were processed as previously described by our group [44,45]. At the end of the experiments the rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, ip). The rats were then perfused via the ascending aorta with 100 ml of physiological saline followed by 150 ml of a 10% phosphate buffered formalin (4% formalin, w/v) solution. The rats were perfused with the intracranial drug injectors in place in order to enhance the visibility of the injection tracts in the brain sections. Brains were postfixed for 24 h in phosphate buffered formalin and then stored in 0.1 M phosphate buffered saline until further processing. Sections were cut on a Leica CM3050 S cryostat (coronal sections of 40 μ m at -15° C) and mounted on Superfrost Plus microscope slides. The sections were stained with cresyl violet. The locations of the guide cannulae and injections sites were verified with a Leica DM2500 light microscope and with reference to a stereotaxic atlas of the rat brain (Figs. 1A–D)[46].

2.8. Experimental design

2.8.1. Experiments 1–4, Effects of leptin and insulin in the VTA and Arc on food intake—Prior to the implantation of the cannulae and during the recovery period the rats were fed a standard rodent diet (7912 Teklad LM-485 Mouse/Rat, Harlan Laboratories, Indianapolis, IN, USA). After the recovery period the rats were fed a wet mash (food/water ratio of 1/1.5) that was prepared by processing a standard rodent diet and water with a blender [47]. The mash prevented spillage and allowed accurate food intake measurements. The wet mash was offered in 60 ml glass jars. Each jar was attached to the cage wall (4 cm above bottom) with a hose clamp and a metal strap. Food intake was measured daily immediately before the onset of the dark cycle (9 AM). The rats were fed the wet mash for 10 days prior to the onset of the leptin and insulin injections. The experiments investigated the effects of leptin in the VTA (Exp. 1, $n = 12$), leptin in the Arc (Exp. 2, $n = 13$), insulin in the VTA (Exp. 3, $n = 13$), and insulin in the Arc (Exp. 4, $n = 13$) on food intake. Leptin (15–500 ng VTA; 15–150 ng Arc; unilateral doses) and insulin (0.005–5 mU VTA and Arc, unilateral doses) were administered according to a Latin square design. The drugs were administered bilaterally immediately before the onset of the dark cycle. The highest doses of insulin (5 mU) and leptin (500 ng) were not included in the Latin square and were administered last to prevent these high doses from affecting subsequent treatments. Food intake was recorded 1, 4, 24, 48, and 72 h after the infusions. The body weights were recorded 24, 48, and 72 h after the injections. Leptin and insulin were administered

according to a Latin-square design and there were at least 96 h between injections. At the end of the experiments the rats received a high dose of pentobarbital. After the rats were fully anesthetized they were perfused and the brains were removed to verify the anatomical localization of the injection sites. Histological verification indicated that all the injector tracts ended within the boundaries of the VTA or Arc and therefore all the rats were included in the statistical analyses.

2.8.2. Experiments 5–8, Effects of leptin and insulin in the VTA and Arc on brain reward function—After recovery from the electrode and cannula implantations, the rats were trained on the ICSS procedure. When stable baseline brain reward thresholds were achieved, defined as less than 10% variation within a 5 day period, the injections with leptin and insulin started. Brain reward thresholds and response latencies were assessed daily throughout the experiment between 9:00 AM and 12:00 noon. Drug naive rats were used in all the experiments. The experiments investigated the effects of leptin in the VTA (Exp. 5, $n = 9$), leptin in the Arc (Exp. 6, $n = 10$), insulin in the VTA (Exp. 7, $n = 10$), and insulin in the Arc (Exp. 8, $n = 9$) on brain reward thresholds and response latencies. Leptin (15–500 ng, unilateral dose) and insulin (0.005–0.5 mU, unilateral dose) were administered bilaterally according to a Latin-square design. The highest dose of leptin (500 ng) was not included in the Latin square and was administered last to prevent this high dose from affecting subsequent treatments. The rats were placed in the ICSS test chambers 30 minutes after the administration of insulin or leptin. The minimum time-interval between the injections was at least 96 h. At the end of the experiments the anatomical localization of the injection sites were verified and they were found to be within the boundaries of the VTA or Arc.

2.9. Statistical analyses

The effects of insulin and leptin in the VTA and the Arc on food intake and changes in body weights were analyzed with one-way repeated-measures analyses of variance (ANOVA) with the dose of insulin or leptin as the within-subjects factor. Because there is a large variation in absolute brain reward thresholds between animals, ICSS parameters (brain reward thresholds and response latencies) were expressed as a percentage of the pre-test day values [48]. Percent changes in ICSS parameters were analyzed using one-way repeated-measures ANOVAs with the dose of insulin or leptin as the within-subjects factor. Newman-Keuls post hoc tests were conducted when the ANOVA revealed statistically significant effects. For all the experiments, the criterion for significance was set at 0.05. The statistical analyses were performed using PASW Statistics 18 for Windows software.

