CONTROL OF FOOD INTAKE: NEUROBIOLOGICAL ASPECTS

RÉGULATION DE LA PRISE ALIMENTAIRE : ASPECTS NEUROBIOLOGIQUES

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SUMMARY

Feeding, a behaviour vital for survival, is subject to intense regulation by the brain to maintain energy homeostasis. Neural networks located in the hypothalamic nuclei and the dorsal vagal complex in the brainstem play a crucial role, as revealed by the integration of satiety and adiposity signalling. Contrary to the traditional view of a hierarchical model in which the hypothalamus plays the major role, recent results are consistent with a distributed model in the processing of energy balance regulation. Interestingly, network re-organisation and neurogenesis are potentially involved in food intake and body weight regulation, suggesting that neuroplasticity may provide important clues to the understanding of energy balance disorders such as obesity.

Keys words: food intake, body weight, hypothalamus, brainstem, neuroplasticity.

RÉSUMÉ

La prise alimentaire, comportement vital pour la survie, est soumise à une intense régulation de la part du cerveau, afin de maintenir l'homéostasie énergétique. Les réseaux neuronaux, localisés dans les noyaux hypothalamiques et dans le complexe vagal dorsal du bulbe rachidien, jouent un rôle crucial dans l'intégration des signaux de satiété et d'adiposité. Contrairement à la conception classique du modèle hiérarchisé où l'hypothalamus joue le rôle majeur, les résultats actuels sont en faveur d'un modèle distributif. Il est intéressant de noter que la réorganisation des réseaux neuronaux et la neurogenèse sont impliquées dans la régulation de la prise alimentaire et du poids corporel , ce qui suggère que l'étude de la neuroplasticité pourrait ouvrir des pistes originales pour la compréhension des désordres de la balance énergétique tels que l'obésité.

Mots-clés : prise alimentaire, poids corporel, hypothalamus, bulbe rachidien, neuroplasticité.

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INTRODUCTION

Feeding behaviour is critical for survival. Food is necessary to provide most body's macro- and micronutrients. Food is also required to cover the cellular energetic needs necessary for life. Energetic needs are continuous, they serve to supply metabolism at rest, and variable energy expenditures such as those depending on muscular activity and body temperature regulation, a crucial problem for mammals, which must maintain a stable body temperature even in severe climatic conditions.

Food that covers energetic needs is generally not constantly available. In addition, food intake behaviour is not compatible with some physiological states such as sleep. Therefore, food intake in all mammals is a discontinuous behaviour organised in discrete meals. The continuous, but variable, needs of the various tissues are covered by the use of recently ingested calories, during or immediately after a meal, and by the mobilisation of energy stores, principally from adipose tissue, at other moments. Food intake, energy expenditure and energy storage characterise, therefore, nutritional homeostasis.

Although several variables influencing food intake, such as emotions, social factors, time of the day, convenience, cost, etc… are not biologically regulated, in mammals, including humans, eating can be primarily considered as a regulatory behaviour with the primary function of supporting the continuous energy needs of body tissues. Within this perspective of energy homeostasis, food intake is influenced by hunger and satiety, the biological mechanisms that couple eating with internal caloric supplies and a stable body weight.

Since the pioneering work by Hetherington and Ranson (1940), it has been well established that the central nervous system plays a pivotal role in food intake regulation. Indeed, any defect in the functioning of the neuronal networks involved in feeding results in severe morbidity, such as anorexia or obesity. Given the enormous increase in obesity all over the world, morbid overweight with its related disorders actually represents a crucial problem of public health justifying the increasing number of studies in this field.

For the past 50 years, two main concepts have dominated the study of food intake. In the "depletion-repletion" model, it is proposed that a meal is initiated when available energy falls below a threshold value and is terminated when substrate levels are sufficiently replenished. Therefore, this model can account for both meal onset and meal termination. This is well illustrated by Mayer's glucostatic hypothesis (1952), which postulates that small declines in glucose concentrations or utilisation trigger meal initiation. However, although key parameters relating to energy depletion and repletion correlate well with energy intake, they correlate poorly with energy expenditure. Depletion-repletion models therefore cannot explain the matching of energy intake with expenditure that results in the long-term stability of fat stores.
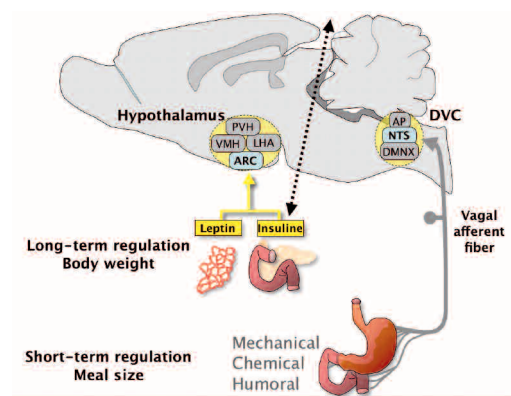

Figure 1: Traditional view of food intake and energy balance control. Depending on the continuous energy needs and discontinuous feeding behaviour, a short-term regulation controlling meal size and a long-term regulation controlling body weight are distinguished. The short-term regulation involves mainly the vagal afferent pathway, activated by various meal-generated signals, i.e. satiety signals, such as gastric distension, macronutrients and hormones. Therefore, the primary integrative site in the brain is the dorsal vagal complex (DVC) formed by the nucleus tractus solitarii (NTS), the dorsal motor nucleus of the vagus (DMNX) and the area postrema (AP). The long-term regulation involves hypothalamic nuclei such as the arcuate (ARC), the ventromedial (VMH) and paraventricular (PVH) nuclei, and the lateral hypothalamic area (LHA). The activity of these nuclei is regulated by leptin and insulin, i.e. the adiposity signalling hormones (see text for more information).

The second type of model links food intake to the amount of stored energy, as fat mass, in the body. This "lipostatic model" originally postulated by Kennedy (1953), proposes that signals proportional to the size of fat stores, termed adiposity signals, become integrated in the brain to regulate food intake. Thus, the onset of eating is not necessarily linked to acute energetic needs and meal termination is not necessarily tied to the replenishment of depleted substrates. Eating may therefore be considered as a prime behaviour unless influenced by inhibitory signals depending on energy stores. Interestingly, since the discovery of leptin in the 1990s, most recent results support the hypothesis that food intake is controlled within a lipostatic system for energy homeostasis.

