How Leptin Controls the Drive to Eat

Christa M. Patterson¹, Martin G. Myers, Jr^{1,2,*}

¹Division of Metabolism, Endocrinology and Diabetes, Department of Internal Medicine; ²Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109, USA

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 LepRb^{NT} cells also innervate HCRT neurons to control *Hcr*t 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- satiety and the incentive to eat. Generally speaking, satiety (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

Corresponding author Martin G. Myers, Jr
Division of Metabolism, Endocrinology and Diabetes, Department of Internal Medicine,
University of Michigan Medical School, 6317 Brehm Tower, 1000 Wall St, Ann Arbor,
MI 48105, USA
Tel +1-734-647-9515 Fax +1-734-232-8175 E-mail mgyers@umich.edu

Copyright © 2015 Korean Society for the Study of Obesity

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

abuse, etc.).⁶ 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.⁷ The central role for leptin in energy balance is apparent from the phenotypes of mice null for leptin (*Lep^{ob/ob}*) or null for the signaling-competent form of its receptor (LepRb; *LepR^{db/db}* 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 *Lep^{ob/ob}* (but not *LepR^{db/db}*) mice.⁸

The decreased energy expenditure and increased appetite of weight-reduced animals and humans is associated with decreased circulating leptin (commensurate with lower fat mass).⁹ 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.¹⁰

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 brain¹¹, 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 *LepR^{db/db}* mice normalizes energy balance.¹²

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.¹³ 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.¹⁴

LepRb in the hypothalamus mediates the majority of leptin action on energy balance.¹⁵ 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.¹⁶

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).¹⁷ Leptin inhibits NPY/AgRP neurons and activates POMC cells.¹⁷ 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.¹⁸ 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^{19,20}; 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.²¹ 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.²²

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.²³ 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.²⁴

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).²⁵ 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.²⁶

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).²⁷ 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).²⁸ 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 pro-

longed 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.²⁹ 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 *Lep^{ob/ob}* 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.^{28,29} Thus, decreased *Th* expression (and consequently lower DA availability within the MLDA) presumably underlies at least part of the decreased activity and motivation of *Lep^{ob/ob}* animals.

Acute changes in leptin concentration affect the MLDA system differently than does the prolonged absolute deficiency of leptin in *Lep^{ob/ob}* mice. Acute leptin treatment of normal animals does not affect VTA *Th* expression, but increases Amphetamine (AMPH)-evoked DA release.³⁰ 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).³⁰ 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 activity³¹, 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.²⁶

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.²⁶ The disruption of LepRb expression in DA neurons does not alter locomotor activity, body weight or feeding, however.³² Additionally, direct VTA leptin administration does not restore VTA *Th* expression in *Lep^{ob/ob}* mice.²⁸

Interestingly, mice lacking LepRb in DA neurons display an anxiogenic phenotype, consistent with changes in DA function distinct from those attributable to alterations in NAc-mediated reward.³² 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.³³ The CeA plays a crucial role in aversive learning and anxiety-like behaviors.³⁴ 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.³⁵ 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.^{28,36} 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.³⁷

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).³⁸ 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.³⁹ Furthermore, LHA self-stimulation is enhanced by negative energy balance, implying a role for the LHA in modulating motivated behavior based upon nutritional status.^{40,41}

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

The LHA contains a large population of LepRb neurons that par-

ticipate in the control of food intake and energy homeostasis.²⁸ 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.²⁸ Intra-LHA leptin decreases feeding and body weight in rats and *Lep^{ob/ob}* mice, consistent with a role for leptin action via LHA LepRb cells in the control of energy balance.²⁸

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)⁴², 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 *Lep^{ob/ob}* mice.²⁸ Thus, LHA leptin acts via LHA LepRb neurons to regulate the MLDA system. Interestingly, activation of the LHA GABA→VTA circuit promotes food seeking⁴³; 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: LepRb^{NT} 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; LepRb^{NT} cells, which are only found in the LHA).⁴² Electrophysiological data suggest that leptin activates the majority of LepRb^{NT} neurons; while leptin does not acutely modulate *Nt* expression, *Nt* expression in these cells is decreased in *Lep^{ob/ob}* mice, suggesting that diminished NT signaling might contribute to the phenotype of leptin-deficient animals.

