Orexin/Hypocretin and Organizing Principles for a Diversity of Wake-Promoting Neurons in the Brain

Cornelia Schöne and Denis Burdakov

Abstract An enigmatic feature of behavioural state control is the rich diversity of wake-promoting neural systems. This diversity has been rationalized as 'robustness via redundancy', wherein wakefulness control is not critically dependent on one type of neuron or molecule. Studies of the brain orexin/hypocretin system challenge this view by demonstrating that wakefulness control fails upon loss of this neurotransmitter system. Since orexin neurons signal arousal need, and excite other wake-promoting neurons, their actions illuminate nonredundant principles of arousal control. Here, we suggest such principles by reviewing the orexin system from a collective viewpoint of biology, physics and engineering. Orexin peptides excite other arousal-promoting neurons (noradrenaline, histamine, serotonin, acetylcholine neurons), either by activating mixed-cation conductances or by inhibiting potassium conductances. Ohm's law predicts that these opposite conductance changes will produce opposite effects on sensitivity of neuronal excitability to current inputs, thus enabling orexin to differentially control input-output gain of its target networks. Orexin neurons also produce other transmitters, including glutamate. When orexin cells fire, glutamate-mediated downstream excitation displays temporal decay, but orexin-mediated excitation escalates, as if orexin transmission enabled arousal controllers to compute a time integral of arousal need. Since the anatomical and functional architecture of the orexin system contains negative feedback loops (e.g. orexin → histamine → noradrenaline/serotonin—orexin), such computations may stabilize wakefulness via integral feedback, a basic engineering strategy for set point control in uncertain environments. Such dynamic

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behavioural control requires several distinct wake-promoting modules, which perform nonredundant transformations of arousal signals and are connected in feedback loops.

Keywords Arousal • Brain state • Control theory • Hypocretin • Hypothalamus • Neurons • Orexin

Contents

1 Postsynaptic Actions of Orexin/Hypocretin Mediate Arousal Control 52
2 Sites and Biophysics of Postsynaptic Actions of Orexin/Hypocretin Neurons 55
3 Which Orexin/Hypocretin-Regulated Sites Are Critical for Wake Stability in the Normal Brain? 61
4 Mapping Orexin/Hypocretin Biology onto Control Operations 64
5 Explanatory and Predictive Value of Viewing Orexin/Hypocretin Actions as Control Computations 65
6 Overview, Omissions and Future Perspectives 67
References 68

1 Postsynaptic Actions of Orexin/Hypocretin Mediate Arousal Control

Efficient behaviour requires optimal adjustment of behavioural state (i.e. arousal, wakefulness, activity, energy expenditure) to perturbations in the environment. The environmental perturbations come in many diverse types, which differ greatly in their speed and predictability, necessitating an evolution of behavioural control systems that can deal with this perturbation diversity. A fundamental example of a slow and predictable perturbation in the environment is the ca. 24 h day-night cycle on Earth. The hypothalamic suprachiasmatic nucleus (SCN) adjusts behaviour to this cycle by emitting sinusoidal (ca. 24 h period) neural signals that schedule behavioural activity for either the day (for diurnal animals) or the night (for nocturnal animals), depending on specific survival advantages afforded to different animals by being active during light or dark. These slow daily rhythms of behavioural activity collapse upon description of the SCN [1].

However, wakefulness also needs to be controlled on a much more rapid and unpredictable timescale than that controlled by the SCN. Most of us take this rapid wakefulness adjustment for granted, assuming that we will not fall asleep in the middle of laughing or talking. This is not so for patients suffering from the sleep-wake disorder narcolepsy, which affects about 1:2000 people and where sleep and paralysis suddenly and uncontrollably intrude into normal wakefulness [2, 3]. Most cases of human narcolepsy are associated with reduced levels of orexin/hypocretin peptides in the CSF and lack of central orexin/hypocretin-producing neurons in the brain [4–7]. Loss of orexin/hypocretin peptides in humans, dogs, mice and rats

impairs arousal control, resulting in abnormally frequent and rapid loss of consciousness ('sleep attacks'). It seems that without the orexin/hypocretin system, wakefulness is prone to instability in the face of rapid perturbations in the environment, and processes enabled by orexin/hypocretin cells keep this instability under control. Orexin/hypocretin and the SCN systems are thus two hypothalamic systems that are essential for appropriate matching of behavioural state to the environment on rapid and slow timescales, respectively.

