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Invited review

To eat or to sleep: That is a lateral hypothalamic question

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HIGHLIGHTS

- The lateral hypothalamus (LH) is important for the regulation of feeding, arousal, and energy balance.
- Newer genetic and electrophysiological tools now permit selective manipulation of discrete LH cell populations.
- We compare and contrast the roles of melanin-concentrating hormone (MCH), orexin/hypocretin, and GABA neurons in the LH.
- We discuss how these three cell populations interact to optimize and coordinate metabolism, sleep, and arousal.

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ABSTRACT

The lateral hypothalamus (LH) is a functionally and anatomically complex brain region that is involved in the regulation of many behavioral and physiological processes including feeding, arousal, energy balance, stress, reward and motivated behaviors, pain perception, body temperature regulation, digestive functions and blood pressure. Despite noteworthy experimental efforts over the past decades, the circuit, cellular and synaptic bases by which these different processes are regulated by the LH remains incompletely understood. This knowledge gap links in large part to the high cellular heterogeneity of the LH. Fortunately, the rapid evolution of newer genetic and electrophysiological tools is now permitting the selective manipulation, typically genetically-driven, of discrete LH cell populations. This, in turn, permits not only assignment of function to discrete cell groups, but also reveals that considerable synergistic and antagonistic interactions exist between key LH cell populations that regulate feeding and arousal. For example, we now know that while LH melanin-concentrating hormone (MCH) and orexin/hypocretin neurons both function as sensors of the internal metabolic environment, their roles regulating sleep and arousal are actually opposing. Additional studies have uncovered similarly important roles for subpopulations of LH GABAergic cells in the regulation of both feeding and arousal. Herein we review the role of LH MCH, orexin/hypocretin and GABAergic cell populations in the regulation of energy homeostasis (including feeding) and sleep-wake and discuss how these three cell populations, and their subpopulations, may interact to optimize and coordinate metabolism, sleep and arousal.

1. Introduction

The mammalian hypothalamus is small brain structure comprising a number of nuclei that play a fundamental role in the homeostatic regulation of internal physiological balance. The hypothalamus maintains the body's internal balance or *milieu interior* through feedback control and the coordinated regulation of endocrine and autonomic outflow. The lateral hypothalamus (LH) is one of the larger and more heterogeneous regions of the hypothalamus and its resident cell populations have been implicated in the regulation of a number of vital physiological processes, including sleep-wake control, feeding and energy metabolism. Here we discuss the contribution of three key lateral hypothalamic cell populations – the melanin-concentrating hormone (MCH), orexin/hypocretin and GABAergic subpopulations – to the regulation of sleep-wake, feeding and energy balance and how interactions among these three cell groups may inextricably link the regulation of behavioral state and energy homeostasis.

2. MCH neuron physiology

MCH was first discovered in the pituitary glands of salmon, where its release through the neurohypophysis functions to concentrate

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melanosomes and lighten the color of fish scales (Kawauchi et al., 1983). MCH was later isolated from rat hypothalami and was shown to be a nonadecapeptide (Vaughan et al., 1989). Its primary structure was subsequently shown to be highly conserved among mammals (Pissios et al., 2006). The first functional role of MCH revealed in mammals was its ability to acutely and robustly stimulate food intake (Qu et al., 1996) whereas chronic administration was later shown to produce body weight gain (Gomori et al., 2003).

It is now known that MCH is synthesized in the hypothalamus of all studied species. MCH neurons themselves are largely restricted to the LH, although a few cells are found within the zona incerta (Bittencourt et al., 1992) and, during lactation, in the medial preoptic area (Knollema et al., 1992). MCH neurons variably co-express multiple chemical messengers, including the neuropeptides nesfatin 1, cocaine and amphetamine regulatory transcript (CART), and galanin (Mickelsen et al., 2017), and almost all (> 90%) MCH neurons synthesize and synaptically release the fast neurotransmitter glutamate (Chee et al., 2015). Synthesis and release of the fast inhibitory neurotransmitter GABA by MCH neurons has also been shown (Jego et al., 2013).

3. MCH neurons and energy homeostasis

In the broadest context, MCH neurons promote positive energy balance. For example, targeted ablation of MCH neurons produces leanness, hyperactivity, and increased energy expenditure (Alon and Friedman, 2006; Vetrivelan et al., 2016; Whiddon and Palmiter, 2013). This is similar to transgenic mouse models of congenital MCH peptide deletion that show a lean, hyperactive, and hypermetabolic phenotype (Kokkotou et al., 2005; Shimada et al., 1998). In contrast, but consistent with the notion that MCH neurons promote positive energy balance, mice that overexpress MCH are prone to diet-induced obesity and are insulin resistant (Ludwig et al., 2001). Interestingly, chemogenetic activation of MCH neurons suppresses locomotor activity (Hausen et al., 2016), yet this acute perturbation produces only mild, if detectable, metabolic changes (Vetrivelan et al., 2016). Hence, MCH neurons likely function in conjunction with other central metabolic circuits and regulators to modulate energy homeostasis. For example, MCH neurons are directly excited by orexin (van den Pol et al., 2004), a known stimulator of food intake, and they are also activated by elevated glucose levels (Burdakov et al., 2005) to sustain feeding states (Fig. 1; Pathways 6 and 7).

The distribution of MCH receptors (MCHR1 in rodents) and the pattern of MCH axon projections do, however, provide clues to its postsynaptic sites of action (Table 1; Fig. 1). For example, in addition to the hypothalamus, MCHR1 is expressed in the nucleus accumbens (NAc) (Chee et al., 2013; Engle et al., 2018) and direct infusion of MCH into the NAc hyperpolarizes the medium spiny neurons (Sears et al., 2010) and stimulates food intake (Georgescu et al., 2005) (Fig. 1; Pathway 1). Just as MCH neurons undergo age-dependent decreases in excitability (Li and van den Pol, 2009; Linehan and Hirasawa, 2018), MCHR1 expression also fluctuates throughout postnatal brain development (Engle et al., 2018). MCHR1 expression in the hypothalamus increases with age, while that in the NAc is strongest at birth (Engle et al., 2018). It is therefore tempting to speculate that MCH is required for the development of neural circuits that enable hedonic feeding but that its role in adulthood links more directly to hypothalamic circuit control of homeostatic feeding.

Recent technical advances such as chemogenetics and optogenetics have helped define the time course and mechanisms leading to the orexigenic (feeding) actions of MCH. These studies have also serendipitously provided anterograde maps of MCH somal efferents, hinting at postsynaptic targets through which MCH may mediate its orexigenic effects. Notably, a recent study showed that chemogenetic activation of MCH neurons leads to MCH release into the cerebrospinal fluid (CSF). The release of MCH into the CSF preceded the increase in food intake and blocking the secretion of MCH suppressed feeding (Noble et al., 2018). This finding suggests the interesting possibility that MCH may produce its orexigenic effects through paracrine/volume transmission, thus the absence of MCH nerve terminals in a brain region would not *a priori* preclude a role for this region in MCH action.

MCH neurons may also play a role in regulating glucose homeostasis and insulin sensitivity. Whereas extracellular glucose activates MCH neurons (Burdakov et al., 2005) and functional expression of K_{ATP} channels is necessary for glucosensing at MCH neurons (Kong et al., 2010), the expression of uncoupling protein 2 (UCP2) (Kong et al., 2010) and insulin receptors (Hausen et al., 2016) on MCH neurons are required for the maintenance of insulin sensitivity. Furthermore, optogenetic activation of MCH neurons suggest that they are capable of discriminating the nutrient value of sugars (Domingos et al., 2013). This may have more to do with chemical messengers, such as glutamate, that are co-released with MCH than MCH itself given that deletion of the MCH peptide is without effects on glucose homeostasis whereas ablation of MCH neurons can improve glucose tolerance (Whiddon and Palmiter, 2013). To this end, preventing glutamate

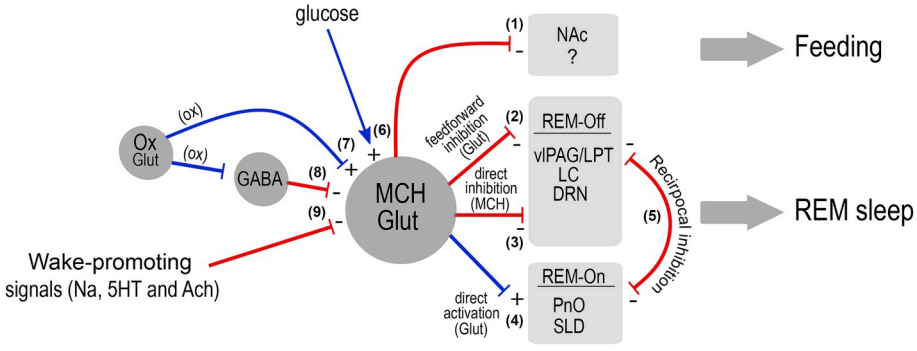

Fig. 1. Melanin-concentrating hormone (MCH) neurons. MCH neurons promote feeding through the nucleus accumbens (NAc) (1) and REM sleep through descending projections to pontine REM-Off and REM-On neurons (2–4). MCH neurons release MCH and glutamate: MCH is an inhibitory peptide whereas glutamate generally excites postsynaptic neurons. It is unclear how MCH neurons promote feeding through the NAc, nor is it clear if release of MCH, glutamate or both underlie the feeding effects by NAc neurons (1). It also remains to be determined whether the feeding effects mediated by MCH require additional pathways beyond the projection to the NAc. With respect to REM sleep regulation, MCH neurons are thought to promote REM sleep by directly activating REM-On neurons of the PnO and SLD, likely through the release of glutamate (4) and/or by inhibiting pontine REM-Off neurons of the vlPAG/LPT, LC and DRN (2–3). Pontine REM-Off neurons could be, for example, inhibited directly by MCH signaling (3) or indirectly by glutamate-mediated feedforward inhibition (2). As described in the text, during REM sleep REM-On neurons inhibit and suppress the activity of REM-Off neurons, whereas upon arousal REM-Off neurons start firing and suppress the activity of REM-On neurons (5). MCH neurons are excited by glucose (6) and orexin (7), which may promote the feeding effects of MCH neurons. Orexin neurons also inhibit MCH neurons through GABA release and this effect depends upon orexin-mediated activation of a GABAergic input which itself may originate from local LH GABAergic neurons or from GABAergic neurons outside of the LH (8). MCH neurons are also inhibited by wake-promoting signals such as noradrenaline, serotonin and acetylcholine (9). Blue and red arrows indicate excitatory and inhibitory inputs, respectively. Line arrow endings indicate synaptic inputs. Arrowhead endings indicate neuromodulation by peripheral signals. Ach, acetylcholine DRN, dorsal raphe nucleus; Glut, glutamate; LC, locus coeruleus; Na, noradrenalin; NAc, nucleus accumbens; NPY, neuropeptide Y; Ox, orexin; PnO, oralis pontine region; SLD, sublaterodorsal nucleus; vlPAG/LPT, ventrolateral periaqueductal grey matter and lateral pontine tegmentum; α-MSH, α-melanocyte-stimulating hormone; 5HT, serotonin.

