Bioscience Reports Immediate Publication. Published on 14 May 2012 as manuscript BSR20120026

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

Zhiping P. Pang[1*], Weiping Han[2,3,4*]

[1]Child Health Institute of New Jersey, Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, 89 French Street, New Brunswick, NJ; [2]Singapore Bioimaging Consortium and Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore; [3]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [4]Cardiovascular and Metabolic Disorders Program, Duke-NUS Graduate Medical School, Singapore

* To whom correspondence should be addressed:
Zhiping Pang: 89 French Street, New Brunswick, NJ 08901; Tel: +1-732-235-8074;
Email: pangzh@umdnj.edu
Weiping Han: #02-02 Helios, 11 Biopolis Way, Singapore 138667; Tel: +65-6478 8721;
Email: weiping_han@sbic.a-star.edu.sg;

Key words: addiction, cognition, diabetes, energy homeostasis, ghrelin, hormone, insulin, leptin, neural circuitry, neurodegeneration, neurotransmission, obesity
Word count: 4,234 (main text excluding references and figure legends); 3 figures.
Disclosure: The authors declare that there is no duality of interest associated with this manuscript.

Abbreviations:
AMPA: 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid; NMDA: N-Methyl-D-aspartate; Kainic acid (KA): (2S,3S,4S)-3-(Carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylic acid; ARC: arcuate nucleus; VMH: ventromedial hypothalamus; DMH: dorsomedial hypothalamus; PVN: paraventricular hypothalamic nucleus; LHA: lateral hypothalamic nucleus; NTS: nucleus tractus solitaries; NAc: nucleus accumbens; PFC: prefrontal cortex; VTA: ventral tegmental area; POMC: proopiomelanocortin; Cart: cocaine- and amphetamine-regulated transcript; AgRP: agouti-related peptide; αMSH: α-melanocyte stimulating hormone; MC: melanocortin; MC3R: melanocortin 3 receptor; NPY: neuropeptide Y; GABA: γ-Amino Butyric acid; PBN: parabrachial nucleus; SV: synaptic vesicles; LDCV: large dense-core vesicles; CAM: cell adhesion molecules; PI3K: phosphatidylinositol 3-kinases; JAK2: janus kinase 2; STAT3: signal transducer and activator of transcription 3; MAPK3: mitogen-activated protein kinase 3; PLC: phospholipase C; PKC: protein kinase C; MEK: MAPK/ERK kinase; PSD: postsynaptic density;

Bioscience Reports Immediate Publication. Published on 14 May 2012 as manuscript BSR20120026

SYNOPSIS

Energy homeostasis, coordinated balance of food intake and energy expenditure, is regulated by the central nervous system (CNS). The past decade has witnessed significant advances in our understanding of metabolic processes and brain circuitry that 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, NMDA and GABA 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.

I. INTRODUCTION

Food intake and energy expenditure, the key determinants of energy homeostasis, are regulated by the central nervous system (CNS). Since the end of the nineteenth 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 onto 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 arcuate nucleus (ARC), ventromedial (VMH), paraventricular (PVN) and dorsal hypothalamus, as important brain regions in the development of hyperphagia and obesity; while lesions in lateral hypothalamus (LHA) resulted in hypophagia and anorexia (Figure 1). These findings led to a simple, yet appealing model: the mediobasal hypothalamic nuclei are the "satiety centers" and the LHA is the "hunger/feeding center" [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 of energy homeostasis [1,3,7-9]. Among all the hormones related to feeding behavior 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 behaviors, 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 signaling cascades of hormones have begun to accumulate and these studies collectively indicate that leptin, insulin and ghrelin play important roles in synaptic functions (Figure 1 & 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.

II. Synaptic regulation of hypothalamic function in energy homeostasis

Synaptic transmission mediates all brain related behavior including food intake and energy expenditure [1,12]. Fast excitatory neurotransmission is mainly mediated by ionotropic glutamate receptors, i.e. AMPA-, Kainic acid- and NMDA-receptors (AMPARs, KARs and NMDARs). AMPARs are tetramers composed of four types of subunits, GluR1-4, and mediate the major excitatory synaptic 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 excitatory postsynaptic currents (EPSCs) and thus cause postsynaptic depolarization. Most AMPARs in CNS contain GluR2 subunit and are permeable to Na^+ and K^+, but not Ca^{2+}, while those AMPARs without GluR2 subunit are permeable to Ca^{2+}, in addition to Na^+ and K^+ [15]. Fast inhibitory neurotransmission is mainly mediated by ionotropic $GABA_A$ receptors, which allow Cl^- influx upon binding to GABA released from presynaptic terminals, and induce inhibitory postsynaptic currents (IPSCs) 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 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 proopiomelanocortin (POMC), the precursor for many active neuropeptides including αMSH (α-melanocyte stimulating hormone). αMSH signals anorexia by binding to melanocortin (MC) receptors (especially MC4R) in several areas of the brain [17,18]; ARC orexigenic (*i.e.* increase feeding) neurons synthesize neuropeptide Y (NPY) [19] and agouti-related peptide (AgRP) [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. reported that body weight, fat stores and food intake were markedly reduced in mice with specific deletion of NMDARs in AgRP neurons [21]. 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-mediated synaptic transmission, and this effect was abolished when NMDARs were eliminated from postsynaptic neurons [21]. In their second study, Vong et al. demonstrated that inhibitory input to POMC neurons was the key modulatory component in energy homeostasis [22]. Food deprivation enhanced excitatory synaptic input in AgRP neurons [22], which was mediated by a presynaptic positive feedback loop involving 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 parabrachial nucleus (PBN) to promote feeding, and that the blockade of GABAergic input to PBN results in anorexia independent of the melanocortin system [25]. This study suggests that loss of GABA signaling 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 nucleus tractus solitaries (NTS) and caudal serotonergic neurons [24].

