Role of orexin receptors in obesity: from cellular to behavioral evidence

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Abstract

The orexin peptides and their two receptors are involved in multiple physiological processes, including energy homeostasis, arousal, stress and reward. Higher signaling of the orexin peptides at the orexin receptors (OXR) protects against obesity, but it is less clear how their activation in different brain regions contributes to this behavioral output. This review summarizes the evidence available for a role of central OXR in energy homeostasis and their contribution to obesity. A detailed analysis of anatomical, cellular and behavioral evidence shows that modulation of energy homeostasis by the OXR is largely dependent upon anatomical and cellular context. It also shows that obesity resistance provided by activation of the OXR is distributed across multiple brain sites with site-specific actions. We suggest that understanding the role of the OXR in the development of obesity requires considering both specific mechanisms within brain regions and interactions of orexinergic input between multiple sites.

Keywords

orexin; orexin receptors; hypothalamus; energy metabolism; diet-induced obesity; spontaneous physical activity

INTRODUCTION

The orexins are two closely related peptides, orexin-A (OXA, hypocretin 1) and orexin-B (OXB, hypocretin 2). In several mammalian species, the majority of the central nervous system orexin peptides are synthesized in neurons located in the lateral hypothalamus (LH) and perifornical area that project to multiple brain regions.[1–7] The orexin peptides exert their biological effects through two G protein-coupled receptors, orexin receptor type-1 (OX1R, hypocretin receptor 1) and orexin receptor type-2 (OX2R, hypocretin receptor 2).[8,9] Both

CONFLICT OF INTEREST

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orexin receptors (OXR) subtypes can bind to OXA and OXB, but with differential affinity; OX1R has a higher affinity for OXA, whereas OX2R has equal affinity for either orexin peptide.[9,10]

Following the discovery of the orexin/hypocretin peptides in 1998,[8,9] research focused on their role in energy metabolism,[11–14] as well as modulation of sleep and arousal.[15–17] By early 2000, the role of the orexin peptides as modulators of acute food intake and arousal had been recognized.[18] Development of the orexin neuron-deficient mice in 2001 defined the orexin peptides as positive modulators of energy expenditure and suggested a role for orexin peptides in development of obesity.[19] Since then, a combination of evidence from pharmacological analysis and genetic models has established orexin peptides and their receptors as central regulators of energy expenditure, which influences energy balance. The aim of this review is to integrate evidence from cellular and behavioral experiments to examine our current understanding of the role of the OXR in energy metabolism and obesity.

Throughout this review, we use the term 'orexin system' to describe neurons that produce orexin peptides (the orexinergic neurons), orexin-responsive neurons (i.e., those expressing OXR) and interactions between them that modulate animal behavior. It is important to remember that some orexinergic neurons can be regulated through the OX2R.[20] Therefore, the categories of orexinergic neurons and orexin-responsive neurons are not mutually exclusive. We use this terminology to be explicit about the fact that behavioral and physiological effects associated with orexin peptides arise from combined action of orexin peptides at OXR-expressing neurons.

The data reviewed in this article suggests that both OXR subtypes can mediate increased energy expenditure and thus, contribute to obesity resistance mediated by the orexin system. However, we argue that the mechanisms by which each orexin receptor subtype acts are largely dependent upon their anatomical and cellular context. Furthermore, we suggest that the effects of the orexin system in energy metabolism result from parallel signaling to multiple brain regions. Thus, proper understanding of the function of the OXR in energy balance and obesity requires studying the anatomical and functional connections between sites that receive orexinergic innervation.

OXR BRAIN EXPRESSION IS PART OF A NETWORK

The orexin system is characterized by the wide expression of the OXR in brain, which matches the pattern of orexinergic neuronal projections.[1–6] A number of anatomical studies in rat brain show an agreement between OXR mRNA and protein distribution.[21–26] Across these studies, the overall conclusion is that there are overlapping, yet distinct, patterns of expression for OX1R and OX2R in brain (Figure 1). However, whether both OXR are co-expressed in the same neurons across different neuronal populations remains unknown.

One important clue about the function of the orexin system comes from the fact that the OXR are expressed in different brain regions that control the same behaviors. For example, several brain regions involved in control of food intake receive orexinergic input and express the OXR, including the arcuate nucleus, ventromedial hypothalamic and the tuberomammillary nuclei.[27,28] This expression pattern suggests the behavioral outcomes of the orexin system are due to simultaneous activation of the OXR in different brain regions. In direct support of this hypothesis, retrograde tracer studies have shown that orexin neurons have collateral projections within the central nervous system,[29–31] trans-synaptically collateral central nervous system efferents[32] or collateral efferents to both, to the central nervous system regions and brown adipose tissue.[33]

Together, the available anatomical evidence supports the hypothesis that the behavioral consequences of the activity of the orexin system are due to parallel signaling to multiple brain regions. The idea that modulation of energy homeostasis is a distributed process through a neuronal network is not without support in the literature.[34,35] This framework suggests that understanding the function of the OXR requires studying them in a brain region-specific basis, as well as understanding the interactions between different brain regions that receive orexinergic input. Fundamental to this idea is whether there are common mechanisms that mediate the OXR function across different brain regions and/or neuronal populations. In the next section, we review cellular evidence to illustrate how the function and regulation of the OXR appears to be cellular type-specific.