Table 1
MCH neuronal pathways responsible for feeding and sleep-wake regulation. DRN, dorsal raphe nucleus; LC, locus coeruleus; LPT, lateral pontine tegmentum; NAc, nucleus accumbens; PnO, oralis pontine region; SLD, sublaterodorsal nucleus; vlPAG/LPT, ventrolateral periaqueductal grey matter and lateral pontine tegmentum.

Origin	Target	Effect on		Effect on	
		Feeding	References	Sleep & Wake	References
LH^{MCH}	NAc	Stimulates food intake	Georgescu et al. (2005), Chee et al. (2013), Engle et al. (2018)		
LH^{MCH}	LC, DRN, vlPAG/LPT			Could silence LC, DRN, and vlPAG/LPT REM-Off neurons during REM sleep	Torterolo et al. (2009), Yoon and Lee (2013), Costa et al. (2018)
LH^{MCH}	SLD, PnO			Could activate SLD and PnO REM-On neurons during REM sleep	Torterolo et al. (2009), Torterolo et al. (2013)
LH^{Ox}	LH^{MCH}	Could promote feeding	van den Pol et al. (2004)		

release from MCH neurons produces similar improvements in glucose homeostasis (Schneeberger et al., 2018).

4. MCH neurons and REM sleep regulation

MCH attracted the attention of the sleep field in the early 2000s. At that time, there was a great deal of interest in the role of lateral hypothalamic orexin neurons in sleep-wake regulation and narcolepsy. While MCH neurons were mainly known for their role in energy homeostasis, they are also intermingled with orexin neurons and shared similar projection patterns (Bittencourt et al., 1992; Peyron et al., 1998; Steininger et al., 2004). In 2003, Verret and colleagues used cFos to show that MCH neurons were active during REM sleep and that central injections of MCH increased NREM and REM sleep. These MCH-driven effects were particularly striking for REM sleep, which was doubled following MCH administration (Verret et al., 2003). Subsequent work confirmed the expression of cFos in MCH neurons in REM sleep (Goutagny et al., 2005; Hanriot et al., 2007; Kitka et al., 2011; Modirrousta et al., 2005; Verret et al., 2003).

In 2009, Hassani and colleagues performed unit recordings in unanesthetized rats to demonstrate that MCH and orexin neurons discharge in a reciprocal pattern, with orexin neurons being primarily active in wakefulness but quiescent in NREM and REM sleep, and MCH neurons being primarily active during REM sleep, firing in a sustained manner and maximally up to 10–20 Hz during spike doublets or triplets. While MCH neurons are virtually silent during wakefulness, they do fire occasionally during NREM sleep, displaying bursts of activity up to 2–3 Hz (Hassani et al., 2009).

In general agreement with the behavioral state unit recording work, MCH levels in the CSF were found to be higher in rats during the rest period (Pelluru et al., 2013) and wake-promoting signals such as noradrenaline, acetylcholine and serotonin inhibit MCH neurons (Bayer et al., 2005; Gao et al., 2003; van den Pol et al., 2004). Curiously, however, orexin directly excites MCH neurons (Apergis-Schoute et al., 2015; van den Pol et al., 2004). Given the foregoing pharmacologic and unit recording data, which did not suggest a major role for MCH in the regulation of REM sleep, it was surprising when an early optogenetic study found that selective activation of MCH neurons produced an increase in both NREM and REM sleep (Konadhode et al., 2013). Somewhat divergent outcomes were reported in later studies which instead found that optogenetic or chemogenetic activations of the MCH neurons increased REM sleep, but not NREM sleep. In fact, the increase in REM sleep occurred at the expense of NREM sleep, which itself was slightly, but significantly, reduced (Tsunematsu et al., 2014; Varin et al., 2018; Vetrivelan et al., 2016). The reduction in NREM sleep was attributed to an increased probability of REM sleep transitions, which resulted in shorter NREM episodes. Using an optogenetic approach Jego and colleagues found that optogenetic stimulation of MCH neurons during NREM sleep doubled the probability of transitions into REM sleep whereas optogenetic stimulation of MCH neurons during REM sleep prolonged the duration of REM sleep episodes (Jego et al., 2013). These results together with those from the chemogenetic activation of MCH neurons are generally most consistent with MCH neurons facilitating the entry into REM sleep and prolonging REM sleep bout duration.

While considerable evidence indicates that MCH neurons can promote REM sleep (i.e., activation thereof is sufficient), it remains unclear whether MCH neurons are actually necessary for spontaneous REM sleep, in particular as ablation, photoinhibition or chemogenetic inhibition of MCH neurons is without effect on REM sleep amount or REM sleep episode duration (Jego et al., 2013; Tsunematsu et al., 2014; Varin et al., 2016, 2018; Vetrivelan et al., 2016). Despite the absence of changes in the amount of REM sleep following acute inhibition or chronic lesions of MCH neurons, some minor effects in the quality of REM sleep have been reported and should be mentioned. Specifically, ablation of MCH neurons results in a small increase in REM amounts during the light period and a small decrease during the dark period, resulting in an increase in REM sleep circadian amplitude (Varin et al., 2016; Vetrivelan et al., 2016). Hence, MCH neurons may function as an effector in the circadian network that regulates REM sleep timing. MCH neurons could, for example, be under temporal regulation by the polysynaptic suprachiasmatic nucleus (SCN)-subparaventricular zone-dorsomedial hypothalamic pathway that appears to regulate the timing of sleep-wake (Saper et al., 2010; Vujovic et al., 2015). It has also been reported that optogenetic silencing of MCH neurons during REM sleep reduced the frequency and amplitude of theta oscillations (Jego et al., 2013), whereas chemogenetic activation of MCH neurons produced an increase in the theta rhythm in REM sleep (Varin et al., 2018). This influence is likely to be mediated through direct projections of MCH neurons to the hippocampus and cortical areas and/or indirectly through the medial septum and the supramammillary nucleus (Bittencourt and Elias, 1998; Pedersen et al., 2017).

In agreement with the results from the acute inhibition or chronic lesions of MCH neurons, MCHR1 null mice exhibit only minor changes in vigilance states (Adamantidis et al., 2008; Willie et al., 2008). Interestingly, under baseline conditions, MCH knockout mice have a similar overall organization of vigilance states as their wild-type littermates yet, under conditions of fasting, MCH knockout mice exhibit hyperactivity, accelerated weight loss and a dramatic suppression of REM sleep (Willie et al., 2008). Because MCH knockout mice have significantly reduced fat mass, the hyperactivity phenotype under fasting conditions may represent compulsive food seeking behavior (Sanathara et al., 2018). Also, the near complete suppression of REM sleep in MCH knock out mice under conditions of food deprivation might represent an energy conserving mechanism given the established energetic cost of REM sleep (Willie et al., 2008).

5. MCH neurons promote REM sleep through multiple neuronal pathways

The postsynaptic targets and synaptic mechanisms through which MCH neurons promote REM sleep also remain incompletely understood. MCH is an inhibitory peptide that acts through both presynaptic and postsynaptic mechanisms, although the effects of MCH are typically presynaptic (Gao, 2009; van den Pol, 2012). Further complicating things is the uncertainty over whether MCH neurons co-release glutamate, GABA or both with MCH. These alternate combinations would produce vastly different membrane effects at the postsynaptic target. MCH neurons contain GAD65 and GAD67, indicating that they are capable of synthesizing GABA, but it remains unclear whether MCH neurons can actually produce synaptic release of GABA. MCH neurons do not express the vesicular GABA transporter (VGAT) or the vesicular monoamine transporter (VMAT) (Chee et al., 2015; Jennings et al., 2015; Mickelsen et al., 2017; Romanov et al., 2017; Schneeberger et al., 2018), one or both of which are typically considered to be necessary for a neuron to produce synaptic release of GABA. Hence, if MCH neurons can synaptically release GABA, they must rely on another, as yet unidentified, GABA vesicular transporter, i.e., not VGAT or VMAT, to package and release GABA. By contrast, the vast majority of MCH neurons express the vesicular glutamate transporter 2 (VGLUT2) and can synaptically release glutamate (Chee et al., 2015).

