



# Regulation of synaptic functions in central nervous system by endocrine hormones and the maintenance of energy homeostasis

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## Synopsis

Energy homeostasis, a co-ordinated balance of food intake and energy expenditure, is regulated by the CNS (central nervous system). The past decade has witnessed significant advances in our understanding of metabolic processes and brain circuitry which responds to a broad range of neural, nutrient and hormonal signals. Accumulating evidence demonstrates altered synaptic plasticity in the CNS in response to hormone signals. Moreover, emerging observations suggest that synaptic plasticity underlies all brain functions, including the physiological regulation of energy homeostasis, and that impaired synaptic constellation and plasticity may lead to pathological development and conditions. Here, we summarize the current knowledge on the regulation of postsynaptic receptors such as AMPA ( $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid), NMDA (*N*-methyl-D-aspartate) and GABA ( $\gamma$ -aminobutyric acid) receptors, and the presynaptic components by hormone signals. A detailed understanding of the neurobiological mechanisms by which hormones regulate energy homeostasis may lead to novel strategies in treating metabolic disorders.

**Key words:** cognition, diabetes, energy homeostasis, ghrelin, hormone, insulin, leptin, neural circuitry, neurodegeneration

Cite this article as: Pang, Z.P. and Han, W. (2012) Regulation of synaptic functions in central nervous system by endocrine hormones and the maintenance of energy homeostasis. *Biosci. Rep.* **32**, 423–432

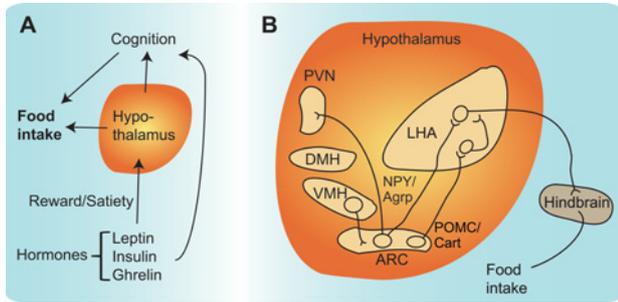
## INTRODUCTION

Food intake and energy expenditure, the key determinants of energy homeostasis, are regulated by the CNS (central nervous system). Since the end of the 19th century, profound intellectual and experimental efforts have been made to understand how the brain regulates glucose and energy homeostasis and how impaired brain functions contribute to the pathogenesis of metabolic diseases. Growing evidence suggests that nutrient and hormonal signals from the periphery, including adipocyte-derived hormone leptin, pancreatic insulin and stomach-secreted ghrelin, converge on to the CNS to modulate nutrient intake and utilization. The CNS integrates the peripheral signals and progressively adapts to the changes to maintain energy balance [1].

In the early 1940s, lesion studies identified the ventromedial nuclei of the hypothalamus, including the ARC (arcuate nucleus), VMH (ventromedial hypothalamus), PVN (paraventricular nucleus) and dorsal hypothalamus, as important brain regions in the development of hyperphagia and obesity, whereas lesions in LHA (lateral hypothalamic area) resulted in hypophagia and anorexia (Figure 1). These findings led to a simple, yet appealing model: the mediobasal hypothalamic nuclei are the ‘satiety centres’ and the LHA is the ‘hunger/feeding centre’ [2,3]. A fundamental breakthrough took place when the adipose-tissue-derived hormone leptin was discovered and found to act via its receptor in the brain to regulate feeding and neuroendocrine functions [4–6]. Ever since, extensive studies coupled with new experimental tools have shed light on the mechanisms underlying the influence of hormonal signals on the brain regarding the neuronal regulation

**Abbreviations used:** AgRP, agouti-related peptide; AMPAR,  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; ARC, arcuate nucleus; CNS, central nervous system; DA, dopaminergic; EPSC, excitatory postsynaptic current; GABA,  $\gamma$ -aminobutyric acid; GABA<sub>A</sub>,  $\gamma$ -aminobutyric acid A; GluR, glutamate receptor; GHSR, growth hormone secretagogue receptor; IPSC, inhibitory postsynaptic current; IR, insulin receptor; JAK2, Janus kinase 2; KA, kainic acid; LepRb, leptin receptor long isoform; LHA, lateral hypothalamic area; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MC, melanocortin; MC3R, melanocortin 3 receptor; MSH, melanocyte stimulating hormone; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptor; NPY, neuropeptide Y; NAc, nucleus accumbens; PBN, parabrachial nucleus; PI3K, phosphoinositide 3-kinase; POMC, pro-opiomelanocortin; STAT3, signal transducer and activator of transcription 3; TH, tyrosine hydroxylase; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