There is however great flexibility in the number and size of the meals, so to maintain energy balance and fat stores, controls must exist that determine the energetic value of a meal once eating has been initiated. In the 1970s, the results of several studies documented the existence of meal-generated signals, called satiety signals, that accumulate during eating and contribute to meal termination, determining meal size (Gibbs *et al.* 1973) *(figure 1)*.

The aim of this short review is to summarise the main mechanisms believed to be involved in meal size and body weight regulation, with a particular focus on the respective role of hypothalamus and brainstem in these mechanisms and on neuroplasticity phenomena, which have been recently demonstrated to intervene in food intake regulation.

REGULATION OF MEAL SIZE

Meal initiation depends on several factors: social, cultural, food intake habits; external factors such as sensory stimuli (olfactory and visual); internal signals of nervous, humoral and metabolic origin. Once the meal is initiated, food comes into contact with the lining of the alimentary canal, providing various stimuli. Orosensorial stimuli are generated through the activation of taste receptors, mechanoreceptors, and thermoreceptors located in the oropharyngeal region, running within the trigeminal (V), facial (VII), glossopharyngeal (IX) and vagal (X) nerves, and projecting to the brainstem, at the level of the nucleus tractus solitarii (NTS) *(figure 2)*. These pre-ingestive orosensorial stimuli provide positive feedback, which maintains the ingestion sequence, as best demonstrated by the continuous ingestion observed in sham feeding experiments on animals equipped with an open oesophageal or gastric fistula (for review, see Smith 2000). By contrast, in rats under real feeding conditions, meal duration rarely exceeds 20 minutes. The rate of ingestion begins to decline within a few minutes of meal onset and steadily declines thereafter until meal termination. The gastro-intestinal tract is richly innervated by mechano- and chemo-sensitive vagal afferent fibres. Moreover, the presence of food in the gastro-intestinal tract triggers the release of various gastro-intestinal peptides as well as other neuroactive substances, which act in a paracrine fashion on vagal afferent fibres and/or in a humoral fashion on more distant targets such as the liver, the pancreas and the brain. Altogether, these nervous and humoral signals play a role in the coordination of the digestion process, by adapting the secretion of digestive enzymes and the gastro-intestinal motility to the type of ingested food. Some of these signals also participate in the regulation of meal size, by generating a feeling of satiety. It is generally agreed that under normal conditions, meal termination results from a combination of gastric and post-gastric signals (for review, see Ritter 2004). Pyloric closure experiments show that satiation produced by food in the stomach depends on mechanical rather than chemical stimulation by meal constituents (Philips & Pawley, 1996; Eisen *et al.* 2002). The level of food intake inhibition depends on the strength of gastric distension. Empirically, humans have been using the satiating effect of gastric distension to deal with hunger, by means of largely diluted soups in the event of food shortage, and in the form of various surgical alterations of the stomach volume to fight obesity. Intestinal infusion experiments demonstrated that intestinal satiation can be elicited by all three macronutrient groups: carbohydrates, amino acids and fat, with uneven potency for the various members of each macronutrient group. Most of this rich chemical sensing is mediated by capsaicin-sensitive vagal afferent fibres.
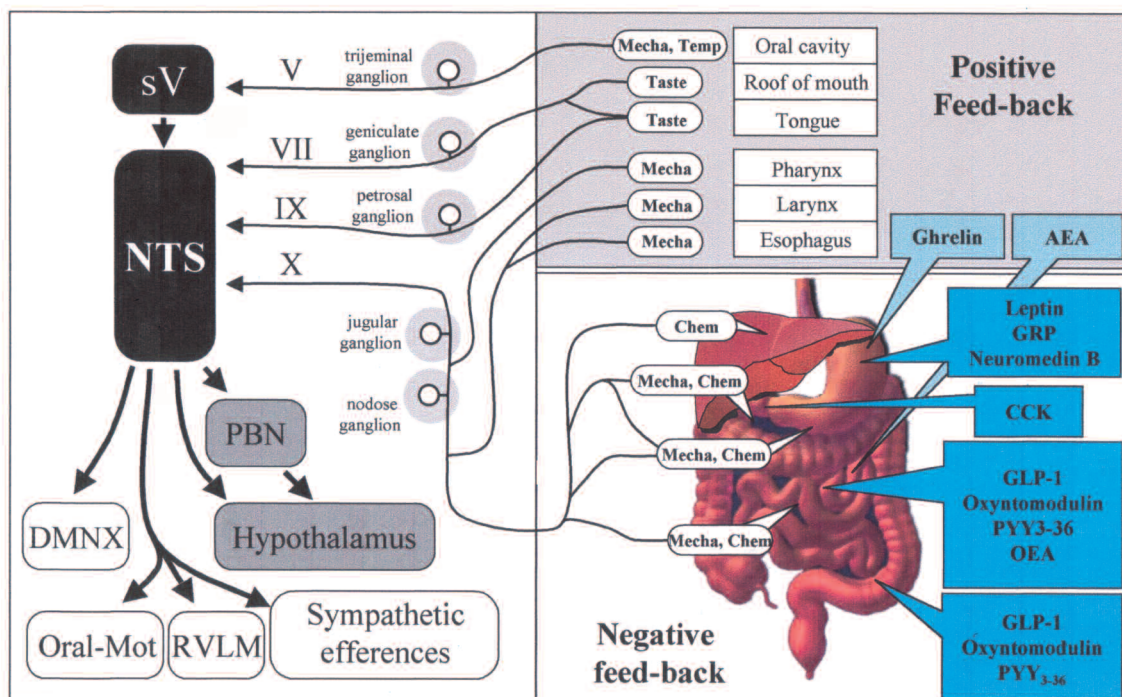

Figure 2: Signals generated by the oro-gastro-intestinal tract.

Various sensory receptors (mechanoreceptors, thermoreceptors, taste receptors, chemoreceptors) are located all along the alimentary canal, transmitting meal-related signals to the brainstem via primary afferent fibres in the trigeminal (V), facial (VII), glossopharyngeal (IX) and vagus (X) nerves. Sensory inputs generated rostrally in the upper part of the tract act as positive feedback to maintain ingestive behaviour, while those generated more caudally act as negative feedback (satiety signals) resulting in meal termination. In addition, various hormones, peptides and other neuroactive substances, are secreted by the gastro-intestinal tract and intervene in food intake control either as anorexigenic or orexigenic signals. The neuroactive substances can act on sensory receptors located on vagal afferent fibres in a paracrine fashion or directly in the brain via a humoral pathway (see text for more details).