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

effects of hormones on synaptic transmission for the understanding of how CNS controls energy homeostasis.

III. Leptin and synaptic transmission

The adipose tissue-derived hormone leptin is a 167 amino acid protein in human [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 is crucial for leptin action [1,3,7,30,31]. Leptin binds to LepRb and activates JAK2/STAT3 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 signaling 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 αMSH 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 dopaminergic (DA) neurons (Figure 2A) [39]. Recently, a subgroup of neurons in LHA was identified to expresses 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 signaling 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), but not K_{ATP} channels, through a PI3-kinase (PI3K) signaling cascade [43]. Elevated leptin level in hippocampal neurons leads to PtdIns(3,4,5)P3 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 signaling attenuates the firing frequency of VTA dopaminergic 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.

III-1. Leptin and AMPA receptors

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

Bioscience Reports Immediate Publication. Published on 14 May 2012 as manuscript BSR20120026

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 NMDA receptor activation and is associated with an increase in cytoplasmic PtdIns(3,4,5)P3 levels through enhanced phosphorylation of the lipid phosphatase PTEN, 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.

III-2. Leptin and NMDA receptors

The number and subunit composition of NMDA receptors at the synapse are under dynamic regulations during synaptic plasticity [47]. Leptin has been shown to facilitate the induction phase of hippocampal LTP [48,49] probably through the activation of NMDA receptors [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/2A-containing NMDA receptors together with LepRb, but not NR1A/NR2A alone [48], indicating that leptin modulates NMDA responses only through LepRb signaling 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 NMDA receptor density at the cell surface [51,52]. The detailed molecular and cellular mechanisms of how leptin regulates NMDA receptor trafficking remain elusive.

III-3. Leptin and GABA receptors

Disruption of leptin signaling 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 signaling in energy homeostasis regulation. A number of studies attempted to identify additional effector neurons for leptin action. Lowell and colleagues recently investigated whether the "first-order" effectors of leptin signaling are excitatory- or inhibitory-neurons [22]. 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 mediate 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.

III-4. Leptin and long-term synaptic plasticity

Long-term synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), 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 leptin-LepRb cascade in synaptic plasticity [58]. NMDARs, but not metabotropic glutamate receptors (mGluRs) mediate leptin-induced LTD in hippocampus. The signaling pathway underlying leptin-induced LTD was independent of the Ras-Raf-MAPK pathway, but

Bioscience Reports Immediate Publication. Published on 14 May 2012 as manuscript BSR20120026

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 NMDA receptors. 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-potentiation 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].

III-5. Leptin, axon guidance and synaptic rewiring

Hypothalamic neurocircuitry undergoes dynamic remodeling including structural and morphological changes of neurons in response to energy status in animals, partially dependent on the leptin signaling 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 onto 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 hours after leptin administration [61], indicating the profound effects of leptin-LepRb signaling cascade on synaptic reorganization including morphological modifications [60]. Indeed, leptin has been reported to exert 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.

IV. 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 remodeling 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 insulin receptors (IRs) to regulate glucose levels and energy balance [70]. Defective insulin signaling in the CNS contributes to obesity and type 2 diabetes. Numerous epidemiology 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 insulin receptors. 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 $GABA_A$ receptors in hippocampal neurons [75]. Although there is no direct evidence to support insulin action on $GABA_A$ receptors in hypothalamic region, insulin-induced hyperphagia in free-moving rats could be blocked by $GABA_A$ receptor antagonists that were applied in the VMH region [76]. This suggests that insulin-induced $GABA_A$ receptor trafficking might at least partially account for the effects of insulin regulation of food intake. Besides its influence on $GABA_A$ receptor recruitments, insulin can also facilitate the internalization of AMPA receptor [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-95 at the postsynapse via the activation of PI3K-AKT-mTOR signaling 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 signaling in synaptic remodeling [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].

V. 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 growth hormone secretagogue receptor (GHSR) 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 $GABA_A$-receptor blockers [93]. Paradoxically, in the presence of NPY- and $GABA_A$-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 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 dopaminergic neurons located in VTA, ghrelin treatment led to increased frequency of miniature EPSCs, but decreased frequency of miniature IPSCs [96]. This was likely 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 channels [97].

VI. Interaction of feeding neural circuitry and reward system

Feeding activity has classically been perceived as an innate behavior to provide energy and building materials to the body, and is under the control of CNS to maintain energy homeostasis. Abnormal feeding behavior 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 dopaminergic neurons integrate information during food intake and drug abuse into an elaborate and complex neural circuitry critical in the regulation of energy homeostasis (Figure 3). Selective deletion of IR in midbrain (including VTA and substantia nigra) tyrosine hydroxylase (TH)-expressing neurons could abolish insulin-mediated increase of firing rate in TH-positive neurons [100]. Furthermore, mice with inactivation of insulin signaling 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 dopaminergic signaling activities, including decreased dopamine release in the Nucleus accumbens (NAc), diminished locomotor response to amphetamine, as well as lacking locomotor sensitization to amphetamine injection. All these deficits in dopaminergic functions could be rescued by leptin administration to VTA [101]. Clearly, leptin has direct effects on mesolimbic system related to both feeding and motivated behaviors [102]. Given the overlap between the circuits involved in regulating energy balance and motivated behaviors and reward, it is becoming increasingly important to understand the neurobiological mechanisms that link addiction and obesity research (Figure 3).