3. Results

3.1. Experiments 1–2: Effects of leptin in the VTA and Arc on food intake

The administration of leptin into the VTA did not affect food intake during the first hour (0–1 h period) after the injection (data not shown). The administration of leptin into the VTA decreased food intake during the 0–4 h period (Fig. 2A; $F_{4,44}=2.94$, $P<0.03$), the 0–24 h period (Fig. 2B; $F_{4,44}=16.27$, $P<0.0001$), the 24–48 h period (Fig. 2C; $F_{4,44}=15.17$, $P<0.0001$), and the 48–72 h period (Fig. 2D; $F_{3,33}=3.14$, $P<0.04$). There was a trend toward a leptin-induced decrease in food intake during the 72–96 h period (data not shown; $F_{3,33}=2.87$, $P<0.051$). The 500 ng dose was not included in statistical analyses for the 48–72 h (Fig. 2D) and the 72–96 h period. The 500 ng dose was administered last and food intake and body weights were not recorded beyond the 48 h time point for this specific dose. The overall ANOVA analysis indicated that leptin decreased food intake over the 0–4 h period but posthoc analysis did not reveal a significant effect of any specific dose of leptin. During the 0–24 h and 24–48 h periods, leptin dose-dependently decreased food intake.

During the 0–24 h period, the 50, 150, and 500 ng doses of leptin decreased food intake and the 500 ng dose was more effective than the 150 ng dose. During the 24–48 h period, the two highest doses, 150 and 500 ng, decreased food intake and the 500 ng dose was more effective than the 150 ng dose at decreasing food intake. These findings indicate that a single injection of leptin in the VTA has a relatively long-term effect on food intake in female rats. The administration of leptin decreased body weight gain during the first 0–24 h (Fig. 3A; $F_{4,44}=6.22$, $P<0.0005$) and 24–48 h period (Fig. 3B; $F_{4,44}=4.51$, $P<0.004$). Posthoc analyses indicated that the 150 and 500 ng doses of leptin decreased body weight gain during the 0–24 h and the 24–48 h periods.

The administration of leptin into the Arc decreased food intake during the 0–24 h period (Fig. 4B; $F_{3,36}=4.94$, $P<0.006$), the 24–48 h period (Fig. 4C; $F_{3,36}=12.16$, $P<0.0001$), and the 48–72 h period (Fig. 4D; $F_{3,36}=3.75$, $P<0.02$). Posthoc analyses indicated that 50 and 150 ng of leptin decreased food intake during the 0–24 h and the 24–48 h period and 150 ng of leptin decreased food intake during the 48–72 h period. The administration of leptin into the Arc did not affect food intake during the 0–1 h period (data not shown) or during the 0–4 h period (Fig. 4A). The administration of leptin into the Arc decreased body weight gain during the 0–24 h period (Fig. 3C; $F_{3,36}=3.69$, $P<0.02$) and did not affect body weights during the 24–48 h period (Fig. 3D). Posthoc analyses indicated that 50 and 150 ng of leptin decreased body weight gain compared to the vehicle group during the 0–24 h period.

3.2. Experiments 3–4: Effects of insulin in the VTA and Arc on food intake

The administration of insulin into the VTA decreased food intake during the 0–24 h period (Fig. 5B; $F_{4,44}=2.94$, $P<0.03$). Posthoc analysis indicated that only the highest dose of insulin, 5 mU, decreased food intake during the 0–24 h period. The administration of insulin into the VTA did not decrease food intake during the 0–1 h period (data not shown), the 0–4 h period (Fig. 5A), or the 24–48 h period (Fig. 5C). The administration of insulin into the VTA did not significantly affect weight gain during the 0–24 h period or during the 24–48 h period (data not shown).

The administration of insulin into the Arc decreased food intake during the 0–24 h period (Fig. 6B; $F_{4,48}=4.83$, $P<0.002$). The administration of insulin into the Arc did not affect food intake during the 0–1 h period (data not shown), the 0–4 h period (Fig. 6A), or the 24–48 h period (Fig. 6C). The administration of insulin into the Arc did not affect body weight gain during the 0–24 h period or the 24–48 h period (data not shown).

3.3. Experiments 5–6: Effects of leptin in the VTA and Arc on brain reward function

The baseline brain reward thresholds and response latencies prior to the onset of the leptin injections into the VTA were 109 ± 13.6 and 3.5 ± 0.3 , respectively. The administration of leptin into the VTA elevated the brain reward thresholds of the rats (Fig. 7A; $F_{4,28}=3.13$, $P<0.03$). Posthoc analysis indicated that only the lowest dose of leptin 15 ng significantly elevated the brain reward thresholds. The administration of leptin into the VTA did not affect the response latencies (Fig. 7B).

The baseline brain reward thresholds and response latencies prior to the onset of the leptin injections into the Arc were 87.6 ± 11.6 and 3.1 ± 0.2 , respectively. The administration of leptin into the Arc did not affect the brain reward thresholds or the response latencies (data not shown).

3.4. Experiments 7–8: Effects of insulin in the VTA and Arc on brain reward function

The baseline brain reward thresholds and response latencies prior to the onset of the insulin injections into the VTA were 117.5 ± 15.8 and 3.1 ± 0.1 , respectively. The administration of

insulin into the VTA elevated the brain reward thresholds of the rats (Fig. 8A; $F_{3,27}=3.59$, $P<0.03$). Posthoc analyses indicated that 0.005 mU of insulin elevated the brain reward thresholds and that higher doses did not affect the brain reward thresholds. The administration of insulin into the VTA did not affect the response latencies (Fig. 8B).

The baseline brain reward thresholds and response latencies prior to the onset of the insulin injections into the Arc were 100.3 ± 12.7 and 3.1 ± 0.2 , respectively. The infusions of insulin into the Arc did not affect the brain reward thresholds or the response latencies (data not shown).

