Orexin and melanin-concentrating hormone (MCH) neurons reside in the lateral hypothalamic area (LHA) and regulate sleep and feeding behaviour in mammals. In rodents, orexin neurons are implicated in the regulation of wakefulness or palatable consumption, whereas MCH neurons are implicated in the regulation of rapid eye movement sleep episode duration or caloric consumption. This review explores the molecular, genetic and neuronal components of orexin and MCH signalling as mediators of arousal state transitions. These peptidergic signalling systems, which interconnect both with sleep centres in the LHA and feeding centres in the arcuate nucleus, may maintain the balance between sleep need and duration with hunger and food foraging.

Key words: orexin, melanin-concentrating hormone, lateral hypothalamic area, arcuate nucleus, sleep, feeding

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Introduction

Hypothalamic regulation of sleep and feeding

Sleep and feeding are goal-directed activities which mammals must perform to survive. Sleep deprivation and hunger are especially suitable for studying in animal models, as sleep and feeding are not evidently reliant on volition, emotion or intelligence (Le Doux, 2012; Sternson, 2013). While the requirement of feeding for survival is self-evident, there is also ample evidence from sleep deprivation studies which indicates that sleep is also required for mammalian survival (Rechtschaffen et al., 1983; Campbell and Tobler, 1984; Bentivoglio and Grassi-Zucconi, 1997). Sleep regulation involves a neuronal circuit known as the ascending arousal system (AAS), which includes projections to the neocortex from the brainstem and subcortical nuclei such as the lateral hypothalamic area (LHA). The importance of LHA neurons for arousal maintenance is demonstrated by the genetic knockout of neuropeptides released from LHA principal neurons, which can cause narcolepsy in many mammalian species (Lin et al., 1999). Feeding regulation also involves a neuronal circuit, here described as the feeding circuit, which includes input and output regions of the arcuate nucleus (ARC). The importance of ARC neurons for feeding behaviour is demonstrated by the ablation of ARC principal neurons, which can cause fatal starvation in adult rats (Luquet et al., 2005). Both the AAS and the feeding circuit are modulated by the neuropeptide-mediated activity of principal neurons in the LHA, namely orexin (also known as hypocretin) and melanin-concentrating hormone (MCH) neurons (see Table 1 for abbreviations). This review proposes that orexin and MCH neurons form significant inputs to the AAS and the feeding circuit to enable crosstalk between sleep and feeding regulation.

Orexin: structure and signalling mechanisms

There are two mammalian peptide isoforms of orexin, orexin-A (OX_A) and orexin-B (OX_B), which are each encoded...
### Table 1. Abbreviations in text and figures