With respect to the postsynaptic targets and circuits through which MCH neurons promote REM sleep, it has been proposed that MCH neurons promote REM sleep through their descending projections to pontine REM sleep circuits (Costa et al., 2018; Luppi et al., 2013; Monti et al., 2013) (Table 1; Fig. 1). To this end, MCH neurons send dense projections to the locus coeruleus (LC), dorsal raphe nucleus (DRN), and regions of the ventrolateral periaqueductal grey matter and lateral pontine tegmentum (vlPAG/LPT) that are implicated in REM sleep regulation (Costa et al., 2018; Torterolo et al., 2009; Yoon and Lee, 2013) (Fig. 1; Pathways 2–3). All of these postsynaptic targets share the common feature of resident cell populations that are active during wakefulness and, upon entry into REM sleep, become silent (Aston-Jones and Bloom, 1981; McGinty and Harper, 1976; Trulson and Jacobs, 1979), i.e., REM-Off neurons (Arrigoni et al., 2016; Fuller et al., 2007). In general, REM-Off neurons are thought to suppress the activity of pontine REM-generating neurons during wakefulness but will cease firing during REM to disinhibit pontine REM-generating neurons in the oralis pontine (PnO) and sublaterodorsal nucleus (SLD). These pontine REM-On neurons project back to the REM-Off neurons and inhibit the firing of the vlPAG/LPT neurons during REM sleep. This reciprocal inhibitory connectivity between the SLD REM-On neurons and the vlPAG/LPT REM-Off neurons has been proposed to ensure rapid transition in and out of REM sleep (Saper et al., 2010) (Fig. 1; Pathway 5). In support of this circuit model, MCH microinjection in the LC or DRN increases the number of REM sleep episodes, and MCH inhibits the firing of DRN neurons (Devera et al., 2015; Lagos et al., 2009; Monti et al., 2015). MCH neurons could therefore inhibit REM-Off neurons through direct MCH signaling (Fig. 1; Pathway 3) or through glutamate by activating an inhibitory interneuron to produce feedforward inhibition of REM-Off neurons (Fig. 1; Pathway 2), as has been previously described (Chee et al., 2015). Moreover, MCH neurons also project directly to the REM-generating region of the PnO and SLD where microinjections of MCH significantly increase REM sleep and decrease the latency to REM sleep onset (Torterolo et al., 2009, 2013)(Table 1; Fig. 1; Pathway 4). MCH neurons also project to the dorsal paragigantocellular reticular nucleus (DPGi) in the rostral medulla (Costa et al., 2018). This medullary region contains neurons that are active in REM sleep (Valencia Garcia et al., 2018; Verret et al., 2005), although it remains to be confirmed whether DPGi neurons, or activation thereof, can produce REM sleep. MCH may directly activate REM-On neurons in the PnO, SLD and DPGi regions through the release of glutamate, although a recent study has found, unexpectedly, that glutamatergic transmission in MCH is not required for the MCH REM-promoting effects (Naganuma et al., 2018).

6. Orexin neuron physiology

Orexin neurons (alternatively called hypocretin neurons) exert their physiological effects through release of two different peptides, Orexin-A and Orexin-B (OxA and OxB or HCRT1 and HCRT2), which are themselves cleavage products of prepro-orexin (de Lecea et al., 1998; Sakurai et al., 1998). The OxA and OxB peptides exhibit high sequence conservation across species and the orexin system is found in most vertebrates including zebrafish (Elbaz et al., 2017). Once transcribed and translated, the OxA and OxB peptides are packaged into dense core vesicles and synaptically released (Blomeley et al., 2018; de Lecea et al., 1998; Muschamp et al., 2014; Schone et al., 2014). OxA and OxB both produce excitatory post-synaptic responses by binding selectively to the Ox1 and Ox2 receptors (Ox1R and Ox2R or HCRTR1 and HCRTR2). Ox1Rs have higher affinity for OxA, whereas Ox2Rs have similar affinity for both orexin peptides (Sakurai et al., 1998; Scammell and Winrow, 2011). The OxRs themselves are G protein–coupled receptors with Ox1Rs signaling through a G_q pathway and Ox2Rs signaling through both G_q and G_i/G_o pathways, ultimately resulting in either activation of membrane Na^+/Ca^{2+} exchangers (Burdakov et al., 2003; Eriksson et al., 2001) or closing of a potassium conductance (Bayer et al., 2002, 2004; Bisetti et al., 2006; Ivanov and Aston-Jones, 2000). In addition to the OxA and OxB peptides, orexin neurons also produce and release glutamate and the inhibitory peptide dynorphin (Chou et al., 2001; Schone et al., 2012; Torrealba et al., 2003). Dynorphin is packaged in the same dense core vesicles that contain orexin and, hence, is typically co-released with orexin (Muschamp et al., 2014), whereas glutamate is packaged separately and can be released independently of orexin (Blomeley et al., 2018; Schone et al., 2014). The net effect of orexin and dynorphin co-release on the postsynaptic membrane is variable, depending upon the relative expression of the orexin and dynorphin receptors in the postsynaptic target membrane, the extent of desensitization and on the state of activation of the postsynaptic neurons (Eriksson et al., 2004; Ferrari et al., 2016; Li and van den Pol, 2006).

7. Orexin in sleep and wake regulation

MCH and orexin neurons are coextensive within the anatomic LH, but they exert divergent physiological effects. For example, orexin neurons are wake-active, wake-promoting and fire maximally during wake and peak extracellular levels of the orexin peptide occur during wakefulness (Kiyashchenko et al., 2002; Lee et al., 2005). The firing rate of orexin neurons also increases during periods of movement and explorative behaviors, whereas they are mostly silent during NREM and REM sleep (Lee et al., 2005; Mileykovskiy et al., 2005), with the exception of occasional discharges during REM sleep when small movements or twitches occur (Lee et al., 2005; Mileykovskiy et al., 2005). Although orexin neurons seldom fire during NREM and REM sleep and the concentration of orexin in the CSF is low during sleep, orexin receptor antagonists given before bedtime are effective in treating insomnia, suggesting that orexin is likely still functionally active during the early subjective night (Norman and Anderson, 2016). Orexin neurons also fire in response to rewarding stimuli (Harris et al., 2005; Martin-Fardon et al., 2018; Tyree and de Lecea, 2017); for example, orexin neurons fire at a much higher frequency in response to a tone associated with sucrose availability than to a tone associated with quinine (Hassani et al., 2016). Orexin neurons are also activated in response to stress and anxiety (Bonnavion et al., 2015; Johnson et al., 2010), fear (Furlong et al., 2016; Soya et al., 2017), pain (Inutsuka et al., 2016) and fasting (Diano et al., 2003; Horvath and Gao, 2005; Yamanaka et al., 2003). Orexin neurons are also activated in anticipation of food, morphine, cocaine, and ethanol (Akiyama et al., 2004;

Boutrel et al., 2005; Harris et al., 2005; Jupp et al., 2011; Martin-Fardon et al., 2018; Mieda et al., 2004), and this has inspired a considerable body of experimental work on role of orexin neurons in drug seeking behaviors and addiction (Giardino and de Lecea, 2014; James et al., 2017; Tyree et al., 2018). Given that all of these conditions occur against a backdrop of heightened behavioral activation, it is conceivable that activation of the orexin system is also a key contributor to general arousal in all of these contexts (Sakurai, 2014).

With respect to wake-promotion, both optogenetic and chemogenetic activation of orexin neurons rapidly rouses mice from sleep, prolongs wakefulness and strongly suppresses REM sleep (Adamantidis et al., 2007; Sasaki et al., 2011). Similarly, intracerebroventricular injection (ICV) of orexin produces long periods of wakefulness and suppresses REM sleep for several hours (Mieda et al., 2011). But the most essential role for orexin neurons is likely that of wake maintenance. For example, selective loss of orexin-producing neurons in humans, as occurs in narcolepsy (Blouin et al., 2005; Crocker et al., 2005), results in excessive daytime sleepiness (Scammell, 2015). Disruption of orexin signaling in mice, rats, and dogs produces a very similar phenotype, including short bouts of wake and frequent transitions between behavioral states (Chemelli et al., 1999; Mochizuki et al., 2004), even though there are only minor reductions in the total amount of wakefulness across the 24 h day (Branch et al., 2016).

Ox2Rs appear to be more important than Ox1Rs in controlling arousal and sleep as deletion of Ox1Rs in mice produces no evident changes in sleep and wakefulness (Abbas et al., 2015; Mieda et al., 2011). Meanwhile, deletion of Ox2Rs produces a phenotype like narcoleptic patients and global restoration of OX2Rs in double OxR knock out mice also restores a normal sleep wake phenotype (Mochizuki et al., 2011; Willie et al., 2003). Interestingly, the excessive sleepiness phenotype of Ox2R knockout mice is relatively mild as compared to that seen in mice lacking the orexin peptide or the orexin neurons, whereas the excessive sleepiness in mice lacking both OxR subtypes is similar to that seen in orexin peptide knock out mice. Hence, it is thought that both OxR subtypes contribute to wake-maintenance with the Ox1R making the lesser contribution to this process (Hara et al., 2001; Mochizuki et al., 2004; Willie et al., 2003).

While the circuit basis by which orexin neurons influence wakefulness remains unresolved, it is generally thought that orexin directly activates key wake-promoting neurons throughout the forebrain and brainstem (Table 2; Fig. 2; Pathway 9). For example, orexin neurons project to wake-associated neurons in the basal forebrain, tuberomammillary nucleus (TMN), ventral tegmental area (VTA), LC, and DRN; *in vitro* electrophysiology recordings have confirmed that orexin activates neurons in all of these regions (Baimel et al., 2017; Brown et al., 2001; Eggermann et al., 2001; Eriksson et al., 2001; Horvath et al., 1999; Peyron et al., 1998). Although there is general consensus that orexin neurons likely activate target neurons through the co-release of orexin, dynorphin and, often, glutamate, the relative contribution of orexin vs dynorphin vs glutamate signaling in driving arousal responses in these regions has yet to be determined. Experimental work to elucidate the respective contributions of orexin, dynorphin, but in particular glutamate, in these responses is eagerly awaited, in particular as a central role for excitatory fast neurotransmission in regulating sleep-wake has become increasingly evident (Saper and Fuller, 2017).

