From Lesions to Leptin: Hypothalamic Control of Food Intake and Body Weight

Review

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Body weight seems to run within families. However, until recently, the contribution of genetics to obesity was not clear. The identification over the last few years of a number of different genetic mutations in signaling molecules and their receptors that result in changes in body weight has redefined this field of neuroscience (Spiegelman and Flier, 1996; Flier, 1998; Friedman and Halaas, 1998; Woods et al., 1998). By exploring the brain systems that employ recently identified signaling molecules, it has been possible to redefine the hypothalamic pathways involved in the regulation of feeding and body weight (Elmquist et al., 1998a; Woods et al., 1998). For a more general discussion of metabolic, autonomic, and endocrine factors affecting feeding, the reader is referred to a recent review by Woods et al. (1998).

The Dual Center Model for Regulation of Feeding
Most neuroscientists have grown up with the classic "dual center" model for understanding the role of the brain in regulation of feeding (Stellar, 1954). This model was derived originally from clinical observations in patients with Fröhlich's syndrome: pituitary tumors associated with excessive subcutaneous fat and hypogonadism (Bramwell, 1888; Fröhlich, 1901). Whether this adiposogenital syndrome was due to injury to the pituitary gland (as Fröhlich concluded at the time) or to the overlying hypothalamus was controversial. Cushing, for example, championed the view that Fröhlich's syndrome was due to hypophysial insufficiency (Crowe et al., 1910). In 1912, Aschner demonstrated in dogs that mere removal of the pituitary gland without damage to the overlying hypothalamus did not result in obesity (Aschner, 1912).

The role of the hypothalamus in regulating food intake and body weight was finally established in 1940 with the classic experiments of Hetherington and Ranson. They used the Horsley–Clarke stereotaxic apparatus to place bilateral electrolytic lesions in the hypothalamus of rats, without disturbing the pituitary gland (Hetherington and Ranson, 1940). They summarized these experiments by stating:

> A condition of marked adiposity characterized by as much as a doubling of body weight and a tremendous increase of extractable body lipids has been produced in rats by the placing of electrolytic lesions in

the hypothalamus. Examination of these lesions has shown them to be very large, but they all have in common extensive bilateral damage to the region occupied by the dorsomedial and ventromedial hypothalamic nuclei, the arcuate nucleus, the fornix, and that portion of the lateral hypothalamic area ventral to it, and probably also the ventral premammillary nuclei.

Conversely, Hetherington and Ranson noted that lesions in the adjacent lateral hypothalamus could lead to decreased food intake (Hetherington and Ranson, 1940; Stevenson, 1970). Anand and Brobeck (1951) pursued this observation in greater detail, demonstrating that lesions of the lateral hypothalamus at the level adjacent to the ventromedial nucleus caused loss of feeding, inanition, and even death by starvation. Thus, the concept arose of the lateral hypothalamic area serving as a "feeding center" and the ventromedial nucleus as a "satiety center" (Figure 1).

This model was attacked over the ensuing years by revisionists using more refined experimental methodologies. Gold and colleagues found that smaller lesions confined to the ventromedial nucleus were not effective in producing hyperphagia but that more dorsally placed lesions, disrupting the connections of the paraventricular nucleus, often were more effective (Gold, 1973). The paraventricular nucleus is a major source of input to the sympathetic and parasympathetic preganglionic neurons controlling the gastrointestinal tract, suggesting that the changes in feeding might be consequent to autonomic alterations (Saper et al., 1976; Swanson and Kuypers, 1980; Saper, 1995). To test this hypothesis, Opsahl and Powley (1974) and several subsequent groups examined the effect of severing the abdominal vagus nerve in rats with large lesions of the ventromedial nucleus, finding that subdiaphragmatic vagotomy prevented the hyperphagia and obesity (Inoue and Bray, 1977; Berthoud and Jeanrenaud, 1979; Gold et al., 1980; Bray et al., 1981; Cox and Powley, 1981; Sclafani et al., 1981). At the same time, several groups pointed out that the lateral hypothalamic lesions that cause hypophagia interrupt the ascending nigrostriatal bundle, resulting in a Parkinsonian syndrome and reduction in virtually all movement and behavior (see Ungerstedt, 1970; Stricker and Zigmond, 1976; Stricker and Verbalis, 1990; Bernardis and Bellinger, 1996).

New Molecular Probes Verify the Presence of Lateral Hypothalamic Phagic Neurons
Observations such as these questioned the validity of the dual center model and the role of the lateral hypothalamic area and the ventrobasal hypothalamus in controlling food intake (Bernardis and Bellinger, 1987, 1996). Nevertheless, as cell-specific lesion methods emerged, they refocused attention on the ventromedial and lateral hypothalamic syndromes. Lateral hypothalamic lesions produced by kainic acid resulted in hypophagia, even when the ascending dopaminergic system was not damaged (Grossman et al., 1978; Stricker et al., 1978). The

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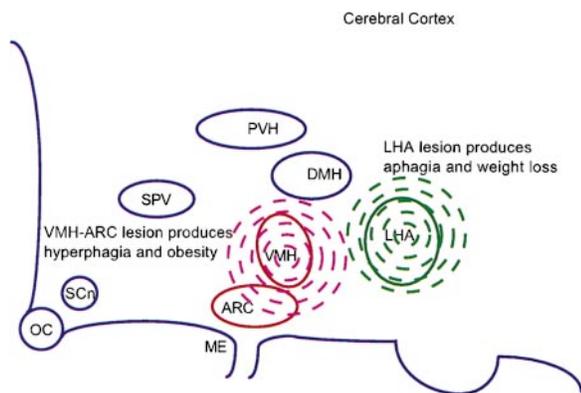
Figure 1. A Schematic Drawing of the Rat Hypothalamus in Sagittal Section Illustrating the Dual Center Hypothesis for Hypothalamic Control of Feeding

The most effective lesions for producing hyperphagia and obesity typically involved both the ventromedial (VMH) and arcuate (ARC) nuclei (illustrated in red, because these pathways normally inhibit eating). Aphagia and weight loss were produced by lateral hypothalamic (LHA) lesions at the same level of the hypothalamus (illustrated in green, because these pathways normally increase feeding). Other abbreviations: DMH, dorsomedial hypothalamic nucleus; ME, median eminence; OC, optic chiasm; PVH, paraventricular hypothalamic nucleus; SCn, suprachiasmatic nucleus; and SPV, subparaventricular zone.