4. Discussion

These studies investigated the role of insulin and leptin in the VTA and Arc in food intake and brain reward function in ad libitum fed female rats. It was shown that the administration of insulin or leptin into the VTA or Arc decreased food intake. The effects of leptin in the VTA and Arc on food intake were of a longer duration than the effects of insulin. Leptin decreased food intake for about 72 h while insulin decreased food intake for 24 h. The ICSS studies indicated that the administration of leptin or insulin into the VTA elevated the brain reward thresholds of the rats. Elevations in brain reward thresholds are indicative of a decrease in brain reward function. It was interesting to note that only the administration of very low doses of insulin or leptin into the VTA elevated the brain reward thresholds. The administration of leptin or insulin into the Arc did not affect brain reward thresholds. The present findings indicate that doses of insulin or leptin that decrease food intake do not affect the state of the brain reward system in female rats. This would suggest that insulin and leptin in the VTA can regulate the state of the brain reward system and food intake independently. These studies also suggest that changes in insulin and leptin levels in the Arc affect food intake but do not affect the state of the brain reward system. It should be noted that the ICSS studies investigated the effects of insulin and leptin on the state of the brain reward system in ad libitum fed rats. The ICSS studies did not investigate the neuronal substrates that signal the rewarding properties of palatable foods. Therefore, these studies do not controvert the hypothesis that insulin and leptin in the VTA and Arc play a role in conveying the hedonic aspects of palatable foods [49].

The first set of experiments investigated the role of leptin in the VTA and Arc in food intake in female rats. It was shown that the administration of leptin into the VTA decreased food intake during the 0–24 h, the 24–48 h, and the 48–72 h period. The administration of leptin into the VTA did not affect food intake during the 0–1 h, 0–4 h, or the 72–96 h period. The administration of leptin into the Arc had the same effect on food intake as the administration of leptin into the VTA. The effect of the administration of leptin into the VTA on food intake is in line with the results of another study [24]. In the present study, it was shown that the administration of 50, 150, and 500 ng of leptin into the VTA decreased food intake during the first 24 h after the injections in female Sprague-Dawley rats. Hommel and colleagues reported that the same doses also decreased food intake in male Sprague-Dawley rats during the first 24 h after the injections [24]. Later time points were not investigated in the aforementioned study. The decreases of food intake were also of a similar magnitude. In the present study, the highest dose of leptin decreased food intake by 27% compared to the vehicle treated controls (vehicle 51.3 g vs. 500 ng of leptin 37.4 g). In the study by Hommel and colleagues, the administration of 500 ng of leptin into the VTA decreased food intake by about 20% compared to vehicle treated controls. In the present study, it was also shown that 50 and 150 ng of leptin in the Arc decreased food intake during the first 24 h after the injection in female Sprague-Dawley rats. The highest dose decreased food intake by about 17% (vehicle 42.1 g vs. 150 ng of leptin 34.8 g). One study has investigated the effects of the administration of leptin into the Arc of male Sprague-Dawley rats on food intake [23].

They investigated the effects of 125, 250, and 500 ng of leptin in the Arc on food intake. Their lowest dose (125 ng), which is relatively close to the highest dose in our study (150 ng), decreased food intake by about 30%. This pattern of results suggest that in the study by Satoh and colleagues, leptin had a greater effect on food intake than in our study [23]. It should be noted, however, that in the experiments by Satoh and colleagues, leptin had a greater effect on food intake than in other studies. For example, they reported that the icv administration of 2 µg of leptin almost completely blocked food intake during the 24 h after the injection (control, ≈ 19 g; leptin ≈ 2 g). In contrast, in a study by another research group, the icv administration of 3.5 µg of leptin induced a much smaller and non significant decrease in food intake (control, ≈ 19 g; leptin ≈ 13 g) during the 24 h after the injection [15].

In the present studies, the administration of leptin into the VTA or the Arc had a long term (> 24 h) effect on food intake. Both in the VTA and Arc experiment, leptin decreased food intake during the 0–24 h, 24–48 h, and 48–72 h periods. After the 72 h time point the effects of leptin on food intake were no longer significant. We are not aware of any other studies that have shown that a single infusion of leptin affects food intake in rodents for 3 days. It might be possible that other studies did not report a long-term effect of a single infusion of leptin on food intake because they recorded food intake only during the first 24 h after the infusion of leptin [15,23,24]. Some studies did not find a long-term effect of a single infusion of leptin on food intake in rodents. One study reported that food intake in rats is decreased 1 h, 4 h, but not 24 h after the icv infusion of leptin in rats [15]. Another study reported that food intake in mice is decreased 0–24 h after the icv administration of 1 µg of leptin but not 24–48 h after the administration of leptin [50]. There are, however, other studies that have reported that leptin can have a long-term effect on food intake. The long term effect of a single infusion of leptin on food intake has been investigated in adult rhesus monkeys. Food intake was not decreased 0–24 h after the infusion. However, the leptin infusion decreased the size of all the meals during the 24–48 h period and the size of 2 of the 5 meals during the 48–72 h period. In another study, the effect of chronic leptin on food intake in rats was investigated [16]. The rats received one injection of leptin, 3.5 µg, per day for 4 days and on these days food intake was decreased. Food intake remained significantly decreased for 48 h after the last leptin injection. Because there was no group that received only one injection, it is not known if this relatively long term effect of leptin on food intake was due to the chronic administration of leptin or due to other experimental factors. There are several possible explanations for the fact that we observed a relatively long-term effect of the administration of leptin into the VTA and Arc on food intake. First, it might be possible that the administration of leptin into the VTA or the Arc has a more prolonged effect on food intake than the administration of leptin into the third ventricle [51]. The local administration of leptin into the VTA or Arc may lead to higher leptin levels in these brain sites than after the icv administration of leptin and therefore more prolonged effects on food intake might be observed. Second, in the present studies the rats were fed a wet mash (food/water ratio of 1/1.5) and the rats in the other discussed studies were fed standard rodent chow. The rats ate about 50 grams of the wet mash per day while rats eat 20–25 grams of standard rodent chow per day. Because the mash has a high moisture content, the rats have to consume a larger volume of the wet mash than regular chow to ingest their daily amount of calories. Previous research has shown that the vagus nerve transmits information about gastric load volume to the nucleus of the solitary tract (NTS) and thereby decreases food intake [52,53,54]. The administration of leptin into the third ventricle increases the sensitivity of the NTS to gastric loads and thereby decreases meal sizes [51]. The volume of the meals of the rats that consume the wet mash is larger than that of rats that consume standard chow. Therefore, the rats that are fed the wet mash might be more sensitive to the effects of leptin on food intake than rats that are fed standard rodent chow.