Abbreviations: AEA, anandamide; CCK, cholecystokinine; Chem, chemoreceptor; DMNX, dorsal motor nucleus of the vagus nerve; GLP-1, glucagon-like peptide-1; GRP, gastrin releasing peptide; Mecha, mechanoreceptor; NTS, nucleus tractus solitarii; OEA, oleylethanolamide; Oro-Mot, brainstem oral motor nuclei; PBN, parabrachial nucleus; PYY3-36, fragment 3-36 of neuropeptide Y; RVLM, rostral ventrolateral medulla; sV, principal sensory nucleus of trigeminal nerve; Taste, taste receptors.

The terminals of vagal afferent fibres do not come into contact with the luminal content and it remains unclear whether they can respond directly to nutrients present in the extracellular space or whether they are activated by peptides and other neuroactive substances secreted from the intestinal epithelium in response to the presence of food. Cholecystokinin (CCK) is secreted by a population of endocrine cells in the duodenal mucosa, in response to the presence of food in the lumen. Two CCK receptors have been characterised, CCK-1R being expressed by a large proportion of vagal afferent fibres. Peripheral treatment with exogenous CCK before a meal reduces meal size in a dose-dependent manner, while treatment with a CCK-1 antagonist increases it. The satiating effect of peripherally applied CCK is almost eliminated by vagotomy. Although conclusive evidence is still lacking, numerous arguments are in favour of a paracrine action of CCK on vagal afferent fibres for mediating the satiation response to intestinal nutrients (for review, see Ritter 2004). Other intestinal peptides display an anorectic action. It is the case for two post-translational derivatives of preproglucagon, oxyntomodulin and glucagon like peptide-1 (GLP-1), secreted by the small intestine and the colon, and for PYY3-36, secreted by the distal intestine and the colon. Apolipoprotein A-IV, a lipoprotein component of chylomicrons, the synthesis of which increases with fat absorption in the intestine, was also shown to be anorexigenic.

More intriguing is the set of meal size affecting peptides secreted by the stomach. Particularly, leptin, the fat cell hormone, is produced by cells of the gastric mucosa and may be released by feeding (Bado *et al.* 1998; Cinty *et al.* 2000; Mix *et al.* 2000; Sobahni *et al.*, 2000), although plasma leptin concentrations in the systemic circulation are not well correlated with meals. Since vagal afferent fibres express the signalling form of leptin receptor (Buyse *et al.* 2001), it appears possible that leptin could act in a paracrine fashion to acutely activate vagal afferent fibres innervating the stomach. Two other anorexigenic peptides are secreted by the stomach: gastrin-releasing peptide, and neuromedin-B.

The stomach is also the main production site of ghrelin, a peptide that stimulates appetite (Tschop *et al.* 2000). Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (GHSR). This receptor is expressed by vagal afferent fibres, and ghrelin suppresses firing of vagal nerves. Moreover, capsaicin treatment blocks stimulation of feeding after peripheral but not central ghrelin treatment (Date *et al.* 2002). Interestingly, ghrelin and leptin plasma concentrations in the systemic circulation show circadian rhythms in phase opposition (Bodosi *et al.* 2004).

Non-peptidic neuroactive substances are also produced in the gastrointestinal tract. Of particular interest are the mono- and polyunsaturated fatty acid ethanol amides (FAE) oleylethanolamide (OEA) and arachidonoylethanolamide (also known as anandamide, AEA). AEA and OEA act on distinct sets of receptors to exert an orexigenic and an anorexigenic effect, respectively, each requiring intact capsaicin-sensitive vagal afferent fibres. Anandamide was the first endogenous ligand for canna-

binoid receptors to be discovered, in 1992 (Devane *et al.* 1992). AEA acts as a partial agonist on the G-protein coupled receptor cannabinoid receptor type 1 (CB1), but is virtually inefficient on CB2. AEA has also been proposed to act as an endogenous full agonist on receptors specific for capsaicin, known as vanilloid type 1 receptors (VR1) (for review, see Dimarzo *et al.* 2001). By contrast, OEA is neither active on CB1 nor on CB2 receptors, but three different targets have been proposed so far for this mono-unsaturated FAE: the nuclear receptor peroxisome-proliferator-activated receptor-α (PPAR-α) (Fu *et al.* 2003); the capsaicine receptor VR1, which can be activated by OEA in a protein kinase C (PKC)-dependent manner (Ahern 2003; Wang *et al.* 2005); and GPR119, a previously orphan G protein-coupled receptor (Overton *et al.* 2006).

Experiments performed on rats decerebrated at the transcollicu-lus level demonstrated that the caudal brainstem is able to integrate positive and negative feedbacks to regulate meal size (Grill & Kaplan, 2002). Whereas the chronic decerebrated rat cannot initiate eating unless liquid food is infused directly into its mouth, it is still able to adapt the duration of the ingestion sequence to the energy density of the proposed diet, until satiety, and then let the infused liquid drip out of its mouth. Visceral afferent fibres from oro-gastro-intestinal origin all project to the NTS in the caudal brainstem. NTS is part of a set of three richly interconnected nuclei, called dorsal vagal complex (DVC), which also comprises the area postrema (AP), a neurohemal organ lining the fourth ventricle and able to detect various signals from blood or cerebro-spinal fluid, and the dorsal motor nucleus of the vagus nerve (DMNX), which contains the perikarya of efferent premotor vagal neurons. The brainstem also contains all the necessary networks for the motor reflexes of the oro-pharyngeal area and of the gastro-intestinal tract, as well as the central pattern generators responsible for the ingestion sequence, such as those of mastication and deglutition (Jean 2001). Therefore, the caudal brainstem plays an important role in food intake regulation, since it contains the whole neuronal circuitry responsible for meal size regulation under the control of gastro-intestinal post-ingestive stimuli.

REGULATION OF BODY WEIGHT

The control of body weight requires that the energy status be sensed and integrated in order to adjust food intake. For instance, the metabolic consequences of transient food deprivation have to be taken into account and translated into increased meal size to replenish energy stores. The decerebrated rat does not increase food intake after food deprivation (Grill & Kaplan, 2002). Therefore, neural interactions between forebrain and brainstem are necessary for a correct adjustment of feeding in response to food deprivation.

