How Leptin Controls the Drive to Eat

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A complex set of brain based systems modulate feeding to maintain constant body weight. The adipose derived-hormone, leptin, plays a crucial role in this control by acting on diverse leptin receptor (LepRb)-expressing neurons in the hypothalamus and brainstem to modify behavior and metabolism. In addition to controlling energy expenditure and satiety, leptin controls motivation and the reward value of food by regulating two interconnected systems: hypocretin (HCRT) neurons and the mesolimbic dopamine (MLDA) system. Modest/acute decreases in leptin levels, as associated with mild caloric restriction, increase MLDA activity and overall food-seeking behavior; in contrast, severe starvation or complete leptin deficiency blunt MLDA activity, along with motivation and associated behaviors. Lateral hypothalamic (LHA) LepRb neurons project to dopamine (DA) neurons in the ventral tegmental area, where neurotensin (NT) release augments MLDA function; these LepRbNT cells also innervate HCRT neurons to control Hcrt expression and inhibit HCRT neurons. Ablation of LepRb in these cells abrogates the control of HCRT cells by leptin and decreases activity and MLDA function. We propose that this neural pathway regulates the MLDA, activity, and motivation in response to leptin and nutritional status.

Key words: Hypothalamus, Dopamine, Ventral tegmental area, Leptin, Neurotensin, Obesity, Feeding

Introduction

Central regulation of energy homeostasis

The amount of energy consumed relative to that expended by an animal dictates the expansion or contraction of its body energy (fat) stores, which are crucial for survival during extended periods without feeding. Body mass and adiposity generally remain within a narrow range over the long term, consistent with the homeostatic control of body adiposity.¹ Indeed, in humans and other animals, insufficient caloric intake to match utilization (which decreases fat stores) promotes hunger and decreases energy expenditure to promote the restoration of adiposity to previous levels.²,³: This response to decreased energy stores underlies the eventual weight regain experienced by the vast majority of overweight individuals who initially lose weight (by dieting).⁴ Conversely, overfeeding (e.g., by the direct infusion of food into the gut in experimental animals) suppresses voluntary food intake and increases energy expenditure, reducing energy stores toward baseline.⁵ Thus, animals possess homeostatic systems that modulate feeding and energy utilization to maintain adiposity within acceptable levels. Obesity, then, must result from overriding the processes that control energy homeostasis.

In animals, the central nervous system (CNS) detects fuel availability and coordinates physiologic and behavioral parameters to maintain long-term energy balance.¹ This homeostatic regulation of energy balance is initiated by specialized neurons in the brainstem and hypothalamus that sense relevant cues, such as fuels and hormones that reflect nutritional status. To control feeding, these neurons modulate two essentially separate parameters- satiation and the incentive to eat. Generally speaking, satiation (which is commonly associated with a feeling of fullness) results from the action of interconnected neural circuits in the brainstem and hypothalamus that sense relevant cues, such as fuels and hormones that reflect nutritional status. To control feeding, these neurons modulate two essentially separate parameters- satiation and the incentive to eat. Generally speaking, satiation (which is commonly associated with a feeling of fullness) results from the action of interconnected neural circuits in the brainstem and hypothalamus that promote meal termination. Dopamine (DA)- containing midbrain neurons that project to limbic regions (the mesolimbic DA (MLDA) system) encode the attractiveness food, as well as other rewards (sex, drugs of
abuse, etc.

The MLDA system interacts with hypothalamic circuits (which are largely distinct from those that encode satiety) to control food seeking, the initiation of feeding, and how hard an animal is willing to work to obtain food.

1. Leptin and the regulation of energy balance

Leptin is among the most crucial physiologic cues that participate in the control of energy homeostasis. Discovered in 1994, leptin is a peptide hormone produced by white adipose tissue; it is released into the circulation in proportion to triglyceride stores. The central role for leptin in energy balance is apparent from the phenotypes of mice null for leptin (LepDob) or null for the signaling-competent form of its receptor (LepRb; LepRb+/+ mice) which exhibit severe hyperphagia and decreased energy expenditure (due to decreased activity, sympathetic tone and thyroid function), and consequent obesity. Leptin suppresses food intake in normal animals and attenuates the phenotype of LepRb (but not LepRb+/+) mice.