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Ascending arousal system</td>
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<tr>
<td>ACh</td>
<td>Acetylcholine</td>
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<tr>
<td>AgRP</td>
<td>Agouti-related peptide</td>
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<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
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<tr>
<td>ARC</td>
<td>Arcuate nucleus</td>
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<tr>
<td>BF</td>
<td>Basal forebrain</td>
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<td>ChR2</td>
<td>Channelrhodopsin-2</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DA</td>
<td>Dopamine</td>
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<tr>
<td>dRN</td>
<td>Dorsal raphe nucleus</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>EOG</td>
<td>Electrococulography</td>
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<tr>
<td>EPSP</td>
<td>Excitatory postsynaptic potential</td>
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<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<tr>
<td>GAD65</td>
<td>Isoform 65 of glutamic acid decarboxylase</td>
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<tr>
<td>GAD67</td>
<td>Isoform 67 of glutamic acid decarboxylase</td>
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<tr>
<td>Glu</td>
<td>Glutamate</td>
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<tr>
<td>GPCRs</td>
<td>G protein-coupled receptors</td>
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<tr>
<td>His</td>
<td>Histamine</td>
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<tr>
<td>hMCHR1</td>
<td>Human melanin-concentrating hormone receptor 1</td>
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<tr>
<td>hMCHR2</td>
<td>Human melanin-concentrating hormone receptor 2</td>
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<tr>
<td>hOX1R</td>
<td>Human orexin 1 receptor</td>
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<tr>
<td>hOX2R</td>
<td>Human orexin 2 receptor</td>
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<tr>
<td>HPF</td>
<td>Highly palatable food</td>
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<tr>
<td>ICV</td>
<td>Intracerebrovascular</td>
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<tr>
<td>LC</td>
<td>Locus coeruleus</td>
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<td>LDT</td>
<td>Laterodorsal tegmentum</td>
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<td>LHA</td>
<td>Lateral hypothalamic area</td>
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<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
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<tr>
<td>N1</td>
<td>Non-rapid eye movement sleep 1; drowsy sleep</td>
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<tr>
<td>N2</td>
<td>Non-rapid eye movement sleep 2</td>
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<tr>
<td>N3</td>
<td>Non-rapid eye movement sleep 3; SWS</td>
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<td>MCH</td>
<td>Melanin-concentrating hormone</td>
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<tr>
<td>MCHR1</td>
<td>Melanin-concentrating hormone receptor 1</td>
</tr>
<tr>
<td>MCHR2</td>
<td>Melanin-concentrating hormone receptor 1</td>
</tr>
<tr>
<td>mEPSCs</td>
<td>Miniature excitatory postsynaptic currents</td>
</tr>
<tr>
<td>mnPO</td>
<td>Medial preoptic area</td>
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<tr>
<td>NA</td>
<td>Noradrenaline</td>
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<tr>
<td>NEI</td>
<td>Neuropeptide E-I</td>
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<tr>
<td>NGE</td>
<td>Neuropeptide G-E</td>
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<tr>
<td>NREMS</td>
<td>Non-rapid eye movement sleep</td>
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<tr>
<td>NTS</td>
<td>Nucleus of the solitary tract</td>
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<tr>
<td>OX_A</td>
<td>Orexin-A</td>
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<tr>
<td>OX_B</td>
<td>Orexin-B</td>
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<tr>
<td>OX/Chr2</td>
<td>Orexin/Channelrhodopsin-2</td>
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<tr>
<td>PAG</td>
<td>Periaqueductal grey</td>
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<tr>
<td>PB</td>
<td>Parabrachial nucleus</td>
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<tr>
<td>P-LC</td>
<td>Pre-locus coeruleus</td>
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<tr>
<td>Pmch</td>
<td>Pro-melanin-concentrating hormone</td>
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<tr>
<td>POMC</td>
<td>Proopiomelanocortin</td>
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<tr>
<td>PPT</td>
<td>Pedunculopontine tegmentum</td>
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<td>PVH</td>
<td>Paraventricular hypothalamus</td>
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<td>PVT</td>
<td>Paraventricular thalamus</td>
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<tr>
<td>REMS</td>
<td>Rapid eye movement sleep</td>
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<tr>
<td>RIP-Cre</td>
<td>Rat insulin promoter-cre</td>
</tr>
<tr>
<td>RPa</td>
<td>Raphe pallidus</td>
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<tr>
<td>SCN</td>
<td>Suprachiasmatic nucleus</td>
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<tr>
<td>SLD</td>
<td>Sublaterodorsal tegmentum</td>
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<tr>
<td>SWS</td>
<td>Slow-wave sleep; N3</td>
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<tr>
<td>TMN</td>
<td>Tuberomammillary nucleus</td>
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<tr>
<td>TRPMS</td>
<td>Long transient receptor potential channel 5</td>
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<tr>
<td>vIPAG</td>
<td>Ventrolateral periaqueductal grey</td>
</tr>
<tr>
<td>vIPPO</td>
<td>Ventrolateral preoptic area</td>
</tr>
<tr>
<td>vPAG</td>
<td>Ventral periaqueductal grey</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-Hydroxytryptamine</td>
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by one of the two exons of the prepro-orexin gene (Fig. 1 A–B) and formed after alternative splicing of the prepro-orexin peptide in mice and humans (Miyoshi et al., 2001). Both OXₐ and OX₈ can bind to orexin receptor 1 (OX₁R) and orexin receptor 2 (OX₂R) in vitro (Fig. 1D–E), which are G protein-coupled receptors (GPCRs) that can couple to multiple Gα-subtypes including Gαs, Gαi, Gαo and Gαq (Sakurai et al., 1998; Kim et al., 2004; Karteris et al., 2005). The orexin receptors have different affinity profiles for orexin peptide isoforms (Fig. 1C): OX₁R is selective by one order of magnitude for OXₐ over OX₈, whereas OX₂R has equal affinity for OXₐ and OX₈ (Sakurai et al., 1998). Orexin can facilitate excitatory postsynaptic potentials mediated by glutamate release from orexin neurons (Zhu et al., 2003) and can depolarize orexin neurons ex vivo (Yamanaka et al., 2010). Thus, orexin generally potentiates excitatory transmission mediated by orexin neuronal terminals.