Figure 2. MCH–Immunoreactive and Agouti-Related Protein–Immunoreactive Neurons in the Hypothalamus of the Human Brain

Note the presence in (A) of a cluster of MCH-IR neurons draped over the fornix (fx) and others spread among the fibers of the lenticular fasciculus (lf). In another section from the same brain (B), AgRP-IR cell bodies are seen in the arcuate nucleus (Arc). AgRP-IR axons run dorsally and laterally in a dense bundle into the region of the lateral hypothalamus containing the MCH and ORX neurons. Scale, 2 mm.

possibility that the lateral hypothalamus was involved in feeding was further supported by neuroanatomical studies showing a lateral hypothalamic cell system with direct projections both to the cerebral cortex and to the autonomic and motor systems of the brainstem and the spinal cord. This extensive system with both ascending and descending connections was predicted to have the necessary anatomical range to support a lateral hypothalamic phagic function (Saper, 1985; Saper et al., 1986; Bittencourt et al., 1992). This prediction was confirmed by the subsequent discovery that lateral hypothalamic neurons contain two newly recognized neuropeptides, melanin concentrating hormone (MCH) (Bittencourt et al., 1992) and the orexins (ORX) (Sakurai et al., 1998), also called hypocretin (de Lecea et al., 1998). MCH and ORX are found in separate but spatially overlapping populations of neurons in the perifornical region, lateral hypothalamic area, and zona incerta in the rodent and human brain (Broberger et al., 1998; Elias et al., 1998a; Peyron et al., 1998; see Figures 2A and 3A). Both cell groups appear to contribute to the entire range of lateral hypothalamic neuronal projections, from the cerebral cortex to the spinal cord (Bittencourt et al., 1992; Broberger et al., 1998; Elias et al., 1998a; Peyron et al., 1998; see Figure 4). For both of these peptides, the levels of mRNA are increased by starvation (Qu et al., 1996; Sakurai et al., 1998), when leptin levels rapidly fall (Frederich et al., 1995; Ahima et al., 1996), and the MCH mRNA levels are normalized by refeeding or by leptin administration (Qu et al., 1996). Injection of either MCH or ORX into the lateral ventricle causes rats to eat (Qu et al., 1996; Sakurai et al., 1998). Moreover, targeted deletion of the MCH gene results in decreased food intake and body weight compared to wild-type control mice (Shimada et al., 1998). Hence, it now seems likely that these specific populations of peptidergic neurons represent the substrate for Anand and Brobeck's lateral hypothalamic feeding center.

The specific CNS sites targeted by MCH and ORX neurons that are involved in feeding are not yet known. It therefore will be interesting to determine whether they include key autonomic regions such as the medial prefrontal cortex (infralimbic and prelimbic areas), the insular cortex, the parabrachial nucleus, and the dorsal vagal complex, as well as premotor neuronal pools in the brainstem and the spinal cord, which may control stereotyped feeding motor patterns (Bittencourt et al., 1992; Sawchenko, 1998).

Recent evidence suggests that neuropeptides that decrease food intake and body weight may also be expressed in the lateral hypothalamic area, and these may play a counterregulatory role, opposing the systems that increase food intake. One such peptide, CART (cocaine and amphetamine regulated transcript) is expressed in the lateral hypothalamic area and decreases food intake when centrally administered (Koylu et al., 1997; Kristensen et al., 1998). In addition, corticotropin releasing hormone mRNA is induced in lateral hypothalamic area neurons that innervate the parabrachial nucleus in a dehydration-induced model of anorexia (Kelly and Watts, 1996; Kelly and Watts, 1998). As corticotropin releasing hormone is thought to be anorectic, it is likely that lateral hypothalamic area neurons are influenced by physiological stimuli to integrate body weight and food intake. It is not known whether the same lateral hypothalamic neurons can express corticotropin releasing hormone during dehydration and MCH or ORX during starvation (see Sawchenko, 1998). If that were so, and the same lateral hypothalamic neurons were able to produce different neurotransmitters with distinct effects under differing conditions, it would represent a completely novel mechanism for the regulation of feeding.
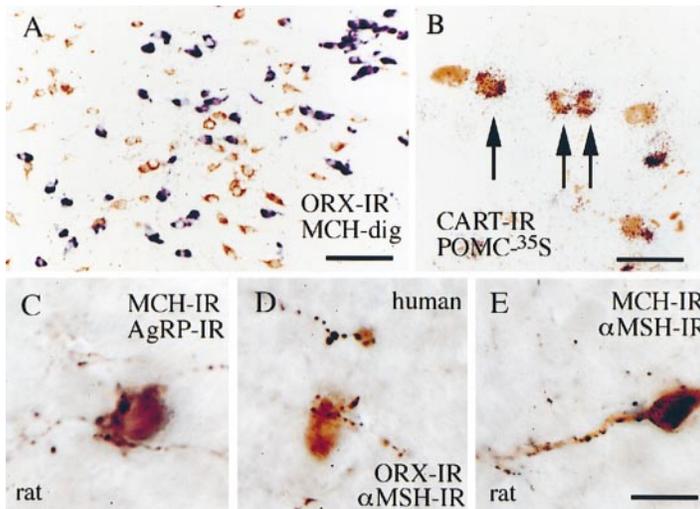
Figure 3. Dual Localization of Putative Neurotransmitters Involved in the Regulation of Feeding