Recent work using optogenetic manipulations of orexin efferent pathways as well as focal restoration of orexin signaling in discrete brain regions has begun to shed light on the neurocircuitries by which orexin produces its wake-promoting effects. For example, optogenetic stimulation of orexin terminals in the LC promotes awakening, suggesting that orexinergic input to LC is sufficient to reproduce the arousal responses of activating the orexin neurons themselves (Carter et al., 2012). Interestingly, optogenetic stimulation of orexin neurons can still promote arousal in animals lacking central histamine, suggesting that histamine neurons are not a necessary signaling element underlying the arousal effects of orexin (Carter et al., 2009). In contrast, two other studies showed that central administration of orexin significantly increases wakefulness in wild type mice, but not in histamine receptor 1 knock-out mice (Huang et al., 2001) and that genetic reconstitution of Ox2Rs in the posterior hypothalamic region rescued sleepiness in narcoleptic mice (Mochizuki et al., 2011). In this latter study, re-expression of the Ox2Rs in TMN histamine neurons was hypothesized to form the basis of this phenotypic rescue (Mochizuki et al., 2011). While involvement of TMN histamine neurons in orexin-mediated arousal responses is plausible, another possibility is that reconstitution of Ox2Rs in glutamatergic/Nos1-expressing supramammillary neurons, which were recently described as potent cellular mediator of arousal, may also underlie, at least in part, the phenotypic rescue (Pedersen et al., 2017). Using a similar approach to Mochizuki et al. Hasegawa and colleagues showed that focal restoration of Ox1Rs in the LC region improved wake maintenance, whereas restoration of Ox2Rs in the DRN had no effect on vigilance of the mice, but did improve cataplexy (Hasegawa et al., 2014). Collectively, these results indicate that orexin signaling through the LC and posterior hypothalamus is sufficient to improve narcoleptic symptoms and, furthermore, that orexin neurons promote arousal and prevent cataplexy through separate pathways.

As indicated, orexin neurons exhibit a pronounced reduction in their firing rate during quiet wakefulness and are virtually silent during NREM sleep. But how are these state-dependent changes in firing rate mediated? One putative mechanism by which orexin neurons may become inhibited during NREM and REM sleep is via long-range GABAergic inputs. To this end, GABA release in the LH is higher during NREM and REM sleep (Nitz and Siegel, 1996) and blocking GABAergic signals in the LH during the light (i.e., sleep) period activates orexin neurons (Alam et al., 2005). With respect to the source of GABAergic inputs, possibilities include neurons in the medial preoptic area (mPOA), median preoptic (MnPO), and ventrolateral preoptic (VLPO) areas (Fig. 2; Pathway 1). All of these regions 1) project to orexin neurons (Saito et al., 2013, 2018; Yoshida et al., 2006), 2) contain GABAergic neurons that are active during NREM and/or REM sleep (Alam et al., 2014; Chung et al., 2017; Sherin et al., 1996) and 3) promote NREM sleep (Benedetto et al., 2012; Chung et al., 2017; Kaushik et al., 2011; Vetrivelan et al., 2012). In addition to long-range inputs, local LH GABAergic sleep-active neurons, which in fact are intermingled with LH orexin neurons, may also contribute to the inhibition of orexin neurons during NREM and REM sleep (Alam et al., 2002; Hassani et al., 2010; Koyama et al., 2003). While many of these GABAergic LH sleep-active neurons send descending projections to the brainstem (Clement et al., 2012; Verret et al., 2006), they also provide local collaterals (Bonnavion et al., 2015; Ferrari et al., 2018; Hassani et al., 2010) that could inhibit surrounding orexin neurons during NREM and REM sleep. A recent paper has also identified orexin-projecting, sleep-promoting GABAergic neurons in the dorsally adjacent zona incerta as another potential source of GABAergic input that may contribute to the inhibition of the orexin neurons during NREM and REM sleep (Liu et al., 2017b).

8. Orexin and REM sleep

Activation of orexin neurons, central administration of orexin and overexpression of the orexin peptide all result in reductions in REM sleep (Mieda et al., 2011; Sasaki et al., 2011; Willie et al., 2011), likely through activation of both Ox1Rs and Ox2Rs (Mieda et al., 2011). Hence, humans lacking orexin, such as narcoleptic patients, exhibit short latency to REM sleep and naps often include bouts of REM sleep, regardless of the time of day (Andlauer et al., 2013; Dantz et al., 1994). Similar to narcoleptic patients, mice lacking orexin neurons and orexin receptors are unable to suppress REM sleep during their active phase, suggesting that high endogenous orexin ‘tone’ during the active phase may be necessary for REM suppression and may underlie the circadian

Table 2
Orexin neuronal pathways responsible for feeding and sleep-wake regulation. DRN, dorsal raphe nucleus; LC, locus coeruleus; LepRb, leptin receptors; Lhx6; LIM homeodomain factor Lhx6; LPT, lateral pontine tegmentum; mPOA, medial preoptic area; MnPO, median preoptic area; NAc, nucleus accumbens; PnO, oralis pontine region; PVH, paraventricular hypothalamic nucleus; SLD, sublaterodorsal nucleus; TMN, tuberomammillary nucleus; vlPAG/LPT, ventrolateral periaqueductal grey matter and lateral pontine tegmentum; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area; ZI, zona incerta.

Origin	Target	Effect on		Effect on	
		Feeding	References	Sleep & Wake	References
LH^{Ox}	LC			Promotes arousal and suppresses REM	Bourgin et al. (2000), Chen et al. (2010), Carter et al. (2012), Choudhary et al. (2014), Hasegawa et al. (2014)
LH^{Ox}	TMN			Suppresses REM sleep, but may not mediate the arousal responses of orexin in the posterior hypothalamic mamillary region	Carter et al. (2009), Mochizuki et al., 2011, Schwartz et al. (2016), Huang et al. (2001)
LH^{Ox}	DRN			Prevents cataplexy	Luppi et al. (2013)
LH^{Ox}	SLD, PnO			Suppresses REM sleep	Thakkar et al. (1999)
LH^{Ox}	vlPAG, LPT			Suppreses REM and prevents cataplexy	Boissard et al. (2003), Lu et al. (2006), Chen et al. (2013)
LH^{Ox}	$ARC^{NPY/AgRP}$	Activates NPY neurons and promotes feeding	Dube et al. (2000), Jain et al. (2000), Yamanaka et al. (2000), van den Pol (2012), Valencia Garcia et al., 2018		
LH^{Ox}	ARC^{POMC}	Inhibits POMC neurons and reduces melanocortin production	Ma et al. (2007), Morello et al. (2016)		
LH^{Ox}	PVH	Might promote feeding	Edwards et al. (1999)		
LH^{Ox}	LH^{MCH}	Could promote feeding	van den Pol et al., 2004		
LH^{LepRb}	LH^{Ox}	Could inhibit feeding	Leinninger et al. (2009), Leinninger et al. (2011), Goforth et al. (2014)	Might facilitate entry into NREM sleep	Bonnavion et al. (2015), Herrera et al. (2016)
$mPOA^{GABA}$, $MnPO^{GABA}$, $VLPO^{GABA}$	LH^{Ox}			Could silence orexin neurons during NREM and REM sleep	Yoshida et al. (2006), Saito et al. (2013), Saito et al. (2018)
LH^{VGAT}	LH^{Ox}			Could silence orexin neurons during NREM and REM sleep	Hassani et al. (2010), Ferrari et al. (2018)
$ZI^{Lhx6/GABA}$	LH^{Ox}			Promotes sleep	Liu et al. (2017b)