Genetic ablation of LepRb in LepRb^{NT} cells (LepRb^{NT}KO mice), leads to mild obesity in association with decreased metabolic rate and decreased volitional activity. In addition to reduced baseline activity, LepRb^{NT}KO mice exhibit a blunted AMPH-induced locomotor response, indicating altered MLDA function.⁴² Indeed, NAc DAT activity (which controls the locomotor response to AMPH) is decreased in LepRb^{NT}KO mice. Thus, the absolute lack of leptin action via LepRb^{NT}

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^{ob/ob}* 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 rodents⁴⁴, 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.^{45,46} Furthermore, activation of LHA NT neurons promotes NT efflux in the VTA, which in turn increases extracellular DA concentration in the NAc.⁴⁷

NTR1 null (NTR1KO) mice exhibit increased consummatory activity and susceptibility to high-fat feeding-induced obesity.⁴⁶ NTR1KO mice also display altered responses to intra-LHA leptin administration—exhibiting increased feeding and decreased VTA *Th* expression (the opposite of the effect seen in *Lep^{ob/ob}* mice). This reversal of LHA leptin action in NTR1KO mice suggests the presence of additional neuropeptides and/or transmitters in LHA LepRb neurons that act distinctly from NT in their regulation of the MLDA system by leptin. Indeed, subsets of LHA LepRb neurons express *Galanin* (*Gal*), *Tachykinin-1* (*Tac1*, which encodes the precursor for substance P), and *corticotropin-releasing hormone* (*Crh*).^{48,49} Most of the peptides overlap to some extent with NT, suggesting that many individual LHA LepRb neurons contain multiple neuropeptides.

10. HCRT and MCH neurons of the LHA

The LHA also contains non-LepRb neurons, each of which plays specific roles in behavior and the regulation of the MLDA system. In addition to non-LepRb GABA neurons and non-LepRb NT neurons, important non-LepRb LHA neurons include distinct sets of cells that express the neuropeptides hypocretin (HCRT, also known as orexin) or melanin-concentrating hormone (MCH).⁵⁰

ICV MCH administration increases food intake and transgenic overexpression of MCH results in hyperphagia and obesity in mice,

while *Pmch*-null mice are hypophagic and lean, suggesting an important role for MCH in promoting food intake.⁵⁰ 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.⁵¹ Fasting increases *Pmch* expression, and exogenous leptin suppresses this effect.⁵² A variety of indirect data suggest that leptin may influence MCH neurons via ARC NPY/AgRP and POMC cells.⁵³

LHA HCRT neurons also project widely throughout the brain, but densely innervate the VTA, SN, and DR in particular.⁵⁴ HCRT neurons promote arousal, locomotor activity, and motivation—in part through an excitatory connection to VTA DA neurons.⁵⁵ Acute administration of HCRT in mice increases activity, along with food-seeking behavior and feeding, suggesting that HCRT could promote obesity.^{55,56} Mice lacking HCRT, HCRT neurons, or HCRT receptors are obese due to decreased locomotor activity and energy expenditure, however.⁵⁷ 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.^{57,58}

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 *Hcr*t expression.^{42,59} Thus, leptin may promote the expression of *Hcr*t 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.²⁸ 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.⁶⁰ 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

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 LepR^{bNT} neurons.

HCRT neurons do not contain NT receptors and are not inhibited by NT application, suggesting that other neurotransmitters in LHA LepRb^{NT} neurons must mediate the inhibition of HCRT cells by leptin.⁴⁶ While LHA LepRb neurons (including LepRb^{NT} 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.⁶⁰ 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.⁴⁸ Indeed, Gal inhibits HCRT neurons in slice preparations, and Gal receptor antagonist blocks the inhibition of HCRT neurons by leptin.⁶⁰ Thus, the Gal-expressing subset of LHA LepRb^{NT} neurons

likely inhibits HCRT cells by the local release of Gal.

Conclusion

Proposed model of LHA leptin action: control of HRCT 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-

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. Neurotensin- (NT)-containing LepRb (LepRb^{NT}) 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 LepRb^{NT} 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^{NT}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^{NT} 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.

Acknowledgements

Supported by NIH DK078056, the Michigan Diabetes Research Center (DK020572), and the Marilyn H Vincent Foundation. We thank David Olson, MD, PhD, and current and former members of the Myers and Olson labs for their discussion and input.