**MCH: structure and signalling mechanisms**

Human MCH is one of three neuropeptides encoded by the Pmch gene (Pedeutour, Szpirer and Nahon, 1994) and can

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**Figure 1.** Orexin signalling. (A) Both orexin-A (OXₐ) and orexin-B (OX₈) are encoded by a single exon of the prepro-orexin gene. (B) Unlike OX₈, OXₐ has two disulphide bonds (black lines). Almost half (46%, black circles) of OX₈ peptide sequence is conserved with OXₐ peptide sequence. (C) OXₐ and OX₈ act via two GPCRs named OX₁R and OX₂R. OXₐ has twice the affinity for OX₁R than for OX₂R, whereas OX₈ has equal affinity for both OX₁R and OX₂R. (D) There are unique (white circles), conserved (black circles) and insertion (grey circles) regions in the peptide sequences of human OX₁R (hOX₁R) and OX₂R (hOX₂R). (E) Potential signalling mechanisms of rat OX₁R/OX₂R. Adapted from Tsujino and Sakurai (2013).
bind to two related GPCRs (Hawes et al., 2000; Wang et al., 2001), MCH receptor 1 (MCHR1) and MCH receptor 2 (MCHR2) (Fig. 2A–D). Only ‘higher order’ mammals such as ferrets, dogs and primates express functional MCHR2 (Tan et al., 2002). Rodent MCHR1 utilizes primarily Goi/o signalling but also other unidentified Go signalling pathways (Hawes et al., 2000) (Fig. 2E). All MCH neurons contain mRNA for isoform 67 of glutamic acid decarboxylase (GAD67) (Harthoorn et al., 2005; Elias et al., 2008; Rondini et al., 2010; Sapin et al., 2010), and in vitro MCH signalling decreases the frequency of miniature excitatory postsynaptic potentials (mEPSCs) (Gao and van den Pol, 2001). Thus, MCH potentiates inhibitory GABAergic transmission mediated by MCH neuronal terminals (Fig. 2).

**Figure 2.** MCH signalling. (A) The human prepro-MCH (PMCH) gene encodes neuropeptide G-E (NGE), neuropeptide E-I (NEI) and MCH. (B) NGE and NEI have no effect on MCH signalling; MCH is a non-adecapeptide containing one disulphide bond. (C) MCH binds with relatively similar binding affinity to both MCHR1 and MCHR2. (D) There are unique (white dots), conserved (black dots) and insertion (grey dots) sequences in the peptide sequences of human MCHR1 (hMCHR1) and MCHR2 (hMCHR2). (E) Potential signalling mechanisms of rat MCHR1, hMCHR1 and hMCHR2.
Sleep regulation

Definitions of sleep and the arousal circuit: AAS

Sleep is a global state that involves major changes in behaviour, consciousness and cognition. In mammals, sleep is composed of four distinct stages defined by cortical electroencephalography (EEG) activity: rapid-eye movement sleep (REMS) and three stages of non-rapid eye movement sleep (NREMS) named NREMS 1 (N1), NREMS 2 (N2) and NREMS 3 (N3) sleep (Fig. 3). The timing of REMS and NREMS is regulated by two general processes, homeostatic and circadian processes (Borbély, 1982). Homeostatic processes govern the rise of sleep pressure during wakefulness and the dissipation of sleep pressure during sleep. Homeostatic processes also govern sleep rebound, which is a compensatory increase in NREMS or REMS duration following sleep deprivation. Circadian processes govern sleep onset relative to an internal 24 h clock-like rhythm maintained by the suprachiasmatic nucleus (SCN), which can be entrained by cyclic environmental cues including light exposure.

Vertebrates can transition from one arousal state (wakefulness, NREMS or REMS) to another in response to changes in activity within the AAS (Saper, Scammell and Lu, 2005). Most AAS regions are either wake promoting or sleep promoting, with the latter class subdivided into those which promote REMS (REMS-on) and those which promote NREMS (REMS-off). The AAS is divided into thalamic and extrathalamic routes (Fig. 4): pedunculopontine (PPT) and laterodorsal tegmentum (LDT) projections to the thalamus, or midbrain projections through the reticular formation to the basal forebrain (BF) (Fuller and Lu, 2009). The extrathalamic system consists of the dorsal raphe nucleus (dRN), locus coeruleus (LC), LHA, tuberomammillary nucleus (TMN), ventral (vPAG) and ventrolateral (vlPAG) periaqueductal grey, and the ventrolateral (vlPO) and medial (mnPO) preoptic nucleus (see Table 1 for abbreviations). The extrathalamic route