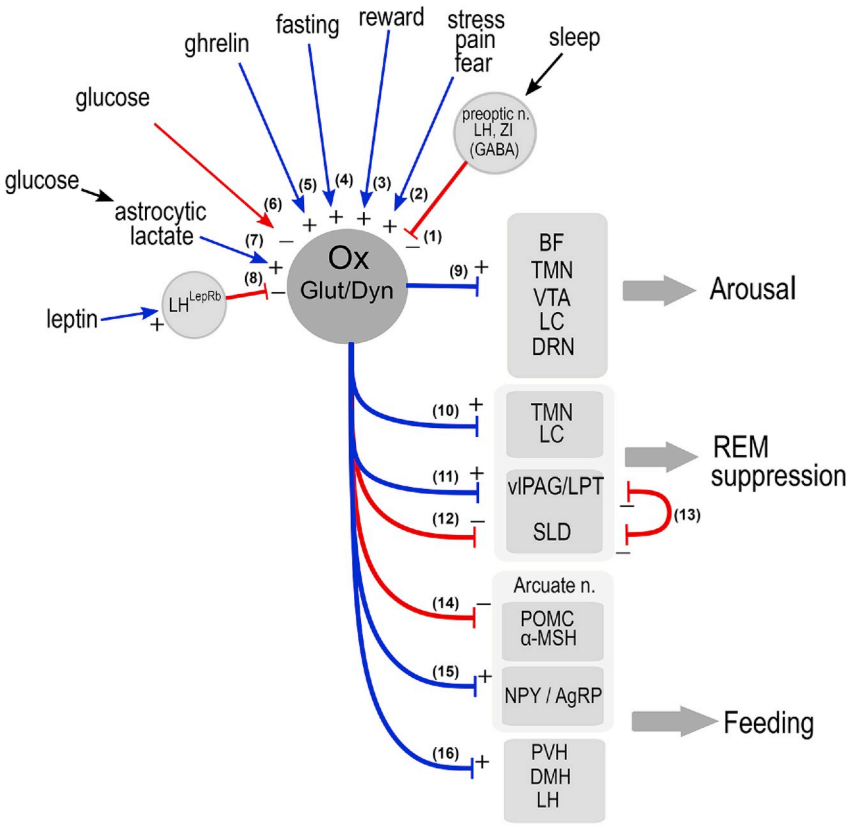

Fig. 2. Orexin neurons. Orexin neurons are activated in response to reward stimuli, stress, fear and pain, and when activated promote a high level of arousal (2,3). The circuit basis by which these stimuli activate orexin neurons remains unresolved but remains active areas of experimental inquiry. During sleep, orexin neurons are silent, and they are thought to be inhibited by local and long-range GABAergic inputs. Sources of sleep-active GABAergic inputs includes neurons of the preoptic region (mPOA, MnPO, VLPO areas), GABAergic neurons in the ZI and local LH GABAergic neurons (1). With respect to feeding behaviors, negative energy states activate orexin neurons to ensure high levels of arousal for food seeking. Orexin neurons are activated by fasting (4) and directly by ghrelin (5), whereas they are inhibited by glucose (6) and leptin (8). The effect of leptin on orexin neurons is indirect, occurring through the activation of local GABA neurons in the LH that express LepRbs (8). The effect of glucose on orexin neurons is complicated: it appears that rapid rises in extracellular concentration of glucose inhibits orexin neurons (6), but glucose levels are also controlled by local astrocytes that convert glucose into lactate, which in turn excites orexin neurons (7). Orexin neurons may stimulate feeding through intra-hypothalamic projections to the arcuate nucleus, PVH, DMH and locally within the LH (16). Orexin peptide directly excites arcuate NPY neurons (15) and indirectly inhibits arcuate POMC neurons (14) through an increase of GABAergic afferent input and a concomitant reduction of glutamatergic afferent input. With respect to arousal control, orexin may directly activate key wake-promoting neurons in the forebrain and brainstem (e.g., basal forebrain, TMN, VTA, LC, and DRN) through orexin and/or glutamate signaling (9). In fact, orexin neurons project to all of these regions and *in vitro* electrophysiology recordings have confirmed that neurons within these regions are directly excited by orexin. Orexin neurons may also suppress REM sleep through the activation (by orexin and/or glutamate release) of the TMN and LC (10) as well as by acting on the pontine REM circuit (11–12). In the pons, orexin neurons are thought to directly activate vlPAG/LPT REM-Off neurons (11) and to inhibit, likely through feed-forward inhibition, the SLD and PnO REM-On neurons (12). As discussed in the text, the pontine REM-On neurons of the SLD and the REM-Off neurons of the vlPAG/LPT are reciprocally inhibited through GABAergic projections (13). This reciprocal connectivity ensures rapid transition in and out of REM sleep. Blue and red arrows indicate excitatory and inhibitory inputs, respectively. Line arrow endings indicate synaptic inputs. Arrowhead endings indicate neuromodulation by peripheral signals or by behavioral/physiological states. AgRP, agouti-related peptide; BF, basal forebrain; DMH, dorsomedial hypothalamic nucleus; DRN, dorsal raphe nucleus; Dyn, dynorphin; Glut, glutamate; LC, locus coeruleus; LepRb, leptin receptors; LH, lateral hypothalamus; Ox, orexin; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVH, paraventricular hypothalamic nucleus; SLD, sublaterodorsal nucleus; TMN, tuberomammillary nucleus; vlPAG/LPT, ventrolateral periaqueductal grey matter and lateral pontine tegmentum; VTA, ventral tegmental area; ZI, zona incerta; α-MSH, α-melanocyte-stimulating hormone.

rhythm of REM sleep (Beuckmann et al., 2004; Chemelli et al., 1999; Hara et al., 2001; Kantor et al., 2009; Roman et al., 2018).

While the circuit mechanisms by which the orexin system suppresses REM sleep remain incompletely understood, a recent study has shown that orexin's REM suppressing effects might occur via inputs to the LC and TMN (Bourgin et al., 2000; Chen et al., 2010; Choudhary et al., 2014; Schwartz et al., 2016) (Table 2; Fig. 2; Pathway 10). While it is known that both the LC and TMN receive strong innervation from orexin neurons and that both LC and TMN neurons are directly excited by orexin (Eriksson et al., 2001; Horvath et al., 1999; Peyron et al., 1998), the precise mechanism by which orexin might inhibit REM sleep through these regions remains to be elucidated. Orexin neurons also project to pontine REM sleep generator neurons (Torterolo et al., 2013; Zhang et al., 2004) and antisense knockdown of Ox2Rs in this region results in more than a doubling of REM sleep in both the light and dark periods (Thakkar et al., 1999). Pharmacological studies have, however, produced conflicting results as injections of orexin into the same pontine region can also produce arousal responses, rather than suppression of REM sleep (Xi and Chase, 2010). One possible explanation for this apparent discrepant finding is spillover of orexin into neighboring regions of the pons that are potently wake-promoting, including the LC and parabrachial nucleus (PB) (Fuller et al., 2011; Gompf et al., 2015; Qiu et al., 2016).

Orexin neurons may also suppress REM sleep through the release of glutamate or orexin onto GABAergic neurons located in the pontine PnO and SLD regions (Torterolo et al., 2013; Zhang et al., 2004) (Table 2; Fig. 2; Pathway 12). Alternatively, or in combination, orexin neurons may suppress REM sleep by activating REM-Off neurons in the vlPAG/LPT (Boissard et al., 2003; Lu et al., 2006) (Fig. 2; Pathway 11). To this end, disruption of Ox2R signaling in the vlPAG/LPT region produces a ca. 30% increase in REM sleep and unusually long REM sleep episodes (Chen et al., 2013). Orexin projections to the vlPAG/LPT region are also thought to be particularly important during wakefulness in preventing activation of the pontine REM sleep muscle atonia circuit (Lu et al., 2006; Luppi et al., 2006). For example, in narcoleptic patients, a lack of excitatory orexin input to the vlPAG/LPT region may be responsible for the reduction of inhibitory tone onto SLD REM atonia neurons, in turn facilitating inappropriate activation of SLD neurons during wake, ultimately leading to the loss of postural muscle tone, i.e., cataplexy (Burgess and Scammell, 2012; Lu et al., 2006; Luppi et al., 2006).

9. Orexin promotes feeding and increases energy expenditure

Orexin neurons increase their firing in response to conditions of negative energy balance (Burdakov et al., 2005; Yamanaka et al., 2003) and central injection of orexin increases food intake even in sated animals (Dube et al., 2000; Ida et al., 1999; Jain et al., 2000; Lubkin and Stricker-Krongrad, 1998; Sakurai et al., 1998; Sartin et al., 2001; Yamanaka et al., 2000). Orexin's effects on feeding are comparable in

magnitude to the feeding responses elicited by ICV injections of MCH (Edwards et al., 1999; Ida et al., 1999; Sakurai et al., 1998). Administration of Ox1R antagonists reduces feeding in wild type mice and obese *ob/ob* mice, suggesting that orexin acts primarily via Ox1Rs to induce feeding (Haynes et al., 2000; Rodgers et al., 2001; White et al., 2005).

In addition to increasing food intake, orexin also increases energy expenditure, with the latter playing the apparent dominant role in determining overall energy balance. Increases in orexin signaling (*vis a vis* overexpression of Ox2R or using selective Ox2R agonist) also result in decreased body weight and prevent both diet-induced obesity and late-onset obesity in orexin double receptor knock out mice (Funato et al., 2009; Irukayama-Tomobe et al., 2017). In contrast, mice with disruption of the orexin system develop obesity despite eating less than their wild type littermates, suggesting reduced energy expenditure and/or a lower metabolic rate in these mice (Fujiki et al., 2006; Funato et al., 2009; Hara et al., 2001, 2005). Energy expenditure is comprised of three major components: basal metabolic rate, exercise (activity), and non-exercise thermogenesis. Mice that lack orexin neurons exhibit low physical activity (Hara et al., 2001) and central administration of OxA increases metabolic rate (Lubkin and Stricker-Krongrad, 1998), increases activity and non-exercise thermogenesis (Coborn et al., 2017), the latter of which may link to activation of a local GABAergic circuit (Kosse et al., 2017). Furthermore, consumption of a high-fat diet increases the bout lengths of inactivity and decreases spontaneous physical activity (i.e., unconscious non-volitional movements like fidgeting in humans), as well as attendant thermogenesis from these activities. Chemogenetic activation of orexin neurons prevents periods of inactivity following a high fat diet meal and can increase non-exercise thermogenesis during periods of spontaneous physical activity (Bunney et al., 2017). In agreement with the foregoing animal studies, human studies have found that narcoleptic patients eat less but tend to gain weight, and have a higher incidence of obesity compared to the average population (Schuld et al., 2000). Thus, while deficits in orexin signaling produces hypophagia, concomitant reductions in energy expenditure and metabolic rate that accompany low orexin tone are off-setting and lead to body weight gain and late-onset obesity.

10. Orexin promotes feeding through multiple neuronal pathways

Orexin neurons may stimulate feeding through intra-hypothalamic connections to the arcuate nucleus, paraventricular hypothalamic nucleus (PVH), dorsomedial hypothalamic nucleus (DMH), and within the LH (Date et al., 1999; Peyron et al., 1998) (Table 2; Fig. 2; Pathway 14–16). Although orexin infusion into all these regions stimulates feeding (Dube et al., 1999; Edwards et al., 1999; Muroya et al., 2004; Thorpe et al., 2003), the role of the arcuate nucleus in mediating orexin-induced feeding is arguably the best characterized (Fig. 2; Pathways 14–15).