The implication of the hypothalamus in food intake regulation was clearly demonstrated in the 1950s, by the use of electrolytic lesions or electric stimulations experiments (Anand & Brobeck, 1951). Stellar's dual-center hypothesis (Stellar, 1954) stated that food intake regulation depends on interactions between two anta-

gonistic centres: a "feeding centre", localised in the lateral hypothalamic area (LHA), and a "satiety centre", localised in the ventromedial hypothalamus (VMH). Indeed, bilateral lesion of the LHA leads to aphagia, whereas stimulation of LHA in a satiated animal triggers food intake. Conversely, a bilateral lesion of the VMH leads to hyperphagia and obesity, whereas stimulation of the VMH suppresses food intake. While appealing in its simplicity, the dual-centre hypothesis had many critics and was finally dropped, and the list of hypothalamic nuclei important for food intake regulation enlarged with the paraventricular (PVH), arcuate (ARC) and dorso-medial (DMH) nuclei.

The hypothalamus lipostat: the classical view in the post-leptin era

A fundamental breakthrough was made with the discovery of leptin in 1994 (Zhang *et al.* 1994) and the cloning of its receptor one year later (Tartaglia *et al.* 1995). Leptin is a circulating hormone, produced by white adipose tissue in proportion to fat stores, which signals through the long form of the leptin receptor (ObRb) *via* the JAK/STAT-3 pathway to modulate gene expression. Deficits

in genes encoding either leptin (in ob/ob mice) or its receptor (in db/db mice) lead to profound obesity. The ARC is thought to be critical in mediating leptin actions *(figure 3)* (for review, see Schwartz 2000; Woods *et al.* 1998; Cowley 2003). The ARC contains two distinct populations of neurons that exert opposing regulatory functions: one maintains an orexigenic tone, whereas the other maintains an anorexigenic tone. The anorexigenic neurons express pro-opio-melanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). The orexigenic neurons express neuropeptide Y (NPY) and agouti-related protein (AgRP). These two neuronal populations provide overlapping projections to other key parts of the hypothalamus, notably the PVH, DMH, VMH and LHA (Bagnol *et al.* 1999; Xu *et al.* 2003). Among the melanocortin peptides derived from POMC, α-melanocyte-stimulating hormone (α-MSH) plays a major role in maintaining the anorexigenic tone, by acting on melanocortin-4 receptors (MC4-R). Orexigenic neurons antagonise the anorexigenic POMC neurons at two different levels: in the ARC through axo-somatic synapses using both NPY and GABA as inhibitory neurotransmitters, and at the level of target cells expres-
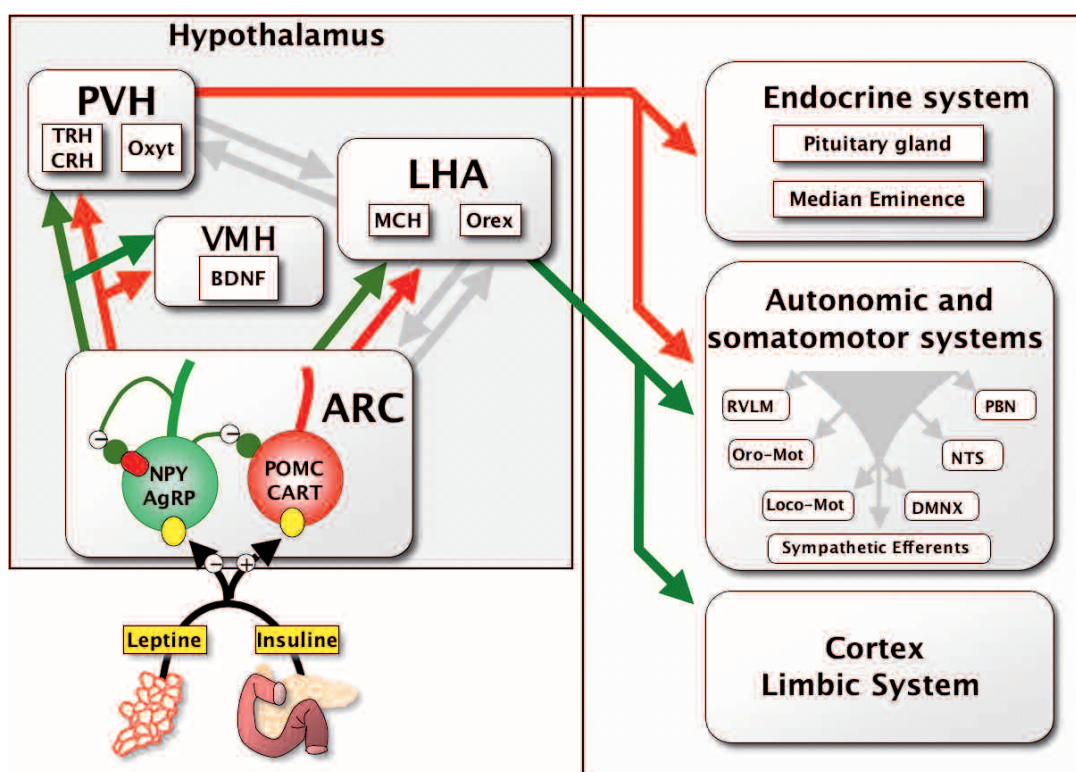

Figure 3: Signals generated by the oro-gastro-intestinal tract.
The adiposity signals, leptin and insulin, secreted proportionally to fat storage, modulate food intake by acting on two distinct populations of neurons within the arcuate nucleus (ARC). One population, activated by adiposity signals, is formed by anorexigenic neurons (in red), which express pro-opio-melanocortin (POMC) and cocaine- and amphetamine-related transcript (CART). Another population, inhibited by adiposity signals, is composed of orexigenic neurons (in green) expressing neuropeptide Y (NPY) and Agouti gene-related peptide (AgRP). Arcuate NPY/AgRP and POMC/CART neurons project to several brain nuclei, in particular to hypothalamic nuclei such as the ventromedial (VMH) and paraventricular (PVH) nuclei and the lateral hypothalamic area (LHA). Neurons within these nuclei synthesise anorexigenic transmitters such as corticotrophin releasing hormone (CRH), thyrotropin releasing hormone (TRH), oxytocin (Oxyt), brain-derived neurotrophic factor (BDNF) or orexigenic transmitters such as hypocretin/orexin (Orex) and melanin-concentrating hormone (MCH). The anorexigenic and orexigenic efferent pathways project both to the autonomic and somatomotor systems in the brainstem, and to the cortex and the limbic system in the forebrain. Lipostat output signals can also control the endocrine system via the pituitary and the median eminence (see text for more details).