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**Figure 1 Hypothalamic neurocircuitry regulates food intake**

(A) Hypothalamus is the primary brain region that responds to peripheral signal such as leptin, insulin and ghrelin to regulate feeding behaviour. The hypothalamic brain region also interacts with higher brain regions that control cognition and the latter also play important roles in food intake. (B) Diagram showing the major hypothalamic nuclei and wirings between the nuclei. DMH, dorsomedial hypothalamus.

of energy homeostasis [1,3,7–9]. Among all the hormones related to feeding behaviour and cognition, leptin, insulin and ghrelin are among the best characterized.

Synapses are specialized structures on the neuronal cell membrane that mediate rapid and highly efficient information transmission from a neuron to its target cells in a highly plastic manner. Synaptic plasticity is known to play a central role in a range of brain-related behaviours, such as learning, memory and addiction [10,11]. However, such synaptic plasticity has not been considered previously as a critical regulator of energy homeostasis. Recent studies have revealed that synaptic vesicle release [12] and continual plasticity in the feeding circuits may be a key component in energy balance control [13]. Detailed understanding of intracellular signalling cascades of hormones have begun to accumulate, and these studies collectively indicate that leptin, insulin and ghrelin play important roles in synaptic functions (Figures 1 and 2) [3]. In this review, we begin with the current view of synaptic regulation of hypothalamic function in energy homeostasis, then focus on the cellular mechanisms underlying hormonal regulation of synaptic transmission, and conclude by discussing how hormones function in the regulation of feeding- and reward-neural circuitry.

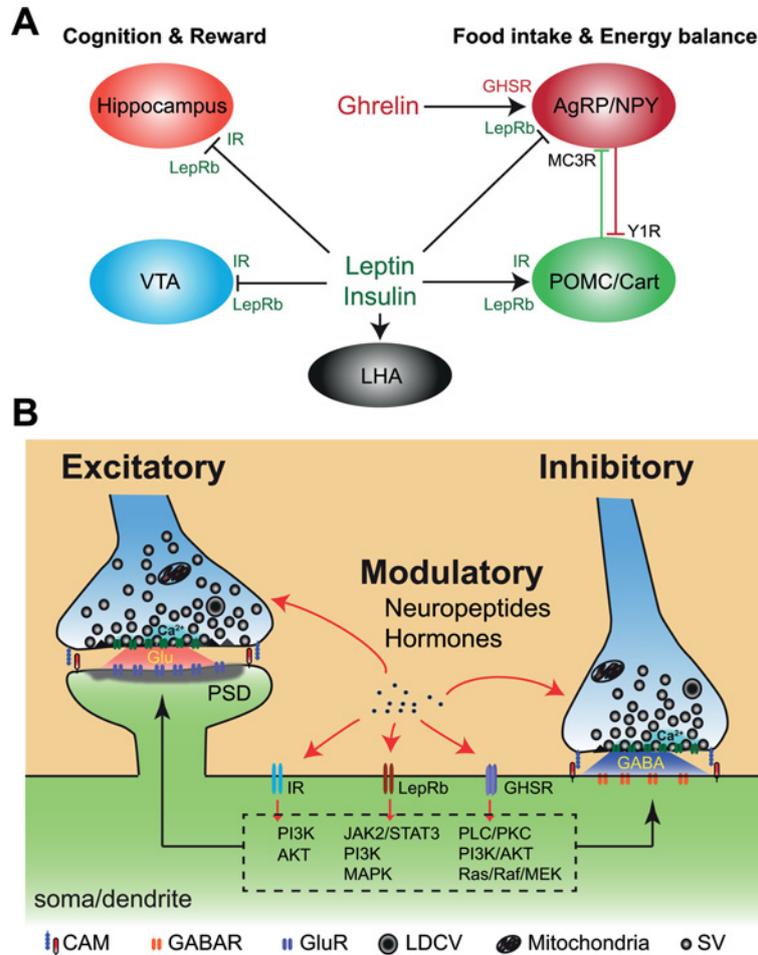
## SYNAPTIC REGULATION OF HYPOTHALAMIC FUNCTION IN ENERGY HOMOEOSTASIS

Synaptic transmission mediates all brain-related behaviour, including food intake and energy expenditure [1,12]. Fast excitatory neurotransmission is mainly mediated by ionotropic glutamate receptors, i.e. AMPARs ( $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors), KARs (kainic acid receptors) and NMDARs [NMDA (*N*-methyl-D-aspartate) receptors]. AMPARs are tetramers composed of four types of subunits, GluR1–GluR4 (glutamate receptor 1–4), and mediate the major excitatory syn-