Figure 3. Sleep: polysomnography, hypnogram, orexin and MCH neuronal recordings in a healthy individual. Wakefulness involves gamma (25–80 Hz) and beta (14–25 Hz) EEG waves; conscious relaxation involves alpha (8–13 Hz) waves. There are four stages of sleep across REMS and NREMS. During NREMS 1 (N1), theta waves (4–7 Hz) predominate, accompanied with a partial loss of consciousness. During NREMS 2 (N2), sleep spindles (series of 11–16 Hz waves which are usually maximal nearest the median wave) and K-complexes (sharp bi- or tri-phasic waveforms) predominate. During NREMS 3 (N3), also known as slow-wave sleep, slow, high amplitude delta (0–4 Hz) waves predominate. During REMS (grey column), also known as paradoxical sleep as (desynchronized) waves resemble wakefulness, distinct sawtooth wave (serrated 2–6 Hz waves), rapid eye movements (EOG) and motor atonia (EMG) recordings appear. Orexin neurons are active only during wakefulness. MCH neurons are active only during sleep, preferentially during REMS. No wake-to-REMS transitions occur in humans without narcolepsy.
contains regions essential for non-vegetative levels of arousal: cholinergic projections from the parabrachial nucleus (PB) and pre-locus coeruleus region (P-LC) (Fuller et al., 2011). Moreover, photostimulation of the BF evokes immediate transitions from only NREMS to either wakefulness or REMS (Han et al., 2014). Therefore, the extrathalamic route appears to be more involved with arousal state transitions than the thalamic route (Fig. 4). All arousal state transitions require activity from multiple AAS regions, as rodents with lesions of any single AAS region still experience wakefulness, NREMS and REMS (Fuller et al., 2011). In this manner, LHA neurons may synchronize the discharge of multiple AAS neuronal populations to increase the likelihood of arousal state transitions at times which are favourable to the homeostatic and circadian processes of sleep.

Orexin and MCH neurons as inputs of AAS flip-flop switches

Functional models of AAS circuitry have been used to explain arousal state transitions in mammals. The wake–sleep switch describes transitions between wakefulness and sleep (NREMS) (Saper, Chou and Scammell, 2001), and the REMS–NREMS switch describes transitions between NREMS and REMS during sleep (Lu et al., 2006). These models consist of mutually inhibitory connections, as seen in an electrical flip-flop circuit, which enable dichotomous transitions between arousal states.

The wake–sleep switch (Fig. 5A) consists primarily of reciprocal GABAergic inhibition between sleep-promoting preoptic nuclei (vIPo and mnPO) and wake-promoting cholinergic (LDT and PPT) and glutamatergic (P-LC and PB) brainstem nuclei, with additional wake-promoting input from monoaminergic nuclei (dRN, LC, TMN and vPAG). The wake–sleep switch may incorporate homeostatic sleep pressure in the form of the sleep–wake and REMS–NREMS switches.
of vlPO activity. Upon waking, wake-promoting regions directly inhibit the vlPO (Chou et al., 2002) and are activated concurrently by orexin neurons (Estabrooke et al., 2001). During sustained wakefulness, homeostatic sleep pressure may rise in the form of excitatory input from the mnPO to the vlPO (Gvilia et al., 2006; Suntsova et al., 2007; Saper et al., 2010; Hsieh et al., 2011), until the collective activity of sleep-promoting preoptic regions supersedes those of wake-promoting regions to evoke a wake-to-NREMS transition. The wake–sleep switch may also incorporate circadian processes in the form of projections from the SCN to both orexin neurons (Abrahamson, Leak and Moore, 2001) and preoptic area neurons (Deurivel and Semba, 2005). Orexin neurons may form an input and output of the SCN, as SCN ablation induces fluctuations in orexin cerebrospinal fluid (CSF) levels (Zhang et al., 2004) and OX₄₃ inhibits SCN neurons in vitro (Belle et al., 2014). Thus, orexin neurons may promote wakefulness by interacting with both homeostatic and circadian processes of sleep.

The REMS–NREMS switch (Fig. 5B) consists primarily of reciprocal GABAergic inhibition between REMS-off neurons in the vIPAG and REMS-on neurons in both the P-LC and the sublaterodorsal nucleus (SLD) (Lu et al., 2006). Here, SLD neurons disinhibit P-LC neurons to promote REMS-associated EEG (Fig. 5B). Moreover, SLD neurons also disinhibit medullary interneurons to promote REMS-associated motor atonia. Arousal state transitions within sleep are currently attributed to MCH neurons, which can inhibit vIPAG REMS-off neurons to initiate NREMS-to-REMS transitions (Saper et al., 2010; Jego et al., 2013).

The LHA has three distinct populations of principal neurons: orexin neurons, MCH neurons and neurons containing isoform 65 of glutamic acid decarboxylase (GAD₆₅) named ‘GAD₆₅ neurons’ (Karnani et al., 2013). Orexin neurons fire only during wakefulness (Lee et al., 2005), whereas GAD₆₅ and MCH neurons fire only during sleep and fire maximally during REMS (Hassani, Lee and Jones, 2009; Hassani et al., 2010). These firing profiles may be enforced by tonic activity from wake-promoting and sleep-promoting AAS regions, as histamine can inhibit MCH neurons potently in vitro (Parks et al., 2014). These firing profiles of LHA neurons enable a simplification of the flip-flop switches. Wake-to-NREMS transitions result from a flip in dominant activity from wake-promoting orexin neurons (wakefulness need) to sleep-promoting MnPO/vlPO neurons (sleep need) (Fig. 5A). NREMS-to-REMS transitions result from a flip in dominant activity from REMS-off vlPO neurons to REMS-on P-LC/SLD neurons and MCH neurons (Fig. 5B). Thus, orexin neurons prolong the duration of wakefulness and MCH neurons prolong the duration of REMS within the flip-flop models of AAS circuitry.