OXR CAN INDUCE NEURONAL DEPOLARIZATION THROUGH MULTIPLE, CELL-SPECIFIC MECHANISMS

In vitro and *in vivo* studies suggest that both the OXR subtypes can couple to multiple G-proteins. Depending on the cell system for heterologous expression or tissue of choice, the OXR can couple with G_q, $G_{I/O}$ and G_S subunits.[36–47] Interestingly, in whole hypothalamic samples, food restriction has been demonstrated to increase coupling of either orexin receptor subtype to G_q, G_S and G_O, and decrease coupling to G_i. However, in the same study, the opposite effects were observed in adrenal cortex.[48] Collectively, these data show that regulation of OXR signaling in response to energy status change is tissue-specific. These data also suggest that regulation of OXR signaling is cell type-specific.

Activation of the OXR causes depolarization and increases neuronal firing by four possible mechanisms (Figure 2): (1) activation of non-specific cationic currents, (2) activation of the Na^+/Ca^{2+} exchanger, (3) phosphorylation-dependent inhibition of inwardly rectifying K^+ channels, and (4) increase in $[Ca^{2+}]_i$ through activation of L- and N-type Ca^{2+} channels.[43,49–59] The type of mechanism appears to be cell dependent. For example, in nucleus accumbens and nucleus of the solitary tract, OXR-mediated depolarization requires a simultaneous decrease in K^+ conductance and increase in non-specific cationic currents.[51,60] In GABAergic neurons from the arcuate nucleus, it occurs through a decrease in K^+ conductance and activation of the Na^+/Ca^{2+} exchanger.[61] Finally, there are differences in the temporal profile of $[Ca^{2+}]_i$ increase after OXR activation between neurons from the dorsal raphe and laterodorsal tegmentum areas.[62,63]

In summary, both OXR subtypes can couple to multiple G-proteins and can thus induce neuronal depolarization through many mechanisms engaged in a cell-specific manner. In the next section, we review the literature about the role of OXR subtypes in control of spontaneous physical activity (SPA) and food intake. Together with cellular data discussed in this section, we interpret this evidence in favor of the idea that function of the OXR in energy metabolism is dependent on the anatomical and cellular context, and argue against generalization of the function of OXR.

ROLE OF THE OXR IN ENERGY METABOLISM AND OBESITY

Early pharmacological studies have demonstrated that intracerebro ventricular injections of either OXA or OXB increased food intake and locomotor activity.[9,11,13,17,64] Although these data alone would suggest that both OXR subtypes would always mediate the same type of behaviors, the evidence discussed below supports the idea that the function of OXR subtypes is brain site-specific.

First, we summarize key evidence from mouse genetic models to review the general role of the orexin system in energy balance. Next, we discuss pharmacological and genetic data

specific to the OXR and their function in energy metabolism, and its implications for obesity. Finally, we briefly review how the role of the orexin system in sleep might contribute to its influence on energy balance.

Role of the orexin system in energy metabolism and obesity

This section reviews key findings from genetic models to illustrate both the general role and important details of the function of the orexin system in control of energy metabolism.

In 2001, Hara *et al.*[19] developed a mouse model that lacks orexin neurons (*OX-AT3*). These mice express the *ataxia3* gene under the control of orexin promoter, which leads to loss of the orexin neurons postnatally due to expression of the toxic protein ataxin3.[19] The OX-AT3 mice showed both hypophagia and lower levels of SPA, but the energetic consequences of lower activity were greater as they developed late-onset obesity when fed a regular diet.[19,65] This result suggests that although activation of the orexin system can increase both SPA and food intake, its primary function is to promote an increase in energy expenditure. This hypothesis is supported by studies showing that chronic orexin intrathecal injections do not result in body weight gain.[66,67] Further support comes from a mouse model, in which the *pre-proorexin* gene is under the control of the β-actin/cytomegalovirus (*CAG*) promoter (*CAG-OX*), leading to overexpression of the orexin peptides.[68] Consistent with the role of orexin in promoting energy expenditure, the *CAG-OX* mice show resistance to obesity induced by consumption of a high-fat diet.[69]

It has been shown that genetic background and sex contribute to the role of the orexin system in energy balance. For example, the late spontaneous obesity observed in the OX-AT3 mice, but not the higher weight gain on a high-fat diet, was dependent on genetic background.[70] Additional studies found that both female *OX-AT3* and *OX*–/– mice (from both C57BL/6 and mixed genetic background) gained more body weight gain on a standard diet compared with male mice.[71] Together, these studies suggest that activation of the orexin system increases energy expenditure, which results in protection against development of obesity. Recent evidence shows that orexin responsive neurons in raphe pallidus stimulate brown adipose tissue thermogenesis, which provides another mechanism by which elevated orexin system activity may elevate energy expenditure.[72,73] The mechanisms by which genetic background, gender and diet modulate the activity of the orexin system remain unclear.