aptic transmission in the brain [14]. Upon activation by glutamate released from presynaptic nerve terminals, postsynaptic AMPARs and NMDARs mediate non-selective influx of cations, which result in inward EPSCs (excitatory postsynaptic currents) and thus cause postsynaptic depolarization. Most AMPARs in CNS contain GluR2 subunit and are permeable to  $\text{Na}^+$  and  $\text{K}^+$ , but not  $\text{Ca}^{2+}$ , whereas those AMPARs without GluR2 subunit are permeable to  $\text{Ca}^{2+}$ , in addition to  $\text{Na}^+$  and  $\text{K}^+$  [15]. Fast inhibitory neurotransmission is mainly mediated by ionotropic GABA<sub>A</sub> ( $\gamma$ -aminobutyric acid A) receptors, which allow  $\text{Cl}^-$  influx upon binding to GABA released from presynaptic terminals, and induce IPSCs (inhibitory postsynaptic currents) and consequently hyperpolarization of postsynaptic neurons.

Recent development in mouse genetic tools has made it possible for detailed analysis of the involvement of both excitatory and inhibitory synaptic transmission in regulating body weight especially in the ARC. There are two major groups of neurons located in ARC (for review see [16]): the anorexigenic (i.e. inhibit feeding and weight gain) neurons synthesize POMC (pro-opiomelanocortin), the precursor for many active neuropeptides including  $\alpha$ -MSH (melanocyte-stimulating hormone).  $\alpha$ -MSH signals anorexia by binding to MC (melanocortin) receptors (especially MC4R) in several areas of the brain [17,18]; ARC orexigenic (i.e. increase feeding) neurons synthesize NPY (neuropeptide Y) [19] and AgRP (agouti-related peptide) [20]. Using genetic tools, two recent elegant papers from the Lowell Laboratory highlighted the importance of synaptic transmission in regulating food intake. In the first study, Liu et al. [21] reported that body weight, fat stores and food intake were markedly reduced in mice with specific deletion of NMDARs in AgRP neurons. Interestingly, the deletion of NMDARs in POMC neurons had no effect on energy homeostasis. Furthermore, they showed that fasting activated AgRP neurons and increased the synaptic strength due to increased AMPA ( $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid)-mediated synaptic transmission, and this effect was abolished when NMDARs were eliminated from postsynaptic neurons [21]. In their second study, Vong et al. [22] demonstrated that inhibitory input to POMC neurons was the key modulatory component in energy homeostasis. Food deprivation enhanced excitatory synaptic input in AgRP neurons [22], which was mediated by a presynaptic positive feedback loop involving AMPK (AMP-activated protein kinase) [23]. Other recent studies also support the significance of synaptic transmission in energy homeostasis regulation [23–26]. For example, GABAergic AgRP neurons project to PBN (parabrachial nucleus) to promote feeding, and that the blockade of GABAergic input to PBN results in anorexia independent of the MC system [25]. This study suggests that loss of GABA signalling from AgRP neurons to PBN unmasks an excitatory input to PBN, which in turn leads to reduced feeding. The excitatory input to PBN comes from the glutamatergic neurons in NTS (nucleus tractus solitaries) and caudal serotonergic neurons [24].

Collectively, these latest studies in cellular and circuitry analysis reveal the involvement of synaptic regulation in feeding



**Figure 2** Endocrine hormones regulate neuronal function in the brain

(A) Leptin and insulin bind to their specific receptors to regulate brain functions. Leptin and insulin activate POMC neurons and inhibit AgRP/NPY neurons to suppress feeding behaviour. Ghrelin on the other hand, activates AgRP/NPY neurons to stimulate feeding behaviour. Leptin and insulin also regulate neuronal functions within brain regions that are important components for cognition and reward behaviours such as the hippocampus and VTA. (B) Neuromodulators including peptide hormones regulate both excitatory and inhibitory synaptic functions. The modulatory functions can be both presynaptic and postsynaptic origins. Hormones such as insulin, leptin and ghrelin bind to their corresponding receptors and activate second messenger cascades to influence synaptic function. Note that the signalling cascades depicted in the postsynaptic compartment also apply to the presynapse. Abbreviations: CAM, cell-adhesion molecules (e.g. neuexins and neuroigins); Cart, cocaine- and amphetamine-regulated transcript; GABAR, GABA receptors; GluR, glutamate receptors including both NMDAR and AMPAR; LDCV, large-dense core vesicle; MEK, MAPK/extracellular-signal-regulated kinase kinase; PKC, protein kinase C; PLC, phospholipase C; PSD, postsynaptic density; SV, synaptic vesicle X1R, neuropeptide X1 receptor.