The decreased energy expenditure and increased appetite of weight-reduced animals and humans is associated with decreased circulating leptin (commensurate with lower fat mass). Treatment with exogenous leptin to restore leptin concentrations to pre-weight-loss levels blunts the hunger and reduced energy expenditure that accompanies weight reduction, thus revealing the crucial role for (low) leptin in the homeostatic response to weight loss.

2. Leptin action through LepRb in the brain

Receptors for leptin have been identified in many tissues throughout the body; the majority of leptin’s actions on energy homeostasis are attributable to effects mediated by LepRb in the brain, however. Intracerebroventricular (ICV) leptin is as effective as intraperitoneal (IP) leptin at decreasing appetite and increasing energy expenditure in rodents. Furthermore, CNS-specific disruption of leptin action (by ablation of LepRb) results in hyperphagia and obesity, and CNS-restricted restoration of LepRb in LepRb+/+ mice normalizes energy balance.

Within the CNS, multiple groups of anatomically and functionally distinct neurons express LepRb. Most of these LepRb neurons lie within nuclei of the hypothalamus and brainstem that have known roles in energy balance. Commensurate with the diverse processes regulated by leptin, each set of anatomically and molecularly distinct LepRb neurons appears to play a unique role in energy balance. For instance, ablation of LepRb in the hindbrain nucleus tractus solitarius (NTS; which plays an important role in satiety) results in increased meal size and reduced sensitivity to peripheral satiety signals, but does not change energy expenditure, suggesting that reduced leptin action on these cells in weight-reduced animals specifically decreases satiety to promote increased food intake.

LepRb in the hypothalamus mediates the majority of leptin action on energy balance. As elsewhere, however, deletion of LepRb within specific hypothalamic regions produces more circumscribed effects, consistent with distinct roles for subgroups of hypothalamic LepRb cells. For example, ablation of LepRb from the ventromedial hypothalamic nucleus (VMH, which controls sympathetic tone and energy expenditure) blunts the increased energy utilization associated with high fat diet-induced obesity, but does not alter feeding.

Other important sets of hypothalamic LepRb neurons include arcuate nucleus (ARC) neuropeptide-Y (NPY)/agouti-related peptide (AgRP) neurons (which stimulate feeding and inhibit energy expenditure) and pro-opiomelanocortin (POMC) neurons (which decrease feeding and increase energy utilization). Leptin inhibits NPY/AgRP neurons and activates POMC cells. While NPY/AgRP and POMC cells are crucial for energy balance, disruption of LepRb from both cell types only modestly alters feeding and energy homeostasis, indicating that additional sets of LepRb neurons must play important roles in controlling feeding and body weight.

LepRb neurons that express neuronal nitric oxide synthase (Nos1) or the vesicular gamma amino butyric acid (GABA) transporter (Vgat) are required for the control of feeding and energy balance by leptin; these hypothalamic Nos1- and Vgat-expressing LepRb neurons may each act in part by indirectly controlling NPY/AgRP and POMC cells, however.

In addition to modulating energy expenditure, the circuit containing NPY/AgRP and POMC neurons plays a crucial role in the control of satiation. In addition to controlling hindbrain satiety systems indirectly, by acting on brainstem-projecting neurons in the paraventricular nucleus of the hypothalamus (PVH), AgRP and POMC cells send direct projections to brainstem satiety centers.

3. Leptin, energy balance, and the control of incentive and reward

While one often-quoted theory holds that obesity results from “leptin resistance” (a failure of LepRb signaling correlated with increased adiposity), many data contravene this model. Indeed, the elevated circulating leptin that results from increased adiposity in obesi-
ty augments hypothalamic LepRb signaling in diet-induced obese animals relative to lean controls. Thus, rather than overriding LepRb signaling, the ubiquity of palatable food in the developed world may override the systems that restrain the incentive value of food, resulting in hedonic overfeeding. Indeed, satiety signals appear largely intact in obesity, while motivation for highly palatable/rewarding food remains high.