Evidence for a brain region-specific contribution of the OXR to locomotor activity and energy metabolism

Shortly after discovery of the orexins, a number of studies showed that intra-cerebro ventricular injections of either OXA or OXB increased energy expenditure, physical activity and food intake.[25,64,74–76] However, the broad expression of the OXR subtypes[21,23] and the redundancy of brain control mechanisms observed in energy metabolism[35] suggest that intra-cerebro ventricular injections may not provide accurate information about the physiological role of the OXR subtypes.

Injection of OXA in specific brain regions has shown that activation of the OXR can increase SPA and/or food intake with region-specific changes in the magnitude of the effects (Figure 3). The increase in food intake mediated by OXA injections provides a good example of brain regional specificity. Injection of OXA in the perifornical area, the rostral portion of LH (RLH), and the paraventricular nucleus of the hypothalamus (PVN) and nucleus accumbens (NAC), but not in the substantia nigra increases food intake.[13,77–80] The orexin peptides modulate the activity of orexinergic neurons through activation of both OXR subtypes in glutamatergic interneurons[81] and cell-intrinsic effects through the OX2R.[20] It is

likely that injection of OXA in the perifornical area is activating orexinergic neurons themselves and increasing orexin signaling through multiple brain sites, leading to the observed increase in food intake. OXA injected in the RLH increases food intake as well as locomotor activity,[82] and most orexinergic neurons are located in the dorsal region of the LH, indicating this effect is not mediated by their activation.

Figure 3 summarizes the results of site-specific injections of OXA in SPA in a subset of brain regions for which there is detailed pharmacological information regarding contributions of the OXR. Injection of OXA in PVN increases both SPA and energy expenditure, and these effects are blocked by pre-treatment with an OX1R antagonist.[83] However, this same drug proves ineffective in blocking the increase in SPA resulting from OXA injection in the NAC.[84] This example clearly shows that both OX1R and OX2R can modulate the same type of behavior through different brain regions. However, the magnitude of effect is different; the effects of OXA injection in SPA are similar in RLH, PVN and substantia nigra, followed by NAC. To date, there is no information about the relative contribution of the OXR subunits to effects of OXA in SPA and food intake through substantia nigra. The differences in magnitude across the brain regions could be caused by different mechanisms that mediate OXR-induced neuronal depolarization (see section 'OXR can induce neuronal depolarization through multiple, cell-specific mechanisms'), differences in density of orexinergic innervation and/or destination of the efferent projections. Together, the data summarized in Figure 3 provides proof-of-concept that the effects of the orexin system in energy metabolism are distributed across multiple regions.

In summary, the evidence discussed is consistent with activation of both OXR subtypes increasing SPA, energy expenditure and food intake. It shows that multiple brain regions (including NAC, PVN, RLH and substantia nigra) contribute to the behavioral output of the orexin system. Furthermore, the specific mechanisms by which each orexin receptor subunit modulates changes in SPA seem to depend on its cellular and anatomical context. These data are consistent with the idea that the influence of the orexin system in animal behavior is mediated by parallel signaling to multiple brain regions, which is also supported by anatomical data (section 'OXR brain expression is part of a network').

If we consider the orexin system as a network, it becomes clear that the interactions between the simultaneous OXR activation in these brain sites are important for understanding how the orexin system drives SPA and increases energy expenditure. Currently, there is no information about interactions between the orexinergic inputs across multiple brain regions. Evaluation of anatomical connections between orexin-responsive neurons across different brain regions (i.e., using *in situ* hybridization coupled with retrograde tracer injections) will be an important first step towards understanding the interactions between brain regions that receive orexinergic input.

Evidence from obesity models and OXR knock-out mice

A polygenic obesity model, the obesity-prone and obesity-resistant (OR) rats has provided some insight in the role of the OXR in obesity. The obesity-prone and OR rats were derived by inbreeding from Sprague Dawley rats that show high or low body weight gain when fed a high-fat diet.[85] The OR phenotype of the OR rats is associated with higher levels of SPA and increased OX1R and OX2R expression in the RLH.[86] Long-term analysis of body composition of OR and Sprague Dawley rats showed that up to 18 months of age, the OR rats show lower fat mass and higher lean mass compared with Sprague Dawley rats (Figure 4).[87] Together, these results agree with genetic models (section 'Role of the orexin system in energy metabolism and obesity') and support the view that higher orexinergic signaling provides resistance to the development of obesity.

As injection of OXA in multiple brain sites (with different levels of OXR subtypes expression) increases SPA (section 'Role of the OXR in energy metabolism and obesity'), it is tempting to speculate that adaptations of the orexin system in other brain regions in addition to RLH may contribute to the OR phenotype of the OR rats. One possibility is that long-term potentiation due to activation of either OXR subtype[88–90] can increase the gain of orexin signaling in OXR-expressing neurons, contributing to higher levels of SPA and energy expenditure in brain sites known to mediate the effects of the orexin system in SPA, such as RLH, NAC or PVN.