References

- Berthoud HR. Interactions between the “cognitive” and “metabolic” brain in the control of food intake. *Physiol Behav* 2007;91:486-98.
- Levin BE, Keesey RE. Defense of differing body weight set points in diet-induced obese and resistant rats. *Am J Physiol* 1998;274(2 Pt 2):R412-9.
- Hill JO, Anderson JC, Lin D, Yakubu F. Effects of meal frequency on energy utilization in rats. *Am J Physiol* 1988;255(4 Pt 2):R616-21.
- Kramer FM, Jeffery RW, Forster JL, Snell MK. Long-term follow-up of behavioral treatment for obesity: patterns of weight regain among men and women. *Int J Obes* 1989;13:123-36.
- Seeley RJ, Matson CA, Chavez M, Woods SC, Dallman MF, Schwartz MW. Behavioral, endocrine, and hypothalamic responses to involuntary overfeeding. *Am J Physiol* 1996;271(3 Pt 2):R819-23.
- Kenny PJ. Reward mechanisms in obesity: new insights and future directions. *Neuron* 2011;69:664-79.
- 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;372:425-32.
- Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;395:763-70.
- Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995;1:1155-61.
- Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 1999;341:879-84.
- Myers MG Jr. Outstanding Scientific Achievement Award Lecture 2010: deconstructing leptin: from signals to circuits. *Diabetes* 2010;59:2708-14.
- Chua SC Jr, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, et al. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science* 1996;271:994-6.
- Patterson CM, Leshan RL, Jones JC, Myers MG Jr. Molecular mapping of mouse brain regions innervated by leptin receptor-expressing cells. *Brain Res* 2011;1378:18-28.
- Hayes MR, Skibicka KP, Lechner TM, Guarnieri DJ, DiLeone RJ, Bence KK, et al. Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. *Cell Metab* 2010;11:77-83.
- Elmquist JK. Anatomic basis of leptin action in the hypothalamus. *Front Horm Res* 2000;26:21-41.
- Dhillon H, Zigman JM, Ye C, Lee CE, McGovern RA, Tang V, et al. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron* 2006;49:191-203.
- Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Cen-

- tral nervous system control of food intake. *Nature* 2000;404:661-71.
18. Balthasar N, Coppari R, McMinn J, Liu SM, Lee CE, Tang V, et al. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron* 2004;42:983-91.
 19. Leshan RL, Greenwald-Yarnell M, Patterson CM, Gonzalez IE, Myers MG Jr. Leptin action through hypothalamic nitric oxide synthase-1-expressing neurons controls energy balance. *Nat Med* 2012;18:820-3.
 20. Vong L, Ye C, Yang Z, Choi B, Chua S Jr, Lowell BB. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. *Neuron* 2011;71:142-54.
 21. Zheng H, Patterson LM, Rhodes CJ, Louis GW, Skibicka KP, Grill HJ, et al. A potential role for hypothalamomedullary POMC projections in leptin-induced suppression of food intake. *Am J Physiol Regul Integr Comp Physiol* 2010;298:R720-8.
 22. Bouret SG, Draper SJ, Simerly RB. Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. *J Neurosci* 2004;24:2797-805.
 23. Myers MG Jr, Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab* 2010;21:643-51.
 24. la Fleur SE, Vanderschuren LJ, Luijendijk MC, Kloeze BM, Tiesjema B, Adan RA. A reciprocal interaction between food-motivated behavior and diet-induced obesity. *Int J Obes (Lond)* 2007;31:1286-94.
 25. Berridge KC. 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. *Physiol Behav* 2009;97:537-50.
 26. Figlewicz DP, Benoit SC. Insulin, leptin, and food reward: update 2008. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R9-19.
 27. Beninger RJ. The role of dopamine in locomotor activity and learning. *Brain Res* 1983;287:173-96.
 28. Leininger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, et al. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab* 2009;10:89-98.
 29. Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, et al. Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 2006;51:811-22.
 30. Perry ML, Leininger GM, Chen R, Luderman KD, Yang H, Gnengy ME, et al. Leptin promotes dopamine transporter and tyrosine hydroxylase activity in the nucleus accumbens of Sprague-Dawley rats. *J Neurochem* 2010;114:666-74.
 31. Figlewicz DP, Patterson TA, Johnson LB, Zavosh A, Israel PA, Szot P. Dopamine transporter mRNA is increased in the CNS of Zucker fatty (fa/fa) rats. *Brain Res Bull* 1998;46:199-202.
 32. Liu J, Perez SM, Zhang W, Lodge DJ, Lu XY. Selective deletion of the leptin receptor in dopamine neurons produces anxiogenic-like behavior and increases dopaminergic activity in amygdala. *Mol Psychiatry* 2011;16:1024-38.
 33. Leshan RL, Opland DM, Louis GW, Leininger GM, Patterson CM, Rhodes CJ, et al. Ventral tegmental area leptin receptor neurons specifically project to and regulate cocaine- and amphetamine-regulated transcript neurons of the extended central amygdala. *J Neurosci* 2010;30:5713-23.
 34. Davis M. The role of the amygdala in fear and anxiety. *Annu Rev Neurosci* 1992;15:353-75.
 35. Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, et al. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 2006;51:801-10.
 36. Berthoud HR, Münzberg H. The lateral hypothalamus as integrator of metabolic and environmental needs: from electrical self-stimulation to opto-genetics. *Physiol Behav* 2011;104:29-39.
 37. Olds J, Milner P. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 1954;47:419-27.
 38. Saper CB, Swanson LW, Cowan WM. An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. *J Comp Neurol* 1979;183:689-706.
 39. Kempadoo KA, Tourino C, Cho SL, Magnani F, Leininger GM, Stuber GD, et al. Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. *J Neurosci* 2013;33:7618-26.
 40. Fulton S, Woodside B, Shizgal P. Potentiation of brain stimulation reward by weight loss: evidence for functional heterogeneity in brain reward circuitry. *Behav Brain Res* 2006;174:56-63.
 41. Fulton S, Woodside B, Shizgal P. Modulation of brain reward circuitry by leptin. *Science* 2000;287:125-8.
 42. Leininger GM, Opland DM, Jo YH, Faouzi M, Christensen L, Cappellucci LA, et al. Leptin action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy balance. *Cell Metab* 2011;14:313-23.
 43. Nieh EH, Matthews GA, Allsop SA, Presbrey KN, Leppla CA,