(A) shows that the neurons in the lateral hypothalamic area that contain mRNA for MCH (stained with a blue histochemical reaction for digoxigenin label [dig]) are entirely distinct from the population of neurons that contain ORX (stained immunocytochemically [IR] with a brown label). Conversely, in (B), nearly all of the CART-IR neurons (stained brown) in the lateral arcuate nucleus are overlaid by autoradiographic silver grains, indicating hybridization for pro-opiomelanocortin (POMC) mRNA. (C), (D), and (E) demonstrate lateral hypothalamic neurons in the rat (C and E) and human (D) brain, stained brown with an immunocytochemical reaction for MCH or ORX and apposed by black immunostained axons that have been labeled with antisera against agouti-related protein (AgRP) or α-melanocyte stimulating hormone (αMSH). Scale bars, 250 μm (A); 100 μm (B); and 50 μm (C–E).

Leptin Action Illuminates a Medial Hypothalamic Satiety Network

Two earlier lines of evidence had focused attention on the medial hypothalamic ventromedial and arcuate nuclei in integrating circulating metabolic signals and producing satiety. First, in the early 1960s, Debons et al. (1962) found that systemic injections of gold thioglucose (a toxic glucose analog) caused injury to the arcuate and ventromedial nuclei, producing overeating and obesity (Debons et al., 1962). In the late 1960s, Olney and colleagues systemically administered monosodium glutamate to young mice while exploring the toxicity of food additives (Olney, 1969). They found a pattern of obesity and hypothalamic damage that was similar to the lesions produced by gold thioglucose (and produced some of the first evidence of glutamate excitotoxicity).

These findings raised the question: why are the lesions from these systemically administered toxins so localized? Although it is protected from circulating toxins by the blood–brain barrier, the brain maintains specific "windows on the circulation," the circumventricular organs, for interchanging signaling molecules with the bloodstream (Broadwell and Brightman, 1976). One of these windows, which is used by the hypothalamus to secrete pituitary releasing hormones into the hypophysial portal circulation, is the median eminence. The ventromedial and arcuate nuclei directly overlie the median eminence, and the experiments of Broadwell and Brightman (1976) demonstrated that even rather large proteins such as horseradish peroxidase can enter the medial hypothalamus from the median eminence.

The median eminence is also a candidate site at which circulating hormones, such as leptin, may enter the brain (Banks et al., 1996). Leptin is a peptide hormone made by white adipose cells (Zhang et al., 1994) during times of adequate metabolic substrate. Leptin is also produced under certain conditions by other cells such as those of the placenta (Bi et al., 1997; Masuzaki et al., 1997), gastric parietal cells (Bado et al., 1998), and skeletal muscle cells (Wang et al., 1998). The existence of a protein like leptin was hypothesized by Coleman and colleagues (Coleman and Hummel, 1969; Coleman, 1973), who studied two mutant mouse strains, the *ob/ob* mice and *db/db* mice, each of which had a phenotype of massive overeating, obesity, and delay of sexual maturation, similar to the patients noted clinically by Fröhlich and Cushing. Coleman performed parabiosis experiments, establishing cross-circulation between *ob/ob*
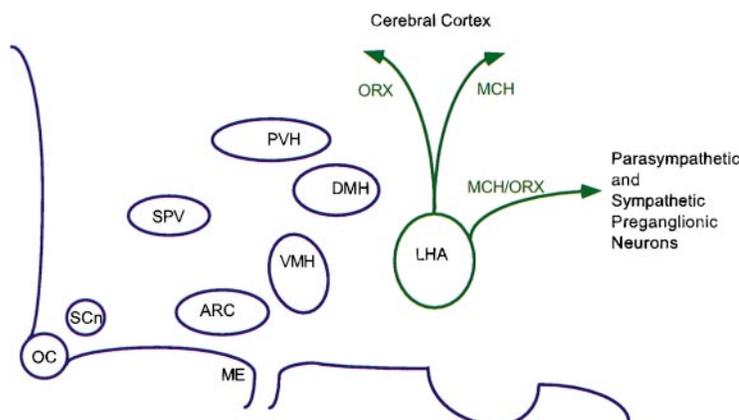
Figure 4. A Schematic Drawing Illustrating the Divergent Projections from the ORX and MCH Neurons in the Lateral Hypothalamus, Ascending to the Cerebral Cortex and Descending to the Brainstem and Spinal Cord

These neurons are activated during starvation or leptin deprivation, and knockout of the MCH gene produces hypophagia and leanness, suggesting that these peptides play a role in the regulation of feeding. Other abbreviations are the same as in Figure 1.

and *db/db* animals or normal mice. His results suggested that the gene containing the *ob* mutation encoded a secreted peptide, whereas the *db/db* mouse had a defect in the corresponding receptor.

These predictions were borne out first by the cloning of leptin (Zhang et al., 1994) and subsequently its receptor (Tartaglia et al., 1995). It was quickly found that administration of leptin to *ob/ob* mice resulted in marked decreases in food intake, body weight, and normalized body temperature and neuroendocrine status (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Moreover, mutations of the long form of the leptin receptor (which include the intracellular signaling portion of the molecule) were found to underlie the phenotype of the *db/db* mouse (Chen et al., 1996; Lee et al., 1996) and the Zucker fatty rat (White et al., 1997).