behaviour, and highlight the importance of investigating the effects of hormones on synaptic transmission for the understanding of how CNS controls energy homeostasis.

## LEPTIN AND SYNAPTIC TRANSMISSION

The adipose-tissue-derived hormone leptin is a 167-amino-acid protein in humans [27]. Circulating leptin plays a pivotal role in regulating energy homeostasis by communicating the body energy status to the CNS to suppress feeding and promote energy

utilization (Figure 2) [8,28,29]. There are multiple leptin receptor isoforms, among which LepRb (leptin receptor long isoform) is crucial for leptin action [1,3,7,30,31]. Leptin binds to LepRb and activates JAK2 (Janus kinase 2)/STAT3 (signal transducer and activator of transcription 3) signal cascade and exert downstream functions (Figure 2B, also see [3] for details). Loss-of-function mutations in leptin or leptin receptor, such as *ob/ob* and *db/db*, cause morbid obesity in rodents [32–34] and humans [35]. Many effects of leptin signalling are attributed to its actions in the CNS, especially in the hypothalamus (Figure 1A), in which LepRb is highly expressed [36]. In the ARC, leptin differentially regulates catabolic/anorexigenic and anabolic/orexigenic neurons. Leptin acts via LepRb to increase the firing of



anorexigenic LepRb/POMC neurons, POMC expression and  $\alpha$ -MSH secretion, and, to suppress the firing of orexigenic LepRb/NPY-expressing neurons, secretion of NPY and AgRP (Figure 1B) [21,37,38]. The response to leptin in ARC neurons mainly contributes to satiety. Leptin can also directly regulate mesolimbic VTA (ventral tegmental area) DA (dopaminergic) neurons (Figure 2A) [39]. Recently, a subgroup of neurons in LHA was identified to express LepRb, but not orexin/hypocretin [40,41]. These LepRb neurons project to VTA, whereas LHA orexin-expressing neurons are known to project to the hindbrain region [40]. It is likely that LHA neurons are key effectors of leptin signalling in the regulation of energy homeostasis. However, not all the LepRb-expressing neurons in LHA respond to leptin in the same fashion: one-third of LepRb-expressing LHA neurons are depolarized by leptin; another third are hyperpolarized by leptin and the remaining third does not respond to leptin [40]. The molecular and cellular nature for the differential effects of leptin is not known.

Besides the hypothalamus, LepRb is present in several brain regions related to cognition [42]. In hippocampus, leptin can hyperpolarize hippocampal neurons by activating large conductance  $Ca^{2+}$ -activated  $K^{+}$  (BK channels), but not  $K_{ATP}$  channels, through a PI3K (phosphoinositide 3-kinase) signalling cascade [43]. Elevated leptin level in hippocampal neurons leads to  $PtdIns(3,4,5)P_3$  increase, which has been shown to promote actin rearrangement and BK channel trafficking in the hippocampal synapses. The fact that leptin could reduce the excitability and inhibit the action potential generation in hippocampal neurons led to the studies that examined leptin as an anti-convulsion candidate in epilepsy animal models [44]. Interestingly, leptin has also been shown to increase the excitability of neurons in the somatomotor cortex. More recently, leptin receptor expression has been detected in mesolimbic dopamine neurons, and the activation of leptin signalling attenuates the firing frequency of VTA DA neurons [39]. Again, these opposing functions suggest that leptin acts on neuronal excitability in a region- and/or neuron-dependent manner. However, the biological basis for the opposing effects remains to be determined.