**Evidence for the role of orexin in sleep regulation**

Narcolepsy is a sleep disorder affecting ~1 in 2000 people worldwide and is characterized by excessive daytime sleepiness and recurrent wake-to-REMS transitions which rarely occur in normal individuals. Narcolepsy is due to a deficiency of orexin signalling, usually because of a loss of orexin neurons during late adolescence by an unknown (possibly autoimmune) mechanism. The majority of narcoleptic cases involve cataplexy, a symptom which involves a transient loss of muscle tone and consciousness following strong emotional responses. As cataplexy can be triggered by environmental cues and there is presently no evidence indicating that narcoleptic bouts have a circadian rhythm, this suggests that orexin mediates the homeostatic control of sleep.

What evidence suggests that narcolepsy is a behavioural manifestation of orexin deficiency? First, orexin deficiency produces narcoleptic phenotypes in many organisms. For example, there is a significant loss of orexin neurons in the brains of human narcoleptic patients (Peyron et al., 2000; Thannickal et al., 2000; Thannickal, Neinhuis and Siegel, 2009). Narcolepsy may occur due to a loss of orexin peptide signalling, as loss-of-function mutations of OX₂R DNA account for natural and experimental phenotypes of canine narcolepsy (Lin et al., 1999). Similarly, prepro-orexin−/− and OX₂R−/− mice have narcoleptic phenotypes (Chemelli et al., 1999; Willie et al., 2003). Furthermore, the overexpression of prepro-orexin induces an insomnia-like phenotype in zebrafish (Prober et al., 2006), whereas the ablation of zebrafish orexin neurons induces a narcolepsy-like phenotype (Elbaz et al., 2012). Second, an orexin CSF level lower (≤110 pg/mL) than normal (<200 pg/mL) is a cardinal symptom for human narcolepsy (Nishino et al., 2000), and orexin CSF concentrations correlate with the number of intact orexin neurons in rodents (Gerashchenko et al., 2003). Third, the onset of narcolepsy is correlated with a decrease in orexin CSF levels and weight gain, which suggests that the loss of orexin neurons leads to arousal and feeding disturbances in narcolepsy (Savvidou et al., 2013). In summary, narcolepsy demonstrates the requirement for orexin neurons to maintain wakefulness in mammals, including humans (van den Pol, 2000).

Orexin neurons have been studied in vivo via the photostimulation of channelrhodopsin-2 (ChR2) in transgenic rodent orexin neurons which co-express ChR2 (Ox/ChR2). The photostimulation of Ox/ChR2 neurons can activate fast AMPA receptor-mediated transmission at 40% of TMN neurons, which may govern the temporal precision of the wake-sleep switch (Schöne et al., 2012). Orexin neurons can induce awakening as the photostimulation of Ox/ChR2 neurons during the circadian inactive period (day) significantly decreases the latency between sleep onset and NREMS-to-wake or REMS-to-wake transitions relative to controls (Adamantidis et al., 2007), so long as rats are not sleep deprived for longer than 2 h (Carter et al., 2009). Moreover, no NREMS or REMS rebound occurs in sleep fragmented by Ox/ChR2 neuron photostimulation (Rolls et al., 2011). This favours the interpretation that orexin neurons ‘lower the arousal threshold’—the level of wake-promoting activity required for transitions from sleep to wakefulness—which
may be overridden by homeostatic sleep pressure (Adamantidis, Carter and de Lecea, 2010). This applies at least to orexin neuron projections to the LC, as the photoinhibition of LC neurons can prevent sleep-to-wake transitions evoked by the photostimulation of Ox/ChR2 neurons (Carter et al., 2012). In summary, optogenetic studies have confirmed the wake-promoting activity of orexin neurons.

Evidence for the role of MCH in sleep regulation

As MCH neurons fire predominantly during REMS (Hassani, Lee and Jones, 2009), they may be involved in prolonging REMS duration. This is supported by the hyperactive phenotype of MCHR−/− mice (Shimada et al., 1998), which exhibit a significant decrease in total REMS duration and REMS episode duration during fasting relative to controls (Willie et al., 2008). Moreover, the duration of REMS episodes is increased by MCH intracerebrovascular (ICV) injections, in a dose-dependent manner (Verret et al., 2003). In particular, MCH neurons appear to mediate homeostatic REMS rebound, as there is an increased c-Fos expression in MCH neurons in rats undergoing total (Modirrousta et al., 2005) or REMS-specific (Hanriot et al., 2007; Kitka et al., 2011) sleep deprivation relative to controls.

