

Leptin Signaling in the Medial Nucleus Tractus Solitarius Reduces Food Seeking and Willingness to Work for Food

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The adipose-derived hormone leptin signals in the medial nucleus tractus solitarius (mNTS) to suppress food intake, in part, by amplifying within-meal gastrointestinal (GI) satiation signals. Here we show that mNTS leptin receptor (LepRb) signaling also reduces appetitive and motivational aspects of feeding, and that these effects can depend on energy status. Using the lowest dose that significantly suppressed 3-h cumulative food intake, unilateral leptin (0.3 µg) administration to the mNTS (3 h before testing) reduced operant lever pressing for sucrose under increasing work demands (progressive ratio reinforcement schedule) regardless of whether animals were energy deplete (food restricted) or replete (*ad libitum* fed). However, in a separate test of food-motivated responding in which there was no opportunity to consume food (conditioned place preference (CPP) for an environment previously associated with a palatable food reward), mNTS leptin administration suppressed food-seeking behavior only in chronically food-restricted rats. On the other hand, mNTS LepRb signaling did not reduce CPP expression for morphine reinforcement regardless of energy status, suggesting that mNTS leptin signaling differentially influences motivated responding for food vs opioid reward. Overall results show that mNTS LepRb signaling reduces food intake and appetitive food-motivated responding independent of energy status in situations involving orosensory and postingestive contact with food, whereas food-seeking behavior independent of food consumption is only reduced by mNTS LepRb activation in a state of energy deficit. These findings reveal a novel appetitive role for LepRb signaling in the mNTS, a brain region traditionally linked with processing of meal-related GI satiation signals.

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INTRODUCTION

Leptin, secreted primarily from white adipose cells, acts on receptors (leptin receptor, LepRb) in the brain to reduce body weight by suppressing food intake and increasing energy expenditure. Historically, attention has been directed toward leptin's action in the hypothalamus, particularly the arcuate hypothalamic nucleus (ARC). More recent findings reveal that leptin's powerful control over energy balance is anatomically distributed (Grill, 2010; Grill and Kaplan, 1990; Grill and Kaplan, 2002a) as it involves contributions from midbrain and forebrain regions such as the ventral tegmental area (Fulton *et al*, 2006; Hommel *et al*, 2006) and the hippocampus (Kanoski *et al*, 2011) that are associated with the control of motivational, learned, and rewarding aspects of food intake, as well as in extra-ARC hypothalamic nuclei (Leininger *et al*, 2009; Zhang *et al*, 2011) and hindbrain nuclei (Grill *et al*, 2002b; Huo *et al*, 2007; Schwartz and Moran, 2002; Skibicka and Grill, 2009)

whose neural processing is typically associated with the control of need-based food intake (ie, feeding driven by energy deficit). Within the hindbrain, the LepRb is most densely expressed in the medial nucleus tractus solitarius (mNTS) of the dorsal vagal complex (Caron *et al*, 2010; Elias *et al*, 2000; Huo *et al*, 2006; Li *et al*, 1999; Patterson *et al*, 2011), which is the first CNS site to receive vagal afferent-mediated gastrointestinal (GI) signals and is a critical nucleus for satiation signal processing and meal size control (Grill and Hayes, 2009; Schwartz, 2010). Indeed, LepRb signaling in mNTS neurons potently reduces food intake by amplifying the food intake-suppressive effects of various within-meal GI-derived satiation signals, including the intestinally secreted peptide cholecystokinin (Hayes *et al*, 2010), mechanical distention of the stomach (Huo *et al*, 2007), and intestinal delivery of a mixed nutrient meal (Kanoski *et al*, 2012b). These findings have identified mNTS neurons as a key site of integration between leptin signaling and GI satiation signals in the control of meal size (for review, see Grill, 2010; Grill and Hayes, 2012).

Here we examine a novel role for mNTS LepRb signaling in the control of food intake. Experiments described directly test the hypothesis that the anorectic effects triggered by mNTS LepRb signaling are not exclusively mediated through amplification of within-meal GI-derived satiation signals, but also involve the suppression of appetitive and

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motivational aspects of feeding, which include: (a) an animal's willingness to work for palatable food, and (b) food seeking independent of orosensory and/or postingestive contact with food. Leptin or vehicle are infused intra-mNTS before behavioral tests of learned food-motivated responding using a dose of leptin (0.3 μ g) that was first determined to be the lowest effective dose for significant short-term (3 h) food intake suppression in free-feeding rats. Willingness to work for food is examined using an operant lever-pressing paradigm with a progressive ratio (PR) reinforcement schedule in which the number of lever presses required to obtain food reinforcement increases exponentially. Food-seeking behavior is assessed using a conditioned place preference (CPP) paradigm in which freely moving rats chose between a location/environment where palatable food (high in saturated fat and sucrose) was previously available for consumption, *vs* another location where food was neither presented nor consumed.