### Leptin and AMPARs

At the molecular level, leptin inhibits AMPAR-mediated excitatory synaptic transmission in mouse hippocampal slices, but not in *db/db* hippocampal slices [45]. Further studies reveal that JAK2–PI3K pathways are involved in leptin actions on AMPARs [45]. However, unlike the transient synaptic depression elicited by leptin in juvenile hippocampus [45], leptin can increase the excitatory synaptic strength in adult hippocampus through preferential up-regulation of the cell surface expression of GluR1 and the synaptic density of GluR2-lacking AMPARs. This effect of leptin requires NMDAR activation and is associated with an increase in cytoplasmic  $PtdIns(3,4,5)P_3$  levels through enhanced phosphorylation of the lipid phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10), which inhibits PTEN function [46]. The different effects of leptin on excitatory synaptic transmissions indicate that leptin actions on synaptic transmission are probably developmentally regulated.

### Leptin and NMDARs

The number and subunit composition of NMDARs at the synapse are under dynamic regulations during synaptic plasticity [47]. Leptin has been shown to facilitate the induction phase of hippocampal LTP (long-term potentiation) [48,49] probably through the activation of NMDARs [50]. Acute application of leptin-enhanced NMDAR-mediated EPSCs in hippocampal slices [48]. *In vitro* studies using *Xenopus* oocytes showed that NMDAR response was modulated by leptin only in cells expressing NR1A/NR2A-containing NMDARs together with LepRb, but not NR1A/NR2A alone [48], indicating that leptin modulates NMDA responses only through LepRb signalling pathways [51]. Leptin facilitation of NMDA responses was observed over the entire dose–response curve, including the maximal responses, suggesting that leptin acts through LepRb to increase the NMDAR density at the cell surface [51,52]. The detailed molecular and cellular mechanisms of how leptin regulates NMDAR trafficking remain elusive.

### Leptin and GABA receptors

Disruption of leptin signalling in neurons by deleting LepRb in hypothalamic neurons only resulted in mild obesity [53–55], indicating that hypothalamic neurons cannot be the only site of action by leptin signalling in energy homeostasis regulation. A number of studies attempted to identify additional effector neurons for leptin action. Lowell and co-workers [22] recently investigated whether the ‘first-order’ effectors of leptin signaling are excitatory- or inhibitory-neurons. In this elegant study, they made use of vGluT2-ires-Cre and vGAT-ires-Cre with specific expression in excitatory and inhibitory neurons respectively. Surprisingly, they found that the vast majority of leptin’s anti-obesity effects were mediated by GABAergic neurons, and glutamatergic neurons played only a minor role [22]. Although this study did not pinpoint where the critical inhibitory neurons that regulate body weight were located, it provided a first direct evidence that leptin directly acts on presynaptic GABAergic neurons and reduces inhibitory tone to postsynaptic POMC neurons, and thus prevents animals from over-feeding.

### Leptin and long-term synaptic plasticity

Long-term synaptic plasticity, including LTP and LTD (long-term depression), is a molecular mechanism underlying learning and memory [11]. Growing evidence suggests that endocrine hormones, particularly leptin, play pivotal roles in human cognition (for review, see [56,57]). Leptin facilitates the induction phase of hippocampal LTP presumably through the enhancement of NMDAR activation [58]. In addition, LepRb-deficient animals have impaired synaptic plasticity in hippocampus, supporting the involvement and function of the leptin/LepRb cascade in synaptic plasticity [58]. NMDARs, but not metabotropic glutamate receptors, mediate leptin-induced LTD in the hippocampus. The signalling pathway underlying leptin-induced LTD was independent of the Ras/Raf/MAPK (mitogen-activated protein kinase) pathway, but was markedly enhanced following inhibition of either

PI3K or protein phosphatases 1 and 2A [58,59]. Recently, it was shown that leptin could reverse hippocampal LTP through a postsynaptic mechanism that required the activation of NMDARs. Interestingly, activation of the calcium/calmodulin-dependent protein phosphatase calcineurin in the postsynapse was required for leptin function on reversing LTP. Moreover, the leptin-induced de-potentialization was accompanied by a reduction in AMPAR rectification, which normally mediates EPSC during LTP through GluR1 insertion in the absence of GluR2s. This suggests that leptin function in the hippocampus may be through the regulation of internalization of GluR1 homomeric AMPARs [58].