VII. Challenges and emerging new methodology to study the synaptic function in feeding behavior

For the understanding of synaptic mechanisms by hormone regulation of CNS functions in feeding and motivated behaviors 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; second, detailed cellular and molecular mechanisms of hormone regulation of synaptic functions; third, synaptic alterations under pathologic states such as insulin- and leptin-resistance, and whether synaptic mechanisms contribute to the pathogenesis of diabetes and obesity; and fourth, 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 behavior. Their studies provided direct evidence that AgRP neurons were sufficient to orchestrate feeding behavior [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 pseudorabies 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 behavior 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 LHA neurons while hyperpolarizes another one third of LepRb-expressing neurons [112]. Within VTA dopaminergic 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 behavior and metabolism. We believe 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.

ACKNOWLEDGEMENTS

Research in the authors' laboratories is supported by Brain & Behavior Research Foundation (Z.P.P), Robert Wood Johnson Foundation to Child Health Institute of New Jersey (Z.P.P.) and A*STAR Biomedical Research Council (W.H.). We thank Dr. Nan Yang at Stanford University for critical reading of the initial version of the manuscript.

FIGURE LEGENDS

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 behavior. 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. ARC:

arcuate nucleus; DMH: dorsomedial hypothalamus; LHA: lateral hypothalamic area; PVH: paraventricular hypothalamic nucleus; VMH: ventromedial hypothalamic nucleus.

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 behavior. Ghrelin on the other hand, activates AgRP/NPY neurons to stimulate feeding behavior. Leptin and insulin also regulate neuronal functions within brain regions that are important components for cognition and reward behaviors 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 signaling cascades depicted in the postsynaptic compartment also apply to the presynapse. CAM: cell-adhesion molecules (*e.g.* neurexins and neuroligins); GABAR: GABA receptors; GluR: Glutamate receptors including both NMDA and AMPA receptors; LDCV: Large-dense core vesicle; PSD: postsynaptic density; SV: synaptic vesicle.

Figure 3. Interaction between food intake neurociruitry and reward neurocircuitry
Diagram showing interactions between the brain regions involved in the regulation of food intake with those involved in motivated behavior. ARC: arcuate nucleus; LHA: lateral hypothalamic area; NAc: nucleus accumbens; PFC: prefrontal cortex; VTA: ventral tegmental area.

REFERENCES

1. Morton, G.J., Cummings, D.E., Baskin, D.G., Barsh, G.S., and Schwartz, M.W. 2006. Central nervous system control of food intake and body weight. *Nature*. 443:289-295.
2. Stellar, E. 1954. The physiology of motivation. *Psychol Rev*. 61:5-22.
3. Gao, Q., and Horvath, T.L. 2007. Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci*. 30:367-398.
4. Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T., and Collins, F. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science*. 269:540-543.
5. Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., and Friedman, J.M. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science*. 269:543-546.
6. Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K.J., Smutko, J.S., Mays, G.G., Wool, E.A., Monroe, C.A., and Tepper, R.I. 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell*. 83:1263-1271.
7. Friedman, J.M. 2002. The function of leptin in nutrition, weight, and physiology. *Nutr Rev*. 60:S1-14; discussion S68-84, 85-17.

8. Friedman, J.M., and Halaas, J.L. 1998. Leptin and the regulation of body weight in mammals. *Nature*. 395:763-770.

9. Woods, S.C., Schwartz, M.W., Baskin, D.G., and Seeley, R.J. 2000. Food intake and the regulation of body weight. *Annu Rev Psychol*. 51:255-277.

10. Citri, A., and Malenka, R.C. 2008. Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology*. 33:18-41.

11. Kauer, J.A., and Malenka, R.C. 2007. Synaptic plasticity and addiction. *Nat Rev Neurosci*. 8:844-858.

12. Zhang, G., Bai, H., Zhang, H., Dean, C., Wu, Q., Li, J., Guariglia, S., Meng, Q., and Cai, D. 2011. Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. *Neuron*. 69:523-535.

13. Dietrich, M.O., and Horvath, T.L. 2009. Feeding signals and brain circuitry. *Eur J Neurosci*. 30:1688-1696.

14. Wisden, W., and Seeburg, P.H. 1993. Mammalian ionotropic glutamate receptors. *Current opinion in neurobiology*. 3:291-298.

15. Hollmann, M., Hartley, M., and Heinemann, S. 1991. Ca2+ permeability of KA-AMPA--gated glutamate receptor channels depends on subunit composition. *Science*. 252:851-853.

16. Belgardt, B.F., Okamura, T., and Bruning, J.C. 2009. Hormone and glucose signalling in POMC and AgRP neurons. *The Journal of physiology*. 587:5305-5314.

17. Raffin-Sanson, M.L., de Keyzer, Y., and Bertagna, X. 2003. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. *Eur J Endocrinol*. 149:79-90.