If injections of OXA and OXB can increase both food intake and SPA, one might expect that increased OXR expression would lead to hyperphagia and obesity instead of an OR phenotype. However, as discussed earlier, orexinergic activity has a net effect of increasing energy expenditure (section 'Role of the orexin system in energy metabolism and obesity'). In summary, increased orexin responsivity in OR rats shows that activation of both the OXR subtypes can have a protective effect against obesity and suggests that at least in RLH, both OXR subtypes can drive the same behavioral response, which in this case is an increase in SPA.

The role of the OXR in obesity has been studied using genetic mouse models. When crossing the *CAG-OX* mice (section 'Role of the orexin system in energy metabolism and obesity') with the *OX1R–/–* and the *OX2R–/–* mice, only the *OX1R–/–/CAG-OX* mice were resistant to diet-induced obesity. This result was interpreted as evidence that signaling through OX2R mediates orexin system defense against diet-induced obesity.[69] A key prediction based on this would be that *OX2R–/–* mice would have a higher sensitivity to diet-induced obesity, but there were no differences in body weight gain or fat mass between the wild type and either *OX1R–/–* or *OXR2R–/–* fed a low-fat or a high-fat diet,[69] which precludes definitive conclusions regarding receptor subtype roles in obesity resistance.

Although the studies on the *OX2R–/–/CAG-OX* mice may implicate the OX2R in the resistance against diet-induced obesity, lack of higher sensitivity to high-fat diet in the *OX2R–/–* mice can be interpreted as evidence against an exclusive role of the OX2R in this phenomenon. For example, both OXR subtypes are expressed in PVN (Figure 1) and daily intra-PVN injections of OXA in male rats decreased body weight under a standard diet.[66] Because OXA can activate both OX1R and OX2R, this suggests that both OXR subtypes are involved in the obesity-protective effects of orexin signaling, at least through PVN. However, to confirm this hypothesis, experiments using OXR antagonists in PVN are needed. These results highlight two important aspects of research into the function of the orexin system. First, that action of a particular OXR subtype depends on its anatomical and cellular context, and second, that a full understanding of the function of the orexin system will require use of multiple techniques that include pharmacological, genetic and optogenetic approaches.

In summary, the evidence reviewed in this section is most consistent with involvement of both OX1R and OX2R in energy balance. The data emphasize the multiplicity of factors important to defining the contribution of the orexin system in control of energy metabolism. Although evidence from genetic models is limited, our interpretation of the available evidence is that both OXR subtypes are involved in the physiological control of energy metabolism and resistance to diet-induced obesity.

Orexin system influence on sleep and arousal, and its relationship to energy balance

In addition to orexinergic control of energy expenditure by its effects on food intake and physical activity, the orexin system also controls arousal and behavioral state stabilization. In this section, we briefly discuss how orexin system modulation of arousal and behavioral

state stabilization might contribute to its effect on energy expenditure. The inverse effect, how alterations in sleep and wake behavior affect energy metabolism, and the role of the orexin system in these processes, are beyond the scope of this section, and thus, we refer the reader to excellent reviews about this topic.[91–94]

It is well established that activation of the orexin system promotes wakefulness and its stability. The OX–/–, OX-ATX3 and double OX1R–/–; OX2R–/– mice display a phenotype similar to narcolepsy-cataplexy,[16,19,95] which is characterized by frequent transitions from awake to sleep state during the animal's active phase and by severe sleep fragmentation during the rest phase.[96] Additionally, the orexin neurons show higher activation during the active (i.e., awake) phase of the light cycle,[97] OXA cerebrospinal fluid levels peak during the active phase[98] and the neuronal activity of orexin neurons is higher during awake episodes.[99–101] Finally, optogenetic stimulation of orexin neurons directly increases the probability of transition from sleep to awake states and duration of awake periods.[102] Together, this evidence suggests the orexin system has increased activity during the active/ awake phase and contributes to behavioral state stability. These effects are mediated by orexinergic modulation of neuronal activity across regions with a known role in control of sleep. These include the dorsal raphe, locus coeruleus, tuberomammillary nucleus, medial preoptic area and laterodorsal tegmental nucleus/ pedunculopontine tegmental nucleus.[12,59,103–110] Therefore, in parallel to its modulation of energy expenditure and SPA, control of sleep and arousal by the orexin system also appears to be distributed across multiple brain sites.

Shorter sleep time and sleep stability are associated with development of obesity in humans.[111,112] Similarly, narcolepsy in humans and mouse models is associated with severe sleep/wake fragmentation, reduced energy expenditure and higher body mass index.[18,19,70,96,113] As mentioned before, narcolepsy is characterized by inability to consolidate sleep or wake into long bouts, resulting in behavioral instability.[114,115] It was suggested that sleep fragmentation at rest phase results in intrusion of sleep during active phase, and hence absence of consolidated wake episodes. This leads to reduced locomotor activity and energy expenditure resulting in weight gain.[96] Two models of differential sensitivity to obesity based on the orexin system also show differences in their sleep quality; the OX-ATX3 mice, which develop spontaneous obesity, also show a higher number of wake--sleep transitions.[19] The OR rats, which are resistant to diet-induced obesity compared with non-selectively bred Sprague Dawley rats, show better sleep quality, prolonged wakefulness and higher orexin signaling in brain regions involved in arousal. It is possible these differences directly contribute to their OR phenotype.[116] This evidence suggests that positive modulation of arousal and behavioral state stabilization by the orexin system contributes to an OR phenotype.