Abbreviations: DMNX, dorsal motor nucleus of the vagus nerve; Loco-Mot, spinal motoneurons; NTS, nucleus tractus solitarii; Oro-Mot, brainstem oral motor nuclei; PBN, parabrachial nucleus; RVLM, rostral ventrolateral medulla.

sing MC4-R, through the release of AgRP, which acts as an endogenous antagonist of α- MSH on central melanocortin receptors. The location of NPY/AgRP and POMC/CART neurons in the ventromedial ARC, near the median eminence, a neurohemal structure, is believed to allow the reception of circulating metabolic signals. Indeed, most of NPY/AgRP and POMC/CART neurons express ObRb, and leptin induces the expression of suppressor of cytokine signaling-3 (SOCS3) in these two neuronal populations, indicative of STAT3 activation, but increases c-fos expression only in POMC/CART neurons, suggesting that, consistent with its anorexigenic role, leptin activates POMC/CART neurons, but inhibits NPY/AgRP neurons. While leptin is the prototypical adiposity signal, it is not the only one. Insulin, the secretion of which by pancreatic β-cells is known to increase in response to hyperglycemia, displays basal plasma levels directly proportional to fat mass. Arcuate NPY/AgRP and POMC/CART neurons also express the receptor for insulin.

Arcuate NPY/AgRP and POMC/CART neurons project to many areas inside and outside the hypothalamus. Intrahypothalamic projections comprise nuclei involved in food intake regulation such as PVH, the perifornical area (PFA) and LHA, DMH and, perhaps to a less extent, VMH (Bagnol *et al.* 1999; Zigman & Elmquist, 2003; Xu *et al.* 2003). PVH participates in food intake moderation, since stimulation of PVH inhibits food intake, whereas bilateral lesions of this nucleus lead to hyperphagia and obesity. Moreover, haploinsufficiency in the gene encoding Sim1 (single-minded 1), a transcription factor that controls development of the PVH, also leads to obesity (Michaud *et al.* 2001). At least three neuropeptides synthesised in the PVH decrease food intake and body weight gain when administered centrally: corticotropin releasing hormone (CRH), thyrotropin releasing hormone (TRH) and oxytocin. LHA, the so-called feeding centre in Stellar's dual-centre hypothesis, contains two groups of orexigenic peptide-expressing neurons: the hypocretin/orexin neurons and the melanin-concentrating hormone (MCH) neurons. Both MCH and hypocretin/orexin have been implicated in the regulation of food intake and arousal. Expression of MCH is increased by fasting, and MCH knockout mice display a lean phenotype. Central administration of hypocretin/orexin induces arousal and hyperphagia, whereas invalidation of their encoding genes leads to narcolepsia. VMH was formerly presented as the satiety centre on the basis of the massive obesity produced by its lesion. It has recently been shown that knockout mice for the gene encoding steroidogenic factor-1 (SF-1), a transcription factor implicated in the normal development of the VMH, are obese (Majdic *et al.* 2002). A recently identified anorexigenic signal produced in the VMH is the brain derived neurotrophic factor (BDNF). This neurotrophin mainly signals through its high affinity receptor TrkB. Central administration of BDNF induces hypophagia and weight loss, whereas genetic models with altered BDNF/TrkB signalling are hyperphagic and obese (Pelleymounter *et al.* 1995; Kernie *et al.* 2000; Rios *et al.* 2001; Xu *et al.* 2003). BDNF is expressed at high levels in the VMH, where its expression is regulated by nutritional status and by MC4-R signalling (Xu *et al.*, 2003).

Altogether, arcuate NPY/AgRP and POMC/CART neurons are first-order leptin-sensitive neurons, which constitute the core of a hypothalamic lipostat. The intrahypothalamic second-order neurons of this lipostat are at the origin of two major hypothalamic efferent systems, an orexigenic one through LHA (MCH and hypocretin/orexin) neurons, and an anorexigenic one through PVH (CRH, TRH, oxytocin) neurons. These two efferent systems project to the somatomotor and visceromotor systems in the brainstem, and also to the limbic system and cerebral cortex (Berthoud, 2002). Arcuate POMC/CART neurons also send direct projections to some brainstem nuclei, notably the NTS.

Intrahypothalamic distribution of first-order leptin-sensitive neurons

With the recent availability of genetic tools allowing for gene deletion or re-expression in specific neuronal populations, the physiological meaning of redundancy in first-order leptine-sensitive neurons or their downstream second order counterparts has been called into question. As mentioned earlier, ObRs are expressed outside the hypothalamus, notably in the NTS. In the hypothalamus, ObRs are also expressed in several nuclei other than ARC, particularly VMH and DMH. The significance of these first-order leptine-sensitive neurons in body weight regulation has attracted attention quite recently. Mice lacking leptin receptors only in POMC neurons display mild obesity, the severity of which is about one-fifth that observed in db/db mice (Balthasar *et al.* 2004). These results clearly show that leptin receptors on POMC neurons are required, but not solely responsible for the regulation of body weight homeostasis by leptin. VMH is one non-arcuate site where leptin action has been studied in detail, taking advantage of the restricted expression of SF1 in this nucleus (Dhillon *et al.* 2006). In brain slice preparations, leptin was shown to depolarise and increase the firing rate of SF1-GFP labelled neurons in the VMH. Mice lacking leptin receptors only in SF1-positive neurons displayed mild obesity, similar to that observed in mice lacking leptin receptors only in POMC neurons. Moreover, it was shown that ablation of leptin receptors both in SF1-positive neurons and POMC neurons resulted in an additive effect on body weight, which was still less marked than that observed in mice totally devoid of leptin receptors. Determining the role of ObRs expressed in other hypothalamic nuclei such as DMH will require further investigation.

Hierarchical and/or distributed integration between the hypothalamus and the brainstem

Given the ability of decerebrated rats to regulate meal size under oro-gastro-intestinal feedback controls – not in response to food deprivation on the one hand and the core hypothalamic circuitry capable of sensing adiposity signals on the other – it is tempting to speculate that in the neurologically intact rat, regulation of food intake could result from a hierarchical functioning of the two major integrative centres, the hypothalamus integrating adiposity signals would influence the brainstem integrator of satiety reflex (DVC), so as to adjust meal size as a function of fat stores. Consistent with this view, leptin is known to

modulate food intake by regulating meal size, without modifying meal frequency. Moreover, it has been shown that leptin and insuline both increase the satiating effects of CCK (Woods *et al.* 1998), and that leptin amplifies the activating effect of CCK on NTS neurons (Schwartz *et al.* 2000). These results suggest that the integration of adiposity signals (in the hypothalamus) could lead to the modulation of the satiety signals integration process (in the brainstem) *(figure 4A)*.