18. Huszar, D., Lynch, C.A., Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, L.R., Gu, W., Kesterson, R.A., Boston, B.A., Cone, R.D., Smith, F.J., Campfield, L.A., Burn, P., and Lee, F. 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell*. 88:131-141.

19. Allen, J.M., McGregor, G.P., Woodhams, P.L., Polak, J.M., and Bloom, S.R. 1984. Ontogeny of a novel peptide, neuropeptide Y (NPY) in rat brain. *Brain research*. 303:197-200.

20. Shutter, J.R., Graham, M., Kinsey, A.C., Scully, S., Luthy, R., and Stark, K.L. 1997. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev*. 11:593-602.

21. Liu, T., Kong, D., Shah, B.P., Ye, C., Koda, S., Saunders, A., Ding, J.B., Yang, Z., Sabatini, B.L., and Lowell, B.B. 2012. Fasting activation of AgRP neurons requires NMDA receptors and involves spinogenesis and increased excitatory tone. *Neuron*. 73:511-522.

22. Vong, L., Ye, C., Yang, Z., Choi, B., Chua, S., Jr., and Lowell, B.B. 2011. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. *Neuron*. 71:142-154.

23. Yang, Y., Atasoy, D., Su, H.H., and Sternson, S.M. 2011. Hunger states switch a flip-flop memory circuit via a synaptic AMPK-dependent positive feedback loop. *Cell*. 146:992-1003.

24. Wu, Q., Clark, M.S., and Palmiter, R.D. 2012. Deciphering a neuronal circuit that mediates appetite. *Nature*. 483:594-597.

25. Wu, Q., and Palmiter, R.D. 2011. GABAergic signaling by AgRP neurons prevents anorexia via a melanocortin-independent mechanism. *Eur J Pharmacol*. 660:21-27.

26. Wu, Q., Whiddon, B.B., and Palmiter, R.D. 2012. Ablation of neurons expressing agouti-related protein, but not melanin concentrating hormone, in leptin-deficient mice restores metabolic functions and fertility. *Proc Natl Acad Sci U S A*. 109:3155-3160.

27. Cohen, S.L., Halaas, J.L., Friedman, J.M., Chait, B.T., Bennett, L., Chang, D., Hecht, R., and Collins, F. 1996. Human leptin characterization. *Nature*. 382:589.

28. Elmquist, J.K., Maratos-Flier, E., Saper, C.B., and Flier, J.S. 1998. Unraveling the central nervous system pathways underlying responses to leptin. *Nat Neurosci*. 1:445-450.

29. Bates, S.H., and Myers, M.G., Jr. 2003. The role of leptin receptor signaling in feeding and neuroendocrine function. *Trends Endocrinol Metab*. 14:447-452.

30. Cohen, P., Zhao, C., Cai, X., Montez, J.M., Rohani, S.C., Feinstein, P., Mombaerts, P., and Friedman, J.M. 2001. Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest*. 108:1113-1121.

31. de Luca, C., Kowalski, T.J., Zhang, Y., Elmquist, J.K., Lee, C., Kilimann, M.W., Ludwig, T., Liu, S.M., and Chua, S.C., Jr. 2005. Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. *J Clin Invest*. 115:3484-3493.

32. Hummel, K.P., Dickie, M.M., and Coleman, D.L. 1966. Diabetes, a new mutation in the mouse. *Science*. 153:1127-1128.

33. Bahary, N., Leibel, R.L., Joseph, L., and Friedman, J.M. 1990. Molecular mapping of the mouse db mutation. *Proc Natl Acad Sci U S A*. 87:8642-8646.

34. Friedman, J.M., Leibel, R.L., Siegel, D.S., Walsh, J., and Bahary, N. 1991. Molecular mapping of the mouse ob mutation. *Genomics*. 11:1054-1062.

35. Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J.M., Basdevant, A., Bougneres, P., Lebouc, Y., Froguel, P., and Guy-Grand, B. 1998. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*. 392:398-401.

36. Elmquist, J.K., Bjorbaek, C., Ahima, R.S., Flier, J.S., and Saper, C.B. 1998. Distributions of leptin receptor mRNA isoforms in the rat brain. *J Comp Neurol*. 395:535-547.

37. Elias, C.F., Aschkenasi, C., Lee, C., Kelly, J., Ahima, R.S., Bjorbaek, C., Flier, J.S., Saper, C.B., and Elmquist, J.K. 1999. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron*. 23:775-786.

38. Elmquist, J.K., Elias, C.F., and Saper, C.B. 1999. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron*. 22:221-232.

39. Hommel, J.D., Trinko, R., Sears, R.M., Georgescu, D., Liu, Z.W., Gao, X.B., Thurmon, J.J., Marinelli, M., and DiLeone, R.J. 2006. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron*. 51:801-810.

40. Leinninger, G.M., Jo, Y.H., Leshan, R.L., Louis, G.W., Yang, H., Barrera, J.G., Wilson, H., Opland, D.M., Faouzi, M.A., Gong, Y., Jones, J.C., Rhodes, C.J., Chua, S., Jr., Diano, S., Horvath, T.L., Seeley, R.J., Becker, J.B., Munzberg, H., and Myers, M.G., Jr. 2009. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab*. 10:89-98.