The incentive value of food (and other rewards) is encoded by the MLDA system. At the core of this system lie ventral tegmental area (VTA) DA neurons that project to and release DA in the nucleus accumbens (NAc). Several lines of evidence demonstrate the modulation of reward and the MLDA system by feeding status and leptin. For instance, the reinforcing properties of drugs of abuse and rewarding brain stimulation (which are encoded by effects in the MLDA system) are augmented by food restriction and blunted by leptin treatment.

4. Acute and chronic modulation of incentive and reward by energy balance and leptin

The control of MLDA function and reward by nutritional status is complex, displaying distinct responses depending upon the duration and severity of the caloric deficit. Short-term or moderate caloric restriction increases the incentive value of food (including the amount of work an animal is willing to expend to obtain food), and also increases locomotor activity (which is a DA-dependent motivated behavior required for foraging). Intuitively, this makes sense, because increased food seeking by animals with moderate caloric deficits should augment their chances of discovering and acquiring food, thus increasing feeding and restoring adiposity to appropriate levels.

In contrast to moderate caloric deficits, prolonged starvation that reduces energy stores to near zero decreases locomotor activity (including foraging) and diminishes the amount of work an animal will perform to obtain food (although feeding is increased over baseline if food is available with minimal work). Presumably, this reflects the likelihood that there is little or no food to be found in the environment under circumstances where energy stores have fallen to near zero, and that the animal is more likely to survive by conserving energy stores until such time as food availability increases (rather than by expending remaining energy in a fruitless search for essentially non-existent food).

5. Modulation of the MLDA system by leptin

Consistent with a role for very low leptin in the response to prolonged starvation, genetically leptin- or LepRb-deficient animals exhibit very low locomotor activity and, despite consuming more food when it is freely available, will perform less work (e.g., fewer lever presses) to obtain food. Thus, lifelong, absolute leptin deficiency mimics the behavioral response to severe prolonged starvation, decreasing foraging and the motivation to acquire food when obtaining food required substantial work. Importantly, leptin-deficient Lepobob mice exhibit decreased expression of tyrosine hydroxylase (Th; the rate-limiting enzyme in DA synthesis) in the VTA, with consistent decreases in DA stores; chronic leptin treatment ameliorates these effects. Thus, decreased Th expression (and consequently lower DA availability within the MLDA) presumably underlies at least part of the decreased activity and motivation of Lepobob animals.

Acute changes in leptin concentration affect the MLDA system differently than does the prolonged absolute deficiency of leptin in Lepobob mice. Acute leptin treatment of normal animals does not affect VTA Th expression, but increases Amphetamine (AMPH)-evoked DA release. AMPH reverses the synaptic DA reuptake transporter, DAT, to release cellular DA into the synaptic cleft, and the acute effect of leptin on the AMPH response reflects increased DAT activity in NAc tissue (rather than increased DA stores). These findings are consistent with the notion that acute/modest decreases in leptin decrease DAT activity, prolonging and increasing DA concentration within the synapse, thereby increasing locomotor activity and motivation. Indeed, moderate fasting decreases NAc DAT activity, presumably increasing extracellular DA and thus promoting DA-dependent locomotor activity and motivation to feed. Similarly, the increased NAc DAT activity of leptin-treated animals correlates with their decreased motivation for rewards such as sucrose or high-fat food.

6. Direct effects of leptin on the mesolimbic dopamine system

A subset (approximately 5%) of DA neurons in the VTA and the neighboring substantia nigra (SN) express LepRb. The disruption of LepRb expression in DA neurons does not alter locomotor activity, body weight or feeding, however. Additionally, direct VTA leptin administration does not restore VTA Th expression in Lepobob mice.