Leptin administration to *ob/ob* mice not only decreases food intake and body weight but also corrects neuroendocrine abnormalities including hypogonadism, hypercorticosteronemia, and low levels of thyroid hormone. Leptin-deficient *ob/ob* mice do not undergo normal puberty, unless they are given exogenous leptin (Barash et al., 1996; Chehab et al., 1996). In normal female mice, leptin administration from the time of weaning accelerates the onset of puberty (Ahima et al., 1997; Chehab et al., 1997; Cheung et al., 1997a). Patients presenting with Frölich's syndrome, as well as ventromedial hypothalamus–lesioned animals, in fact have hypogonadism. Taken together, these data suggest that leptin is essential for normal functioning of the reproductive system (Barash et al., 1996; Chehab et al., 1996, 1997; Ahima et al., 1997; Cheung et al., 1997a; Montague et al., 1997). These observations are consistent with the suggestion by Flier and colleagues that the predominant role of leptin may be to signal the brain about the overall nutritional state of the individual, rather than to serve as a short-term inhibitor of feeding (Flier, 1998).

The leptin receptor has been identified in the human brain (Couce et al., 1997). Mutations in human leptin (Montague et al., 1997; Strobel et al., 1998) and the leptin receptor (Clement et al., 1998) result in morbid obesity and neuroendocrine derangements, including failure to undergo puberty. Therefore, in humans as well as rodents, intact leptin and its long-form receptor are necessary for leptin signaling and are essential in the maintenance of body weight and energy homeostasis.

The long form of the leptin receptor is expressed in a relatively restricted distribution in the hypothalamus (Mercer et al., 1996a; Schwartz et al., 1996; Cheung et al., 1997b; Fei et al., 1997; Guan et al., 1997; Elmquist et al., 1998b). The highest levels of leptin receptor mRNA and protein are found in the ventrobasal hypothalamus. Is this the site where leptin acts upon the brain to reduce feeding, increase energy expenditure, and regulate activities such as sexual reproduction and generalized activity level? Systemic injection of radiolabeled leptin results in leptin binding in the choroid plexus, which contains high levels of the short form of the leptin receptor (Tartaglia, 1997). Leptin can enter the brain at the circumventricular organs, but the highest level of leptin binding in the brain parenchyma is observed in the structures surrounding the median eminence (Banks et al., 1996), which may be critical in responding to circulating
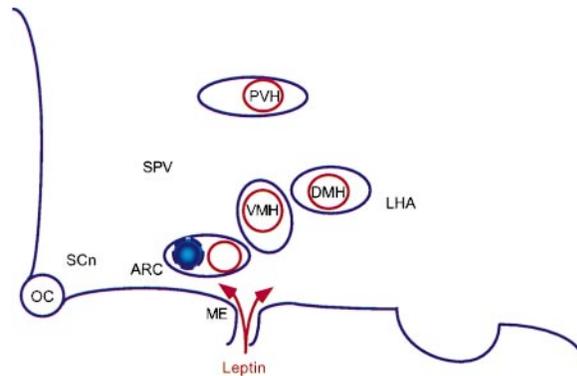
Figure 5. A Schematic Drawing Illustrating Cell Groups in the Hypothalamus that Respond to the Administration of Leptin as Demonstrated by Changes in Gene Expression

Leptin enters the hypothalamus through the median eminence, where it can diffuse to interact with neurons expressing high levels of leptin receptors in the arcuate (ARC), ventromedial (VMH), and dorsomedial (DMH) nuclei. Leptin suppresses the activity of some neurons in the medial arcuate nucleus (shown by a shaded, blue circle) that demonstrate expression of the signaling molecule SOCS-3 but not Fos. Leptin activates neurons that show both Fos and SOCS-3 expression (illustrated by open, red circles) in the ARC, VMH, DMH, and ventral premammillary (not shown) nuclei. Neurons of the ventral parvocellular part of the paraventricular nucleus (PVH) project selectively to the dorsal vagal complex. Cells in this group have low levels of leptin receptors but do show Fos expression after systemic administration of leptin, suggesting that they have been activated by leptin-responsive neuronal pathways. Other abbreviations are the same as in Figure 1.

leptin and regulating body weight. Evidence for this hypothesis is provided by the observation that treatment of mice with gold thioglucose markedly reduces the mRNA for the long form of the leptin receptor in the hypothalamus (Fei et al., 1997), confirming that this toxin works by destroying the very cells that leptin engages. Interestingly, cells that bear the highest levels of the receptor mRNA are located in the arcuate nucleus and parts of the ventromedial, dorsomedial, and ventral premammillary nuclei (Hakansson et al., 1996, 1998; Mercer et al., 1996a, 1996b; Schwartz et al., 1996; Fei et al., 1997; Elmquist et al., 1998b). These hypothalamic nuclei that cluster around the median eminence constitute exactly the areas where Hetherington and Ranson found electrolytic lesions to cause obesity.

Mapping the Effects of Leptin on the Brain
The expression of immediate-early genes, particularly c-*fos*, by neurons in the brain that have been activated by systemic administration of leptin has allowed important insights into actions of leptin in the CNS (Van Dijk et al., 1996; Woods and Stock, 1996; Elmquist et al., 1997, 1998c). Although not all neurons in the brain that are affected by leptin will necessarily show expression of Fos protein, this method allows the mapping of extended neuronal systems that do respond to leptin administration. Leptin induces Fos expression in key sites surrounding the median eminence, including the lateral arcuate nucleus and adjacent retrochiasmatic area, the dorsomedial part of the ventromedial nucleus, the caudal part of the dorsomedial nucleus, and the ventral
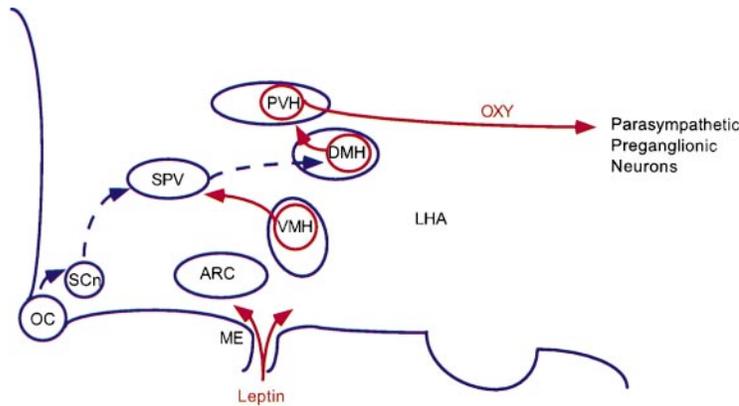
Figure 6. A Schematic Drawing Showing Some of the Leptin-Activated Pathways that Affect the Paraventricular Nucleus of the Hypothalamus