Leptin's control over energy balance is highly dependent on energy status. This is most clearly supported by the fact that levels of endogenous circulating leptin directly correlate with overall adiposity (Ahima *et al*, 2000), and by studies showing that the engagement of intracellular signaling pathways following neuronal LepRb activation is disrupted in the pathophysiological state of obesity (Munzberg *et al*, 2005). Here we determine whether effects of mNTS LepRb activation on food-motivated responding (both for operant responding and CPP) are dependent on energy status by comparing effects in rats whose access to food is chronically restricted (daily rations) *vs* rats given *ad libitum* access to food in the home cage throughout the duration of the experiment. In the latter case, reduction of appetitive responding would suggest that mNTS LepRb activation inhibits food-directed behaviors that are driven by factors other than caloric deficit resulting from food restriction.

Both CPP and operant lever-pressing paradigms have been used to evaluate the effects of both drug and food reward. The common neural and neurochemical controls of these food and drug reward-mediated effects continue to attract attention (eg, Kenny, 2011; Tomasi and Volkow, 2013; Volkow *et al*, 2013a,b). The extent that CNS LepRb signaling reduces appetitive behavior directed toward reinforcers other than food is largely unknown. Here we examine whether mNTS LepRb activation reduces motivated responding for opioid-based reward (CPP for morphine), both in energy-deplete and -replete subjects. The potential for mNTS LepRb signaling to reduce appetitive responding for morphine is supported by findings showing that leptin and opioid agonists (either peripheral or centrally delivered) have opposing effects on mesolimbic dopaminergic signaling (Baldo and Kelley, 2007; De Luca *et al*, 2011; Fulton *et al*, 2006; Hommel *et al*, 2006), suggesting that leptin and morphine may differentially influence motivated behavior by acting on a common neurochemical system. Further, intracerebroventricular leptin administration reduces the food deprivation-induced relapse to heroin seeking (ie, reinstatement of conditioned responding for morphine following extinction) in rats (Shalev *et al*, 2001).

Overall, our results show that mNTS LepRb signaling reduces appetitive responding for palatable food, and these effects can depend on energy status. The reduction

of food-motivated responding by mNTS LepRb activation in the energy replete (nondeprived) state was observed, but only in a test that allowed for consumption of the food reinforcement (operant responding for food reinforcement). By contrast, in the absence of direct contact with food (CPP paradigm) mNTS leptin reduced food-seeking but only under food-restricted conditions. Surprisingly, mNTS leptin signaling had no effect on learned appetitive behavior directed toward opiate reinforcement. These novel findings have implications, not only with respect to the leptinergic control of energy balance, but also regarding food intake control by mNTS neural processing more generally.

MATERIALS AND METHODS

Subjects

Adult male Sprague–Dawley rats (250–300 g upon arrival; Charles River Laboratories, Wilmington, MA), housed individually in hanging metal cages and maintained on a 12:12-h light–dark cycle (lights off at 0900 hours for Exps 1–2; lights on at 0800 hours for Exps 3–4), had *ad libitum* access to rodent chow (5001 Rodent diet; Lab Diets, St Louis, MO) and water unless otherwise noted. All protocols and procedures conformed to the institutional standards of the University of Pennsylvania Animal Care and Use Committee and were approved by the committee.