Orexin induces feeding at the arcuate nucleus by activating orexigenic Neuropeptide Y (NPY) neurons (Fig. 2; Pathway 15). and by inhibiting the anorexigenic proopiomelanocortin (POMC) neurons (Fig. 2; Pathway 14). Central administration of orexin stimulates NPY whereas NPY antagonists reduce orexin-driven food intake (Dube et al., 2000; Jain et al., 2000; Yamanaka et al., 2000). In addition, orexin neurons densely innervate the arcuate nucleus, making appositions onto NPY neurons (Date et al., 1999; Muroya et al., 2004; Peyron et al., 1998), which themselves express Ox1Rs and are directly activated by orexin (Muroya et al., 2004; Suzuki et al., 2002; van den Pol et al., 2009; van den Top et al., 2004). While some studies (Dube et al., 2000; Jain et al., 2000) have found that NPY antagonists abolish the effects of orexin on feeding (i.e., suggesting that orexin mainly acts through an NPY-dependent pathway), other studies find only a partial blocking effect (Yamanaka et al., 2000) thus indicating that additional circuits are involved in mediating the effect of orexin on food intake.

Recent studies have suggested that orexin promotes feeding by inhibiting anorexigenic POMC neurons. For example, orexin-immunoreactive axon terminals innervate POMC neurons (Guan et al., 2001) and orexin inhibits POMC neuron firing in brain slices (Ma et al., 2007; Muroya et al., 2004), although the precise mechanism(s) by which orexin inhibit POMC neurons remains incompletely understood. While, for example, it is long established that POMC neurons express Ox1Rs and are inhibited by orexin (Ma et al., 2007; Muroya et al., 2004; Suzuki et al., 2002), it was initially unclear why POMC neurons express orexin receptors given that orexin acts nearly exclusively presynaptically. We now know that orexin inhibits POMC neurons through an increase of GABAergic afferent input and a concomitant reduction of glutamatergic afferent input (Ma et al., 2007). While orexin-mediated increases in GABA release onto POMC neurons is compatible with the presynaptic effects of orexin at other brain regions, how orexin would mediate the inhibition of glutamatergic input to POMC neurons remains unclear. Interestingly, it was recently shown that orexin activates Ox1Rs in POMC neurons, resulting in the biosynthesis and subsequent release of 2-arachidonoylglycerol (2-AG). Once released, 2-AG activates the endocannabinoid receptor 1 (CB1Rs) on the POMC neurons themselves which leads to a reduction in melanocortin production (Morello et al., 2016). One possibility, therefore, is that orexin inhibits glutamatergic input to POMC neurons through a similar mechanism. In fact 2-AG can act as an inhibitory retrograde messenger (Ohno-Shosaku and Kano, 2014) and it was recently shown that orexin inhibits GABAergic input to LC neurons through the release of 2-AG and the activation of presynaptic CB1R receptors (Kargar et al., 2018).

Orexin neurons may also promote feeding through the PVH (Fig. 2; Pathway 16), which is an important downstream circuit node through which both arcuate POMC and NPY neurons regulate hunger and satiety. For example, orexin neurons project to the PVH and ICV injection of orexin into the PVH robustly activates the PVH and arcuate nucleus (Date et al., 1999; Edwards et al., 1999). However, interpretative caution is warranted as the orexin effects on feeding seem to be primarily mediated by Ox1Rs (Sakurai, 2007) but PVH neurons only express Ox2Rs (Marcus et al., 2001). Hence, how orexin acts in the PVH and whether the PVH could be an additional target region through which orexin could stimulate food intake remains to be determined.

Taken together, the available data suggest that multiple neuronal circuits are likely involved in mediating orexin's feeding responses and that considerable additional work will be required to establish both the relative contribution of the circuits to orexin-driven feeding and the mechanisms by which orexin influences the activity of its postsynaptic target neurons. One common theme that does arise from these studies is that, by promoting both arousal and food intake, orexin helps ensure that appetite is coincident with arousal, the latter being a prerequisite to finding food.

11. Orexin neurons as sensors of metabolic states

Several studies have reported that fasting activates orexin neurons (Diano et al., 2003; Horvath and Gao, 2005; Yamanaka et al., 2003). This is likely the result of the combination of orexin neurons being intrinsically capable of sensing metabolic status (Burdakov et al., 2005; Yamanaka et al., 2003) and to neuronal inputs (Horvath and Gao, 2005) (Fig. 2). For example, results from electrophysiological recordings in brain slices have demonstrated that orexin neurons increase their firing in response to low glucose levels (Burdakov et al., 2005; Sheng et al., 2014; Yamanaka et al., 2003) (Fig. 2; Pathway 6). Orexin neurons directly respond to glucose (Burdakov et al., 2005; Sheng et al., 2014) and they sense glucose independently of glucose metabolism (Gonzalez et al., 2008, 2009). Extracellular glucose directly inhibits orexin neurons through the opening of K^+ conductances, resulting in membrane hyperpolarization and silencing of action potential firing (Burdakov et al., 2006). Additional work has demonstrated that orexin neurons can function as conditional glucosensors: orexin neurons are inhibited by glucose only when their cellular metabolic state is low, whereas their response to glucose is reduced in a dose-dependent manner by raising

intracellular levels of lactate, pyruvate and ATP (Venner et al., 2011). In addition, glucose-mediated inhibition of orexin neurons is transient as orexin neurons adapt quickly to a new glucose level (Williams et al., 2008), suggesting that maybe orexin neurons sense rapid changes in glucose, rather than the absolute concentration of glucose (Fig. 2; Pathways 6). Additional studies have proposed that the spontaneous activity of orexin neurons is maintained indirectly by glucose. Glucose is first metabolized into lactate by astrocytes, then astrocytes supply extracellular lactate which excites orexin neurons through the closure of K_{ATP} channels (Liu et al., 2011; Parsons and Hirasawa, 2010) (Fig. 2; Pathway 7). Importantly, impairing glucose trafficking between astrocytes silence orexin neurons in brain slices and, in whole animal studies, produces sleepiness and fragmentation of wakefulness including frequent transitions into NREM sleep. These results have been attributed to reduced astrocyte-derived lactate supply, in particular as local administration of lactate in the LH reverses these effects (Clasadonte et al., 2017). Another possibility is that glucose sensitivity of orexin neurons is bell-shaped. At levels above 1 mM, orexin neurons are directly inhibited by glucose (Fig. 2; Pathway 6) whereas at lower levels they sense glucose levels indirectly through lactate signaling (Parsons and Hirasawa, 2010) (Fig. 2; Pathway 7).

In addition to direct sensing of extracellular glucose levels, orexin neurons are also sensitive to other peripheral indicators of energy status (Fig. 2; Pathways 4–5 and 8). For instance, the satiety hormone leptin inhibits orexin neurons while the hunger hormone ghrelin activates the same neurons (Yamanaka et al., 2003). The inhibitory effect of leptin on orexin neurons, which do not express leptin receptors, are only indirect (Laque et al., 2013; Louis et al., 2010; Mickelsen et al., 2017; Sheng et al., 2014). Several studies have shown that leptin depresses glutamatergic afferent input to orexin neurons, which in turn reduces their firing (Goforth et al., 2014; Liu et al., 2017a), however the source of glutamatergic afferents that signal hunger to orexin neurons is not known. Furthermore, leptin-mediated inhibition of orexin neurons appears to require the activation of LH neurons that express leptin receptors (LepRb) and neurotensin and, furthermore, appears to be dependent upon galanin signaling (Goforth et al., 2014; Leinninger et al., 2009, 2011). In general support of this hypothesis, LH LepRb-expressing neurons are activated by leptin, some co-express the inhibitory peptide galanin, and their optogenetic activation produces GABA release onto orexin neurons (Bonnavion et al., 2015; Laque et al., 2013; Leinninger et al., 2009, 2011; Louis et al., 2010). Leptin could therefore inhibit orexin neurons by activating local LH LepRb neurons through the release of both GABA and galanin (Fig. 2; Pathway 8).

In contrast to leptin, ghrelin activates orexin neurons. Also unlike leptin, ghrelin appears to activate orexin neurons directly (Sheng et al., 2014; Yamanaka et al., 2003) likely through the activation of a non-selective cation conductance (Osterstock et al., 2010) (Fig. 2; Pathway 5). In addition, whereas leptin reduces the sensitivity of orexin neurons to glucose changes, ghrelin has an enhancing effect (Sheng et al., 2014).

Additionally, activation of the orexin neurons by negative energy balance can be mediated by neural inputs (Table 2). For example, the arcuate nucleus projects back to the orexin field and this pathway could be responsible for keeping hungry mice awake (Danguir and Nicolaidis, 1979; Diano et al., 2003; Sakurai et al., 2005; Yamanaka et al., 2003; Yoshida et al., 2006). Surprisingly, there is dense NPY, AgRP and α-MSH immunoreactive innervation onto orexin neurons but arcuate NPY and POMC neurons have opposite effects on food regulation (Elias et al., 1998). Furthermore, arcuate NPY neurons release GABA and NPY, which inhibits orexin neurons, so it is unlikely that they are responsible for activation of orexin neurons during fasting (Fu et al., 2004). Therefore, although the arcuate nucleus densely innervates the perifornical region where the orexin neurons are located, NPY innervation of the orexin neurons may originate from other brain regions (Elias et al., 1998).

In addition to activation of orexin neurons, fasting increases the expression of orexin mRNA (Cai et al., 1999; Sakurai et al., 1998) and increases glutamatergic afferent input to orexin neurons (Horvath and Gao, 2005) but the source of these glutamatergic afferents are not known. Furthermore, mice in which orexin neurons are ablated respond differently to food deprivation than their wild type littermates. Given that fasting increases wakefulness, reduces NREM sleep and increases exploratory food-seeking behaviors in wildtype, but not orexin neuron-ablated mice, it would appear that orexin neurons are required for driving arousal and foraging behaviors in response to hunger (Yamanaka et al., 2003). It is also interesting that orexin neuron-ablated mice are unable to fully express food anticipatory activity on a restricted feeding schedule (Akiyama et al., 2004; Mieda et al., 2004). Finally, fasting does not induce delays in REM sleep onset in orexin neuron-ablated mice as it does in wildtype mice (Yamanaka et al., 2003).