Ventral parvocellular neurons of the paraventricular nucleus (PVH) containing oxytocin (OXY) innervate vagal preganglionic parasympathetic neurons involved in gastrointestinal control. The caudal part of the dorsomedial nucleus (DMH) provides the major leptin-activated input to the PVH. Leptin-activated cells in the ventromedial nucleus (VMH) project to the subparaventricular zone (SPV), a region that also receives suprachiasmatic nucleus (SCn) inputs and is involved in the circadian regulation of feeding (circadian pathways are illustrated by blue dashed lines). The SPV in turn innervates the leptin-activated part of the DMH. Other abbreviations are the same as in Figure 1.

premammillary nucleus (Van Dijk et al., 1996; Elmquist et al., 1997; see Figure 5). In addition, leptin administration induces Fos expression in the ventral parvocellular part of the paraventricular nucleus (Van Dijk et al., 1996; Elmquist et al., 1997), a cell group that specifically innervates the dorsal vagal complex (Saper et al., 1976; Swanson and Sawchenko, 1983) and that is involved in regulating gastric motility and gastric acid secretion (Rogers and Hermann, 1985).

The significance of this pattern of Fos expression was explored by combining Fos immunocytochemistry with retrograde tracing of neuronal connections (see Figure 6). The Fos-immunoreactive neurons in the caudal dorsomedial nucleus provide the major leptin-responsive input to the paraventricular nucleus (Elmquist et al., 1998c). Fos-immunoreactive neurons in the ventromedial nucleus, by contrast, provide a major input to the subparaventricular area (Elmquist et al., 1998c). The subparaventricular area receives the bulk of the output from the circadian pacemaker, the suprachiasmatic nucleus, and it has been implicated as a key site for integrating circadian rhythms in feeding (Watts et al., 1987).

New Molecular Probes Identify the Arcuate Nucleus as a Key Site in Regulating Feeding

Neuropeptide Y is a widely distributed CNS neuropeptide that has long been considered a major regulator of feeding (Clark et al., 1984; Kalra, 1997). Injection of neuropeptide Y into the region of the paraventricular nucleus was shown to increase feeding and decrease energy expenditure (Stanley and Leibowitz, 1985; Billington et al., 1994). Neurons expressing neuropeptide Y in the medial part of the arcuate nucleus have been implicated as at least one source of the dense innervation of the paraventricular nucleus by neuropeptide Y–containing axons (Sawchenko, 1998). The role of these neurons in regulating feeding was reinforced by the finding that in fasted mice neuropeptide Y mRNA is elevated in the arcuate nucleus. A similar elevation of neuropeptide Y mRNA is found in *ob/ob* and *db/db* mice. The overexpression of neuropeptide Y mRNA is blunted in starved and leptin-deficient mice by leptin administration (Stephens et al., 1995; Ahima et al., 1996; Schwartz et al., 1996). As expected, neuropeptide Y neurons were

shown to express leptin receptors (Mercer et al., 1996b). However, they do not show Fos after leptin administration, presumably because they are inhibited rather than excited by leptin administration.

Instead, the action of leptin on neuropeptide Y cells in the arcuate nucleus has been suggested by the expression of the suppressor of cytokine signaling 3 (SOCS-3) mRNA throughout the arcuate nucleus after leptin administration (Bjorbaek et al., 1998). The SOCS proteins are rapidly induced by activation of the type I cytokine family of receptors, which includes the leptin receptor as well as the interleukin 6 receptor (Tartaglia, 1997; White et al., 1997). These receptors engage the JAK tyrosine kinases, leading to phosphorylation of STAT protein transcription factors. SOCS proteins are thought to act as intracellular regulators to inhibit STAT activation induced by activation of cytokine receptors (Endo et al., 1997; Naka et al., 1997; Starr et al., 1997). Specifically, SOCS-3 mRNA is rapidly induced following leptin administration in cell groups that express the long form of the leptin receptor (Bjorbaek et al., 1998). Furthermore, SOCS-3 protein blocks leptin-induced phosphorylation of long-form leptin receptors. The lack of Fos and the presence of SOCS-3 expression by medial arcuate neurons suggests that the leptin receptor has been activated, even though the net effect may be inhibition of firing and thus expression of c-*fos*.

However, targeted gene deletion experiments have made it clear that regulation of neuropeptide Y systems cannot account for the full effect of leptin. Deletion of the neuropeptide Y gene does not decrease food intake or body weight or the response to starvation (Erickson et al., 1996a, 1996b). When neuropeptide Y–deficient mice are crossed with leptin-deficient (*ob/ob*) mice, the neuropeptide Y knockout only partially corrects the obese phenotype. Not only do the resultant mice remain overweight (although not as extreme as the *ob/ob* mice), but they also demonstrate neuroendocrine abnormalities, indicating that other signaling pathways are also involved in obesity induced by leptin deficiency.