Interestingly, mice lacking LepRb in DA neurons display an anxious phenotype, consistent with changes in DA function distinct from those attributable to alterations in NAc-mediated reward. Indeed, tracing specifically from VTA LepRb neurons (approximately 75% of which are DA neurons) revealed their dense innervation of the
central nucleus of the amygdala (CeA) and associated structures, but few projections to the NAc. The CeA plays a crucial role in aversive learning and anxiety-like behaviors. Collectively, these data are consistent with the notion that direct leptin action on VTA DA neurons controls CeA-mediated anxiety-related behaviors. The existence of such a system makes teleological sense, since the increased foraging activity that is stimulated by negative energy balance/low leptin exposes animals to the increased risk of predation, requiring increased anxiety/vigilance to ensure survival.

In contrast to the lack of effect of LepRb ablation from DA neurons on energy balance, acute VTA leptin administration decreases food intake and interference with leptin action in all VTA LepRb neurons promotes reward-driven feeding. Since not all VTA LepRb neurons contain DA (approximately 25% contain GABA), non-DA LepRb cells in the VTA might participate in controlling reward in response to leptin. The responses observed to pan-VTA LepRb modulation do not approach the full effect of systemic leptin on MLDA function and motivation, however, implying that leptin must regulate most aspects of MLDA function via a different pathway.

7. The lateral hypothalamic area as a relay center to the MLDA system

The lateral hypothalamic area (LHA) links the interoceptive homeostatic circuits of the hypothalamus to the MLDA system. Positioned around the fornix, the LHA is ideally located to integrate nutritional, endocrine and autonomic information from neighboring hypothalamic sites for relay to the MLDA system. The LHA was originally described as a “feeding center” because its electrolytic ablation abrogates the motivation to feed, resulting in starvation.

The LHA sends dense rostral projections to the septal nuclei and striatum and strongly innervates midbrain sites that include the VTA, SN, and dorsal raphe (DR). Animals will self-administer activation of the LHA projections that specifically innervate the VTA, revealing the importance of the LHA—VTA circuit for this reward signaling. Furthermore, LHA self-stimulation is enhanced by negative energy balance, implying a role for the LHA in modulating motivated behavior based upon nutritional status.

8. Lateral hypothalamic leptin action and the control of MLDA function

The LHA contains a large population of LepRb neurons that participate in the control of food intake and energy homeostasis. All LHA LepRb neurons contain the GABA-synthesizing enzyme, glutamate decarboxylase-1 (GAD1), as well as the vesicular GABA transporter, vGAT, suggesting their potential use of GABAergic (inhibitory) neurotransmission. Intra-LHA leptin decreases feeding and body weight in rats and LepRbKO mice, consistent with a role for leptin action via LHA LepRb cells in the control of energy balance.

Although conventional anterograde tracing techniques have demonstrated widespread projections from the LHA (including to the cortex, striatum, midbrain, and hindbrain), cell-specific viral tracers reveal that (beyond their projections within the LHA) LHA LepRb neurons project primarily to midbrain centers (including the VTA, SN, and DR), suggesting that projections to these structures might contribute to leptin action on the MLDA system. Indeed, intra-LHA (but not intra-VTA) leptin injection restores VTA Th expression in LepRbKO mice. Thus, LHA leptin acts via LHA LepRb neurons to regulate the MLDA system. Interestingly, activation of the LHA GABA—VTA circuit promotes food seeking; although LepRb neurons represent only a subpopulation of LHA GABA cells, it is tempting to speculate that LHA LepRb neurons participate in this effect.

9. Neurochemical subpopulations of LHA LepRb neurons:

LepRbNT neurons

In addition to GABA, LHA LepRb neurons contain several other neurotransmitters, each of which presumably plays a distinct role in the function of these neurons and in overall energy homeostasis. The largest identified subpopulation of LHA LepRb neurons (approximately 60% of LHA LepRb cells) contains the neuropeptide neurotensin (NT; LepRbNT cells, which are only found in the LHA). Electrophysiological data suggest that leptin activates the majority of LepRbNT neurons; while leptin does not acutely modulate NT expression, NT expression in these cells is decreased in LepRbKO mice, suggesting that diminished NT signaling might contribute to the phenotype of leptin-deficient animals.