The orexin/hypocretin cells act as controllers rather than critical generators of wakefulness: with them, the average amount of arousal (sleep and waking) does not change, but the ability to control arousal based on salient environmental set point is impaired [8, 9]. Coordination of many other behaviours, such as reward-seeking, also critically relies on orexin/hypocretin neurons [10–12], but since these behaviours are wakefulness dependent, it is often unclear to what extent these effects are secondary to wakefulness control. Since the discovery of orexin/hypocretin neurons, a key question has been why they are so critical for arousal control, considering the numerous other arousal-controlling neurons in the brain (e.g. noradrenaline, histamine, acetylcholine, serotonin cells). Here, we review the biological properties of brain orexin/hypocretin circuits related to wakefulness/arousal, with particular emphasis on postsynaptic actions of orexin/hypocretin peptides and from a viewpoint of basic principles of signal processing and dynamic set point control. For more comprehensive overviews of orexin/hypocretin physiology, we refer the reader elsewhere [10, 13].

In this article, we will take the view that orexin/hypocretin neurons signal arousal need (or, in control system language, arousal error – see below). We define arousal need as a need to counteract actual or potential dangers such as low energy levels, high CO_2 levels, or potentially threatening sensory stimuli (e.g. sudden sounds, presence of another animal). Orexin neurons sense all these signals (Fig. 1), and thus their activity represents a sum of diverse 'arousal demands' (e.g. they are inhibited by glucose but excited by H_3O^-/CO_2) [8, 14–18]. Orexin/hypocretin neurons are also inhibited by at least some of the other wakefulness-promoting transmitters such as serotonin and noradrenaline, i.e. transmitters that may represent the actual level of arousal [19–21] (discussed below). Thus, orexin/hypocretin cell output may represent an 'arousal error' (actual arousal minus required arousal), thereby signalling how much arousal should be increased. In the absence of these orexin/hypocretin signals, arousal is no longer appropriately coupled to internal and external environment, which is an alternative way of describing sleep-wake instability.

The wake-sleep instability seen upon loss of orexin/hypocretin-producing neurons is recapitulated by the loss of orexin/hypocretin type 2 receptors or of orexin/hypocretin peptides [22–24]. This suggests that postsynaptic actions of orexin/hypocretin peptides on orexin/hypocretin type 2 G-protein-coupled receptors are responsible for arousal control mediated by orexin/hypocretin cells. This also suggests that orexin type 2 receptor-independent actions of orexin/hypocretin cells, such as those mediated by their other transmitters (glutamate, dynorphin, Narp) or by orexin/hypocretin via type 1 receptors, are insufficient to achieve