Surgery

Rats were injected with ketamine (90 mg/kg; Bulter Animal Health Supply, Dublin, OH), xylazine (2.7 mg/kg; Anased, Shenandoah, IA), and acepromazine (0.64 mg/kg; Bulter Animal Health Supply) anesthesia and analgesia (2 mg/kg Metacam; Boehringer Ingelheim Vet-medica, St Joseph, MO) for all surgeries. Bilateral guide cannulae (26-gauge; Plastics One, Roanoke, VA; 1.5 mm spacing between cannulae) were implanted with the tip stereotaxically positioned above the mNTS target injection site using one of two coordinate systems (coordinates varied by surgeon to maximize overall accuracy): (1) midline, 1.0 mm posterior to occipital crest, 6.7 mm ventral from skull surface, injection targeted 2.0 mm below end of guide cannula; or (2) midline, 2.0 mm anterior to occipital crest, 6.8 mm ventral from skull surface using a 15-degree angle (negative slope in anterior to posterior direction), injection targeted 1.5 mm below end of guide cannula. Procedures for unilateral mNTS injections (right side) were based on our previous work (eg, Hayes *et al*, 2009, 2011; Kanoski *et al*, 2012a,b; Spaeth *et al*, 2012) and involve 100 nl infusion (delivery rate of 5 ml/min via Harvard Apparatus PHD 2000 infusion pump; 28 gauge indwelling injector cannulae) followed by a 30- to 40-s diffusion period where the injectors are left in place. Intended anatomic positions of mNTS injection sites were evaluated 1 week post-surgery by measurement of the sympathoadrenal-mediated glycemic response to an injection of 5-thio-D-glucose (24 μ g/100 nl unilateral mNTS injection; Ritter *et al*, 1981). A post-injection elevation in baseline plasma glucose level of 100% or greater was required for subject inclusion (assessed via tail nick at baseline, 30 min, and 60 min following 5TG infusion). Cannula placement was further confirmed

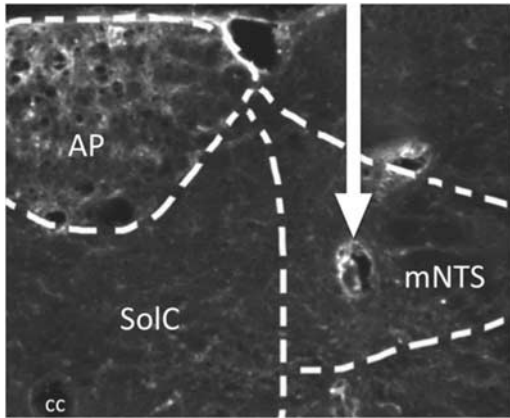


Figure 1 Representative mNTS injection site.

postmortem via bilateral histological location of 100 nl volume injection of Chicago sky blue ink. A representative injection placement is depicted in the right mNTS in Figure 1 (Note: vehicle and leptin were administered unilaterally for all experiments). Animals without confirmation of functional and anatomical injection placement were removed from analyses. Overall, four animals were excluded from Exp. 2 analyses, four animals were excluded from Exp. 3 analyses, and five animals were excluded from Exp. 4 analyses.

Procedures

Experiment 1: free-feeding food intake assessment. Naive rats were unilaterally infused intra-mNTS unilaterally with 0.0 μ g (vehicle; sodium bicarbonate), 0.1, 0.3, or 0.6 μ g leptin (100 nl volume) immediately before dark onset using a within-subjects design. Treatments were separated by 2–3 days. The rats were individually housed in wire-bottom cages with an access hole to a cup containing powdered rodent chow (5001 Rodent diet; Lab Diets) that rested on an electronic scale. The food cup weight was monitored by computer software (LabView). This automated-feeding system tracks cumulative food intake analysis via custom software over each 24-h period without disturbing the natural-feeding cycle of the rats (ie, eliminates experimenter interruption).

Experiment #2a: PR operant responding for sucrose in chronically food-restricted rats. Naive rats ($n = 11$) were given operant lever press training for sucrose reinforcement as previously described (Davis *et al*, 2011; Kanoski *et al*, 2013). Before training rats were given daily chow rations to gradually (over 5–7 days) reduce body weight to 85% of an *ad libitum* body weight established before training. Daily rations were given throughout training and testing to maintain this 85% body weight criterion. Training was carried out over 6 days with a 1-h session each day in conditioning boxes (Med Associates; MedPC IV Software, St Albans, VT). During the first 2 days, a fixed ratio autoshaping procedure was used where each lever press earned a 45-mg sucrose pellet (Bio-Serv, Frenchtown, NJ) (FR1); a free sucrose pellet was also dispensed for every 600 s that elapsed without operant-based reinforcement. The animals then received 2 days of an FR1 schedule with

no autoshaping component followed by 2 days of FR3 training. For all procedures, the right lever was the ‘active’ lever; a left ‘inactive’ lever served as a control (dummy lever) for non-conditioned elevations in responding.