### Leptin, axon guidance and synaptic rewiring

Hypothalamic neurocircuitry undergoes dynamic remodelling including structural and morphological changes of neurons in response to energy status in animals, partially dependent on the leptin signalling cascade [60]. As described above, leptin acts on NPY/AgRP and POMC neurons [3]. Leptin-deficient *ob/ob* mice differed from wild-type mice in the numbers of excitatory and inhibitory synapses on to NPY and POMC neurons, thus the EPSCs and IPSCs of NPY and POMC neurons [61]. Essentially, more excitatory synapses accompanied by fewer inhibitory synapses are formed on NPY neurons and more inhibitory synapses are formed on POMC neurons in the *ob/ob* mice. These changes involve both structural and functional modifications [61]. The resulting synaptic profiles of the NPY and POMC neurons may in part account for the increased food intake in the *ob/ob* mice [61]. Strikingly, the balance of synaptic inputs of NPY and POMC neurons in the *ob/ob* mice was restored as early as 6 h after leptin administration [61], indicating the profound effects of the leptin/LepRb signalling cascade on synaptic reorganization, including morphological modifications [60]. Indeed, leptin has been reported to exert a trophic action on hypothalamic neurons [62,63]. Moreover, synaptic contacts within the hypothalamic region may selectively go through dynamic alterations in response to changes in food intake [64]. For example, a recent report suggests that fasting causes increased dendritic spines, and consequently enhanced glutamatergic inputs in AgRP, but not POMC, neurons [21]. As leptin levels decrease drastically upon fasting, the study further supports leptin's involvement in the regulation of synaptic reorganization [65]. We anticipate that the action of leptin on fast rewiring of synaptic connections will prove to be an exciting and fruitful research area in the near future.

## INSULIN AND SYNAPTIC TRANSMISSION

Insulin, the major anabolic hormone, is a polypeptide of 51 amino acids secreted from the pancreatic islets of Langerhans [66]. It is one of the key regulators of glucose homeostasis, and like leptin, is also involved in the regulation of synaptic remodelling and energy homeostasis [3,67]. Previous studies have shown that the

effect of insulin on glucose and energy homeostasis is at least in part mediated by the CNS [68,69]. Circulating insulin can penetrate the blood–brain barrier and bind to IRs (insulin receptors) to regulate glucose levels and energy balance [70]. Defective insulin signalling in the CNS contributes to obesity and Type 2 diabetes. Numerous epidemiological studies suggest that insulin resistance, along with chronic inflammation, may be underlying links between diabetes and dementia and neurodegeneration [71,72].

Insulin exerts its biological functions via activation of IR located in hypothalamic nuclei (Figure 2). POMC neurons are critical regulators of energy balance and glucose homeostasis, and express both leptin and IRs. Insulin directly inhibits the firing of a subpopulation of POMC neurons [54]. Interestingly, leptin also regulates the same group of neurons. Unlike insulin, however, leptin increases their firing rate. Although both insulin and leptin activate the same intracellular enzyme, PI3K, their impacts on POMC neurons differ dramatically [73]. Moreover, high-fat feeding in mice activates IR-PI3K to inhibit steroidogenic factor 1 expressing VMH neurons [74], which in turn reduces the excitatory strength from VMH to ARC [64] and thus contributes to obesity development.

Insulin rapidly recruits functional GABA<sub>A</sub> receptors in hippocampal neurons [75]. Although there is no direct evidence to support insulin action on GABA<sub>A</sub> receptors in the hypothalamic region, insulin-induced hyperphagia in free-moving rats could be blocked by GABA<sub>A</sub> receptor antagonists that were applied in the VMH region [76]. This suggests that insulin-induced GABA<sub>A</sub> receptor trafficking might at least partially account for the effects of insulin regulation of food intake. Besides its influence on GABA<sub>A</sub> receptor recruitments, insulin can also facilitate the internalization of AMPAR [77–80], resulting in LTD in hippocampal neurons. Moreover, insulin has been indicated to potentiate NMDAR activities [81,82] and to stimulate the translocation of PSD (postsynaptic density)-95 at the postsynapse via the activation of PI3K/Akt/mTOR (mammalian target of rapamycin) signalling pathway [83]. The effects of insulin on membrane trafficking likely contribute to the modulation of synaptic function in the hippocampus, and may be an underlying mechanism of insulin functions in cognition.

Besides its regulation of synaptic transmission, experimental evidence also supports a crucial role of insulin signalling in synaptic remodelling [84]. For example, reduced IR functions through dominant-negative IR expression caused reduced synaptic density and miniature EPSC frequency, and altered experience-dependent dendritic arbor structural plasticity in *Xenopus* tadpole tectal neurons [67].