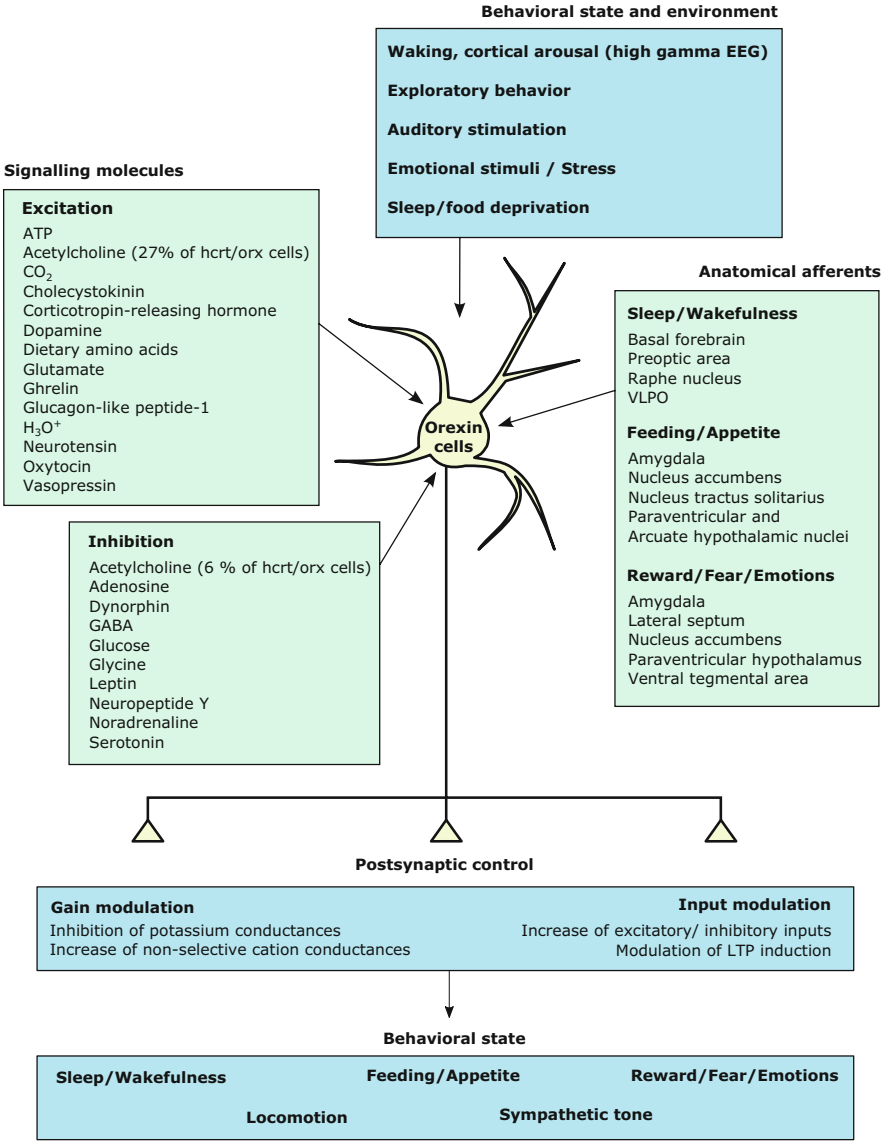

Fig. 1 Input and outputs of orexin/hypocretin cells. Orexin/hypocretin neurons are activated during high vigilance states associated with high gamma EEG, requiring increased arousal such as exploratory behaviour or upon sensory or emotional stimulation. A multitude of excitatory and inhibitory substances modulates orexin/hypocretin cell activity. These include hormones, neuropeptides and small molecule transmitters as well as homeostatic signals. Orexin/hypocretin neurons receive direct inputs from brain areas involved in sleep/wake control, appetite control and reward. Orexin/hypocretin neurons integrate this information via the release of orexin peptides, affecting postsynaptic gain and synaptic drive in target neurons. Orexin/hypocretin activity may thereby gate relevant information based on environmental and homeostatic needs [14, 16, 124–129]

proper arousal control without orexin/hypocretin action of type 2 orexin/hypocretin receptors. The latter point may seem surprising, considering that some arousal-promoting neurons, such as noradrenaline neurons of the LC which are important for orexin/hypocretin-induced wakefulness [25], are excited by orexin/hypocretin via type 1 rather than type 2 OX receptors [26]. In contrast, other arousal-promoting neurons, such as histamine cells of the tuberomammillary hypothalamus, are excited by orexin/hypocretin via type 2 receptors [26, 27]. Below, we catalogue this biological complexity of postsynaptic actions of orexin/hypocretin cells in more detail and comment on some functional biophysical implications of this complexity. Then, we propose a framework that simplifies and generalizes the diversity of postsynaptic orexin/hypocretin actions into a control systems model that accounts for general dynamic features of orexin/hypocretin-dependent arousal and offers an organizing principle for the puzzling diversity of wakefulness-promoting neurons in the brain.

2 Sites and Biophysics of Postsynaptic Actions of Orexin/Hypocretin Neurons

From their location in the lateral hypothalamus, orexin/hypocretin cells project axons to the entire brain [28, 29] (Fig. 2). The anatomical distribution of these projections largely mirrors that of two G-protein-coupled receptors for orexin [30]. Increased firing rate of orexin/hypocretin neurons produces awakening [31], and most of the brain's classical arousal-related systems are innervated by orexin/hypocretin axons and excited by orexin/hypocretin peptides (see Table 1, which lists many key findings alongside corresponding references [19, 26, 27, 32–106]). Orexin/hypocretin peptides also modulate neuronal activity in brain areas related to eating, emotion, autonomic function and motor control (Table 1). Here, we only list (Table 1) but do not discuss the latter actions of orexin/hypocretin in detail, since this has been covered extensively in recent publications (e.g. [10, 107]). We would just like to note that combined activation of arousal and reward systems may ensure that a heightened arousal accompanies reward-seeking, thereby increasing probability of reward discovery and of avoiding danger while exploring for rewards. Also, arousal and exploration may require motivational signals, since these behaviours are not intrinsically rewarding but are energy expending and potentially dangerous. Orexin/hypocretin may provide this motivation [10, 108], at least until reward consumption beings [109].

From a biophysical perspective on signal processing, the excitatory/depolarizing actions of orexin/hypocretin on central neurons (Table 1) can be divided into those increasing membrane conductance (e.g. activation of non-selective cation currents) and those decreasing it (e.g. inhibition of K^+ currents). This has profound implications for input processing capabilities of orexin/hypocretin-modulated neurons. The ability of a current input (I) to change membrane potential (V) is inversely related to