The administration of leptin into the VTA and Arc led to a non-significant decrease in food intake during the 0–4 h period. The administration of leptin (500 ng, unilateral dose) into the VTA decreased food intake by 3.6 grams (control 16.9 g vs. leptin 13.3 g) over the 0–4 h period. The administration of leptin (500 ng, unilateral dose) into the Arc decreased food intake by 1.8 grams (control 13.6 g vs. leptin 11.8 g) over the 0–4 h period. A previous study showed that the bilateral administration of leptin into the VTA (500 ng, unilateral dose) decreases food intake during the 4 h after the infusion in male rats [55]. In the aforementioned study, the decrease in food intake over the 0–4 h period was about 2.8 grams. Therefore, in the present study the administration of leptin into the VTA has a slightly greater effect on food intake than in the study by Morton and colleagues (3.6 vs. 2.8 grams). Despite the fact that the effect was larger in the present study, the statistical analysis did not reveal a significant effect of leptin in the VTA on food intake over the 0–4 h period. It is most likely that we did not detect a statistical effect because many low doses of leptin (15, 50, 150 ng) that did not affect food intake during the 0–4 h period were included in the overall analysis.

Previous research by other groups has shown that acute and chronic icv administration of insulin decreases food intake in rats [10,56]. The acute administration of insulin into the third ventricle (4 mU and 8 mU) of male rats decreases the 4 h and 24 h intake of lab chow [10]. In a more recent experiment, the role of insulin in specific brain sites on operant responding for a 5% sucrose solution was investigated [22]. The bilateral administration of insulin into the Arc (5 mU, unilateral dose), but not into the VTA, decreased sucrose self-administration in male rats. In a follow-up experiment, it was shown that the administration of the mu-opioid receptor agonist DAMGO into the VTA increased the self-administration of the sucrose solution and this effect was blocked by the co-administration of insulin (5 mU, unilateral dose). In the present studies, we investigated the effects of the administration of insulin (0.005–5 mU, unilateral dose) into the VTA and the Arc on consumption of lab chow in ad libitum fed female rats. It was shown that the administration of 5 mU of insulin into the VTA or the Arc decreased the consumption of lab chow. The study confirms and extends previous studies by demonstration that insulin in the VTA and Arc not only plays a role in the consumption of palatable foods and mu-opioid induced consumption of palatable foods but also in the consumption of regular lab chow in ad libitum fed rats.

In the present studies, the body weights of the rats were recorded daily after the administration of insulin and leptin into the VTA and the Arc. It was shown that the administration of leptin into the VTA and the Arc had a significant effect on body weight gain. The administration of leptin into the VTA decreased body weight gain during the 0–24 h and the 24–48 h period. The administration of leptin into the Arc decreased body weight gain during the 0–24 h period and did not affect body weight gain during the 24–48 h period. The effects of leptin in the VTA on body weights are in line with a previous study by Morton and colleagues [55]. They demonstrated that the bilateral administration of 500 ng (unilateral dose) of leptin into the VTA decreases the body weight of male rats by about 5 grams over a 24 h period when compared to their pretreatment body weight and by about 2.5 grams when compared to the vehicle treated controls. The long-term effects on food intake were not reported. The present study extends and corroborates the aforementioned study by demonstrating that a single infusion of leptin also affects body weights during the 24–48 h period. We also showed that the administration of 500 ng of leptin into the Arc led to a 4 gram decrease in body weight compared to the body weights of the vehicle treated control rats during the 0–24 h period. Another study reported that the administration of the same dose of leptin into the Arc led to a 35 gram decrease in body weights in male rats [23]. It is unclear why in the aforementioned study [23] the administration of leptin caused such a dramatic effect on body weights compared to our study. It should be noted, however, that they also reported extremely large effects of icv leptin on food intake and body weights

compared to other studies [15,23]. In the present studies, the administration of insulin into the VTA or Arc did not affect body weights. At this point, we are not aware of any studies that investigated the effects of the acute administration of insulin into the ventricles or a specific brain site on body weights. The chronic administration of insulin into the third ventricle or into the ventromedial hypothalamus has been shown to decrease body weights in rats [56,57]. The present studies suggest that the acute administration of insulin into the VTA or Arc does not affect body weights. Additional studies are needed to investigate if the chronic administration of insulin into these brain sites affects body weights. The half-life of insulin is very short and chronic infusions might be necessary to detect an effect of the administration of insulin in the brain on body weight [58].