41. Myers, M.G., Jr., Munzberg, H., Leinninger, G.M., and Leshan, R.L. 2009. The geometry of leptin action in the brain: more complicated than a simple ARC. *Cell Metab*. 9:117-123.

42. Scott, M.M., Lachey, J.L., Sternson, S.M., Lee, C.E., Elias, C.F., Friedman, J.M., and Elmquist, J.K. 2009. Leptin targets in the mouse brain. *J Comp Neurol*. 514:518-532.

43. Shanley, L.J., Irving, A.J., Rae, M.G., Ashford, M.L., and Harvey, J. 2002. Leptin inhibits rat hippocampal neurons via activation of large conductance calcium-activated K+ channels. *Nat Neurosci*. 5:299-300.

44. Shanley, L.J., O'Malley, D., Irving, A.J., Ashford, M.L., and Harvey, J. 2002. Leptin inhibits epileptiform-like activity in rat hippocampal neurones via PI 3-kinase-driven activation of BK channels. *J Physiol*. 545:933-944.

45. Xu, L., Rensing, N., Yang, X.F., Zhang, H.X., Thio, L.L., Rothman, S.M., Weisenfeld, A.E., Wong, M., and Yamada, K.A. 2008. Leptin inhibits 4-aminopyridine- and pentylenetetrazole-induced seizures and AMPAR-mediated synaptic transmission in rodents. *J Clin Invest*. 118:272-280.

46. Moult, P.R., Cross, A., Santos, S.D., Carvalho, A.L., Lindsay, Y., Connolly, C.N., Irving, A.J., Leslie, N.R., and Harvey, J. 2010. Leptin regulates AMPA receptor trafficking via PTEN inhibition. *J Neurosci*. 30:4088-4101.

47. Lau, C.G., and Zukin, R.S. 2007. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat Rev Neurosci*. 8:413-426.

48. Shanley, L.J., Irving, A.J., and Harvey, J. 2001. Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. *J Neurosci*. 21:RC186.

49. Oomura, Y., Hori, N., Shiraishi, T., Fukunaga, K., Takeda, H., Tsuji, M., Matsumiya, T., Ishibashi, M., Aou, S., Li, X.L., Kohno, D., Uramura, K., Sougawa, H., Yada, T., Wayner, M.J., and Sasaki, K. 2006. Leptin facilitates learning and memory performance and enhances hippocampal CA1 long-term potentiation and CaMK II phosphorylation in rats. *Peptides*. 27:2738-2749.

50. Irving, A.J., Wallace, L., Durakoglugil, D., and Harvey, J. 2006. Leptin enhances NR2B-mediated N-methyl-D-aspartate responses via a mitogen-activated protein kinase-dependent process in cerebellar granule cells. *Neuroscience*. 138:1137-1148.

51. Moult, P.R., and Harvey, J. 2009. Regulation of glutamate receptor trafficking by leptin. *Biochem Soc Trans*. 37:1364-1368.

52. Harvey, J., Shanley, L.J., O'Malley, D., and Irving, A.J. 2005. Leptin: a potential cognitive enhancer? *Biochem Soc Trans*. 33:1029-1032.

53. Balthasar, N., Coppari, R., McMinn, J., Liu, S.M., Lee, C.E., Tang, V., Kenny, C.D., McGovern, R.A., Chua, S.C., Jr., Elmquist, J.K., and Lowell, B.B. 2004. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron*. 42:983-991.

54. Hill, J.W., Elias, C.F., Fukuda, M., Williams, K.W., Berglund, E.D., Holland, W.L., Cho, Y.R., Chuang, J.C., Xu, Y., Choi, M., Lauzon, D., Lee, C.E., Coppari, R., Richardson, J.A., Zigman, J.M., Chua, S., Scherer, P.E., Lowell, B.B., Bruning, J.C., and Elmquist, J.K. 2010. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. *Cell Metab*. 11:286-297.

55. van de Wall, E., Leshan, R., Xu, A.W., Balthasar, N., Coppari, R., Liu, S.M., Jo, Y.H., MacKenzie, R.G., Allison, D.B., Dun, N.J., Elmquist, J., Lowell, B.B., Barsh, G.S., de Luca, C., Myers, M.G., Jr., Schwartz, G.J., and Chua, S.C., Jr. 2008. Collective and individual functions of leptin receptor modulated neurons controlling metabolism and ingestion. *Endocrinology*. 149:1773-1785.

56. Zupancic, M.L., and Mahajan, A. 2011. Leptin as a neuroactive agent. *Psychosom Med*. 73:407-414.

57. Davis, J.F. 2010. Adipostatic regulation of motivation and emotion. *Discov Med*. 9:462-467.

58. Moult, P.R., Milojkovic, B., and Harvey, J. 2009. Leptin reverses long-term potentiation at hippocampal CA1 synapses. *J Neurochem*. 108:685-696.

59. Durakoglugil, M., Irving, A.J., and Harvey, J. 2005. Leptin induces a novel form of NMDA receptor-dependent long-term depression. *J Neurochem*. 95:396-405.

60. Horvath, T.L., Sarman, B., Garcia-Caceres, C., Enriori, P.J., Sotonyi, P., Shanabrough, M., Borok, E., Argente, J., Chowen, J.A., Perez-Tilve, D., Pfluger, P.T., Bronneke, H.S., Levin, B.E., Diano, S., Cowley, M.A., and Tschop, M.H. 2010. Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. *Proc Natl Acad Sci U S A*. 107:14875-14880.