However, several results show that adiposity signal sensing is not restricted to the ARC. It is noticeable that ObRs are expressed in a number of extrahypothalamic sites, notably the NTS
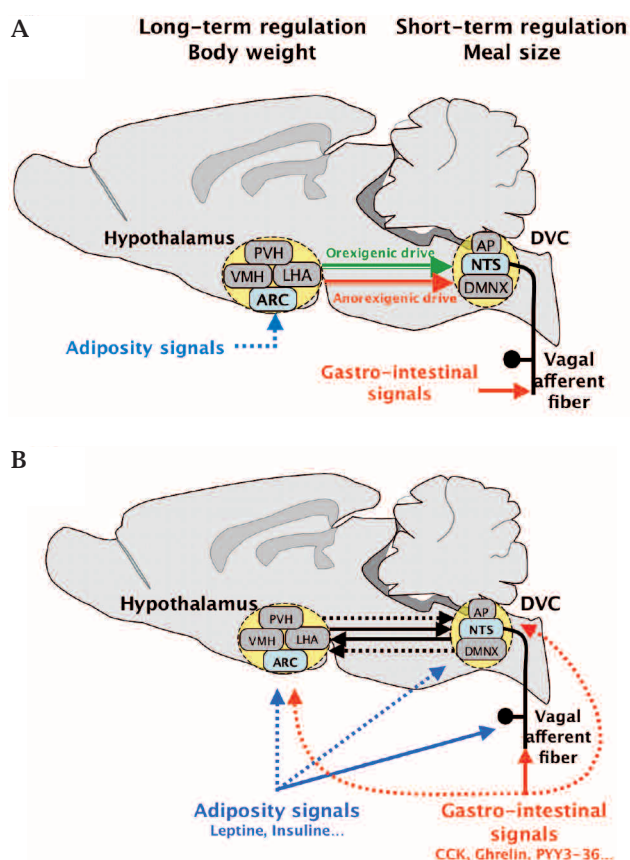

Figure 4 : Models of the interactions between the hypothalamus and the brainstem for the control of food intake and energy balance.

A: Hierarchical model. In this model, adiposity signals (leptin, insulin) are integrated within the hypothalamic nuclei, resulting in a descending drive, either orexigenic or anorexigenic, depending on the inputs from the adiposity signals. The hypothalamic outputs can modulate the integration of gastro-intestinal signals within the brainstem DVC. Therefore, increased inputs from adiposity signals reduce meal size by increasing brainstem responses to satiety signals, whereas reduced inputs have the opposite effect.

B: Distributed model. In fact, adiposity and gastro-intestinal signals can act either via a neural pathway, by stimulating sensory receptors located on vagal afferent fibres, or via an humoral pathway, by stimulating neuronal receptors within the hypothalamic nuclei and the brainstem DVC. In this model, meal size and body weight regulation depend on the interplay between the hypothalamus and the brainstem through central direct or multisynaptic connections between these two structures (see text for more details).

Abbreviations: AP, area postrema; ARC, arcuate nucleus; DVC, dorsal vagal complex; DMNX, dorsal motor nucleus of the vagus nerve; LHA, lateral hypothalamic area; NTS, nucleus tractus solitarii; PVH, paraventricular nucleus; VMH, ventromedial nucleus.

in the brainstem (Hosoi *et al.* 2002; Grill *et al.* 2002). Moreover, administration of leptin to the fourth ventricle and to the DVC parenchyma reduces food intake (Grill *et al.* 2002). Interestingly, recent results show that leptin applied locally within the DVC inhibits swallowing (Félix *et al.* 2006). Vagal afferent fibres themselves are also sensitive to leptin, as described above. These afferent fibres display integrative properties, being able to detect simultaneously mechanical signals elicited by distension of the digestive tract and paracrine signals such as CCK. Moreover, it has been demonstrated that their sensitivity to leptin is modulated by CCK (Gaigé *et al.* 2002).

In addition, the melanocortinergic signalling pathway, the principal integrating arm of the above model of hypothalamic lipostat, displays a striking redundancy in the brainstem. There are only two central locations of POMC expression: the ARC in the hypothalamus and the commissural nucleus tractus solitarii (cNTS) in the DVC. In addition, MC4-R is densely expressed in the DVC where it participates in food intake regulation, as has been demonstrated by intraparenchymal injection of MC4-R agonists and antagonists in the DVC (Williams *et al.* 2001). There are also descending projections from arcuate POMC neurons within the NTS. This brainstem branch of melanocortinergic system plays a role in the satiety reflex, since it has been shown that the POMC neurons of cNTS are activated by CCK and activation of the brainstem neuronal MC4-R is required for CCK-induced suppression of feeding (Fan *et al.* 2004). This does not exclude a role of brainstem MC4-R in long-term regulation of feeding however. Indeed, peripheral administration of leptin was recently shown to induce the phosphorylation of STAT3 in about half of cNTS POMC neurons (Ellacott *et al.* 2006; but see Huo *et al.* 2006). BDNF, a downstream effector of melanocortinergic signalling in the VMH, was recently shown to play a role as an anorexigenic factor in the DVC (Bariohay *et al.* 2005). Indeed, intraparenchymal infusion of exogenous BDNF within the DVC induces anorexia and weight loss, whereas endogenous BDNF protein content in the DVC is modulated by nutritional status and peripheral administration of leptin or CCK.

Other evidence indicates that the integration of at least some hormonal signals of gastro-intestinal origin is also found in hypothalamus, brainstem and vagal afferent fibres. Several data support such a distributed integration process for ghrelin. In the ARC, most NPY/AgRP neurons express GHS-R and are activated by ghrelin in slice preparations (Riediger *et al.* 2003). The DVC is also a direct target for ghrelin, as demonstrated by the hyperphagic response to its intraparenchymal administration in this brainstem site (Faulconbridge *et al.*, 2003). Vagal afferent fibres also express GHS-R and ghrelin suppresses firing of vagal nerve (Date *et al.* 2002). Central action of ghrelin was shown to be mediated, at least in part, by NPY (Chen *et al.*, 2004). Selective forebrain or brainstem delivery of ghrelin in rats with occluded cerebral aqueduct revealed that the hyperphagic response requires contrasting activation of different NPY receptors subsets in the forebrain and/or brainstem, depending on the location of GHS-R stimulation (Faulconbridge *et al.* 2005).