Genetic ablation of LepRb in LepRbNT cells (LepRbNTKO mice) leads to mild obesity in association with decreased metabolic rate and decreased volitional activity. In addition to reduced baseline activity, LepRbNTKO mice exhibit a blunted AMPH-induced locomotor response, indicating altered MLDA function. Indeed, NAc DAT activity (which controls the locomotor response to AMPH) is decreased in LepRbNTKO mice. Thus, the absolute lack of leptin action via LepRbNT
neurons appears to control DAT activity (which correlates with acutely decreased leptin action in normal animals), rather than altering Th expression or DA content as in Lep"shb" animals. These findings imply a potential specificity of the LepRb"NT" neuron for the control of DAT, rather than VTA Th expression, suggesting a potential role for these cells in the response to acute changes in energy balance. Differences between the responses to acute and chronic leptin deficiency may be a result of the specific LepRb neurons and pathways affected, rather than from differences in the duration or amplitude of leptin deficiency.

NT itself contributes to MLDA control by LHA leptin action. Not only does acute ICV administration of NT reduce feeding in rodents, but also NT participates in the overall control of the MLDA system: DA neurons in the VTA and SN express NT receptor-1 (NTR1), NT activates VTA DA neurons, and VTA-administered NT increases locomotor activity and suppresses reward-associated feeding. Furthermore, activation of LHA NT neurons promotes NT efflux in the VTA, which in turn increases extracellular DA concentration in the NAc. (ICV MCH administration increases food intake and transgenic KO mice fail to display fasting-induced hyperphagia and lean, suggesting an important role for MCH in promoting food intake. LHA MCH neurons also modulate the MLDA system: Although they project widely, the NAc shell represents a major target, and MCH in the NAc promotes feeding. Fasting increases Pmch expression, and exogenous leptin suppresses this effect. A variety of indirect data suggest that leptin may influence MCH neurons via ARC NPY/AgRP and POMC cells.

LHA HCRT neurons also project widely throughout the brain, but densely innervate the VTA, SN, and DR in particular. HCRT neurons promote arousal, locomotor activity, and motivation- in part through an excitatory connection to VTA DA neurons. Acute administration of HCRT in mice increases activity, along with food-seeking behavior and feeding, suggesting that HCRT could promote obesity. Mice lacking HCRT, HCRT neurons, or HCRT receptors are obese due to decreased locomotor activity and energy expenditure, however. The findings that fasting activates HCRT cells and HCRT is required for fasting-induced locomotor activity suggest that, rather than being strictly obesogenic or anti-obesogenic, HCRT is required to support general levels of motivated behaviors (including locomotor activity) at baseline, and also plays a crucial role in promoting the locomotor activation associated with increased food-seeking during moderate caloric restriction.

11. Another link to the MLDA system: LHA LepRb neurons and the control of HCRT signaling

Leptin controls HCRT neurons by several mechanisms: leptin inhibits the fasting-induced activation of these cells, but also increases Hcrt expression. Thus, leptin may promote the expression of Hcrt to support generalized locomotor activity, while inhibiting HCRT neurons acutely to attenuate the locomotor activation and foraging that are stimulated by negative energy balance.

HCRT neurons do not contain LepRb, so leptin must regulate HCRT cells indirectly; indeed LHA LepRb neurons innervate and regulate local HCRT neurons. The use of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) has allowed for targeted remote pharmacological activation or inactivation of specific neurons of interest. DREADD-induced activation of LHA NT neurons leads to hyperpolarization and reduced action potential firing of HCRT neurons in slice preparations. Also, the inhibition of HCRT neurons by leptin in slice preparations is abolished in LepRb"NT"KO mice. Consistently, LepRb"NT"KO mice fail to display fasting-induced

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activation of HCRT neurons, and leptin does not increase Hcrt expression in these animals (as it does in controls). Thus, leptin inhibits HCRT cells via its actions on LepRbNT neurons.