61. Pinto, S., Roseberry, A.G., Liu, H., Diano, S., Shanabrough, M., Cai, X., Friedman, J.M., and Horvath, T.L. 2004. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science*. 304:110-115.

62. Bouret, S.G., Draper, S.J., and Simerly, R.B. 2004. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science*. 304:108-110.

63. Bouret, S.G., and Simerly, R.B. 2004. Minireview: Leptin and development of hypothalamic feeding circuits. *Endocrinology*. 145:2621-2626.

64. Sternson, S.M., Shepherd, G.M., and Friedman, J.M. 2005. Topographic mapping of VMH --> arcuate nucleus microcircuits and their reorganization by fasting. *Nat Neurosci*. 8:1356-1363.

65. Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., and Flier, J.S. 1996. Role of leptin in the neuroendocrine response to fasting. *Nature*. 382:250-252.

66. Katsoyannis, P.G., Tometsko, A.M., Ginos, J.Z., and Tilak, M.A. 1966. Insulin peptides. XI. The synthesis of the B chain of human insulin and its combination with the natural a chain of bovine insulin to generate insulin activity. *J Am Chem Soc*. 88:164-166.

67. Chiu, S.L., Chen, C.M., and Cline, H.T. 2008. Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo. *Neuron*. 58:708-719.

68. De Souza, C.T., Araujo, E.P., Bordin, S., Ashimine, R., Zollner, R.L., Boschero, A.C., Saad, M.J., and Velloso, L.A. 2005. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology*. 146:4192-4199.

69. Woods, S.C., Porte, D., Jr., Bobbioni, E., Ionescu, E., Sauter, J.F., Rohner-Jeanrenaud, F., and Jeanrenaud, B. 1985. Insulin: its relationship to the central nervous system and to the control of food intake and body weight. *The American journal of clinical nutrition*. 42:1063-1071.

70. Banks, W.A. 2006. Blood-brain barrier and energy balance. *Obesity*. 14 Suppl 5:234S-237S.

71. Han, W., and Li, C. 2010. Linking type 2 diabetes and Alzheimer's disease. *Proc Natl Acad Sci U S A*. 107:6557-6558.

72. Takeda, S., Sato, N., Uchio-Yamada, K., Sawada, K., Kunieda, T., Takeuchi, D., Kurinami, H., Shinohara, M., Rakugi, H., and Morishita, R. 2010. Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and Abeta deposition in an Alzheimer mouse model with diabetes. *Proc Natl Acad Sci U S A*. 107:7036-7041.

73. Williams, K.W., Margatho, L.O., Lee, C.E., Choi, M., Lee, S., Scott, M.M., Elias, C.F., and Elmquist, J.K. 2010. Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *J Neurosci*. 30:2472-2479.

74. Klockener, T., Hess, S., Belgardt, B.F., Paeger, L., Verhagen, L.A., Husch, A., Sohn, J.W., Hampel, B., Dhillon, H., Zigman, J.M., Lowell, B.B., Williams, K.W., Elmquist, J.K., Horvath, T.L., Kloppenburg, P., and Bruning, J.C. 2011. High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. *Nat Neurosci*.

75. Wan, Q., Xiong, Z.G., Man, H.Y., Ackerley, C.A., Braunton, J., Lu, W.Y., Becker, L.E., MacDonald, J.F., and Wang, Y.T. 1997. Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. *Nature*. 388:686-690.

76. Kamatchi, G.L., Bhakthavatsalam, P., Chandra, D., and Bapna, J.S. 1984. Inhibition of insulin hyperphagia by gamma aminobutyric acid antagonists in rats. *Life Sci*. 34:2297-2301.

77. Man, H.Y., Lin, J.W., Ju, W.H., Ahmadian, G., Liu, L., Becker, L.E., Sheng, M., and Wang, Y.T. 2000. Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. *Neuron*. 25:649-662.

78. Wang, Y.T., and Linden, D.J. 2000. Expression of cerebellar long-term depression requires postsynaptic clathrin-mediated endocytosis. *Neuron*. 25:635-647.

79. Huang, C.C., You, J.L., Lee, C.C., and Hsu, K.S. 2003. Insulin induces a novel form of postsynaptic mossy fiber long-term depression in the hippocampus. *Mol Cell Neurosci*. 24:831-841.

80. Huang, C.C., Lee, C.C., and Hsu, K.S. 2004. An investigation into signal transduction mechanisms involved in insulin-induced long-term depression in the CA1 region of the hippocampus. *J Neurochem*. 89:217-231.

81. Liu, L., Brown, J.C., 3rd, Webster, W.W., Morrisett, R.A., and Monaghan, D.T. 1995. Insulin potentiates N-methyl-D-aspartate receptor activity in Xenopus oocytes and rat hippocampus. *Neurosci Lett*. 192:5-8.

82. Liao, G.Y., and Leonard, J.P. 1999. Insulin modulation of cloned mouse NMDA receptor currents in Xenopus oocytes. *J Neurochem*. 73:1510-1519.

83. Lee, C.C., Huang, C.C., Wu, M.Y., and Hsu, K.S. 2005. Insulin stimulates postsynaptic density-95 protein translation via the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway. *J Biol Chem*. 280:18543-18550.

84. Chiu, S.L., and Cline, H.T. 2010. Insulin receptor signaling in the development of neuronal structure and function. *Neural Dev*. 5:7.