The rats were given two tests (within-subjects design, 2 rest days intervened between tests) using a PR reinforcement schedule. The unilateral mNTS injections (100 nl vehicle or 0.3 μ g leptin; order counterbalanced) were given 3 h before each test session. The 3-h timing (used for Exps 2–4) was chosen based on results of Exp. 1 showing this dose to be the lowest effective dose for significant food intake suppression 3 h after injections. After injections, the animals were returned to their home cages for the 3-h time period between injections and PR testing. The response requirement of the PR schedule increased progressively as previously described (Davis *et al*, 2011; Kanoski *et al*, 2013), using the following formula: $F(i) = 5e^{0.2i-5}$, where $F(i)$ is the number of lever presses required for next pellet at i = pellet number. The breakpoint for each animal was defined as the final completed lever press requirement that preceded a 20-min period without earning a reinforcer.

Experiment #2b: PR operant responding for sucrose in *ad libitum*-fed rats. Exp. 2b was conducted in a separate group of rats ($n = 11$). Procedures were identical to Exp. 2a, except that the rats were not food restricted (*ad libitum* access to chow in home cage) throughout the entire experiment, except during the 3-h period between mNTS injections and PR testing when food was removed from the home cage.

Experiment #3a: CPP for food reward in chronically food-restricted rats. Before and throughout training and testing, naive rats ($n = 13$) were given the 85% body weight food restriction regime described in Exp. 2a. The procedures for appetitive CPP were as we have previously described (Kanoski *et al*, 2011). All CPP training and testing procedures were conducted in a dimly lit room. The procedures were conducted in two identical conjoined plexiglass CPP compartments (74 cm long, 57.4 cm wide, and 24.7 cm wall height) with a removable divider wall in the center. The two sides (henceforth referred to as contexts or locations) of the CPP chamber were made distinguishable by varying wall color (white vs black plexiglass), floor texture (plexiglass vs steel grid), and orientation of stripes (adhesive tape applied vertically vs horizontally) on the walls. Rats were first allowed to freely explore the CPP chamber during one 10-min habituation session in which the divider wall was removed and the time spent in each of the two contexts was recorded by an experimenter. For each rat, the context that was least preferred during this habituation session was assigned as the food-paired context for subsequent training, whereas the more preferred side was never paired with food. CPP training consisted of six, 20-min sessions (one session per day): three sessions where the rat was isolated in the food-paired context and three sessions isolated in the nonfood-paired context. During food-paired training sessions, a total of 5 g of a high-fat/high-sucrose diet (Research Diets; D12492) was divided into five aliquots that were scattered throughout the context, whereas no food was given during nonfood-paired sessions.

All rats consumed the entire 5 g during each food-paired session. The training order was randomized and pair-matched across groups. The first three and last three sessions occurred on continuous days; sessions 1–3 and 4–6 were separated by two intervening days.

Testing occurred 2 days after the sixth training session using a between-subjects design. Rats were assigned to groups matched for baseline context preference ($n=7$ for leptin, $n=6$ for vehicle). For each rat, a unilateral mNTS injection of either vehicle or leptin (0.3 μg) was given 3 h before a 15-min video-recorded test session in which the center divider was removed and no food was given. The rats were returned to the home cage for the 3 h between injections and CPP testing. The time spent in each context during the test was later calculated from analysis of the video recording made by an experimenter blind to the group assignments and context food assignments. The dependent variable used for analysis was the percentage shift in preference score for the food-associated context from the baseline session compared with the test session.

Experiment #3b: CPP for food reward in ad libitum-fed rats. Our pilot work (data not shown) showed that using the same training regime as Exp. 3a resulted in unreliable establishment of CPP in non-restricted (*ad libitum* fed) rats using the same training regime as Exp. 4. Thus, the number of training sessions was increased from 6 (3 per context) to 16 (8 per context) to ensure baseline shift in CPP in vehicle-treated *ad libitum*-fed rats. All other procedures were identical to Exp. 4 except that the rats were not food restricted at any point throughout the experiment (except for during the 3-h period between mNTS injections and CPP testing). Four training sessions were given each week with no more than two intervening days between sessions.