## GHRELIN AND SYNAPTIC TRANSMISSION

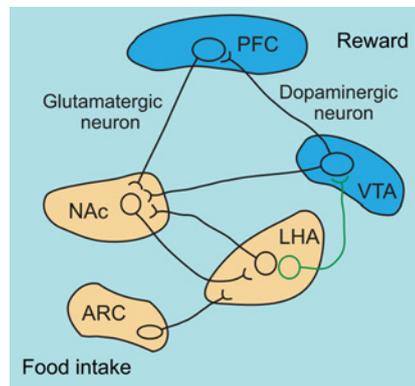
Ghrelin is an acylated polypeptide of 28 amino acids secreted from the upper tract of intestine [85,86] and some hypothalamic

neurons [87]. Although ghrelin-producing neurons are restricted in the hypothalamus, ghrelin receptors are expressed in various regions of the brain. It is known that ghrelin stimulates the release of growth hormone from the pituitary [88], and is involved in feeding regulation and energy homeostasis via activation of GHSR (growth hormone secretagogue receptor) in the hypothalamus [89,90]. NPY and AgRP neurons are primary targets in ghrelin-mediated regulation of feeding [91]. As discussed earlier, NPY/AgRP-producing neurons express LepRb and are regulated by leptin, although in the opposite manner from ghrelin. Leptin inhibits ghrelin-induced feeding activity, and ghrelin substantially attenuates the anorexic effect of leptin, thus forming a pair of Yin-Yang partnership in feeding regulation [92–95]. In hypothalamus (Figure 2), ghrelin axon terminals innervate NPY/AgRP and POMC neurons. Ghrelin directly stimulates the depolarization of NPY/AgRP neurons, but hyperpolarizes POMC neurons [93]. The decreased firing rate of POMC neurons appears to be a result of presynaptic activation of GABAergic NPY/AgRP neurons, since the inhibitory effects of ghrelin on POMC neurons could be blocked by NPY- and GABA<sub>A</sub>-receptor blockers [93]. Paradoxically, in the presence of NPY- and GABA<sub>A</sub>-receptor blockers, ghrelin increases the firing rate of POMC neurons by depolarizing POMC neurons [93]. Furthermore, ghrelin potentiates the dopamine neurons in VTA to promote appetite in animals [96].

The effect of ghrelin on synaptogenesis was first revealed when the application of ghrelin on hypothalamic slices resulted in increased frequency of spontaneous IPSCs in POMC neurons [93], which receive presynaptic input and the inhibitory neurotransmitter GABA from NPY neurons [92]. In DA neurons located in VTA, ghrelin treatment led to increased frequency of miniature EPSCs, but decreased frequency of miniature IPSCs [96]. This was probably due to some presynaptic effects; however, the detailed mechanisms are unclear. In supraoptic magnocellular neurons, ghrelin potentiates miniature EPSCs through a presynaptic mechanism that appears to involve TRPV (transient receptor potential vanilloid) channels [97].

## INTERACTION OF FEEDING NEURAL CIRCUITRY AND REWARD SYSTEM

Feeding activity has classically been perceived as an innate behaviour to provide energy and building materials to the body, and is under the control of CNS to maintain energy homeostasis. Abnormal feeding behaviour can cause anorexia or hyperphagia, an effect that is shared by drug addiction in human and animal models [98,99]. The biological mechanisms of feeding and addiction have overlapped throughout evolution. The best-established commonality of the mechanisms for food intake and drug abuse is their ability to activate the dopamine-containing link in the brain reward circuitry. Midbrain DA neurons integrate information during food intake and drug abuse into an elaborate and complex neural circuitry critical in the regulation of energy



**Figure 3 Interaction between food intake neurocircuitry and reward neurocircuitry**

Diagram showing interactions between the brain regions involved in the regulation of food intake with those involved in motivated behaviour. PFC, prefrontal cortex.

homeostasis (Figure 3). Selective deletion of IR in midbrain (including VTA and substantia nigra) TH (tyrosine hydroxylase)-expressing neurons could abolish insulin-mediated increase of firing rate in TH-positive neurons [100]. Furthermore, mice with inactivation of insulin signalling in TH-expressing neurons exhibited reduced locomotor activity induced by cocaine [100]. Dopamine neurons in VTA express LepRb, and leptin treatment decreases the firing rate of dopamine neurons and suppresses food intake. When LepRb expression was selectively reduced in VTA, increased food intake, locomotor activity and sensitivity to highly palatable food were observed [39]. *Ob/ob* mice have deficient mesoaccumbens DA signalling activities, including decreased dopamine release in the NAc (nucleus accumbens), diminished locomotor response to amphetamine, as well as lacking locomotor sensitization to amphetamine injection. All these deficits in DA functions could be rescued by leptin administration to VTA [101]. Clearly, leptin has direct effects on mesolimbic system related to both feeding and motivated behaviours [102]. Given the overlap between the circuits involved in regulating energy balance and motivated behaviours and reward, it is becoming increasingly important to understand the neurobiological mechanisms that link addiction and obesity research (Figure 3).