85. Sakata, I., Nakamura, K., Yamazaki, M., Matsubara, M., Hayashi, Y., Kangawa, K., and Sakai, T. 2002. Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract. *Peptides*. 23:531-536.

86. Dornonville de la Cour, C., Bjorkqvist, M., Sandvik, A.K., Bakke, I., Zhao, C.M., Chen, D., and Hakanson, R. 2001. A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. *Regul Pept*. 99:141-150.

87. Lu, S., Guan, J.L., Wang, Q.P., Uehara, K., Yamada, S., Goto, N., Date, Y., Nakazato, M., Kojima, M., Kangawa, K., and Shioda, S. 2002. Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus. *Neurosci Lett*. 321:157-160.

88. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 402:656-660.

89. Howard, A.D., Feighner, S.D., Cully, D.F., Arena, J.P., Liberator, P.A., Rosenblum, C.I., Hamelin, M., Hreniuk, D.L., Palyha, O.C., Anderson, J., Paress, P.S., Diaz, C., Chou, M., Liu, K.K., McKee, K.K., Pong, S.S., Chaung, L.Y., Elbrecht, A., Dashkevicz, M., Heavens, R., Rigby, M., Sirinathsinghji, D.J., Dean, D.C., Melillo, D.G., Patchett, A.A., Nargund, R., Griffin, P.R., DeMartino, J.A., Gupta, S.K., Schaeffer, J.M., Smith, R.G., and Van der Ploeg, L.H. 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science*. 273:974-977.

90. Guan, X.M., Yu, H., Palyha, O.C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J., Smith, R.G., Van der Ploeg, L.H., and Howard, A.D. 1997. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res*. 48:23-29.

91. Nakazato, M., Murakami, N., Date, Y., Kojima, M., Matsuo, H., Kangawa, K., and Matsukura, S. 2001. A role for ghrelin in the central regulation of feeding. *Nature*. 409:194-198.

92. Cowley, M.A., Smart, J.L., Rubinstein, M., Cerdan, M.G., Diano, S., Horvath, T.L., Cone, R.D., and Low, M.J. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*. 411:480-484.

93. Cowley, M.A., Smith, R.G., Diano, S., Tschop, M., Pronchuk, N., Grove, K.L., Strasburger, C.J., Bidlingmaier, M., Esterman, M., Heiman, M.L., Garcia-Segura, L.M., Nillni, E.A., Mendez, P., Low, M.J., Sotonyi, P., Friedman, J.M., Liu, H., Pinto, S., Colmers, W.F., Cone, R.D., and Horvath, T.L. 2003. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron*. 37:649-661.

94. Horvath, T.L., Diano, S., Sotonyi, P., Heiman, M., and Tschop, M. 2001. Minireview: ghrelin and the regulation of energy balance--a hypothalamic perspective. *Endocrinology*. 142:4163-4169.

95. Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., and Wakabayashi, I. 2001. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes*. 50:2438-2443.

96. Abizaid, A., Liu, Z.W., Andrews, Z.B., Shanabrough, M., Borok, E., Elsworth, J.D., Roth, R.H., Sleeman, M.W., Picciotto, M.R., Tschop, M.H., Gao, X.B., and Horvath, T.L. 2006. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest*. 116:3229-3239.

97. Yokoyama, T., Saito, T., Ohbuchi, T., Suzuki, H., Otsubo, H., Okamoto, T., Fujihara, H., Nagatomo, T., and Ueta, Y. 2009. Ghrelin potentiates miniature excitatory postsynaptic currents in supraoptic magnocellular neurones. *J Neuroendocrinol*. 21:910-920.

98. Dagher, A. 2009. The neurobiology of appetite: hunger as addiction. *International journal of obesity*. 33 Suppl 2:S30-33.

99. Kenny, P.J. 2011. Common cellular and molecular mechanisms in obesity and drug addiction. *Nature reviews. Neuroscience*. 12:638-651.

100. Konner, A.C., Hess, S., Tovar, S., Mesaros, A., Sanchez-Lasheras, C., Evers, N., Verhagen, L.A., Bronneke, H.S., Kleinridders, A., Hampel, B., Kloppenburg, P., and Bruning, J.C. 2011. Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. *Cell Metab*. 13:720-728.

101. Fulton, S., Pissios, P., Manchon, R.P., Stiles, L., Frank, L., Pothos, E.N., Maratos-Flier, E., and Flier, J.S. 2006. Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron*. 51:811-822.

102. Cota, D., Barrera, J.G., and Seeley, R.J. 2006. Leptin in energy balance and reward: two faces of the same coin? *Neuron*. 51:678-680.

103. Fenno, L., Yizhar, O., and Deisseroth, K. 2011. The development and application of optogenetics. *Annual review of neuroscience*. 34:389-412.

104. Aponte, Y., Atasoy, D., and Sternson, S.M. 2011. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nature neuroscience*. 14:351-355.

105. Atasoy, D., Aponte, Y., Su, H.H., and Sternson, S.M. 2008. A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 28:7025-7030.

106. Miyamichi, K., Amat, F., Moussavi, F., Wang, C., Wickersham, I., Wall, N.R., Taniguchi, H., Tasic, B., Huang, Z.J., He, Z., Callaway, E.M., Horowitz, M.A., and Luo, L. 2011. Cortical representations of olfactory input by trans-synaptic tracing. *Nature*. 472:191-196.