Experiment #4a: CPP for morphine reward in chronically food-restricted rats. Naive rats ($n=18$) were maintained on 85% food restriction regime as described for Exps 2a and 3a. The procedures for morphine CPP were modified from Harris *et al* (2007) and used the same CPP apparatuses as Exp. 3. A 15-min habituation session was first conducted as described for Exp. 3, and the morphine-associated side was assigned for each rat based on their least preferred location during the habituation session. Three days later, training began and continued across 3 consecutive training days. For each training day, rats were given IP injections of either saline (vehicle) or morphine (10 mg/kg) immediately before being confined to the appropriately assigned compartment for 30 min. Morphine and saline sessions alternated between the morning and afternoon with a 4-h interval between sessions. Rats given morphine before the morning training session were given saline before being placed in the opposite chamber in the afternoon, and on subsequent days received saline in the morning and morphine in the afternoon (ABA order). The number of rats in which the morning training session on the first training day involved morning saline vs morphine injections was counterbalanced across the two drug groups. Two days after the last training session, the rats received either intra-mNTS unilateral vehicle or leptin (0.3 μg) injections 3 h before a 15-min

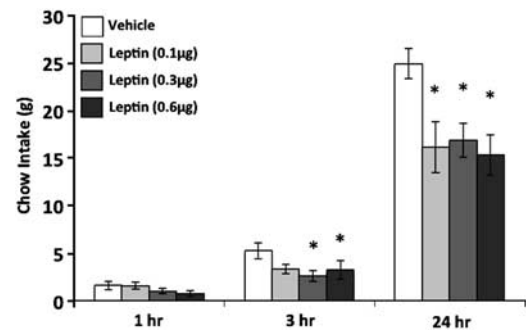


Figure 2 Cumulative home cage food intake in non-restricted rats following mNTS leptin administration (means \pm SEM; *indicates P -value < 0.01).

CPP test in which no prior IP morphine or saline injections were given and the divider wall was removed. Drug groups ($n=8$ for vehicle, $n=10$ for leptin) were assigned according to baseline preference and body weight. The amount of time spent in each of the two separate contexts during testing was calculated via analysis of video recording by an experimenter blind to the treatment groups and the assigned drug locations.

Experiment #4b: CPP for morphine reward in ad libitum-fed rats. A separate group of naive rats ($n=18$) were separated into vehicle or morphine (IP; 10 mg/kg/day) groups ($n=9$ per group) and underwent the same procedures as Exp. 4a except they had *ad libitum* access to rat chow (except for the 3-h period between mNTS leptin injection and CPP testing).

Data and Statistical Analyses

Data from Exp. 1 were analyzed via ANOVA using drug treatment as a repeated-measure variable. When significant overall main effects of drug were found, individual drug dose treatments were compared with vehicle treatment using Tukey's *post hoc* comparisons. Repeated-measures ANOVA was also used to compare drug conditions in the PR experiments (Exps 2a and 2b), whereas one-way ANOVA was used for the between-subjects CPP experiments (3a, 3b, 4a, and 4b). Alpha level for significance was set at 0.05 for all described experiments.

RESULTS

Exp. 1: mNTS Leptin Administration Reduces Chow Intake in Free-Feeding Rats

The two higher doses of leptin (0.3 and 0.6 μg) suppressed cumulative chow intake relative to vehicle at 3 h after drug administration, whereas all three doses suppressed intake at 24 h (Figure 2). The main effect of leptin was significant at 3 h ($F(3,24)=5.08$, $P<0.01$) and 24 h ($F(3,24)=5.01$, $P<0.01$). Although the main effect of leptin was also significant at 1 h ($F(3,24)=3.27$, $P<0.05$), no individual leptin treatment significantly differed from vehicle in the *post hoc* Tukey's comparisons.

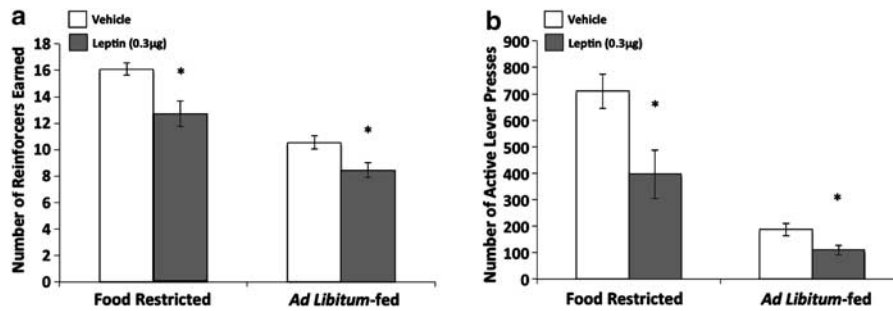


Figure 3 Operant lever pressing for sucrose in a progressive ratio reinforcement test for both food-restricted and non-restricted rats, shown as: (a) the number of reinforcers (45 mg sucrose pellets) earned before reaching breakpoint criterion, and (b) the number of active lever presses performed before reaching breakpoint criterion (means \pm SEM; *indicates P -value < 0.01).