The goal of the ICSS studies was to investigate the effects of insulin and leptin in the VTA and Arc on the state of brain reward system. The studies were conducted to determine whether insulin and leptin in the VTA and Arc affect food intake by altering the state of the brain reward system. The present studies demonstrated that the administration of insulin or leptin into the VTA elevated the brain reward thresholds, which is indicative of decreased brain reward function. It is interesting to note that only relatively low doses of insulin and leptin in the VTA elevated the brain reward thresholds. The doses of insulin and leptin that elevated the brain reward thresholds did not affect food intake. Conversely, the high doses of insulin and leptin that decreased food intake did not affect brain reward thresholds. Leptin may have elevated brain reward thresholds by decreasing dopaminergic transmission. Previous research has shown that systemically administered leptin decreases the firing rate of dopaminergic neurons in the VTA [24]. In addition, centrally administered leptin decreases dopamine levels in the nucleus accumbens [59]. Decreased firing rates of dopaminergic neurons in the VTA and low dopamine levels in the nucleus accumbens have been associated with negative mood states and elevated brain reward thresholds [60,61,62,63]. The administration of insulin or leptin into the Arc did not affect brain reward thresholds. In contrast, insulin and leptin in the Arc decreased food intake. Previous studies have reported that the icv administration of insulin and leptin elevates the brain reward thresholds of rats in a rate-frequency ICSS procedure [33,34]. The present findings would suggest that these effects are at least partly mediated by the stimulation of insulin and leptin receptors in the VTA. The present study would also suggest that it is unlikely that icv administered insulin and leptin elevated brain reward thresholds by stimulating insulin or leptin receptors in the Arc [33,34].

It should be noted that some caution is warranted when comparing the effects of insulin and leptin on food intake and brain reward thresholds. The duration of the ICSS studies and food intake studies were very different. The ICSS sessions were completed within about 1 h and food intake was investigated over a 72 h period. The different duration of these studies could possibly explain some of the differential effects of leptin and insulin in the VTA on brain reward thresholds and food intake. A low dose of leptin and insulin in the VTA elevated brain reward thresholds while higher doses decreased food intake. The elevations in brain reward thresholds may have been due to the acute effects of insulin and leptin while the decrease in food intake may have been caused by relatively long-term insulin and leptin-induced neuroadaptations. The half-life of insulin is about 8 minutes and the half life of leptin is about 30 minutes [64,65]. Therefore, insulin and leptin were inactivated at the time points that food intake was decreased. Higher doses of insulin and leptin may have been required to induce the long-term adaptations that led to the decrease in food intake. Previous research supports the hypothesis that leptin induces neuronal adaptations that lead to a decrease in food intake. A single injection of leptin decreases the number of synapses on orexigenic NPY neurons in the hypothalamus and this change occurs prior to the effects of leptin on food intake [66]. Therefore, different neuronal mechanisms

may have mediated the effects of insulin and leptin in the VTA on brain reward thresholds and food intake.

In conclusion, the present findings indicate that the administration of insulin and leptin in the VTA and the Arc decreases food intake in ad libitum fed female rats. The administration of low doses of insulin and leptin into the VTA, but not into the Arc, led to a decrease in brain reward function. The present studies suggest that levels of insulin and leptin in the VTA that affect food intake do not affect the state of the brain reward system. Insulin and leptin in the Arc affect food intake but not the state of the brain reward system. Negative affective states increase the risk for overeating and episodes of binge eating in humans [67,68]. Therefore, experimental studies that investigate the interplay between mood states and food intake may contribute to the development of novel treatments for obesity.

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References

1. Popkin BM, Doak CM. The obesity epidemic is a worldwide phenomenon. *Nutr. Rev.* 1998; 56(4 Pt 1):106–114. [PubMed: 9584495]
2. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999–2008. *JAMA.* 2010; 303(3):235–241. [PubMed: 20071471]
3. Woods SC, Seeley RJ, Porte D Jr, Schwartz MW. Signals that regulate food intake and energy homeostasis. *Science.* 1998; 280(5368):1378–1383. [PubMed: 9603721]
4. Woods SC, Schwartz MW, Baskin DG, Seeley RJ. Food intake and the regulation of body weight. *Annu. Rev. Psychol.* 2000; 51:255–277. [PubMed: 10751972]
5. Shioda S, Funahashi H, Nakajo S, Yada T, Maruta O, Nakai Y. Immunohistochemical localization of leptin receptor in the rat brain. *Neurosci. Lett.* 1998; 243(1–3):41–44. [PubMed: 9535108]
6. Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. *J. Comp Neurol.* 1998; 395(4):535–547. [PubMed: 9619505]
7. Figlewicz DP, Evans SB, Murphy J, Hoen M, Baskin DG. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res.* 2003; 964(1):107–115. [PubMed: 12573518]
8. Havrankova J, Roth J, Brownstein M. Insulin receptors are widely distributed in the central nervous system of the rat. *Nature.* 1978; 272(5656):827–829. [PubMed: 205798]
9. Werther GA, Hogg A, Oldfield BJ, McKinley MJ, Figdor R, Allen AM, Mendelsohn FA. Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. *Endocrinology.* 1987; 121(4):1562–1570. [PubMed: 3653038]
10. Air EL, Benoit SC, Blake Smith KA, Clegg DJ, Woods SC. Acute third ventricular administration of insulin decreases food intake in two paradigms. *Pharmacol. Biochem. Behav.* 2002; 72(1–2):423–429. [PubMed: 11900815]
11. Woods SC, Lotter EC, McKay LD, Porte D Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature.* 1979; 282(5738):503–505. [PubMed: 116135]
12. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature.* 2000; 404(6778):661–671. [PubMed: 10766253]
13. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994; 372(6505):425–432. [PubMed: 7984236]
14. Chua SC Jr, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science.* 1996; 271(5251):994–996. [PubMed: 8584938]

15. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. *J. Clin. Invest.* 1996; 98(5):1101–1106. [PubMed: 8787671]
16. Seeley RJ, van DG, Campfield LA, Smith FJ, Burn P, Nelligan JA, Bell SM, Baskin DG, Woods SC, Schwartz MW. Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats. *Horm. Metab Res.* 1996; 28(12):664–668. [PubMed: 9013738]
17. Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurink A, Kahn SE, Baskin DG, Woods SC, Figlewicz DP. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology.* 1992; 130(6):3608–3616. [PubMed: 1597158]
18. Schwartz MW, Marks JL, Sipols AJ, Baskin DG, Woods SC, Kahn SE, Porte D Jr. Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. *Endocrinology.* 1991; 128(5):2645–2647. [PubMed: 2019270]
19. Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc. Natl. Acad. Sci. U. S. A.* 1991; 88(23):10931–10935. [PubMed: 1961764]
20. Sahu A, Kalra SP. Neuropeptidergic regulation of feeding behavior Neuropeptide Y. *Trends Endocrinol. Metab.* 1993; 4(7):217–224. [PubMed: 18407159]
21. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nat. Neurosci.* 2002; 5(6):566–572. [PubMed: 12021765]
22. Figlewicz DP, Bennett JL, Aliakbari S, Zavosh A, Sipols AJ. Insulin acts at different CNS sites to decrease acute sucrose intake and sucrose self-administration in rats. *Am. J. Physiol Regul. Integr. Comp Physiol.* 2008; 295(2):R388–R394. [PubMed: 18525010]
23. Satoh N, Ogawa Y, Katsuura G, Hayase M, Tsuji T, Imagawa K, Yoshimasa Y, Nishi S, Hosoda K, Nakao K. The arcuate nucleus as a primary site of satiety effect of leptin in rats. *Neurosci. Lett.* 1997; 224(3):149–152. [PubMed: 9131658]
24. Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M, DiLeone RJ. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron.* 2006; 51(6):801–810. [PubMed: 16982424]
25. Davis JF, Choi DL, Schurdak JD, Fitzgerald MF, Clegg DJ, Lipton JW, Figlewicz DP, Benoit SC. Leptin Regulates Energy Balance and Motivation Through Action at Distinct Neural Circuits. *Biol. Psychiatry.* 2010
26. Lutter M, Nestler EJ. Homeostatic and hedonic signals interact in the regulation of food intake. *J. Nutr.* 2009; 139(3):629–632. [PubMed: 19176746]
27. Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High glycemic index foods, overeating, and obesity. *Pediatrics.* 1999; 103(3):E26. [PubMed: 10049982]
28. Lissner L, Heitmann BL. Dietary fat and obesity: evidence from epidemiology. *Eur. J. Clin. Nutr.* 1995; 49(2):79–90. [PubMed: 7743988]
29. Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.* 1992; 13(5):177–184. [PubMed: 1604710]
30. Wise RA, Rompre PP. Brain dopamine and reward. *Annu. Rev. Psychol.* 1989; 40:191–225. [PubMed: 2648975]
31. Figlewicz DP, Bennett J, Evans SB, Kaiyala K, Sipols AJ, Benoit SC. Intraventricular insulin and leptin reverse place preference conditioned with high-fat diet in rats. *Behav. Neurosci.* 2004; 118(3):479–487. [PubMed: 15174925]
32. Figlewicz DP, Bennett JL, Naleid AM, Davis C, Grimm JW. Intraventricular insulin and leptin decrease sucrose self-administration in rats. *Physiol Behav.* 2006; 89(4):611–616. [PubMed: 17045623]
33. Carr KD, Kim G, Cabeza d V. Hypoinsulinemia may mediate the lowering of self-stimulation thresholds by food restriction and streptozotocin-induced diabetes. *Brain Res.* 2000; 863(1–2): 160–168. [PubMed: 10773204]
34. Fulton S, Woodside B, Shizgal P. Modulation of brain reward circuitry by leptin. *Science.* 2000; 287:125–128. [PubMed: 10615045]