107. Luo, L., Callaway, E.M., and Svoboda, K. 2008. Genetic dissection of neural circuits. *Neuron*. 57:634-660.

108. Wickersham, I.R., Lyon, D.C., Barnard, R.J., Mori, T., Finke, S., Conzelmann, K.K., Young, J.A., and Callaway, E.M. 2007. Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron*. 53:639-647.

109. Wickersham, I.R., Finke, S., Conzelmann, K.K., and Callaway, E.M. 2007. Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nature methods*. 4:47-49.

110. Lammel, S., Hetzel, A., Hackel, O., Jones, I., Liss, B., and Roeper, J. 2008. Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron*. 57:760-773.

111. Lammel, S., Ion, D.I., Roeper, J., and Malenka, R.C. 2011. Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. *Neuron*. 70:855-862.

112. Leinninger, G.M., and Myers, M.G., Jr. 2008. LRb signals act within a distributed network of leptin-responsive neurones to mediate leptin action. *Acta Physiol (Oxf)*. 192:49-59.

113. Pang, Z.P., Yang, N., Vierbuchen, T., Ostermeier, A., Fuentes, D.R., Yang, T.Q., Citri, A., Sebastiano, V., Marro, S., Sudhof, T.C., and Wernig, M. 2011. Induction of human neuronal cells by defined transcription factors. *Nature*.

114. Chambers, S.M., and Studer, L. 2011. Cell fate plug and play: direct reprogramming and induced pluripotency. *Cell*. 145:827-830.

115. Dolmetsch, R., and Geschwind, D.H. 2011. The Human Brain in a Dish: The Promise of iPSC-Derived Neurons. *Cell*. 145:831-834.

116. Czupryn, A., Zhou, Y.D., Chen, X., McNay, D., Anderson, M.P., Flier, J.S., and Macklis, J.D. 2011. Transplanted hypothalamic neurons restore leptin signaling and ameliorate obesity in db/db mice. *Science*. 334:1133-1137.

Bioscience Reports Immediate Publication. Published on 14 May 2012 as manuscript BSR20120026

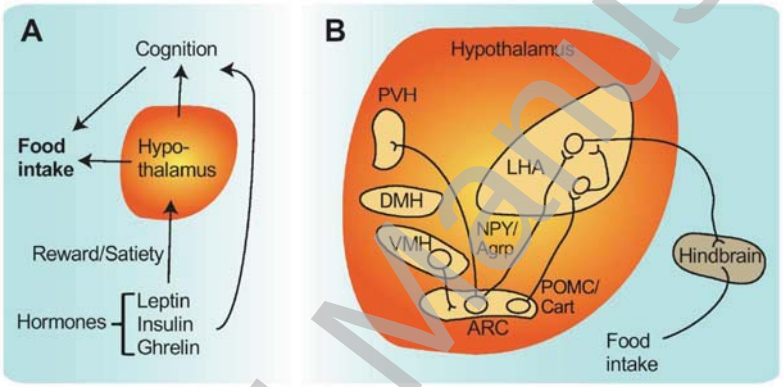

Figure 1

Bioscience Reports Immediate Publication. Published on 14 May 2012 as manuscript BSR20120026

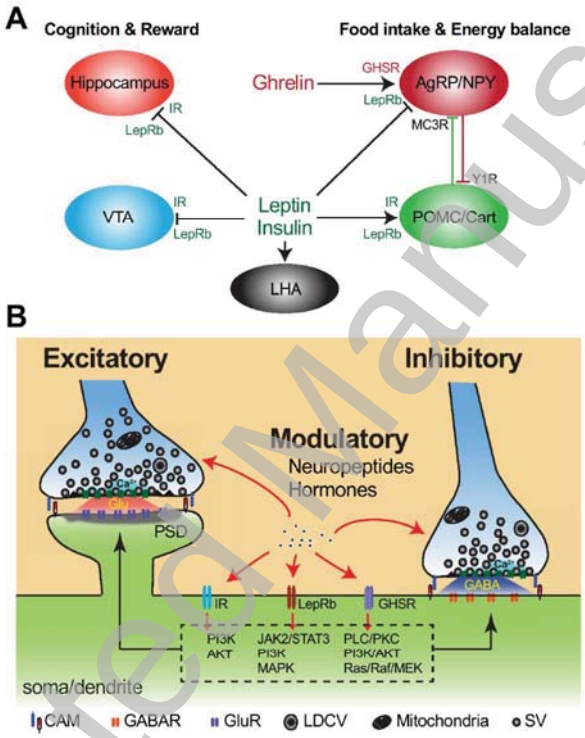

Figure 2

Bioscience Reports Immediate Publication. Published on 14 May 2012 as manuscript BSR20120026

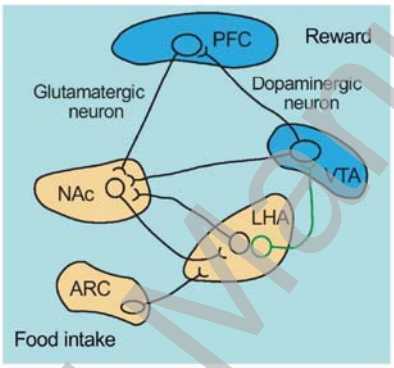

Figure 3