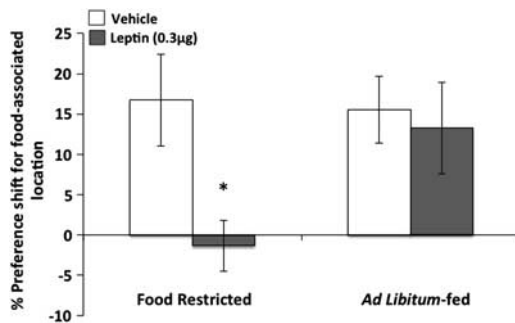


Figure 4 Conditioned place preference expression for an environment previously associated with consuming palatable food (high fat and high sucrose) for both food-restricted and non-restricted rats (means \pm SEM; *indicates P -value < 0.01).

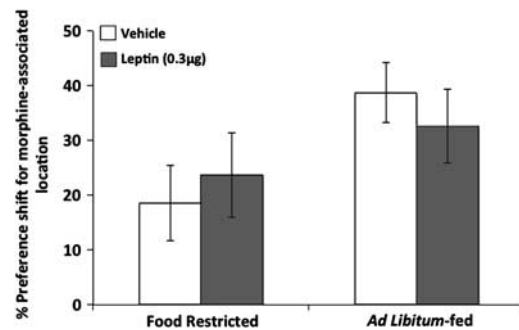


Figure 5 Conditioned place preference expression for a location previously associated with morphine reinforcement for both food-restricted and non-restricted rats (means \pm SEM).

Exps 2a and 2b: mNTS Leptin Administration Reduces Operant Responding for Sucrose in Both Chronically Food-Restricted and Free-Feeding Rats

Leptin (0.3 µg) delivered to the mNTS 3 h before testing significantly suppressed the breakpoint for lever press responding for sucrose under a PR schedule for both food-restricted (Exp. 2a) and non-restricted, *ad libitum*-fed rats (Exp. 2b). This conclusion is supported by significant drug effects for the number of reinforcers earned before breakpoint (Figure 3a), ($F(1, 10) = 10.50$, $P < 0.01$ for Exp. 2a; $F(1, 10) = 11.21$, $P < 0.01$ for Exp. 2b), the breakpoint (data not shown), ($F(1, 10) = 10.97$, $P < 0.01$ for Exp. 2a; $F(1, 10) = 9.65$, $P < 0.05$ for Exp. 2b), and the number of lever presses on the active lever before breakpoint (Figure 3b), ($F(1, 10) = 9.33$, $P < 0.05$ for Exp. 2a; $F(1, 10) = 7.68$, $P < 0.05$ for Exp. 2b). For both Exps 2a and 2b, the number of lever presses on the inactive control lever was not influenced by mNTS leptin delivery (data not shown), ($F(1, 10) < 3.17$, $P > 0.1$).

Exps 3a and 3b: CPP for Food Reward is Reduced by mNTS Leptin Delivery in Food-Restricted, but Not *Ad Libitum*-Fed Rats

Unlike vehicle-treated rats, food-restricted rats infused intra-mNTS w/0.3 µg leptin before CPP testing did not display a shift in preference from baseline (before training) for the food-associated environment (Exp. 3a; Figure 4).

However, mNTS leptin delivery did not impact the expression of the food reward CPP relative to vehicle treatment for non-restricted rats (Exp. 3b; Figure 4). The main effect of drug was significant for CPP testing in Exp. 3a ($F(1, 11) = 8.36$, $P < 0.05$), but not for Exp. 3b ($F(1, 23) = 0.10$).

Exps 4a and 4b: CPP for Morphine-Associated Environment is Not Affected by mNTS LepRb Activation

Relative to vehicle treatment, 0.3 µg leptin delivery to the mNTS did not impact the expression of CPP for an environment associated with morphine reinforcement (Figure 5). This was the case for both food restricted (Exp. 4a), ($F(1, 16) = 0.23$), and non-restricted rats (Exp. 4b), ($F(1, 16) = 0.50$).