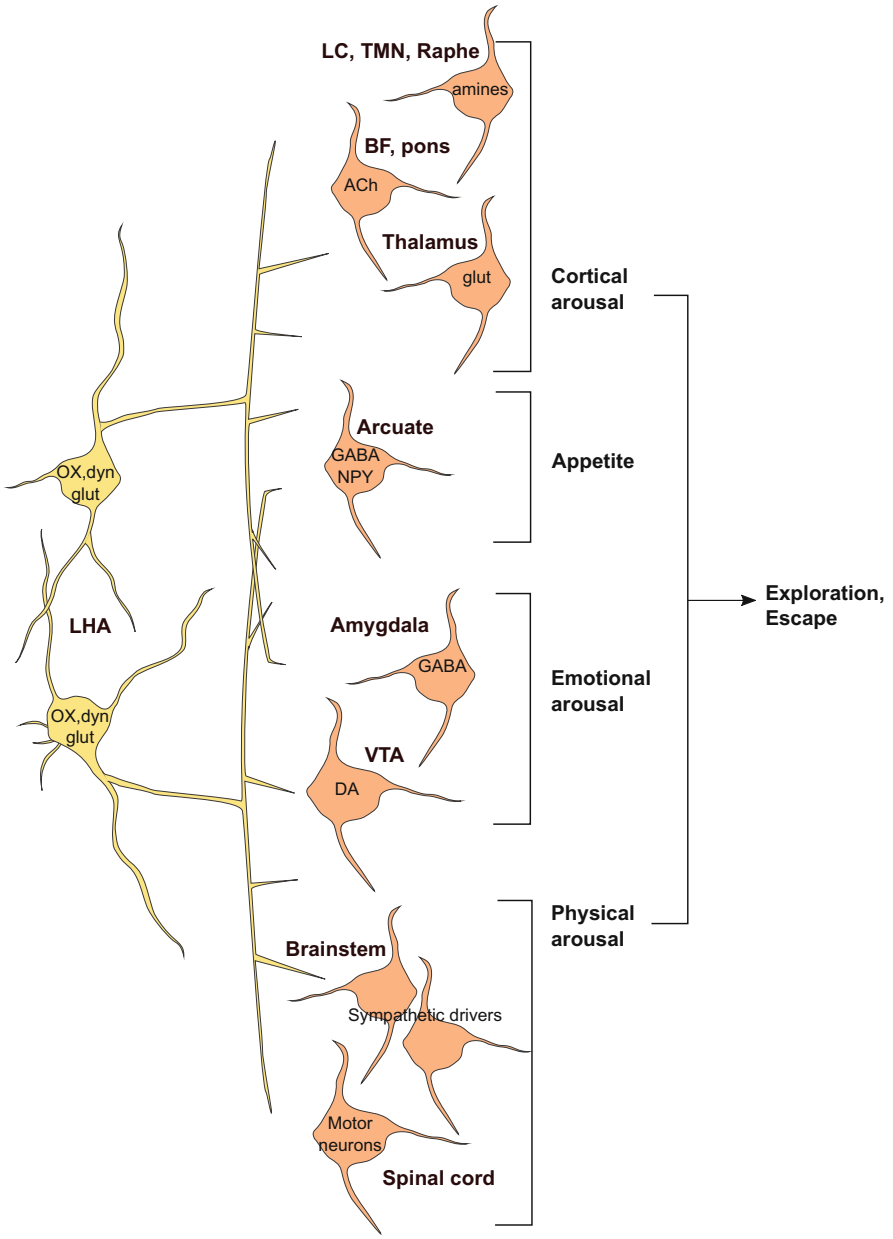

Fig. 2 Main anatomical targets for orexin/hypocretin control of cortical arousal, appetite, emotional arousal and physical arousal. Together, orexin/hypocretin control of these targets would facilitate exploratory or escape behaviour

Table 1 Postsynaptic effects of orexin/hypocretin neurons on sleep-/wakefulness-/arousal-related targets

Brain area	Nucleus	Targets	Receptor/ligand	Mechanism of action	References
Cerebral cortex	PFC		OR1/orexin	Excitation: presynaptic excitation of boutons from excitatory thalamocortical projecting neurons in layer V	[40–42]
	Visual cortex	Layer 6b pyramidal	OR2/orexin	Excitation: TTX insensitive	[43]
		Other layers		Increase synaptic inputs, improved spike-timing of layer VIa responses to rhomboid nucleus	[43–44]
Hippo-campus	DG		OR1/orexin	OR1 mediates induction of LTP in dentate gyrus of freely moving rats, Orexin A enhances LTP in dentate gyrus of urethane anaesthetized rats	[45–46]
	CA1		OR1/orexin A	Orexin A but not B induced LTP in Schaffer collateral – CA1 synapses in adult, while it induced LTD in juvenile mouse hippocampal sections	[47]
Cerebral nuclei	SI, MPO	Cholinergic neurons	OR2/orexin	Excitation: inhibition of constitutively active inward rectifier K+ current; potentiation of evoked EPSCs	[32–34]
			k-opioid/Dyn	Inhibition: activation of inward rectifier potassium current, inhibition of voltage sensitive calcium current, presynaptic depression of glutamatergic inputs	[35]
		Wake-active GABAergic	OR2/orexin	Excitation: unknown mechanism, potentiation of evoked EPSCs	[33]
			Dyn	No effect	[33]
		Sleep-active GABAergic	Orexin	No effect	[33]
			k-opioid/Dyn	Inhibition: subset of non-cholinergic neurons, suppression of evoked EPSCs	[33]
	Medial septum, DBB	Septo-hippo-campal cholinergic	OR2/orexin	Excitation: two ionic mechanisms with 80% overlap: inhibition of an inwardly rectifying K+ conductance and activation of an Na+/Ca2+ exchanger; Inhibition: increased GABA release (spike dependent, via activation of interneuron)	[36, 37]
		Septo-hippocam-pal GABAergic	OR2/orexin	Excitation, activation of sodium–calcium exchanger; Inhibition by increasing local, spike dependent GABA release	[37]
	NA	Accumbens shell	OR2/orexin	Excitation: increase of nonselective cationic conductance and decrease of K(+) conductance; Inhibition: decrease of NMDA currents and increased GABA currents in dissociated cells	[38, 39]
	BNST		OR1/orexins	Excitation: subset of neurons; Inhibition: OR1 dependent depression of excitatory transmission	[49, 50]