CONCLUSION AND FUTURE PERSPECTIVES

During the last decade, different analyses of the orexin system suggest that it drives an increase in energy expenditure through maintenance and/or increase of SPA levels. The evidence suggests that the magnitude and OXR subtype contribution to the behavioral output is entirely dependent on its anatomical location. We suggest that the density of orexin innervation, differences in intracellular signaling pathways, mechanisms of neuronal depolarization and efferent projections contribute to the site-specific actions of the OXR.

Figure 5 summarizes our current understanding of the role of the orexin system in energy metabolism. A key conclusion is that behavioral effects of the orexin system are mediated by parallel signaling through multiple brain regions, in which site-specific activation of either orexin receptor subtype results in an increase in either food intake and/or SPA. In

addition, the orexin neurons also project to brain sites that increase arousal and behavioral wake stabilization. Orexin signaling throughout multiple brain sites affect changes in behavior (feeding, activity and arousal) and sympathetic nervous system activity, which together increase energy expenditure.

Brain site-specific action of OXR subtypes is a defining feature of the orexin system, and future studies of OXR in brain need to consider both regional specificity of their contribution to behavior, as well as interaction between brain sites receiving orexinergic input. We suggest that experiments with double site-specific OXA or OXB injections with and without specific OXR subtype antagonists will be useful in uncovering how parallel orexin signaling through different brain sites contributes to the increase in SPA and energy expenditure associated with activation of the orexin system.

Central to this discussion are the consequences of obesity on orexinergic signaling. A consistent point across the evidence reviewed here is that higher orexin signaling provides resistance to the development of obesity. It is possible that differences in synaptic strength, an increase in synthesis or release of orexin peptides or changes in expression of the OXR could contribute to this particular phenotype. Several studies have shown that development of obesity has a negative impact on neural plasticity across different neuronal populations.[117–120] Therefore, it could be proposed that throughout development of obesity, there are changes in orexinergic signaling, perhaps reducing orexinergic signaling gain by changes in synaptic plasticity, which continue to change orexin system responsiveness and promote further development of obesity. As the function of the OXR subtypes in brain has regional specificity, this suggests that the mechanisms by which obesity affects orexinergic signaling and the associated changes will be brain region-specific.

In summary, the evidence related to OXR subtypes contribution to SPA and energy balance supports the idea of a system in which both OXR subtypes have a positive influence on SPA, but the mechanisms are dependent upon the anatomical and cellular context.