Two photostimulation studies have supported the ability of MCH neurons to promote REMS in vivo. Firstly, MCH neuron photostimulation at the onset of an REMS episode prolongs its duration (~45%), whereas MCH neuron photostimulation at the onset of an NREM episode increases the likelihood (~80%) of NREM-to-REMS transitions (Jego et al., 2013). Further investigation showed that REMS episode duration can be increased by the photostimulation of MCH terminals in the TMN and medial septum, which indicates that MCH neurons inhibit certain wake-promoting monoaminergic regions during REMS. Secondly, MCH neuron photostimulation during the circadian active period (night) significantly decreases the duration of wakefulness and increases the frequency of all transitions between arousal states other than REMS-to-NREMS transitions (Konadhode et al., 2013). In summary, optogenetic studies have indicated that MCH neuron signalling can potently increase the duration and frequency of REMS episodes.

Feeding behaviour

The feeding circuit

Feeding regulation involves ARC neuron responses to input signals such as leptin, as congenital leptin resistance is correlated with human obesity (Montague et al., 1997), and leptin can depolarize ARC neurons ex vivo (Cowley et al., 2001). Foraging, taste aversion and caloric intake involve a neuronal circuit here described as the feeding circuit (Fig. 6A–C), which includes connections to and from the three GABAergic neuronal populations of the ARC: agouti-related peptide/neuropeptide Y (AgRP) neurons, proopiomelanocortin (POMC) neurons and rat insulin promoter-cre (RIP-Cre) neurons.

The photostimulation of AgRP neurons evokes foraging and voracious feeding behaviour in rats, which indicates that these neurons may determine hunger (Aponte, Atasoy and Sternson, 2011). To date, six axon projections are known to evoke feeding when photostimulated (Fig. 6B), four of which are inhibitory AgRP neuronal projections to other feeding circuit regions (Betley et al., 2013). The largest subpopulation of AgRP neurons projects to oxytocinergic neurons of the paraventricular hypothalamus (PVH). As food intake quantities evoked by AgRP neuron photostimulation are similar to those evoked by the chemogenetic silencing of PVH neurons, the connectivity between AgRP and PVH neurons may be particularly important for feeding (Atasoy et al., 2012). This is supported by the additional presence of projections from the PVH to the AgRP, which evoke feeding when chemogenetically and optogenetically stimulated (Krashes et al., 2014). The photostimulation of AgRP neuronal projections to the LHA can also evoke feeding; however, the LHA neuronal populations involved remain unidentified (Betley et al., 2013).

Adult rats undergo fatal starvation following AgRP neuron ablation (Luquet et al., 2005), which signifies the
requirement of AgRP neurons for feeding. Fatal starvation due to AgRP neuron ablation requires PB activity, as the pharmacological inhibition of PB neurons following AgRP neuron ablation enables a full recovery of food intake and body weight (Wu, Boyle and Palmiter, 2009; Wu, Clark and Palmiter, 2012). The ablation of AgRP neurons may disinhibit PB neurons, as AgRP neurons inhibit oxytocinergic PVH neurons during food deprivation (Atasoy et al., 2012), and oxytocinergic PVH neurons mediate leptin-induced weight loss via projections to the PB (Perello and Raingo, 2013). Therefore, AgRP input to the PVH may be critical to the regulation of body weight (Fig. 6B–C). The ablation of AgRP neurons may also disinhibit POMC neurons—the second neuropeptidergic population of ARC neurons—as AgRP neuron photostimulation elicits a strong GABAergic inhibition of POMC neurons (Atasoy et al., 2012). The photostimulation of POMC neurons can decrease food intake and body weight (Aponte, Atasoy and Sterrnson, 2011), which indicates that like the PB, POMC neurons mediate taste aversion, perhaps in response to excessive food quantities or toxins (Jensen et al., 1990; Halatchev and Cone, 2005; Niikura et al., 2013).

The photostimulation of RIP-Cre neurons can increase energy expenditure (oxygen intake and weight loss) without altering food intake significantly (Kong et al., 2012). This increase in energy expenditure involves a change in brown adipose tissue (BAT) activity mediated by RIP-Cre neurons, which monosynaptically innervate PVH neurons projecting to the nucleus of the solitary tract (NTS) (Fig. 6C). As the raphe pallidus (RPa) regulates BAT activity (Morrison and Nakamura, 2011), RIP-Cre neurons may coordinate autonomic responses associated with feeding via NTS input to RPa.