- Wichmann R, et al. Decoding neural circuits that control compulsive sucrose seeking. *Cell* 2015;160:528-41.
44. Luttinger D, King RA, Sheppard D, Strupp J, Nemeroff CB, Prange AJ Jr. The effect of neurotensin on food consumption in the rat. *Eur J Pharmacol* 1982;81:499-503.
 45. Sotty F, Brun P, Leonetti M, Steinberg R, Soubri P, Renaud B, et al. Comparative effects of neurotensin, neurotensin(8-13) and [D-Tyr(11)]neurotensin applied into the ventral tegmental area on extracellular dopamine in the rat prefrontal cortex and nucleus accumbens. *Neuroscience* 2000;98:485-92.
 46. Opland D, Sutton A, Woodworth H, Brown J, Bugescu R, Garcia A, et al. Loss of neurotensin receptor-1 disrupts the control of the mesolimbic dopamine system by leptin and promotes hedonic feeding and obesity. *Mol Metab* 2013;2:423-34.
 47. Patterson CM, Wong JM, Leininger GM, Allison MB, Mabrouk OS, Kasper CL, et al. Ventral tegmental area neurotensin signaling links the lateral hypothalamus to locomotor activity and striatal dopamine efflux in male mice. *Endocrinology* 2015;156:1692-700.
 48. Laque A, Zhang Y, Gettys S, Nguyen TA, Bui K, Morrison CD, et al. Leptin receptor neurons in the mouse hypothalamus are colocalized with the neuropeptide galanin and mediate anorexigenic leptin action. *Am J Physiol Endocrinol Metab* 2013;304:E999-1011.
 49. Allison MB, Patterson CM, Krashes MJ, Lowell BB, Myers MG Jr, Olson DP. TRAP-seq defines markers for novel populations of hypothalamic and brainstem LepRb neurons. *Mol Metab* 2015;4:299-309.
 50. DiLeone RJ, Georgescu D, Nestler EJ. Lateral hypothalamic neuropeptides in reward and drug addiction. *Life Sci* 2003;73:759-68.
 51. Bittencourt JC, Elias CF. Melanin-concentrating hormone and neuropeptide EI projections from the lateral hypothalamic area and zona incerta to the medial septal nucleus and spinal cord: a study using multiple neuronal tracers. *Brain Res* 1998;805:1-19.
 52. Tritos NA, Mastaitis JW, Kokkotou E, Maratos-Flier E. Characterization of melanin concentrating hormone and preproorexin expression in the murine hypothalamus. *Brain Res* 2001;895:160-6.
 53. Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, et al. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* 1999;23:775-86.
 54. Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci U S A* 1999;96:748-53.
 55. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998;92:573-85.
 56. Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, et al. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res* 2000;873:181-7.
 57. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999;98:437-51.
 58. Diano S, Horvath B, Urbanski HF, Sotonyi P, Horvath TL. Fasting activates the nonhuman primate hypocretin (orexin) system and its postsynaptic targets. *Endocrinology* 2003;144:3774-8.
 59. Louis GW, Leininger GM, Rhodes CJ, Myers MG Jr. Direct innervation and modulation of orexin neurons by lateral hypothalamic LepRb neurons. *J Neurosci* 2010;30:11278-87.
 60. Goforth PB, Leininger GM, Patterson CM, Satin LS, Myers MG Jr. Leptin acts via lateral hypothalamic area neurotensin neurons to inhibit orexin neurons by multiple GABA-independent mechanisms. *J Neurosci* 2014;34:11405-15.