(continued)

Table 1 (continued)

Brain area	Nucleus	Targets	Receptor/ligand	Mechanism of action	References
Thalamus	MTN		OR2/orexin	Excitation: closure of potassium channels and activation of nonselective cation channels	[48, 51–53]
	IGL	NPY+ and – neurons	OR1/2/orexin	Excitation (58% of neurons): direct; Inhibition: increased inhibitory synaptic inputs	[54]
	VGN	NO+ and – neurons	OR1/2/orexin	Excitation	[55]
	CLN, PFN, MDT, TRN		OR1/2/orexin	Excitation of subset of neurons: inhibition of K+ current	[56]
	LP, LD P, LGN			No effect	[56]
Hypo-thalamus	TMN	Histamine	OR2/orexin	Excitation: activation of sodium–calcium exchanger, inhibition of G-protein coupled inward rectifier channel in cell culture; Inhibition: presynaptic increase GABA inputs	[27, 57, 59, 60, 61]
			AMPA/Glut	Excitation	[61]
	POA	GABAergic	Orexin	No effect	[32]
	SCN		OR1/2/orexins	Two subsets of cells showing either direct excitation or increase in GABAA mediated synaptic inputs during the day, and during the night direct inhibition via activation of leak-potassium conductance	[62]
	LHA	MCH	OR2/orexin	Excitation: direct inward current, inhibition of K+ current, increase excitatory synaptic inputs Inhibition: activate inhibitory GABAA-mediated synaptic currents	[76]
		Orexin	OR2/orexin	Excitation: increase of excitatory synaptic inputs	[73]
	Arc	GABA neurons	OR2/orexin	Excitation: activation of sodium–calcium exchanger	[78]
		NPY /AGRP neurons	OR1/2/orexin	Excitation: activation of inward current, phospholipase C dependent increase in intracellular calcium	[79, 77, 80]
		POMC neurons	OR2/orexin	Inhibition: decrease intracellular calcium, increase of inhibitory synaptic tone, decrease excitatory synaptic tone	[42, 79, 81]
	PVN	Type 1 and type 2 neurons	OR2/orexin B	Excitation: 50–80% of neurons	[82]
	VMH	Glucose-responsive neurons	OR1/orexins	Inhibition: decrease intracellular calcium	[79]

Cerebellum	CIN		OR2/orexins	Excitation: 67% of neurons	[105]
Midbrain	PPT		OR1/orexins	Excitation: decrease of potassium current, increase of nonselective cationic current	[65]
	DR	Serotonergic	OR2/orexins	Excitation: activation of sodium–potassium nonselective cation channels, protein kinase C dependent enhancement of Ca2+ transients mediated by L-type Ca2+ channels	[63, 68, 72, 73, 88]
				Inhibition: enhanced spontaneous IPSCs, suppression of evoked EPSCs via retrograde endocannabinoid release and stimulation of postynaptic orexin receptors (activation of PLC and DAG lipase signalling pathways)	[73, 74]
		GABA neurons	OR1/2/orexins	TTX insensitive excitation	[42, 73]
	VTA	Dopamine neurons	OR1/2/orexins	Excitation: increase of intracellular calcium via phosphatidylcholine-specific phospholipase C- and protein kinase C- signalling pathways to activate of L- and N-type Ca2+ channels	[42, 83, 84, 86]
			OR2/orexin B	Excitation: protein kinase C dependent postsynaptic potentiation of NMDA receptors; Inhibition: reduction of spike-timing induced LTP	[85]
		Non-dopamine neurons	Orexins	Excitation	[86]
	RMR		OR1/orexins		[42, 89]
	SN-Pars reticulata	GABAergic	OR2/orexins	Excitation: protein kinase A dependent	[104]
	SN-Pars compacta	Dopaminergic	Orexins	No effect	[104]
Pons	LDT	Cholinergic neurons	OR1/orexins	Excitation: activation of noisy cation channels, activation of a protein kinase C (PKC)-dependent enhancement of Ca2+ transients mediated by L-type Ca2+ channels, enhanced spontaneous EPSCs	[26, 64, 88]
		Non-cholinergic	OR1/2/orexins	Excitation: activation of inward current and membrane noise, enhanced spontaneous EPSCs but not IPSC	[26, 42, 64]
	LC	Noradrenergic	OR1/orexins	Excitation: depolarization and reduction in the slow component of the afterhyperpolarization (AHP)	[66]
		LC cells	OR1/orexins	Excitation: augmentation of nonselective cation conductance, inhibition of sustained potassium conductance, inhibition of G-protein coupled inward rectifier channel in cell culture	[59, 67–69, 101]
			Glut	Glutamate co-expression in orexin positive terminals	[71]
	TGMN	Motor neurons	OR1/orexins	Excitation: NMDA dependent excitation	[42, 102]