Orexin and MCH neurons as inputs of the feeding circuit

Contrary to the RIP-Cre neuronal pathway, which may adjust energy expenditure relative to food intake (Fig. 6C), LHA connectivity with the ARC may adjust food intake relative to energy demands (Fig. 5A). First, orexin neurons project directly to the ARC (de Lecea et al., 1998; Date et al., 1998), where many AgRP and POMC neurons co-express the leptin receptor and OX₁R (Funahashi et al., 2003). Orexin neurons can depolarize AgRP neurons (van den Top et al., 2004; Wu et al., 2013) and POMC neurons (Guan et al., 2001; Burdakov, Liss and Ashcroft, 2003; Acuna-Goycolea and van den Pol, 2009), whereas leptin can decrease the firing rate of both ARC (Rauch et al., 2000) and orexin neurons (Jo et al., 2005). Moreover, the overexpression of orexin peptide provides resistance to hyperglycaemia and obesity due to a leptin-dependent increase in energy expenditure (Funato et al., 2009). Thus, the opposition between (orexigenic) orexin and (anorexigenic) leptin signalling to ARC neurons may regulate food intake relative to energy demands. Second, orexin neurons can be hyperpolarized in vitro via glucose (Burdakov et al., 2005, 2006) and MCH neurons can be depolarized in vitro via glucose (Kong et al., 2010), which indicate that rising blood glucose levels may reduce feeding via the hyperpolarization of excitatory (orexin neurons) and depolarization of inhibitory (MCH neurons) input to AgRP neurons. However, it is currently unknown whether MCH neurons project to the ARC. Third, AgRP and POMC neurons project to both orexin and MCH neurons (Elias et al., 1999), and there is in vitro evidence that AgRP neurons inhibit MCH neurons (Hintermann et al., 2001; van den Pol et al., 2004). In summary, the interconnectivity between LHA and ARC neurons indicates that LHA neurons may be significant inputs to the feeding circuit (Fig. 5A).

Orexin neurons motivate palatable consumption

Orexin neurons may not significantly regulate normal feeding, as prepro-orexin⁻/⁻ and wild-type mice consume similar quantities of chow (Sharf et al., 2010). However, the photostimulation of neuronal terminals from the bed nucleus of the stria terminalis (aBNST) to glutamatergic LHA neurons can evoke the consumption of highly palatable food (HPF) in well-fed mice (Fig. 6B) (Jennings et al., 2013). This indicates that orexin neurons mediate the excessive consumption of HPF. Excessive HPF consumption relies on orexin peptide signalling, as a selective OX₁R antagonist or a dual OX₁R/OX₂R antagonist, but not a selective OX₂R antagonist, can reduce HPF intake significantly without altering chow intake (Piccoli et al., 2012). This is further supported by numerous studies wherein OX₁R antagonists reduce the self-administration of sucrose (Akiyama et al., 2004; Choi et al., 2010; Jupp et al., 2011; Cason and Aston-Jones, 2013). These findings indicate that selective OX₁R antagonists are potential pharmacological candidates for treating compulsive eating disorder, although the neuronal populations or receptor mechanisms by which OX₁R activation mediates excessive HPF consumption remain to be investigated.

Orexin may also modulate arousal in response to HPF consumption. The replacement of regular chow with HPF increases the frequency of cataplexic events in narcoleptic prepro-orexin⁻/⁻ mice (Clark et al., 2009; Oishi et al., 2013). The genetic ablation of orexin neurons in fasting mice causes a decrease in wakefulness during the circadian inactive period (day) relative to fasting wild-type controls (Yamanaka et al., 2003). This suggests a functional overlap between LHA circuits which regulate hunger and sleep. Orexin may maintain arousal in animals starving during circadian inactive periods to prioritize starvation as a greater survival threat than sleep deprivation or an attack from predators (Sakurai, 2007). Orexin may prevent fatal starvation also during hypersomnia, as low glucose levels after prolonged sleep could disinhibit wake-promoting orexin activity to reinstate foraging.

MCH neurons motivate caloric consumption

Equivalent doses of MCH and OX₁R ICV injections evoke similar increases in food intake (Edwards et al., 1999). However, unlike orexin and NPY mRNA, rat MCH mRNA expression
Research article

Bioscience Horizons • Volume 7 2014

does not rise during the consumption of a non-caloric sweetener such as saccharin (Furudono et al., 2005). Moreover, MCH may not reinforce non-caloric feeding, as the systemic administration of an MCHR1 antagonist (GW803430) can reduce glucose-reinforced, but not saccharin-reinforced, lever pressing (Karlsson et al., 2012). This indicates that MCH neurons respond to the caloric density, but not the palatability, of ingested food.