The hypothalamus and the DVC are interconnected directly and indirectly, mainly *via* the parabrachial nuclei (Jean 1991). Hypothalamic nuclei receiving direct projections from the NTS are the ARC, PVH and VMH. Conversely, the NTS receives direct projections from the ARC, LHA, and PVH (for review, see Berthoud 2002). The existence of neurons sensitive to leptin and gastro-intestinal peptides in both the hypothalamus and the DVC, two richly interconnected integrative centres for energy homeostasis regulation, suggests that the integration of internal signals relevant for body weight control could result from a dialogue between the hypothalamus and the DVC.

Altogether these results suggest that control of food intake very probably depends on a distributed processing rather than on a hierarchical treatment *(figure 4B)*.

A split efferent arm in the neuronal network involved in energy balance

The melanocortinergic system has also attracted attention, with the aim of delineating the physiological roles of the various MC4-R expressing neurons in body weight regulation. Activation of MC4-R reduces body weight by decreasing food intake and increasing energy expenditure. The role of MC4-R in the PVH were recently studied using genetically engineered mice expressing MC4-R at physiological levels in Sim1 positive neurons, on an otherwise MC4-R null background (Balthasar *et al.* 2005). Restoration of MC4-R expression in the PVH (and part of the amygdala, where Sim1 is also expressed) prevented obesity by 60% compared to MC4-R null mice. Surprisingly, hyperphagia was totally suppressed while reduced energy expenditure was unaffected. These findings show that MC4-R in the PVH (and/or the amygdala) can control food intake, but that energy expenditure depends on MC4-R localised elsewhere in the brain, revealing a divergence in the melanocortinergic pathways that control energy balance. The neuronal network involved in energy balance comprises an afferent arm receiving inputs from the gastro-intestinal tract and sensing adiposity signals. As described above, this afferent arm is distributed among several hypothalamic nuclei and the brainstem. The integrative component of the network is also most probably distributed in a dialogue between the hypothalamus and the brainstem. The divergence of melanocortin signalling in the control of food intake versus energy expenditure places MC4-Rs on the efferent side of the neuronal network involved in energy homeostasis. Another possible interpretation of these results is that control of food intake and energy expenditure may be disassociated and depend on networks situated in different brain regions.

Plasticity in integrative centres for energy homeostasis regulation

Recent data indicate that the integrative centres for energy homeostasis regulation display neuronal plasticity in the adult: 1) involvement of plasticity-promoting signalling pathways in food intake regulation; 2) involvement of a retrograde neuromodulation system, the endocannabinoid system, in orexigenic

signalling; 3) leptin-dependent rapid synaptic rearrangements; 4) leptin-dependent modulation of intrinsic action potential frequency; 5) neurogenesis increasing leptin responsiveness.

Neurotrophins play important roles in proliferation, differentiation and survival of neurons in the peripheral and central nervous systems during development (Davies 1994; McAllister 2001). In addition, some neurotrophins, and in particular BDNF, also play critical roles in the synaptic activity and plasticity of many groups of mature neurons (McAllister *et al.* 1999; Poo 2001; Lu 2003). The anorexigenic signalling by BDNF might be mediated, at least in part, by neuroplasticity phenomena, although this has not been studied up to now.

The endocannabinoid system consists of the cannabinoid receptors, the endocannabinoids and the enzymes catalysing their biosynthesis and degradation. In the hypothalamus, endocannabinoids levels are increased by fasting and decreased by leptin. The pharmacological activation of CB1 stimulates food intake, whereas its blockade reduces it (for review, see Di Marzo & Mathias, 2005). The endocannabinoid system is known to regulate synaptic transmission mainly in a retrograde manner, where the post-synaptic neuron produces endocannabinoids in an activity-dependent manner and the neurotransmitter release from the pre-synaptic neuron is modulated through activation of CB1 (for review, see Di Marzo *et al.* 1998). In the PVH, it has been shown that endocannabinoids retrogradely inhibit glutamatergic inputs to CRH neurons (Di *et al.* 2003; Di *et al.* 2005). In the periforncal lateral hypothalamus, the endocannabinoid system has been shown to be involved in the retrograde inhibition of GABAergic inputs to, notably but not exclusively, MCH neurons. Moreover, leptin was shown to directly inhibit calcium entry in LH neurons, thereby inhibiting the calcium-dependent endocannabinoid synthesis/release by these neurons (Jo *et al.* 2005).

The synaptic input organisation of chemically defined hypothalamic neurons involved in food intake regulation was recently studied. The results obtained revealed a leptin-dependent plasticity *(figure 5A)*. Leptin deficient ob/ob mice differed from wild type mice in the numbers of excitatory and inhibitory synapses, and in the post-synaptic currents on arcuate NPY/AgRP and POMC/CART neurons. Moreover, peripheral administration of leptin to adult ob/ob mice normalised the synaptic density within a few hours (Pinto *et al.* 2004). Ghrelin was also shown to induce intensive synaptic remodelling on POMC/CART neurons. Similarly, synaptic arrangement plasticity has been observed on hypocretin/orexin neurons in the LHA. Fasting was shown to induce an increase in excitatory inputs on these orexigenic neurons, which was reversed by re-feeding, and blocked by leptin administration (Horvath & Gao, 2005).

Direct measurements of GFP-labelled arcuate NPY/AgRP neuronal activity in acute slices were made by Takahashi and Cone in the presence of picrotoxin and kynurenic acid to isolate the cells from synaptic inputs *via* GABA A and ionotropic glutamate receptors. In wild type mice, nutritional status before slice preparation proved to greatly influence the basal action potential frequency of NPY/AgRP neurons. Fasting induced a fourfold increase in spike

frequency; an effect that was largely blocked by *in vivo* peripheral leptin administration, suggesting that fasting modulates NPY/AgRP neuronal activity through a reduction in circulating leptin levels. Consistent with this, NPY/AgRP neurons from leptin deficient ob/ob mice, displayed an elevated intrinsic activity that was not affected by nutritional status (Takahashi & Cone, 2005). Therefore, besides the synaptic plasticity described above, leptin can also influence neuronal pathways involved in food intake regulation through nonsynaptic plasticity.