(continued)

Table 1 (continued)

Brain area	Nucleus	Targets	Receptor/ligand	Mechanism of action	References
Medulla	ReTN	Acid sensing neurons	OR1/orexins	Excitation	[89–91]
	Pre-Bötz		OR1/2/orexins	Increase in diaphragm electromyographic activity	[90, 92]
	NAc		OR1/2/orexins	Excitation: 90% of NTS cells through inhibition of sustained K+ current, activation of nonselective cationic conductance, hypocretin 2 increased mEPSCs in dorsal NTS neurons	[42, 93, 94]
	NAm	Cardiac vagal neurons	OR2/orexins	In postnatal day 5 rats: inhibition: enhanced GABAergic postsynaptic currents In postnatal day 20 and 30 rats: excitation: inhibition of GABAergic postsynaptic currents	[42, 106]
	RVMM		OR1/orexin A	Excitation: majority of neurons, possible indirect mechanisms; Inhibition: subset of neurons with irregular firing pattern that lack fast afterhyperpolarization, possible indirect action	[95]
	RVLM		OR1<2/orexinz A	Excitation: subset of neurons	[90, 96]
	LVN		OR1/2/orexins	Excitation: activation of sodium–calcium exchanger, block of inward rectifier K+ current	[99]
	IVN		OR1/2/orexins	Direct excitation	[103]
Spinal cord		Sympathetic preganglion neurons	OX1/2/orexins	Excitation, inhibition of potassium conductance via protein kinase A-dependent pathway	[97, 98]
		Phrenic/hypoglossal motor neurons	OR1/2/Orexins	Excitation, persistent in 1 μM tetrodotoxin	[89, 90, 99, 100]

Abbreviations: *Arc* arcuate nucleus, *BNST* bed nucleus stria terminalis, *CeM* central medial amygdala, *CIN* cerebellar interpositus nucleus, *CLN* centrolateral nucleus of the thalamus, *DBB* diagonal band of broca, *DR* dorsal raphe, *IGL* intergeniculate leaflet, *Dyn* dynorphin, *Glut* glutamate, *IVN* inferior vestibular nucleus, *LC* locus ceruleus, *LDT* laterodorsal tegmental nucleus, *LGN* lateral geniculate nuclei, *LHA* lateral hypothalamic area, *LP* lateral posterior geniculate nucleus, *LD* laterodorsal geniculate nucleus, *LVN* lateral vestibular nucleus, *MD* mediodorsal, *MPO* magnocellular preoptic nucleus, *MTN* midline thalamic nuclei, *NAc* nucleus accumbens, *NAm* nucleus ambiguus, *NTS* nucleus tractus solitarius, *OR1/2* orexin receptor 1/2, *P* posterior geniculate nuclei, *PFN* parafascicular nucleus, *PFC* prefrontal cortex, *PPT* pedunculo pontine tegmental nucleus, *Pre-Bötz* pre-Bötzinger complex, *PVN* paraventricular nucleus of the hypothalamus, *ReTN* retro trapezoid nucleus, *RVLM* rostral ventro-lateral medulla, *RVMM* rostral ventro-medial medulla, *RMR* rostral medullary raphe, *SCN* suprachiasmatic nucleus, *SI* substantia innominata, *SN* substantia nigra, *TGMN* trigeminal motor nucleus, *TMN* tuberomammillary nucleus, *TRN* reticular nucleus, *VGN* ventrolateral geniculate nucleus, *VMH* ventromedial hypothalamus, *VTA* ventral tegmental area