Overall, a large number of studies have shown that orexin neurons, vis-a-vis release of orexin, dynorphin and glutamate, are an important cell group for promoting arousal and explorative behaviors in response to negative energy and in anticipation of food availability. Moreover, the arousal-promoting responses of orexin appear to be mediated by Ox2Rs, whereas the role of orexin in feeding seems to be primarily mediated by Ox1Rs.

12. GABAergic neurons in the lateral hypothalamus

The LH contains several functionally heterogeneous GABAergic cell populations, which has been defined, and to a certain extent parsed, on the basis of expression of the GAD65, GAD67 or VGAT genes. Importantly, however, the presence of the GABA-synthesizing enzymes GAD65 and GAD67 are necessary but not sufficient conditions for GABAergic transmission. As reviewed by Munster-Wandowski and collegues, the GABAergic phenotype of neurons is defined by the expression of GAD65 or GAD67 as well as the presence of the GABA vesicular transporter, VGAT, and the plasma membrane GABA transporters, GAT (Munster-Wandowski et al., 2016). Recent findings have however challenged this definition as synaptic GABA release appears to occur even in neurons that lack GAD65, GAD67 and VGAT as these neurons take up GABA from the extracellular space through GATs and package GABA in synaptic vesicles though VMAT (Tritsch et al., 2012, 2014). Hence, the operational definition of a GABA-releasing neurons remains a bit fluid.

Lesions of the LH are known to produce hypersomnia whereas stimulation increases wakefulness, suggesting a primarily arousal-promoting role for the LH (Alam and Mallick, 2008; Choudhary et al., 2014; Gerashchenko et al., 2003). Consistent with this notion, both electrophysiological recordings and cFos studies have shown that a large number of neurons in the LH are active during wakefulness (Alam et al., 2002; Estabrooke et al., 2001; Koyama et al., 2003; Lee et al., 2005; Mileykovskiy et al., 2005). This includes a large percentage of recorded LH neurons that are maximally active during the cortical activation of both wakefulness and REM sleep, and an additional large percentage of LH neurons are maximally active during wakefulness and quiet during both NREM and REM sleep (Alam et al., 2002; Koyama et al., 2003; Lee et al., 2005). While orexin neurons clearly represent a portion of wake-active LH neurons, a considerable percentage of non-orexinergic LH neurons exhibit a wake-active profile as well. More specifically, the proportion of non-orexin vs orexin neurons activated during normal active wakefulness is approximately 1:1, but this increases to 1.3:1 during forced wakefulness and to 2:1 during pharmacologically-induced wakefulness (Estabrooke et al., 2001).

In agreement with these results, recent experimental work has found that LH contains at least two, possibly more, subpopulations of GABAergic wake-active neurons that are non-orexinergic and non-MCH. Chemogenetic activation of the first subpopulation of LH VGAT neurons produces sustained and uninterrupted wakefulness, including during the animals' normal resting phase (Venner et al., 2016), and the authors hypothesized the inhibition of sleep-active VLPO neurons as the

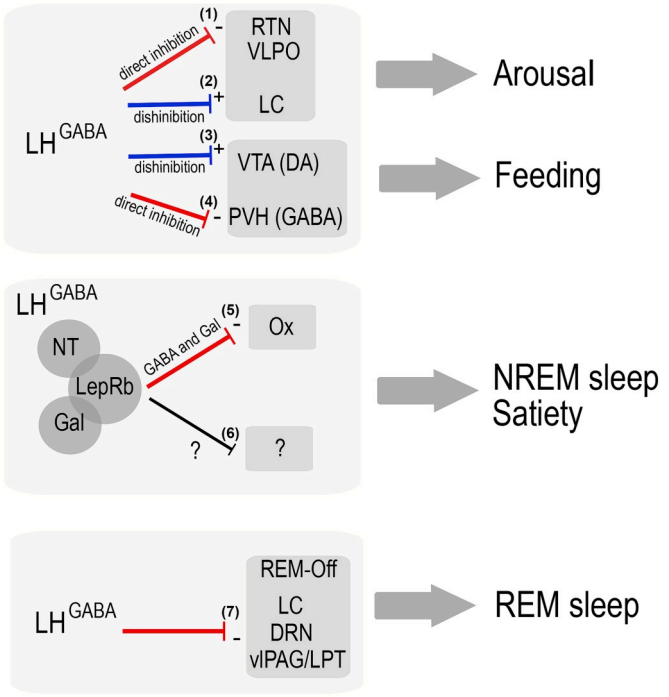

Fig. 3. LH GABAergic neurons. Different populations of LH GABAergic neurons coordinate the control of arousal, feeding and REM sleep. Activation of LH GABAergic neurons can promote arousal and feeding. It is unclear whether these two effects are mediated by the same population of LH GABAergic neurons, although separate populations are indicated. For example, direct inhibition of sleep-promoting VLPO neurons and RTN (1) and/or disinhibition of arousal neurons such as the LC (2) have been suggested as the circuit substrates mediating arousal, whereas feeding responses have been attributed to disinhibition of VTA dopaminergic neurons (3) and/or inhibition of PVH GABAergic neurons (4). The LH also contains GABAergic neurons that are active during sleep and are thought to inhibit wake-promoting neurons, including orexin neurons, through the release of GABA (5–6). These neurons could be the same as those promoting satiety, i.e., LH GABAergic neurons that express LepRb are activated by leptin and these neurons inhibit orexin neurons through GABA and galanin signaling (5). A subset of LH LepRb-expressing neurons also co-express neurotensin, however, and somewhat paradoxically, neurotensin is known to excite orexin neurons. The LH also contains GABAergic neurons that are active during REM sleep, and these neurons are thought to promote REM sleep by inhibiting pontine REM-Off neurons of the LC, DRN and vlPAG/LPT (7). Blue and red arrows indicate excitatory and inhibitory inputs, respectively. DA, dopamine; DRN, dorsal raphe nucleus; Gal, galanin; LC, locus coeruleus; LepRb, leptin receptors; NT, neurotensin; Ox, orexin; PVH, paraventricular hypothalamic nucleus; RTN, thalamic reticular nucleus; vlPAG/LPT, ventrolateral periaqueductal grey matter and lateral pontine tegmentum; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area.

circuit basis for this response (Fig. 3; Pathway 1). Experimental confirmation of this intriguing possibility is eagerly awaited. Optogenetic stimulation of the second subpopulation of GABAergic LH neurons rapidly arouses mice from NREM sleep and anesthesia and these responses were mediated by projections to the thalamic reticular nucleus (RTN) (Herrera et al., 2016) (Fig. 3; Pathway 1). Optogenetic activation of LH GABAergic terminals within the LC also produced rapid arousal from both NREM and REM sleep (Herrera et al., 2016) (Fig. 3; Pathway 2). Most recently, another investigative group found that chemogenetic activation of GAD2 (GAD65+) LH neurons produces 4 h of uninterrupted wakefulness during the inactive phase (Heiss et al., 2018), and that this effect was independent of orexin signaling. As activation of GAD2 LH neurons did not elicit greater cFos expression in orexin, RTN neurons, nor in the recently identified wake-promoting neurons of the supramammillary nucleus (Pedersen et al., 2017), as compared to waking controls, the authors concluded that GAD2 LH neurons must promote arousal through different efferent pathways than orexin, RTN and supramammillary neurons. While a sensible interpretation, it is also the case that chemogenetic activation of wake-promoting neurons might not necessary elicit greater cFos expression in afferent target neurons than seen during normal wakefulness. Therefore, whether LH GAD2 neurons are a distinct population of LH GABAergic neurons that act through different efferent pathways than the previously described wake-promoting LH VGAT neurons (Herrera et al., 2016; Venner et al., 2016) remains to be confirmed. Overall these results show that two or more subpopulations of LH GABAergic neurons, at least one of which expresses VGAT and/or GAD2, play an important role in promoting wakefulness and that the circuit effects mediating this response may be diverse and distributed.

Given the foregoing, it is interesting to note that chemogenetic and optogenetic stimulation of at least one subpopulation of LH VGAT positive neurons during wakefulness promotes feeding and food-seeking behaviors, reinforcing the concept of interactions between arousal and food seeking circuits within the LH (Carus-Cadavieco et al., 2017; Jennings et al., 2015; Navarro et al., 2016) (Table 3; Fig. 3; Pathways 3–4). Recent work has also shown that, while chemogenetic activation of LH VGAT neurons increases both food-seeking and food-consumption behaviors, activation of a galanin-containing subpopulation of LH VGAT neurons increases food-seeking behavior, but not an increase in food intake (Qualls-Creekmore et al., 2017), underscoring the functional diversity among LH VGAT subpopulations. It is still unclear whether arousal and food seeking are functions regulated by the same or different VGAT populations. Additionally, and as proposed recently, these two functions could operate in sequential order, with one subpopulation of VGAT neurons producing arousal and then another population eliciting food intake. Alternatively, activation of LH VGAT population(s) may simultaneously promote arousal, food seeking and consumption (Herrera et al., 2017) (Fig. 3; Pathways 1–4).

LH GABAergic neurons promote food intake through specific efferent pathways (Table 3). For example, optogenetic activation of LH VGAT neurons that project to the VTA increases feeding in satiated animals whereas inhibition of the same pathway reduces feeding in food deprived animals (Nieh et al., 2015, 2016). It is thought that these feeding responses are mediated by disinhibition of VTA dopaminergic neurons (Fig. 3; Pathway 3), possibly through inhibition of local GABAergic interneurons. This same pathway could also promote arousal as direct activation of VTA dopaminergic neurons rapidly arouses mice from sleep, and activation of LH VGAT input to the VTA could potentially disinhibit VTA dopaminergic neurons through the same local GABAergic interneurons (Eban-Rothschild et al., 2016; Nieh et al., 2015). Similarly, LH GABAergic projections to the PVH promote feeding through $GABA_A$ signaling (Wu et al., 2015) (Fig. 3; Pathway 4), but whether stimulation of the same pathway results in arousal responses has yet to be established.