Acknowledgments

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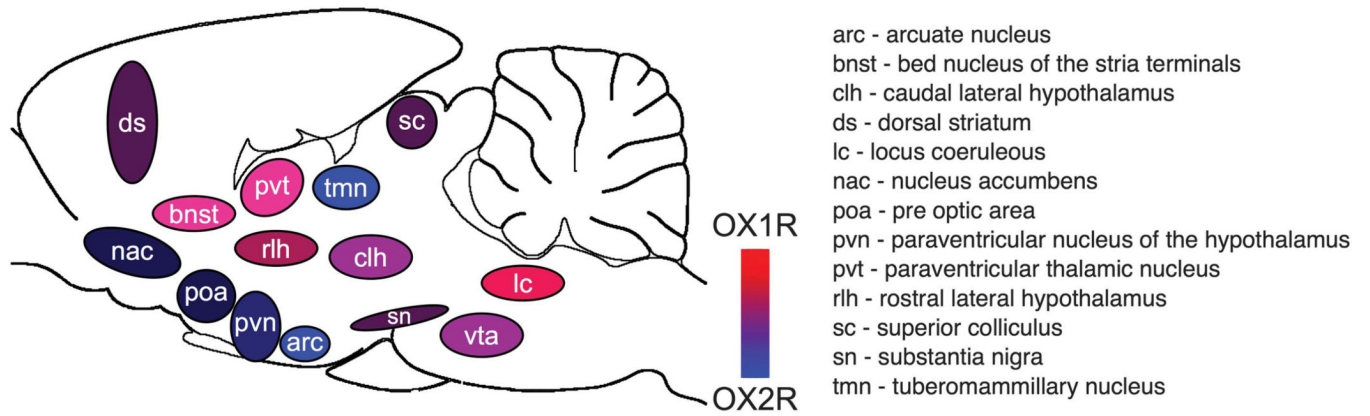
Figure 1.
Expression of the OXR in rat brain. This figure illustrates the expression pattern of the OX1 and OX2 in rat brain in a subset of brain regions that express the OXR. The levels of OXR expression were taken from Johansson *et al*[38]
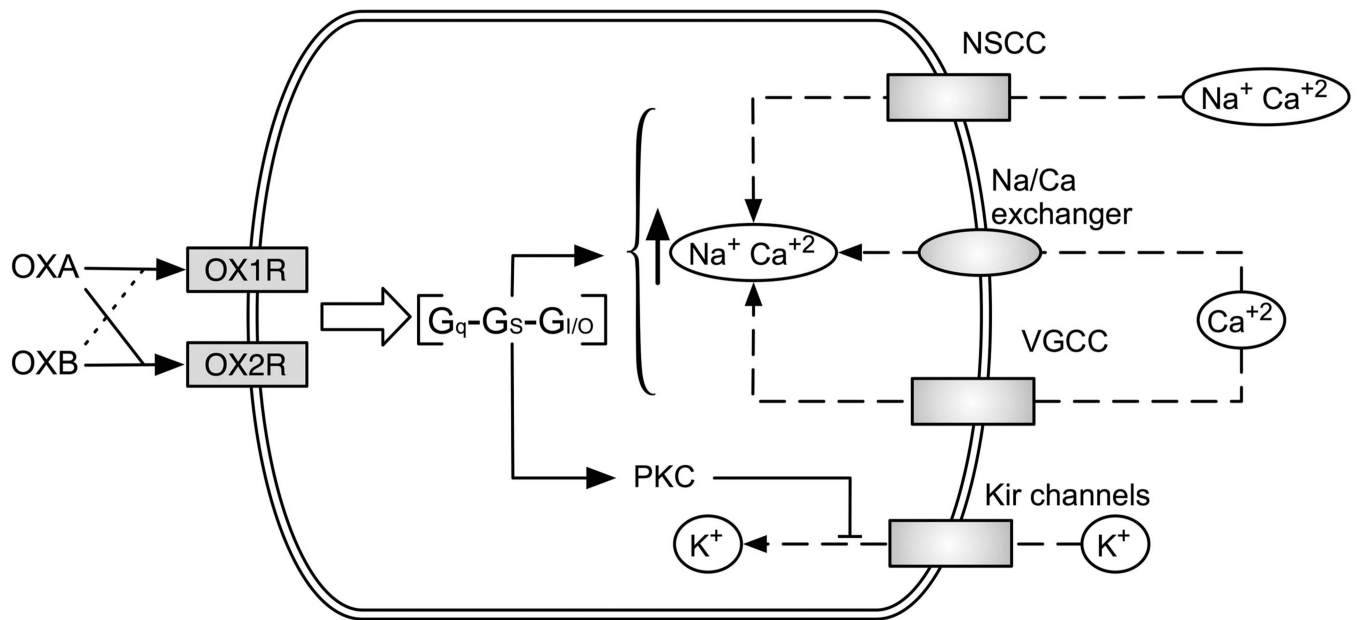
Figure 2.
Diversity of G-protein coupling and mechanisms of neuronal depolarization mediated by the OXR. The orexin peptides (OXA and OXB) differentially activate the OX1R and the OX2R: OX1R has a higher affinity for OXA, whereas OX2R has equal affinity for either orexin peptide. Activation of the OXR leads to an increase in neuronal activity, but the mechanisms are cell-specific. Depending on the particular cell population, the OXR couple to G_q, $G_{I/O}$ or G_S proteins, and modulate different ion channels and exchangers to induce neuronal depolarization. Currently, modulation of non-selective cationic currents, voltage-gated calcium channels (VGCC), the Na/Ca exchanger and Kir channels by the OXR have been described.
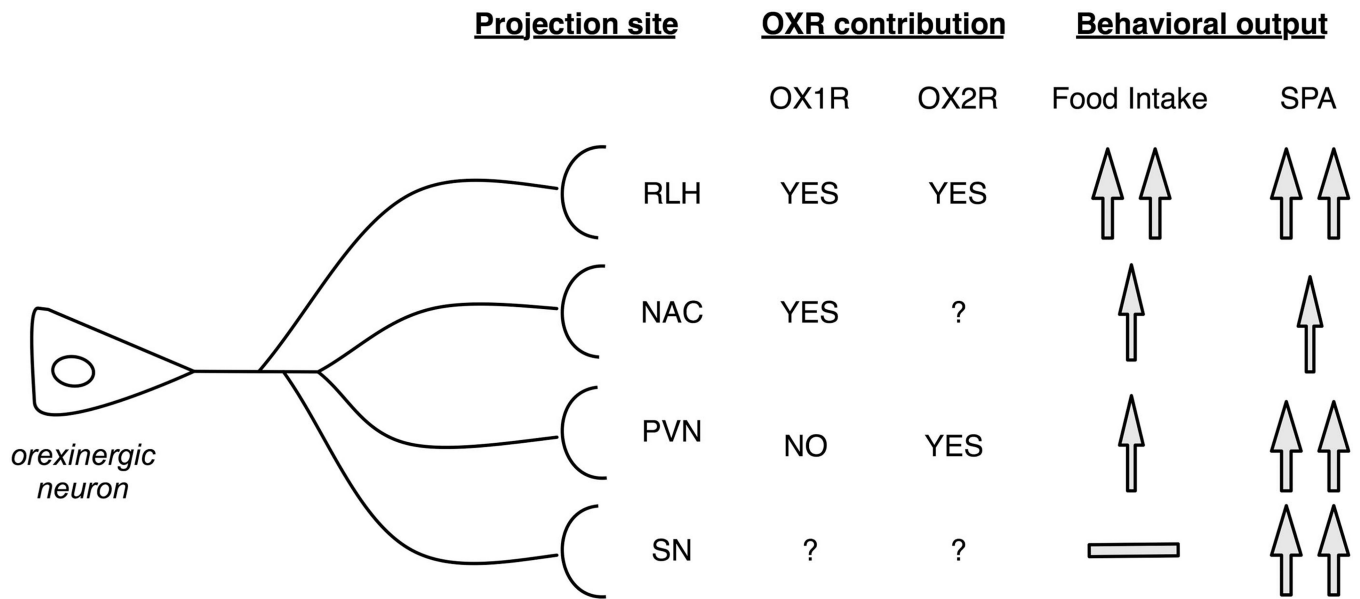
Figure 3.