membrane conductance (g). This dependence is described by Ohm's law, V = I/g. The neuronal firing output depends on the membrane potential (it is increased by depolarization, [110, 111]). Therefore, conductance-increasing actions of orexin/hypocretin will not only depolarize and electrically excite the target neuron but also reduce the sensitivity of the neuron's firing to other inputs. This would effectively lock the neuron in a high-output state, which could be useful for overriding other inputs in times of danger. In turn, conductance-reducing actions of orexin/hypocretin will not only depolarize and excite the neuron but also increase its sensitivity to other inputs. This would enable the neuron to be readily modulated by other inputs (both stimulatory and inhibitory), thus allowing other inputs to either augment or cancel the orexin/hypocretin-induced excitation. Overall, these conductance-related actions of orexin/hypocretin can be viewed as not simply excitatory but also 'gain modulating'. The ability to modulate the input-output gain is an important feature of neural computation [112]. Therefore, the functional/behavioural implications of gain-modulating postsynaptic actions of orexin/hypocretin are an important question for future investigations.

Although the direct postsynaptic actions of orexin/hypocretin are usually excitatory, there are some exceptions. Per1 neurons of the hypothalamic suprachiasmatic nucleus that function as the brain's master circadian clock are inhibited by orexin/hypocretin via activation of leak-like K^+ channels, as well as presynaptically by increasing GABA release [62]. Signal transduction pathways linking orexin/hypocretin receptors to the inhibitory channels remain undefined. The inhibition of mouse Per1 neurons by orexin/hypocretin may enable the circadian clock signals to be overridden by arousal need signalled by orexin/hypocretin cells.

The above summary and classification of postsynaptic actions of orexin/hypocretin highlight the diversity and some general themes of brain-wide orexin/hypocretin signalling. However, such descriptions do not reveal critical components of orexin/hypocretin actions nor why orexins/hypocretins are vital for brain function stability. To achieve the latter insights, it is necessary to understand the functionally critical modules mediating orexin/hypocretin action and the overall control system architecture implemented by these modules. We address this in the next section, continuing to focus on wakefulness control.

3 Which Orexin/Hypocretin-Regulated Sites Are Critical for Wake Stability in the Normal Brain?

The diversity of orexin/hypocretin-excited neurons throughout the brain raises the question of the relative roles of different orexin targets in preventing the narcoleptic instability of wakefulness. An optimal way to deconstruct these natural roles would be to examine the effects on wakefulness stability of targeted, specific and reversible inactivation of orexin receptors in molecularly defined neurons in adult mice. Such technically demanding experiments have not yet been accomplished. The relevant

studies performed to date used other approaches, such as global receptor deletion followed by targeted receptor restoration [113, 114] or experimental stimulation of orexin/hypocretin cells concurrent with experimental silencing of specific downstream targets [25]. These approaches have caveats as far as the natural roles of orexin/hypocretin targets in wake stability are concerned. For example, given the feedback loops in wakefulness circuits (see below), the role of an orexin/hypocretin receptor site in wakefulness control when all other orexin/hypocretin sites are genetically deleted is not the same as its role in the natural brain. In turn, experimental stimulation of orexin/hypocretin cells does not reproduce their natural firing patterns, and the ability of orexin/hypocretin cells to stimulate awakening is not an assay of wakefulness stability. Nevertheless, the existing studies provide fundamental information about causal links between specific neurons and wakefulness, as well as proof-of-concept information relevant to narcolepsy treatment. Therefore, we briefly comment on some of them here (for more in-depth discussions of current literature on this topic, see [2, 115]).

Carter et al. examined the mechanism of orexin-mediated wakefulness by optogenetically stimulating orexin/hypocretin neurons while concurrently optogenetically silencing one of their downstream effectors, the orexin type 1 receptor expressing noradrenaline neurons of the locus coeruleus (LC) [25]. Note that this does not address the question of which orexin/hypocretin targets are critical for orexin/hypocretin-dependent wakefulness stability. They found that when the LC noradrenaline neurons were inactivated, stimulation of orexin/hypocretin neurons no longer produced awakening from sleep. This seminal finding establishes the noradrenaline neurons as critical generators for the orexin/hypocretin-dependent stimulation of wakefulness. However, it remains unclear how these generators are controlled in order to maintain stable wakefulness, especially since the orexin type 2 receptor (not the type 1 expressed by the LC noradrenaline neurons) is essential for the wake stability. In the next sections, we will propose a unifying framework that can reconcile the wake-generator function of the noradrenaline neurons with wake-controller functions of orexin type 2 receptor neurons.

Mochizuki et al. globally deleted orexin type 2 receptors in mice, producing a narcoleptic instability of wakefulness [113]. They then used a viral expression strategy to restore these receptors locally in the tuberomammillary hypothalamus, an area rich in histamine neurons that normally express high levels of orexin type 2 receptors. This local manipulation rescued the wakefulness instability (but interestingly, not sleep instability that also results from loss of orexin/hypocretin function). This suggests that the histamine neurons could be critical for wake-controllers that signal to wake-generators (such as noradrenaline cells) to adjust their signals properly.