The receptor responsible for eliciting the effects of neuropeptide Y on food intake also remains unsettled. The neuropeptide Y type 5 (Y5) receptor was thought to be important in regulating food intake because it is
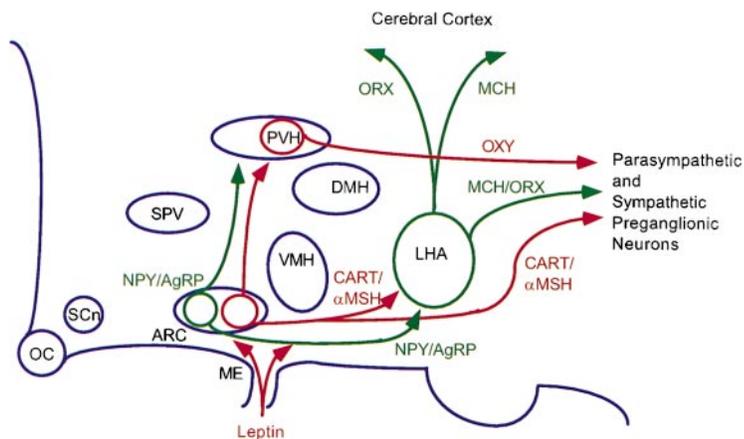
Figure 7. A Schematic Drawing Illustrating the Role of the Leptin-Activated and Leptin-Inhibited Cells in the Arcuate Nucleus of the Hypothalamus in the Regulation of Feeding

Two counterposed populations of neurons are involved. Neurons in the medial part of the arcuate nucleus (ARC) express both neuropeptide Y (NPY) and agouti-related protein (AgRP) (illustrated by green lines), and they are inhibited (show SOCS-3 but not Fos expression) by systemic leptin. A separate population of neurons in the lateral ARC expresses both α-melanocyte stimulating hormone (α-MSH) and CART (illustrated by red lines), and these cells are activated by systemic leptin (show both Fos and SOCS-3 expression). Both populations of ARC neurons project to the paraventricular nucleus (PVH) and the lateral hypothalamic area (LHA). The α-MSH/CART neurons also project to the sympathetic preganglionic cell column in the spinal cord. Other abbreviations are the same as in Figures 1, 4, and 6.

highly expressed in the paraventricular nucleus of the hypothalamus and the lateral hypothalamic area (Gerald et al., 1996). However, targeted deletion of the Y5 receptor does not affect normal food intake and actually leads to adult onset obesity (Marsh et al., 1998). The Y5 knockout mice respond to exogenous leptin and display normal compensatory hyperphagia following starvation. On the other hand, Y5-deficient mice show blunted food intake responses following central injections of neuropeptide Y, suggesting that Y5 receptors can drive feeding (Erickson et al., 1996a, 1996b; Marsh et al., 1998).

A second problem with our understanding of the role of neuropeptide Y in feeding is that the CNS targets of the neuropeptide Y neurons in the medial arcuate nucleus, which are regulated by starvation and leptin, are not known. Although early studies emphasized a projection from neuropeptide Y neurons in the arcuate nucleus to the paraventricular nucleus critical for the regulation of feeding, the paraventricular nucleus actually receives a relatively modest input from the arcuate nucleus (Baker and Herkenham, 1995; C. F. E., J. K. E., and C. B. S., unpublished data). However, the paraventricular nucleus is innervated heavily by neuropeptide Y–containing afferents from the medulla (Sawchenko et al., 1985). Moreover, sites in the perifornical area of the lateral hypothalamus are more sensitive to neuropeptide Y–elicited feeding than is the paraventricular nucleus (Stanley et al., 1993). These observations suggest that although the arcuate neuropeptide Y neurons seem to play an important part in the regulation of feeding, this may not be due entirely to projections to the paraventricular nucleus, as was originally thought.

Mutually Antagonistic Melanocortin Systems from the Arcuate Nucleus

What, then, is the role of the arcuate nucleus in regulating food intake and body weight, and where do leptin-receptive arcuate neurons project? Possible answers have emerged from a recent set of molecular genetic observations on the importance of the central melanocortin systems. The phenotype of the well-known agouti

(A^y/a) mutation includes both a yellow coat and an overeating/obesity syndrome distinct from that seen in ob/ob and db/db mice. The cloning of the agouti gene demonstrated that the phenotype is due to ectopic overexpression of agouti protein throughout the body (see Spiegelman and Flier, 1996; Graham et al., 1997; Ollmann et al., 1997). The agouti protein acts as a natural antagonist at melanocortin receptors, causing the abnormal coat color due to antagonism of the type 1 melanocortin receptor. The observed obesity in the A^y mouse is due to antagonism of CNS type 3 and 4 melanocortin receptors (Fan et al., 1997). The role of the melanocortin systems in regulating feeding further emerged when targeted deletion of the melanocortin 4 receptor gene was found to result in obesity (Huszar et al., 1997). Indeed, mutations in the human melanocortin 4 receptor also induce obesity (Vaisse et al., 1998; Yeo et al., 1998). The melanocortin 4 receptor gene is highly expressed by neurons in the paraventricular nucleus, the dorsomedial hypothalamic nucleus, and the lateral hypothalamic area (Mountjoy et al., 1994). The principle agonist ligand of the CNS melanocortin 4 receptor is α-melanocyte stimulating hormone, a derivative of the pro-opiomelanocortin precursor (Fan et al., 1997). Neurons expressing α-melanocyte stimulating hormone are found in the lateral part of the arcuate nucleus (Watson et al., 1978; Watson and Akil, 1979).