The behavioral effects of the orexin system in SPA and food intake involve multiple brain regions. This figure summarizes the overlap and distinct influence OXR have on food intake and SPA. Pharmacological studies have demonstrated that the contribution of either orexin receptor subtype on behavioral output is based on anatomical location. This would strongly suggest that although there appears to be an overlap in OXR distribution, the mechanisms that are activated could depend upon specific location and orexin innervation or efferent projections.
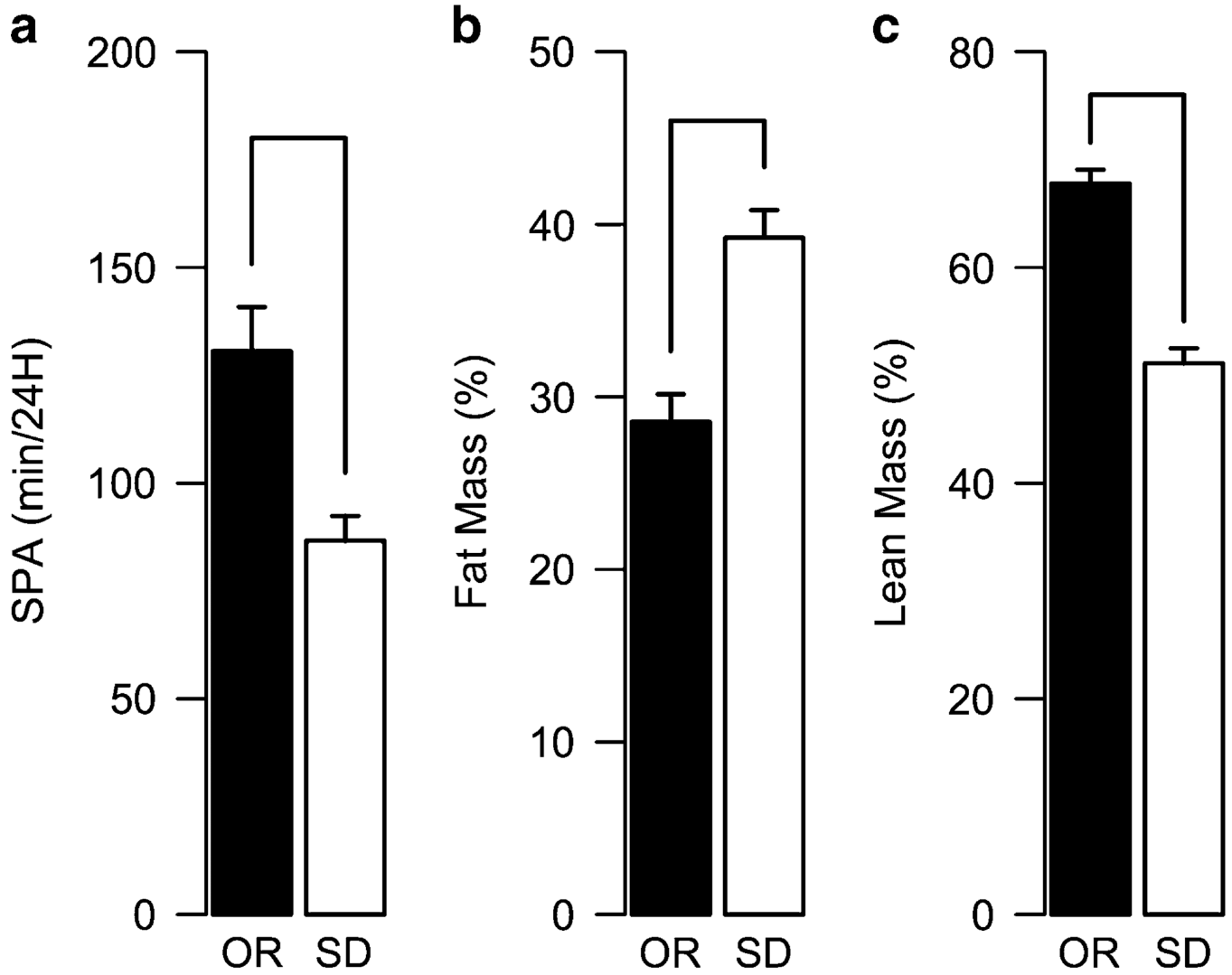
Figure 4.
OR rats remain leaner throughout their lifetime. The OR rats were derived by selective breeding of Sprague Dawley (SD) rats that gain minimal body weight gain when fed a high-fat diet Levin *et al*[85] The OR rats show higher expression of the OXR, higher orexin responsivity through RLH, and higher SPA compared with SD rats (**a**).[86] These higher levels of SPA at an early age (1–2 months) are correlated with lower percent of fat mass (**b**) and higher percent of lean mass (**c**) at 18 months of age.[87] These data illustrate how higher signaling through OXR allows resistance to obesity throughout the lifetime of these animals and supports the view that the overall effect of the activity of the orexin system is to promote negative energy balance. Data is from Teske *et al*[86,87]