35. Licinio J, Negrao AB, Mantzoros C, Kaklamani V, Wong ML, Bongiorno PB, Negro PP, Mulla A, Veldhuis JD, Cernal L, Flier JS, Gold PW. Sex differences in circulating human leptin pulse amplitude: clinical implications. *J. Clin. Endocrinol. Metab.* 1998; 83(11):4140–4147. [PubMed: 9814504]
36. Clegg DJ, Riedy CA, Smith KA, Benoit SC, Woods SC. Differential sensitivity to central leptin and insulin in male and female rats. *Diabetes.* 2003; 52(3):682–687. [PubMed: 12606509]
37. Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. *Science.* 1997; 278:52–58. [PubMed: 9311926]
38. Bruijnzeel AW, Lewis B, Bajpai LK, Morey TE, Dennis DM, Gold M. Severe deficit in brain reward function associated with fentanyl withdrawal in rats. *Biol. Psychiatry.* 2006; 59(5):477–480. [PubMed: 16169528]
39. Bruijnzeel AW, Zislin G, Wilson C, Gold MS. Antagonism of CRF receptors prevents the deficit in brain reward function associated with precipitated nicotine withdrawal in rats. *Neuropsychopharmacology.* 2007; 32(4):955–963. [PubMed: 16943772]
40. Kornetsky C, Esposito RU. Euphorogenic drugs: effects on the reward pathways of the brain. *Fed. Proc.* 1979; 38(11):2473–2476. [PubMed: 488370]
41. Markou A, Koob GF. Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiol Behav.* 1992; 51(1):111–119. [PubMed: 1741436]
42. Markou A, Koob GF. Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology.* 1991; 4(1):17–26. [PubMed: 2003866]
43. Bruijnzeel AW, Markou A. Adaptations in cholinergic transmission in the ventral tegmental area associated with the affective signs of nicotine withdrawal in rats. *Neuropharmacology.* 2004; 47(4):572–579. [PubMed: 15380374]
44. Marcinkiewicz CA, Prado MM, Isaac SK, Marshall A, Rylkova D, Bruijnzeel AW. Corticotropin-releasing factor within the central nucleus of the amygdala and the nucleus accumbens shell mediates the negative affective state of nicotine withdrawal in rats. *Neuropsychopharmacology.* 2009; 34(7):1743–1752. [PubMed: 19145226]
45. Yamada H, Bruijnzeel AW. Stimulation of alpha2-adrenergic receptors in the central nucleus of the amygdala attenuates stress-induced reinstatement of nicotine seeking in rats. *Neuropharmacology.* 2011; 60(2–3):303–311. [PubMed: 20854830]
46. Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates.* San Diego: Academic Press; 1998.
47. Clifton PG, Barnfield AM, Philcox L. A behavioural profile of fluoxetine-induced anorexia. *Psychopharmacology (Berl).* 1989; 97(1):89–95. [PubMed: 2496433]
48. Epping-Jordan MP, Watkins SS, Koob GF, Markou A. Dramatic decreases in brain reward function during nicotine withdrawal. *Nature.* 1998; 393(6680):76–79. [PubMed: 9590692]
49. Figlewicz DP, Benoit SC. Insulin, leptin, and food reward: update 2008. *Am. J. Physiol Regul. Integr. Comp Physiol.* 2009; 296(1):R9–R19. [PubMed: 18945945]
50. Mistry AM, Swick AG, Romsos DR. Leptin rapidly lowers food intake and elevates metabolic rates in lean and ob/ob mice. *J. Nutr.* 1997; 127(10):2065–2072. [PubMed: 9311966]
51. Schwartz GJ, Moran TH. Leptin and neuropeptide y have opposing modulatory effects on nucleus of the solitary tract neurophysiological responses to gastric loads: implications for the control of food intake. *Endocrinology.* 2002; 143(10):3779–3784. [PubMed: 12239088]
52. Kobashi M, Ichikawa H, Sugimoto T, Adachi A. Response of neurons in the solitary tract nucleus, area postrema and lateral parabrachial nucleus to gastric load of hypertonic saline. *Neurosci. Lett.* 1993; 158(1):47–50. [PubMed: 8233072]
53. Phillips RJ, Powley TL. Gastric volume rather than nutrient content inhibits food intake. *Am. J. Physiol.* 1996; 271(3 Pt 2):R766–R769. [PubMed: 8853402]
54. Phillips RJ, Powley TL. Gastric volume detection after selective vagotomies in rats. *Am. J. Physiol.* 1998; 274(6 Pt 2):R1626–R1638. [PubMed: 9608017]
55. Morton GJ, Blevins JE, Kim F, Matsen M, Figlewicz DP. The action of leptin in the ventral tegmental area to decrease food intake is dependent on Jak-2 signaling. *Am. J. Physiol Endocrinol. Metab.* 2009; 297(1):E202–E210. [PubMed: 19435852]

56. Ikeda H, West DB, Pustek JJ, Figlewicz DP, Greenwood MR, Porte D Jr, Woods SC. Intraventricular insulin reduces food intake and body weight of lean but not obese Zucker rats. *Appetite*. 1986; 7(4):381–386. [PubMed: 3539015]
57. McGowan MK, Andrews KM, Kelly J, Grossman SP. Effects of chronic intrahypothalamic infusion of insulin on food intake and diurnal meal patterning in the rat. *Behav. Neurosci.* 1990; 104(2):373–385. [PubMed: 2189441]
58. Katz AI, Rubenstein AH. Metabolism of proinsulin, insulin, and C-peptide in the rat. *J. Clin. Invest.* 1973; 52(5):1113–1121. [PubMed: 4700486]
59. Krugel U, Schraft T, Kittner H, Kiess W, Illes P. Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. *Eur. J. Pharmacol.* 2003; 482(1–3):185–187. [PubMed: 14660021]
60. Diana M, Pistis M, Muntoni A, Gessa G. Profound decrease of mesolimbic dopaminergic neuronal activity in morphine withdrawn rats. *J. Pharmacol. Exp. Ther.* 1995; 272(2):781–785. [PubMed: 7853194]
61. Diana M, Pistis M, Carboni S, Gessa GL, Rossetti ZL. Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. *Proc. Natl. Acad. Sci. U. S. A.* 1993; 90(17):7966–7969. [PubMed: 8367449]
62. Weiss F, Markou A, Lorang MT, Koob GF. Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. *Brain Res.* 1992; 593(2):314–318. [PubMed: 1450939]
63. Wise RA. Addictive drugs and brain stimulation reward. *Annu. Rev. Neurosci.* 1996; 19:319–340. [PubMed: 8833446]
64. Klein S, Coppack SW, Mohamed-Ali V, Landt M. Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes*. 1996; 45(7):984–987. [PubMed: 8666153]
65. Burt RL, Davidson IW. Insulin half-life and utilization in normal pregnancy. *Obstet. Gynecol.* 1974; 43(2):161–170. [PubMed: 4855689]
66. Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science*. 2004; 304(5667):110–115. [PubMed: 15064421]
67. Arnow B, Kenardy J, Agras WS. The Emotional Eating Scale: the development of a measure to assess coping with negative affect by eating. *Int. J. Eat. Disord.* 1995; 18(1):79–90. [PubMed: 7670446]
68. Chua JL, Touyz S, Hill AJ. Negative mood-induced overeating in obese binge eaters: an experimental study. *Int. J. Obes. Relat Metab Disord.* 2004; 28(4):606–610. [PubMed: 14968127]