Several recent observations confirm the role of the pro-opiomelanocortin neurons in regulating food intake and responding to leptin. In a very high percentage of arcuate pro-opiomelanocortin neurons, leptin receptor mRNA is coexpressed (Cheung et al., 1997b). Furthermore, in leptin-deficient ob/ob mice or in fasted rodents (when leptin levels rapidly fall), the pro-opiomelanocortin mRNA is markedly reduced. This decrease in the pro-opiomelanocortin expression is prevented by exogenous leptin administration (Schwartz et al., 1997; Thornton et al., 1997; Mizuno et al., 1998). Furthermore, the decrease in food intake and activation of the sympathetic nervous system caused by leptin administration can be blunted by central coadministration of a melanocortin receptor antagonist (Seeley et al., 1997; Satoh et
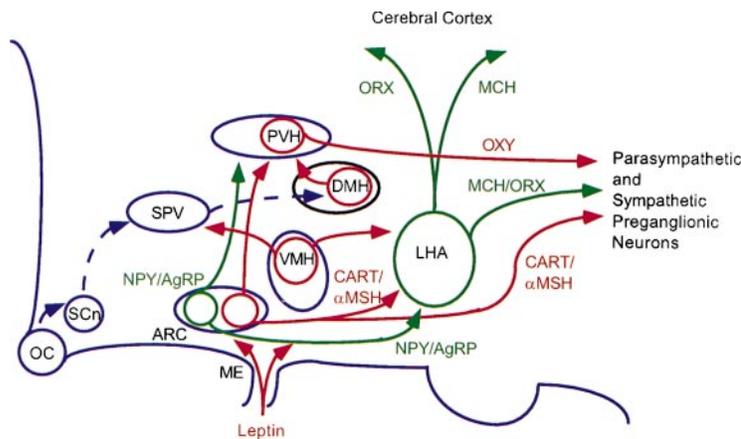
Figure 8. A Schematic Drawing Summarizing Some of the Major Pathways and Neurotransmitters that Have Been Implicated in the Regulation of Feeding

Pathways that are activated by leptin (and therefore presumably have an anorexic influence) are illustrated in red, whereas those that are inhibited by leptin (and are presumed to have a phagic influence) are in green. Circadian influences are illustrated by dashed blue pathways. Leptin is proposed to exert its effects on the hypothalamus by entering through the median eminence. Note that a lesion centered on the ventromedial nucleus (VMH) and arcuate nucleus (ARC), such as that shown in Figure 1, would eliminate leptin influence, resulting in hyperphagia and obesity. A lesion in the lateral hypothalamus (as in Figure 1) that destroys the MCH and ORX cells, which promote feeding, would result in aphagia and inanition. Other abbreviations are the same as in Figures 1, 4, 6, and 7.

al., 1998). The fundamental role of melanocortin systems in regulating body weight in humans has been demonstrated by the discovery of the pro-opiomelanocortin mutations that resulted in early onset obesity (Krude et al., 1998).

Although agouti protein normally is not expressed in the brain, the product of a related gene that is expressed normally in the mammalian brain was recently identified. This agouti-related protein (Ollmann et al., 1997; Shutter et al., 1997) is found primarily in neurons in the medial part of the arcuate nucleus (see Figure 2B). In addition, agouti-related protein is an antagonist at melanocortin 3 and melanocortin 4 receptors. Furthermore, overexpression of this protein results in an obesity syndrome similar to the A^y mouse and the melanocortin 4 receptor knockout mouse (Graham et al., 1997; Ollmann et al., 1997). Moreover, the relative level of arcuate agouti-related protein mRNA is increased with fasting, and agouti-related protein and neuropeptide Y are coexpressed in a high percentage of arcuate neurons (Broberger et al., 1998; Hahn et al., 1998). These observations predict the surprising model that two distinct and counterpoised populations of arcuate neurons, bearing leptin receptors and containing melanocortin 4 receptor agonist and antagonist peptides, might be involved in transmitting the leptin signal to hypothalamic sites such as the lateral hypothalamic area and paraventricular nucleus of the hypothalamus (see Figure 7).

Getting the CART before the Source

The role played by the arcuate nucleus of the hypothalamus in the regulation of feeding has meanwhile been further illuminated by discovery of yet another molecular participant in this process. Investigators interested in the regulation of gene expression induced by drugs of abuse used differential display to examine the genes expressed in the brain after the administration of cocaine and amphetamine. One peptide, dubbed CART (cocaine and amphetamine regulated transcript) was identified (Douglass et al., 1995). As described earlier, neurons expressing CART are found throughout the

CNS, including the arcuate nucleus, the lateral hypothalamic area, the retrochiasmatic area, and the paraventricular hypothalamic nucleus (Couceyro et al., 1997; Koylu et al., 1997, 1998). A role for CART peptides in regulating food intake was provided by the demonstration that injections of CART peptide into the lateral ventricle were found to suppress feeding (Lambert et al., 1997, 1998; Kristensen et al., 1998). Recent evidence indicates that CART mRNA expression also is regulated by leptin. Specifically, like pro-opiomelanocortin, CART mRNA is reduced in the arcuate nucleus in leptin-deficient *ob/ob* mice and fasted rats. Leptin administration elevates arcuate CART mRNA levels, further suggesting that CART systems may play a role in central regulation of feeding and body weight (Kristensen et al., 1998). It is interesting to note that both cocaine and amphetamine are anorexic, and they increase the levels of CART mRNA, although the role of CART in mediating that anorexia remains conjectural.

At present, the sites within the CNS innervated by leptin-regulated CART neurons that may be responsible for inhibition of food intake are not known. CART peptide–immunoreactive terminals innervate central autonomic sites, such as the sympathetic preganglionic neurons in the spinal cord (Koylu et al., 1997, 1998). Injection of retrograde tracer into the spinal cord demonstrates that the CART inputs to the spinal cord originate in part from neurons in the lateral part of the arcuate nucleus and in the adjacent retrochiasmatic area (Elias et al., 1998b; Figure 7). Furthermore, combining these markers with leptin administration demonstrates that there is Fos expression induced specifically in a high proportion of these CART neurons in the arcuate nucleus and the retrochiasmatic area (Elias et al., 1998b). In addition, colocalization studies indicate that virtually all CART neurons in the arcuate and retrochiasmatic area coexpress pro-opiomelanocortin (Figure 3B).