HCRT neurons do not contain NT receptors and are not inhibited by NT application, suggesting that other neurotransmitters in LHA LepRbNT neurons must mediate the inhibition of HCRT cells by leptin. While LHA LepRb neurons (including LepRbNT cells) contain inhibitory GABA, leptin neither requires GABA signaling nor increases GABA transmission onto HCRT neurons, suggesting that GABA does not represent the crucial transmitter by which LHA LepRb neurons inhibit HCRT cells. A subset of LHA LepRb neurons contain the inhibitory neuropeptide Gal, however, and many LHA Gal neurons also contain NT, suggesting a potential role for Gal in the inhibition of HCRT cells by leptin. Indeed, Gal inhibits HCRT neurons in slice preparations, and Gal receptor antagonist blocks the inhibition of HCRT neurons by leptin. Thus, the Gal-expressing subset of LHA LepRbNT neurons likely inhibits HCRT cells by the local release of Gal.

**Conclusion**

Proposed model of LHA leptin action: control of HCRT neurons and the MLDA system

LHA LepRb neurons constitute the major pathway by which leptin controls the MLDA. LHA LepRb neurons mediate this control by at least two mechanisms (Fig. 1). First, LHA LepRb neurons project directly to the VTA, where they release NT and other mediators to modulate the activity of DA neurons. This system might also control the expression of Th and the status of cellular DA stores. Second, LHA LepRb neurons project to and control HCRT neurons. In addition to increasing Hcrt expression (presumably to support general arousal and activity), LHA LepRb neurons inhibit the electrical activity of HCRT cells via Gal neurotransmission. This electrical inhibi-

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**Fig. 1.** Leptin acts on lateral hypothalamic area (LHA) leptin receptor (LepRb) neurons to modulate the mesolimbic dopamine (MLDA) system distinctly in response to mild and severe caloric restriction. Neurtensin- (NT)-containing LepRb (LepRbNT) neurons of the LHA project to and inhibit hypocretin (HCRT) neurons by releasing the neuropeptide galanin (Gal). Short-term fasting activates HCRT neurons (presumably at least in part by the withdrawal of Gal signaling associated with decreased leptin), increasing locomotor activity and the motivation to feed. It is possible that HCRT neurons could contribute to the control of nucleus accumbens (NAc) dopamine transporter (DAT) activity under these circumstances, as well. LHA LepRbNT neurons also project directly to the ventral tegmental area (VTA), where they release NT (and possibly other factors) to modulate dopamine (DA) neuron activity. This pathway might regulate tyrosine hydroxylase (Th) expression to control DA production (although other mechanisms could also be responsible), suppression of VTA Th presumably underlies the decreased activity and motivation that accompany the near-total leptin deficiency of severe starvation.
tion presumably blunts acute activation of HCRT neurons and foraging behavior. Interestingly, the behavioral response to brief fasting correlates with HCRT neuron activation and decreased DAT activity, both of which are dysregulated in LepRb<sup>NT</sup> KO animals; one model for this system is that the acute control of NAc DAT activity could be mediated by the HCRT neuron and its effects on the MLDA system.

Obviously, there are many questions that remain to be answered regarding the mechanisms by which energy balance (and leptin, specifically) controls the MLDA and DA-dependent behavior. In addition to understanding the details of how LepRb<sup>NT</sup> neurons and HCRT may mediate increased locomotor activity and motivation during acute fasting, it will be important to define the changes that mediate the MLDA suppression that occurs with the depletion of energy reserves. This will likely require us to decipher roles for additional subpopulations of LHA LepRb neurons, to define additional neurotransmitters in LHA LepRb cells, and to thoroughly map cellular connectivity within these complicated circuits. In the end, determining the mechanisms by which nutritional cues control MLDA function will reveal potential targets at which to direct therapies that blunt hedonic overfeeding.

**Conflicts of Interest**

The authors declare that they have no competing interests.

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