## CHALLENGES AND EMERGING NEW METHODOLOGY TO STUDY THE SYNAPTIC FUNCTION IN FEEDING BEHAVIOUR

For the understanding of synaptic mechanisms by hormone regulation of CNS functions in feeding and motivated behaviours and cognition, here are some important topics and pressing questions: first, a clear understanding of the complex neural circuitry involved in feeding regulation, motivation and reward and cognitive

functions, and how these circuitries interact with one another; secondly, detailed cellular and molecular mechanisms of hormone regulation of synaptic functions; thirdly, synaptic alterations under pathological states such as insulin- and leptin-resistance, and whether synaptic mechanisms contribute to the pathogenesis of diabetes and obesity; and finally, the cellular and molecular nature of the links between diabetes and obesity with dementia [71].

Since the turn of the century, a growing number of mouse genetic models that express different markers or cre-recombinase in specific neuronal types have become available. The combination of mouse genetics with emerging new techniques such as optogenetics allows us to better address the above questions [103]. By expressing channelrhodopsin in certain types of neurons, one can activate specific synaptic inputs to their target. For example, Sternson's group recently expressed channelrhodopsin 2 in AgRP neurons and then used light to activate these cells to affect feeding behaviour. Their studies provided direct evidence that AgRP neurons were sufficient to orchestrate feeding behaviour [104,105]. Conceivably, the same approach may be used to further dissect individual components within the feeding neural circuitry or to map brain circuits for other functions, such as cognition and reward. Another relevant technical development is the neural-tracing methods using pseudo-rabies viruses [106–109] or micro-fluorescent beads [110,111], which allow tracking of synaptic output of diverse neuronal types. We believe that these new techniques, along with mouse genetic models, will lead to a complete understanding of synaptic mechanisms in feeding and motivated behaviour and cognitive functions, and of regulation of synaptic functions by hormones.

As discussed above, the same group of neurons in the hypothalamus exhibit distinct response to the same hormone regulation, for example, leptin depolarizes one-third of LepRb-expressing neurons, whereas LHA hyperpolarizes another one-third of LepRb-expressing neurons [112]. Within VTA DA neurons, insulin only activates half of the neurons [100]. The understanding of how the same types of neurons respond differently to leptin will likely provide important information on leptin resistance, and thus offer clues in the development of therapeutic strategies against obesity. Recently, high-throughput single-cell gene profiling became possible, such as the use of Fluidigm single-cell gene expression arrays [113]. Single-cell gene profiling, when combined with animal physiology and cellular electrophysiology, will provide definitive answers regarding the cellular and synaptic mechanisms of leptin and insulin resistance.

With the development of modern stem cell biology [113–115], we can now use cell-based models to recapitulate the pathophysiology of hypothalamic neurons in the obese state, and use cell-based therapy to treat feeding disorders at least in animals [116]. The cellular models allow us to identify the mechanism of synaptic function or dysfunction in derived neurons from monogenic forms or common forms of obesity, and examine how they respond to different hormones and therapeutic agents. Our present review is intended to provide the current account of this rapidly evolving research area in understanding the CNS control of feeding behaviour and metabolism. We believe that this is an exciting

topic and the ongoing and future studies using these new technologies aimed at addressing these pressing questions will bring new opportunities and thinking in devising treatment strategies against diabetes, obesity and cognitive impairment.

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#### ACKNOWLEDGEMENT

We thank Dr Nan Yang at Stanford University for critical reading of the initial version of the paper prior to submission.

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#### FUNDING

Our own work was supported by the Brain and Behavior Research Foundation and the Robert Wood Johnson Foundation to Child Health Institute of New Jersey (grant to Z.P.R.), and the A\*STAR Biomedical Research Council (grant to W.H.).

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Received 17 April 2012/9 May 2012; accepted 14 May 2012  
Published as Immediate Publication 14 May 2012, doi 10.1042/BSR20120026