Finally, while adult neurogenesis has long been considered to be confined to the subventricular zone/olfactory bulb (SVZ/OB) system and the subgranular zone of the hippocampal dentate gyrus (Taupin & Gage, 2002; Alvarez-Buylla & Lim, 2004), it was recently demonstrated that the DVC of the adult rat is also a site where neurogenesis occurs (Bauer *et al.* 2005), from intrinsic neural stem cells (Charrier *et al.* 2006). The hypothalamus also displays a low basal neurogenesis rate, which can be revealed by the use of a highly sensitive cell-proliferation labelling protocol (Pencea *et al.* 2001; Kokoeva *et al.* 2005). The potential role of neurogenesis in energy balance has recently been investigated *(figure 5B)* (Kokoeva *et al.* 2005). In wild type mice, Ciliary Neurotrophic Factor (CNTF) was shown to induce a body weight loss that persisted several weeks after the cessation of intracerebroventricular administration. By contrast, CNTF central infusion to leptin-deficient ob/ob mice had a much shorter-term effect

Figure 5: Neuroplasticity in adult feeding networks

A. Neuronal flexibility. Network rewiring, i.e. modification in the number of excitatory and inhibitory synapses, have been demonstrated in the ARC and LHA. In ob/ob mice, arcuate orexigenic NPY neurons (green) and anorexigenic POMC neurons (red) display a contrasted imbalance in their excitatory versus inhibitory synaptic inputs, consistent with hyperphagia. Leptin treatment induces a rapid rewiring, reducing the orexigenic tone and increasing the anorexigenic tone. In wild type mice, similar rapid rewiring has been demonstrated on orexigenic hypocretin/orexin neurons in the LHA, depending on nutritional status.

B. Potential role of neurogenesis in the functioning of feeding network. It has been shown in mice that ciliary neurotrophic factor (CNTF) induces a sustained loss in body weight. CNTF also increases neurogenesis in the hypothalamus. Several newly generated neurons (yellow) show responsiveness to leptin (depicted here as expressing ObR). Co-administration of CNTF and a mitotic blocker suppresses the sustained loss in body weight, suggesting that CNTF-induced neoformation of leptin-sensitive neurons could be responsible for the CNTF-induced sustained body weight loss. In this case, newly generated neurons are very likely included in the feeding network to increase the anorexigenic tone (see text for more details).

on body weight. Correlatively, CNTF treatment induced a five-fold increase in the number of newborn cells in the hypothalamus, and most of these cells survived for weeks after the cessation of treatment. Moreover, many of the newly generated cells expressed neuronal markers and showed responsiveness to leptin. Co-administration of CNTF and a mitotic blocker in wild type mice resulted in a short-term body weight loss, suggesting that CNTF-induced neoformation of leptin-responsive neurons (at least in the hypothalamus) could be responsible for the sustained effect of CNTF on body weight.

Taken together, these results suggest that neuronal plasticity might be a key component in the regulation of energy homeostasis.

CONCLUSION

Feeding, which is essential for the survival of animals, is subject to an intense regulation by the brain to maintain energy homeostasis. Since the discovery of leptin in the early 1990s, and as a consequence of the increasing health problems related to morbid overweight, neural systems controlling food intake and body weight have received considerable attention during the past decade.

Although multiple brain regions are involved in food intake, when focusing on energy homeostasis, two main areas play a major role, namely the hypothalamus and the dorsal vagal complex in the brainstem. The functioning of both hypothalamic and brainstem networks is regulated by short-term and long-term signalling, the so-called satiety and adiposity signals. Therefore, as opposed to a hierarchical model, in which the hypothalamus plays the major role, recent results are consistent with a distributed model for the control of energy balance.

Within these networks, attention has been focused on such transmitters as hormones and neuropeptides, which have been categorised as orexigenic or anorexigenic factors. However, similar to other signalling molecules, the "feeding" transmitters play also important roles in various other functions. Recent results clearly demonstrate that modulatory influences exerted by non-conventional signalling molecules, such as neurotrophins and endocannabinoids, also play a key role in food intake. It is worth noting that other signalling systems, such as bioamines and amino acid transmitters, are also involved in food intake regulation (Van Den Pol 2003; Ramos *et al.* 2005). Understanding the modulation of these transmitter systems may be key to a complete understanding of the control of energy balance.

Recent results have shown that leptin and ghrelin induce contrasting reorganisations of synaptic inputs on arcuate NPY/AgRP and POMC/CART neurons in adults, consistent with the respectively anorexigenic and orexigenic effects of these hormones (Pinto *et al.* 2004). Similarly, the input organisation of hypocretin/orexin neurons in the LHA changes dynamically with nutritional state, at least in part in a leptin-dependent fashion (Horvath & Gao, 2005). Moreover, neurogenesis in the hypothalamus has been shown to be potentially involved in food intake and body weight regulation. Rearrangements of feeding networks, and the possible addition of new neurons within these networks under various
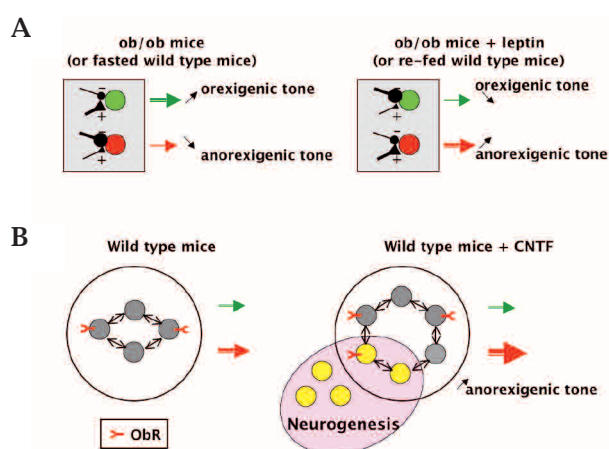
modulatory influences, may provide important clues to the understanding of food intake disorders such as obesity.

Interestingly, it has also been shown that leptin plays a major role in the perinatal maturation of hypothalamic networks involved in food intake regulation (Bouret *et al.* 2004). These results suggest that nutritional perturbations occurring *in utero* or in the neonate may alter the circulating levels of leptin and have long-term consequences on the formation of neural circuits involved in food intake. The rapid increase in the prevalence of obesity in young people these past few decades could well be due to such developmental alterations.

Finally, in addition to the study of the mechanisms that underlie networks controlling energy homeostasis, several new studies have been started to define the neural mechanisms involved in hedonic and addictive components of food intake (Cota *et al.* 2005). Indeed, the rapidly increasing prevalence of obesity in western countries is intimately linked to the recent profound alterations of environment and lifestyle. In this context, it is becoming crucial to identify the mechanisms underlying the rewarding or even addictive properties of feeding behaviour.

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