Connecting the Neuronal Systems for Feeding and Satiety

Recent molecular and anatomic evidence has rejuvenated the once dated dual center hypothesis. However,

several pieces of evidence are lacking before this theory can be considered complete. For example, MCH and ORX systems in the lateral hypothalamic area are regulated by starvation and leptin (or lack of leptin) (Qu et al., 1996; Sakurai et al., 1998). Leptin receptors are present in the lateral hypothalamic area (Fei et al., 1997; Elmquist et al., 1998c; Hakansson et al., 1998); however, the densest collections of leptin receptors are found in mediobasal hypothalamic cell groups, including pro-opiomelanocortin/CART (Cheung et al., 1997b) neurons in the lateral arcuate nucleus and neuropeptide Y/agouti-related protein (Mercer et al., 1996b) neurons in the medial arcuate nucleus. Recent anatomic studies have begun to illuminate the link between leptin-regulated neurons in the arcuate nucleus and MCH and ORX neurons in the lateral hypothalamic area.

Elias and colleagues (1998a) and Broberger and co-workers (1998) have examined the neuropeptide Y–, agouti-related protein–, and α-melanocyte stimulating hormone–immunoreactive axons in the lateral hypothalamic area in mice, rats, and humans. These groups report intense innervation of both MCH and ORX neurons in the lateral hypothalamic area by axons containing all of these peptides (see Figures 1B and 2C–2E). Although electron microscopic confirmation of synaptic contacts is needed, the light micrographs demonstrate MCH- or ORX-immunoreactive cell bodies and dendrites outlined and decorated with extensive pericellular networks of terminals. Direct evidence of neuropeptide Y receptors and melanocortin receptors on MCH and ORX cells is still lacking but seems likely based on available anatomic and genetic evidence (Mountjoy et al., 1994; Huszar et al., 1997; Elias et al., 1998a). The agouti-related protein and α-melanocyte stimulating hormone inputs likely come from the arcuate nucleus, because it is the only source of agouti-related protein in the brain, and α-melanocyte stimulating hormone–containing neurons are likewise found in a very restricted distribution (most are in the lateral arcuate/retrochiasmatic area and a few are found in the nucleus of the solitary tract). Of equal interest is the finding that in the arcuate nucleus there is nearly complete colocalization of neuropeptide Y and agouti-related protein mRNA (Broberger et al., 1998; Hahn et al., 1998). Thus, some of the neuropeptide Y innervation in the lateral hypothalamic area likely comes from the arcuate nucleus. Given that this perifornical region coincides with the site where injection of neuropeptide Y has its greatest effects on feeding, the substrate for that effect may be the neuropeptide Y input to the perifornical MCH and ORX neurons (Stanley et al., 1993).

Consistent with the idea that neurons in the arcuate nucleus convey leptin signals to the lateral hypothalamic area, neuropeptide Y/agouti-related protein neurons in the medial arcuate nucleus (which would be inhibited by leptin) show SOCS-3 but not Fos expression after leptin administration (C. F. E., J. K. E., and C. B. S., unpublished data). Conversely, the CART/pro-opiomelanocortin neurons in the lateral part of the arcuate nucleus and adjacent retrochiasmatic area show Fos protein expression after administration of leptin. Moreover, the lateral arcuate CART neurons that project to the spinal cord are activated by leptin, as judged by Fos expression, and they also may mediate leptin effects on feeding and autonomic response. The regulation of these apparently antagonistic populations of arcuate neurons by leptin and the projections of these cells to the lateral hypothalamic area, to the paraventricular hypothalamic nucleus, and to pools of autonomic preganglionic neurons in the medulla and spinal cord are likely to be critical in the regulation of food intake and body weight.

The Hypothalamic Regulation of Feeding: A New Synthesis

By 1940, Hetherington and Ranson had laid to rest most doubts regarding the importance of the hypothalamus in regulating body weight. However, their electrolytic lesions were inherently crude and started debate about which specific hypothalamic cell groups are critical in the control of feeding and body weight. Despite intense activity, during the 45 years that followed the discovery of the ventromedial nucleus syndrome, we learned relatively little about the actual pathways in the hypothalamus that mediate feeding. Because of the similarity of the leptin deficiency syndrome to the ventromedial nucleus syndrome, the discovery of leptin and its receptors revitalized the field. In fact, the original observations of Hetherington and Ranson, implicating the "ventromedial nucleus, but also including the adjacent arcuate nucleus and parts of the dorsomedial and ventral premammillary nuclei" in the regulation of feeding, have proven to be both prescient and surprisingly accurate in identifying the hypothalamic cell groups with the highest levels of long-form leptin receptors.

In the 4 years since the discovery of leptin, there has been remarkable progress in studying the effects of starvation, leptin deprivation, and leptin administration on the expression of genes for transcription factors (c-*fos*), signaling molecules (SOCS-3), and neurotransmitters (neuropeptide Y, agouti-related protein, α-melanocyte stimulating hormone, CART, MCH, and ORX) involved in feeding. The availability of these molecular tools, coupled with tract tracing, has resulted in striking progress in dissecting an extensive network of hypothalamic circuitry that regulates feeding (Figure 8). Despite the fact that a number of pieces of this puzzle are still missing, the outline of the hypothalamic system for regulation of feeding is now more clear. In retrospect, the ventromedial nucleus itself is only a part of this network. It sits, however, at the epicenter of a web of pathways, running between the arcuate nucleus and the paraventricular nucleus and lateral hypothalamus, and this epicenter defines the mechanisms of neuronal regulation of feeding.

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