DISCUSSION

Leptin's anorectic effects are mediated, in part, by amplifying the food intake-suppressive effects of GI satiation signals via LepRb signaling in mNTS neurons (Hayes *et al*, 2010; Huo *et al*, 2007; Kanoski *et al*, 2012b). Here we highlight a novel role for mNTS LepRb signaling in the suppression of appetitive responding for palatable food. Results revealed reduced food-motivated responding following mNTS LepRb activation in two appetitive behavioral tests: (1) operant lever pressing for sucrose under a PR

reinforcement schedule, and (2) the expression of a CPP for palatable (high fat and high sucrose) food. The pattern of these effects were dependent on: (1) the presence vs absence of food consumption during testing, (2) the long-term energy status of the animals (chronic food restriction vs *ad libitum*-fed), and (3) nature of the positive reinforcement (palatable food vs morphine). Each of these features of our findings is discussed below.

Effort-based responding for sucrose in the PR lever-pressing test was reduced by mNTS LepRb activation regardless of whether the rats were chronically food restricted (Exp. 2a) or *ad libitum* fed (Exp. 2b). The PR breakpoint test conditions allowed for periodic consumption of the reinforcement after the exponentially increasing operant lever press demands were met (totaling approximately 0.33–0.75 g consumed per rat). Thus, effects on motivation to obtain the reward cannot be separated in terms of appetitive/incentive vs consummatory aspects of the reinforcement. In this sense, the reduced lever press responding for sucrose observed following mNTS LepRb activation can be interpreted as being attributable to effects on orosensory or postingestive processing (eg, amplifying satiation signals or altering taste) as opposed to effects on appetitive, food-seeking behavior *per se*. However, the case for suppression of appetitive behavior independent of orosensory or postingestive processing is supported by Exp. 3a results (CPP in food-restricted rats). In this case, CPP expression for food reward was suppressed by mNTS leptin despite the fact that the animals received no food during the 24-h period preceding CPP testing, nor did they receive food during the CPP test. In addition, these findings highlight a potential CNS site of action contributing to the inhibition of food-seeking/hoarding previously observed in food-restricted hamsters treated with peripheral or ICV leptin (Buckley and Schneider, 2003; Keen-Rhinehart and Bartness, 2008).

Unlike results from PR lever-pressing tests, CPP for food reward was *not* influenced by mNTS LepRb activation in animals that were maintained on an *ad libitum* feeding schedule and were only food restricted for 3 h before and during CPP testing (Exp. 3b). The differential effect of energy status on mNTS leptin-induced inhibition of lever pressing vs CPP may be based, in part, on the different underlying neural substrates between these two behavioral tests. Unlike the PR test, CPP expression requires hippocampal-dependent memory retrieval of external contextual cues. In addition to processing external contextual cues, neural processing in the hippocampus is also required for encoding the relationships between internal states (eg, different levels of food deprivation), the external environment, and goal-directed behavior (Kennedy and Shapiro, 2004). It may be that mNTS LepRb activation inhibits hippocampal-dependent contextual memory retrieval via an ascending polysynaptic pathway (eg, Castle *et al*, 2005), and that activation of this pathway is dependent on internal context (upregulated during energy restriction).

Alternatively, the different pattern of PR vs CPP results may be based on the fact that these two tests differ with regards to the opportunity for the animals to consume the food. For example, mNTS LepRb signaling reduces motivated responding for food reward independent of energy

status when orosensory and postingestive contact with food occurs (PR lever-pressing schedule; Exps 2a and 2b), suggesting that leptin signaling in this brain region may augment postingestive satiation signaling in both an energy-deplete and -replete state. However, in the absence of food intake (CPP for food; Exps 3a and 3b), LepRb activation in the mNTS only reduces motivated responding for food in an energy-deplete state (chronic food restriction). According to Rosenbaum and Leibel (2011), when energy stores drop below a neurally encoded threshold for minimum body fat, a compensatory physiological drive is invoked to help restore adipose stores. Within this framework, low endogenous LepRb activation on mNTS neurons in states of chronic food restriction may serve as a neural signal to engage in food-seeking behavior, and this behavior is suppressed when leptin levels are normalized endogenously (weight or adipose gain) or pharmacologically via mNTS exogenous delivery (Exp. 3a), whereas food seeking that occurs in the absence of metabolic need (Exp. 3b) may be less influenced by mNTS leptin signaling.