Recent developments in mammalian taste perception suggest that MCH neurons regulate a taste-independent preference for caloric feeding. Sweet taste requires receptor signalling by long transient receptor potential channel 5 (TRPM5), as many sweet caloric and non-caloric compounds fail to induce action potentials in major nerves innervating the taste receptors of trpm5−/− mice (Zhang et al., 2003). However, the sweet-blind phenotype of trpm5−/− mice maintains a preference for ingesting (caloric) sucrose over (non-caloric) sucralose which is correlated with a post-ingestion release of dopamine in the ventral tegmental area (de Araujo et al., 2008). A recent optogenetic study has identified that this caloric-specific preference and dopaminergic activity is absent in trpm5−/− mice lacking MCH neurons (Domingos et al., 2013). These results indicate that MCH neurons are essential to the taste-independent reinforcement of caloric intake. In summary, during low blood glucose levels, orexin neurons motivate HPF consumption, whereas MCH neurons motivate ongoing caloric consumption.

Concluding remarks

Future directions

Although the experimental manipulation of orexin and MCH neurons can influence arousal and feeding behaviour (Fig. 7), many details remain unaddressed. Although there is evidence for a direct activity of orexin (Zhu et al., 2003; Yamanaka et al., 2010; Aracri et al., 2013) and MCH (Gao and van den Pol, 2001) in synaptic transmission, it is unknown whether synchronous neurotransmitter release is required for neuropeptide release. A direct quantification of neuropeptide contributions to postsynaptic currents would also be helpful to determine whether they are independently capable of depolarizing postsynaptic neurons. Additionally, the postsynaptic targets of GAD65 neurons and RIP-Cre neurons remain to be characterized.

With regard to sleep, the further use of optogenetic and chemogenetic approaches could enable a more detailed investigation of arousal state transitions. For example, vlPO photostimulation could directly support the wake–sleep switch model (Fig. 5A), and vlPAG photostimulation could directly support the REMS–NREMS switch model (Fig. 5B). The photostimulation of SCN inputs to orexin and MCH neurons could also help determine to what extent the wake–sleep switch and REMS–NREMS switch is influenced by circadian processes (Abrahamson, Leak and Moore, 2001). Furthermore, these methods could test new hypotheses of sleep regulation, such as whether narcoleptic wake–REMS transitions result from the disinhibition of MCH neurons following the loss of orexin neurons. Finally, these methods could study how external stimuli may influence arousal, as rodent LC (Hickey et al., 2014) and dRN (Ito et al., 2013) photostimulation appears to elevate arousal during nociception.

With regard to feeding, ChR2-assisted or chemogenetic-assisted circuit mapping of the interconnectivity between LHA and ARC neurons is required to directly support LHA neurons as inputs to ARC neurons (Fig. 6). The ablation or temporary inactivation of the six axonal projections known to evoke feeding in rodents would clarify which, if any, are required for feeding (Fig. 6B). The anterograde tracing of AgRP neurons may identify which projections to the PB are required for survival. Finally, it is unknown whether the photostimulation of orexin neurons in trpm5−/− sweet-blind mice would be sufficient to induce a preference for HPF diets.

Orexin and MCH neurons regulate arousal with respect to hunger

A healthy animal avoids sleep deprivation and hunger by sleeping soon after eating and eating soon after awakening.
This behavioural strategy may be regulated by orexin and MCH neurons, as these neurons have prominent roles in the neuronal circuits which regulate sleep and feeding. Within the AAS, orexin neurons prolong wakefulness duration (Fig. 5A), whereas MCH neurons prolong REMS duration (Fig. 5B). Within the feeding circuit, orexin and MCH neurons promote feeding via interactions with ARC neurons (Fig. 6) such that orexin neurons motivate palatable food consumption, whereas MCH neurons motivate caloric food consumption. Here, the interconnectivity between the LHA and ARC may together coordinate the duration of sleep and feeding relative to the priority (starvation vs. sleep deprivation) of ongoing threats to the survival of an animal. The reliance of neuronal circuits that govern distinct behavioural programs—sleep and feeding—on regulation by a common set of neuropeptidergic systems—orexin and MCH—may facilitate the crosstalk between these circuits that enables one action to be performed over the other. In conclusion, orexin and MCH neurons may coordinate the alternation between sleep and feeding to minimize the survival threat of falling or remaining asleep during severe starvation.

Author biography

I am a fourth-year M.Sc. Neuroscience student at University College London (UCL) pursuing a career in neuroscience research. In particular, I aim to become familiar with advanced electrophysiological and optical imaging techniques to facilitate hypotheses which probe the neurophysiology of mammalian behaviours, such as sleep and feeding. I am applying for neuroscience PhD programmes.

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