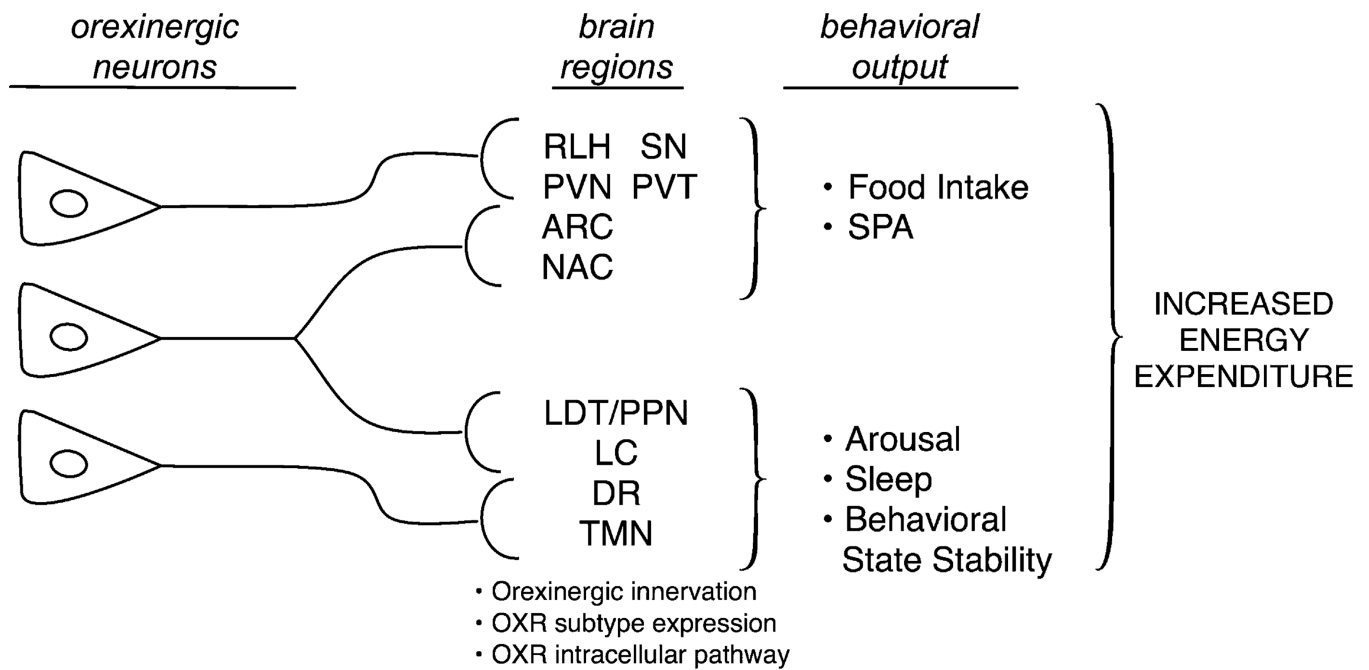
Figure 5.
The orexin system promotes negative energy balance through parallel signaling. This figure summarizes some of the key ideas discussed in this review. The orexin neurons projecting to either single or multiple brain regions can be (albeit somewhat arbitrarily) classified into regions involved in control of SPA, and food intake or control of sleep, arousal and behavioral stability. Within each brain site, there are at least three factors that contribute to the brain site-specific effects of the orexin neurons: (1) expression of the OXR, (2) density of orexinergic innervation and (3) intracellular pathway coupled by each OXR subtype. Our current interpretation of the evidence about the function of the orexin system suggest that it is the combined behavioral output from modulation of SPA, food intake, arousal and sleep that results in a negative energy balance. ARC, arcuate nucleus; PVN, paraventricular hypothalamic nucleus; PVT, paraventricular thalamic nucleus; NAC, nucleus accumbens; SN, substantia nigra; RLH, rostral LH; LC, locus coeruleus; LDT/PPN, laterodorsal tegmental nucleus/pedunculopontine tegmental nucleus; DR, dorsal raphe, TMN, tuberomammillary nucleus.