B

Fig. 1.

Histological overview of bilateral injection sites in the VTA (A) and Arc (C). Drug injections were administered within or at the boundaries of the CeA (−5.20 to −5.60 anterior-posterior) and Arc (−3.60 to −4.16 mm anterior-posterior). The injections sites were evenly distributed over the levels depicted in these figures. The figures are copies from the Paxinos and Watson brain atlas [46]. The photomicrographs depict representative cresyl violet-stained brain section at the level of the VTA (B) and the Arc (D). The photomicrographs show cannulae tracts and injector tracts that end within the VTA and Arc. Abbreviations: MRe; mammillary recess of the third ventricle.

Fig. 2.

Effects of leptin in the VTA on food intake in female rats ($n = 12$). Fig. 2A depicts food intake 0–4 h after leptin infusion; Fig. 2B, 0–24 h after leptin infusion; Fig. 2C, 24–48 h after leptin infusion; Fig. 2D, 48–72 h after leptin infusion. Asterisks (* $P < 0.05$, ** $P < 0.01$) indicate a decrease in food intake compared to the vehicle group (0 ng of leptin). In Fig. 2B, plus signs (++ $P < 0.01$) indicated a decrease in food intake compared to the group treated with 50 ng of leptin. In Fig. 2C, the plus sign (+ $P < 0.01$) indicates a decrease in food intake compared to the group treated with 150 ng of leptin. Leptin is infused bilaterally and the dose is expressed as the unilateral dose. Data are expressed as mean $\pm$ SEM.

Fig. 3.

Effects of leptin in the VTA and Arc on body weights of female rats. Figs. 3A–B depict changes in body weights 0–24 h (A) and 24–48 h (B) after the infusion of leptin into the VTA (n = 12). Figs. 3C–D depict changes in body weights 0–24 h (C) and 24–48 h (D) after the infusion of leptin into the Arc (n = 13). Asterisks (* P<0.05, ** P<0.01) indicate a decrease in body weight compared to the vehicle group (0 ng of leptin). Leptin is infused bilaterally and the dose is expressed as the unilateral dose. Data are expressed as mean $\pm$ SEM.

Fig. 4. Effects of leptin in the Arc on food intake in female rats ($n = 13$). Fig. 4A depicts food intake 0–4 h after leptin infusion; Fig. 4B, 0–24 h after leptin infusion; Fig. 4C, 24–48 h after leptin infusion; Fig. 4D, 48–72 h after leptin infusion. Asterisks (* $P < 0.05$, ** $P < 0.01$) indicate a decrease in food intake compared to the vehicle group (0 ng of leptin). Leptin is infused bilaterally and the dose is expressed as the unilateral dose. Data are expressed as mean $\pm$ SEM.

Fig. 5. Effects of insulin in the VTA on food intake in female rats ($n = 13$). Fig. 5A depicts food intake 0–4 h after insulin infusion; Fig. 5B, 0–24 h after insulin infusion; Fig. 5C, 24–48 h after insulin infusion. Asterisks (* $P < 0.05$) indicate a decrease in food intake compared to the vehicle group (0 mU of insulin). Insulin is infused bilaterally and the dose is expressed as the unilateral dose. Data are expressed as mean $\pm$ SEM.

Fig. 6.

Effects of insulin in the Arc on food intake in female rats ($n = 13$). Fig. 6A depicts food intake 0–4 h after insulin infusion; Fig. 6B, 0–24 h after insulin infusion; Fig. 6C, 24–48 h after insulin infusion. Asterisks (* $P < 0.05$) indicate a decrease in food intake compared to the vehicle group (0 mU of insulin). Insulin is infused bilaterally and the dose is expressed as the unilateral dose. Data are expressed as mean $\pm$ SEM.

Fig. 7. Effects of leptin in the VTA ($n = 9$) on brain reward thresholds (Fig. 7A) and response latencies (Fig 7. B). Brain reward thresholds are expressed as a percentage of the pre-test day values. Asterisks (* $P < 0.05$) indicate elevations in brain reward thresholds compared to those of the vehicle group (0 ng of leptin). Data are expressed as mean $\pm$ SEM.

Fig. 8. Effects of insulin in the VTA ($n = 10$) on brain reward thresholds (Fig. 8A) and response latencies (Fig. 8B). Brain reward thresholds are expressed as a percentage of the pre-test day values. Asterisks (* $P < 0.05$) indicate elevations in brain reward thresholds compared to those of the vehicle group (0 mU of insulin). Data are expressed as mean $\pm$ SEM.