Unlike CPP for food reinforcement, CPP for morphine reinforcement was not affected by mNTS LepRb activation regardless of whether the animals were food restricted or maintained on *ad libitum* feeding. These results were unexpected for several reasons. First, CNS-delivered leptin (forebrain ventricle) reduces self-administration of rewarding electrical stimulation of the lateral hypothalamic area (LHA) in rats (Fulton *et al*, 2000, 2004), suggesting that CNS leptin may reduce reward value more generally. Consistent with these findings, leptin acts on receptors in the VTA to modulate meso-accumbal dopamine transmission and reduce feeding (Baldo and Kelley, 2007; Fulton *et al*, 2006; Hommel *et al*, 2006). One interpretation is that leptin action on receptors in certain forebrain and midbrain nuclei, such as the LHA and VTA, suppresses motivated responding for a range of reinforcers, whereas mNTS LepRb signaling is more specific to food reinforcement. However, there is evidence that LepRb and opioid receptor signaling interact in mNTS neurons based on findings that these systems have opposing effects on vagally mediated excitatory afferent drive. Neurophysiological data from Appleyard, Peters, and others show that while opioid agonists reduce the excitatory vagal afferent drive on mNTS neurons (Appleyard *et al*, 2005; Cui *et al*, 2012), leptin activates cultured nodose ganglia neurons in a cooperative manner with CCK (Peters *et al*, 2004, 2005, 2006). Further, peripheral administration of naloxone, an opioid receptor antagonist, decreases meal size in food-restricted sucrose-fed rats (Glass *et al*, 2001), an effect also observed following CNS or peripheral LepRb agonist delivery (Eckel *et al*, 1998; Kahler *et al*, 1998; Zorrilla *et al*, 2005). Thus, although leptin and opioid receptor activation appear to interact by differentially modulating GI-derived satiation signaling, our CPP results suggest that with regards to appetitive responding, mNTS LepRb signaling may have selective effects on reducing motivation to obtain food reinforcement. However, we note that this hypothesis requires testing across a range of other appetitive reinforcements. An alternative interpretation is that the differential effects of mNTS LepRb activation on appetitive responding for food vs opioid reinforcement is based on differences in reward magnitude. In other words, the putative higher reward magnitude of morphine

compared with food reinforcement may account for the differential effects of mNTS leptin on CPP responding. This interpretation is unlikely, however, given that mNTS leptin reduced CPP for food but not morphine in food-restricted rats despite the fact that the parameters used for CPP training and testing in these two experiments (Exps 3a and 4a) yielded comparable preference shifts following vehicle administration.

Overall results revealed that mNTS LepRb activation reduced food-seeking behavior and effort-based responding for palatable food. These findings alter the conventional wisdom that learned incentive and motivational aspects of feeding are uniquely controlled by neural processing in mesencephalon, diencephalon, and telencephalon regions. Mesolimbic dopaminergic signaling from dopamine-producing VTA neurons to ventral striatal nuclei is generally considered to be a central neural system in controlling incentive and motivational components of ingestion (Baldo and Kelley, 2007; Berridge, 2007; Kelley, 2004; Kenny, 2011; Volkow *et al*, 2011). Indeed, both CPP and operant lever pressing for food reward are modulated by manipulations in striatal DA receptor activity (for review, see Baldo *et al*, 2013). Our results suggest that food-seeking behavior also involves neuroendocrine signaling in the hindbrain, which likely engages ascending neural communication to mesolimbic nuclei that are traditionally linked with appetitive control. Consistent with this notion, ascending neural pathways have been characterized from the mNTS to the VTA (Rinaman, 2010), as well as from the mNTS to the nucleus accumbens (Alhadeff *et al*, 2012; Shekhtman *et al*, 2007). The role of these ascending pathways in the suppression of appetitive responding by mNTS LepRb signaling remains to be determined. A related area for future mechanistic follow-up work is to delineate the intracellular and downstream neurochemical mediators of mNTS LepRb-mediated inhibition of food-motivated behavior. Recent data reveal that GLP-1-producing (preproglucagon (PPG)-expressing) neurons in the mNTS project to the VTA and nucleus accumbens where GLP-1 receptor (GLP1-R) activity modulates feeding and food-motivated responding (Alhadeff *et al*, 2012; Dickson *et al*, 2012; Dossat *et al*, 2011). It may be that LepRb activation on mNTS neurons augments PPG neural transmission to mesolimbic GLP-1R-expressing nuclei via either direct (PPG and LepRb are co-expressed in mNTS neurons for the mouse (Garfield *et al*, 2012; Goldstone *et al*, 1997; Huo *et al*, 2008)) or indirect pathways (PPG and LepRb are not co-expressed in mNTS neurons for the rat; Huo *et al*, 2008).

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