Interestingly, optogenetic activation of LH LepRb-expressing neurons, which comprise a subpopulation of LH VGAT neurons, prolongs NREM sleep bouts (Herrera et al., 2016). A recent study also found that stimulation of LH LepRb neurons reduced corticosterone release in response to stress and this effect was attributed to inhibition of orexin neurons (Bonnavion et al., 2015). Taken together, these finding suggest that activation of LH LepRb neurons after food consumption could alleviate stress responses and promote sleep through mutual inhibition of orexin neurons (Fig. 3; Pathway 5).

While activation of all VGAT neurons has an overall arousal effect, it is conceivable that there are at least two, if not more, LH VGAT neuronal populations that play opposite roles in behavioral state control, and this would likely be mediated by separate efferent projections. Namely, one that is wake-promoting and suppresses the activity of sleep promoting regions or disinhibits wake-promoting neurons (Fig. 3; Pathways 1–2), and another that is sleep active and inhibits wake promoting neurons, including the orexin neurons (Bonnavion et al., 2015; Ferrari et al., 2018) (Fig. 3; Pathways 5–6). In support of this

Table 3
LH GABA neuronal pathways responsible for feeding and sleep-wake regulation. DRN, dorsal raphe nucleus; LC, locus coeruleus; LepRb, leptin receptors; LPT, lateral pontine tegmentum; NAc, nucleus accumbens; PnO, oralis pontine region; PVH, paraventricular hypothalamic nucleus; RTN, thalamic reticular nucleus; SLD, sublaterodorsal nucleus; vlPAG/LPT, ventrolateral periaqueductal grey matter and lateral pontine tegmentum; VLPO, ventrolateral preoptic area; VTA, ventral tegmental area; ZI, zona incerta.

Origin	Target	Effect on		Effect on	
		Feeding	References	Sleep & Wake	References
LH^{VGAT}	Preoptic			Produces uninterrupted wakefulness	Venner et al. (2016)
LH^{VGAT}	RTN			Arouses mice from NREM sleep and anesthesia	Herrera et al. (2016)
LH^{VGAT}	LC			Arouses mice from NREM and REM sleep	Herrera et al. (2016)
LH^{VGAT}	VTA	Promotes feeding	Nieh et al. (2015), Nieh et al. (2016)	Could promote arousal by disinhibiting VTA dopaminergic neurons	Eban-Rothschild et al. (2016)
LH^{VGAT}	PVH	Promotes feeding	Wu et al. (2015)		
LH^{VGAT}	LC, DRN, vlPAG/LPT			Could silence LC, DRN, and vlPAG/LPT REM-Off neurons during REM sleep	Verret et al. (2006), Clement et al. (2012)
LH^{LepRb}	LH^{Ox}	Could inhibit feeding	Leinninger et al. (2009), Leinninger et al. (2011), Goforth et al. (2014)	Might facilitate entry into NREM sleep	Bonnavion et al. (2015), Herrera et al. (2016)
LH^{LepRb}	LC			Might facilitate entry into NREM sleep	Laque et al. (2013), Herrera et al. (2016)

hypothesis, it was reported that optogenetic stimulation of LH VGAT terminals in the LC arouses mice from NREM sleep, likely by disinhibiting noradrenergic LC neurons, whereas stimulation of LH LepRb expressing neurons (cell bodies), which are have been shown to express VGAT (Bonnavion et al., 2015; Vong et al., 2011) and to project directly to noradrenergic LC neurons (Laque et al., 2015), facilitates entry into NREM sleep (Herrera et al., 2016). Hence, at least two LH VGAT subpopulations with different postsynaptic targets exist and they appear to play opposite roles in sleep and wake regulation (Herrera et al., 2016; Laque et al., 2013). Identification of additional cellular markers should permit the functional and anatomic parsing of these two VGAT neuronal populations.

Similar to the brainstem, the LH region contains a high density of neurons that are selectively active during REM sleep (Hassani et al., 2010; Koyama et al., 2003). Among these REM-On neurons some contain MCH and some have been identified as VGAT expressing neurons (Hassani et al., 2009, 2010; Verret et al., 2006). These LH GABAergic REM-active neurons send descending projections to the brainstem and they are thought to be responsible for silencing REM-Off neurons of the LC, DRN and vlPAG (Clement et al., 2012; Verret et al., 2006) (Table 3; Fig. 3; Pathway 7). In support of this model, studies in the late 1990s demonstrated that the LC is inhibited in REM sleep by GABAergic inputs, i.e., GABA release in the LC is increased in REM sleep and blocking GABA transmission prevents the silencing of LC neurons in REM sleep, restoring firing activity in LC noradrenergic neurons similar to that seen in waking (Gervasoni et al., 1998; Nitz and Siegel, 1997). While these data support the concept that a GABAergic input during REM sleep is responsible for silencing LC neurons, whether this is also the case for the DRN and the vlPAG, and whether LH GABAergic neurons are the source of this REM-On input, remains to be determined.

In addition to galanin, some LH VGAT neurons also express neurotensin and some co-express both peptides (Bonnavion et al., 2016; Laque et al., 2013). The neurotensin-expressing LH neurons are an interesting group of neurons, yet little is known about their potential role in sleep and wake regulation. It is known, for example, that LH neurotensin neurons project to and inhibit orexin neurons (Goforth et al., 2014); hence, these neurons could comprise, in part, the LH GABAergic neurons that promote sleep. However, the neurotensin peptide directly excites orexin neurons and neurotensin antagonists decrease wakefulness in wild type, but not in orexin neuron ablated, mice (Furutani et al., 2013). One possible explanation for these apparent conflicting results could be that neurotensin's wake-promoting action is mediated by neurotensin activation of orexin neurons, and that the source of neurotensin lies outside the LH. Alternatively, LH neurotensin neurons might be sleep promoting through the release of GABA or galanin or both (Goforth et al., 2014) (Fig. 3; Pathway 5). A recent study has also shown that chemogenetic inhibition of LH neurotensin neurons drastically reduces the anorectic effects of leptin and decreases motor activity (Brown et al., 2018). Given, however, that a large percentage of orexin neurons themselves express neurotensin (Furutani et al., 2013), it is possible that the effect of neurotensin on motor activity (or any sleep-wake changes) may link to a subpopulation of orexin neurons that express neurotensin (Brown et al., 2018).

13. Interaction among LH neurons

As discussed above, MCH, orexin and LH GABAergic neurons project to and interact with one another, yet the precise synaptic circuitry underlying the interaction among these LH neurons remain incompletely understood. Progress, however, is being made. For example, a recent study has shown that orexin neurons project to and activate LH GAD65 neurons, with activation of this pathway promoting locomotor activity (Kosse et al., 2017). It is tempting to suggest that these LH GAD65 neurons might be the same VGAT LH neurons that produce arousal when optogenetically or chemogenetically activated (Herrera et al., 2016; Venner et al., 2016). As also discussed in the foregoing, LH GABAergic neurons project to and inhibit orexin neurons (Bonnavion et al., 2015; Ferrari et al., 2018). These LH GABAergic neurons are thought to be a different population of LH GABAergic neurons from those promoting arousal and/or locomotor activity, in particular as some of these neurons express LepRb, are involved in satiation and may be sleep-promoting (Bonnavion et al., 2015; Herrera et al., 2016) (Fig. 3; Pathway 5). These LH GABAergic neurons also inhibit orexin neurons, and may also inhibit other wake-promoting neurons during sleep (Laque et al., 2015) (Fig. 3; Pathways 5–6).

The relationship between orexin and MCH neurons also remains somewhat unresolved. For example, MCH neurons are inhibited by most wake-promoting signals, but are excited by orexin (Apergis-Schoute et al., 2015; Bayer et al., 2005; Gao et al., 2003; van den Pol et al., 2004) (Fig. 1; Pathways 7 and 9). Optogenetic activation of orexin neurons inhibits MCH neurons through GABA release, although this effect appears to depend upon orexin-mediated activation of a GABAergic input which itself may originate from local LH GABAergic neurons or from GABAergic neurons outside of the LH (Apergis-Schoute et al., 2015) (Fig. 1; Pathway 8). It was also reported that optogenetic stimulation of orexin neurons inhibits MCH neurons by direct release of

GABA (Apergis-Schoute et al., 2015). This finding was particularly surprising given the absence of evidence that orexin neurons are capable of release, or at least canonical release, of GABA as they don't express VGAT (Mickelsen et al., 2017). Whether orexin neurons express GAT and VMAT and could reuptake GABA from the extracellular as shown in other systems (Tritsch et al., 2012, 2014) has not been established. Hence, additional work will be required to confirm release of GABA from orexin neurons. Moreover, whether and how LH GABAergic neurons control the activity of MCH neurons has yet to be established. Work by Apergis-Schoute and colleagues might suggest that LH GABAergic neurons project to and control the activity of MCH neurons and that LH GABAergic neurons could mediate the inhibition of MCH neurons when orexin neurons are active. As such, this provides additional supporting evidence that the physiological roles of the orexin and MCH systems are opposing (Apergis-Schoute et al., 2015).

14. Conclusion

In summary, it is clear that considerable and laudable strides have been made in the effort to elucidate the circuitry, transmitters and synaptic physiology by which MCH, orexin and GABA neurons of the lateral hypothalamus contribute to the regulation and integration of sleep-wake and energy homeostasis. It is equally clear that much remains unresolved and that additional experimental work directed at dissecting the cellular LH, in particular in the context of homeostatic regulation and integration of sleep-wake and energy metabolism, is eagerly awaited.

Conflicts of interest

The authors declare no competing financial interests.

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