Hasegawa et al. knocked out both types of orexin/hypocretin receptors in mice and then reintroduced both of them at specific brain sites by viral delivery under a non-specific promoter. They found that such receptor overexpression in the LC restored the normal duration and number of wakefulness episodes [114]. It is not clear whether this is the normal function of the orexin signals to the locus coeruleus or an outcome of overexpression of orexin receptors that are not normally there. In contrast, the dual orexin/hypocretin receptor overexpression in the tuberomammillary hypothalamus did not restore the normal duration and number of wakefulness

episodes. This shows that orexin/hypocretin signalling in the tuberomammillary hypothalamus is insufficient for normal wakefulness when all orexin receptors are missing from the locus coeruleus. Together with the data of Mochizuki et al., this can be interpreted to suggest that the tuberomammillary hypothalamus requires an orexin-sensitive downstream wakefulness generator in order to control wakefulness. For effective wakefulness control, orexin/hypocretin may need to alter the activity of both wakefulness regulators and generators in the brain, with different kinetics and via different receptors (see Fig. 3, discussed below).

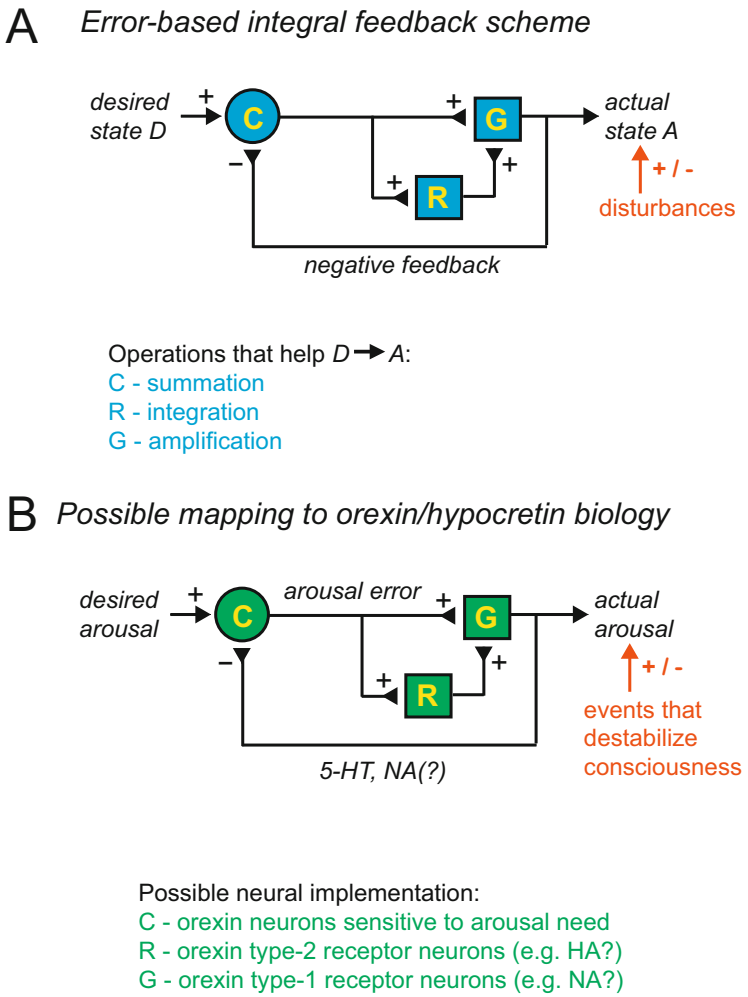

Fig. 3 Brain arousal systems as control modules in a feedback loop. (**a**) A generalized control system architecture (integral feedback loop) for tracking a desired set point (D) despite unpredictable disturbances. After [120]. (**b**) Possible implementation of A by a diversity of wake-promoting neurons in the brain (from more detail, see [18])

4 Mapping Orexin/Hypocretin Biology onto Control Operations

The above-discussed biological measurements define functionally important components of orexin/hypocretin systems and the general signs (plus or minus) for interactions between these components. This knowledge is fundamental, but alone is insufficient to account for control operations performed by orexin/hypocretin to achieve stable wakefulness. To clarify what we mean by control operations, a brief formal definition of tracking and stability is warranted. From a general evolutionary perspective, a highly desirable attribute of arousal control is set point tracking, i.e. the ability to adjust a set point to relevant inputs while rejecting disturbances. A good tracking system will follow salient inputs while resisting disturbances. Disturbance resistance is the ability to protect a set point from irrelevant disturbance, e.g. noise in brain/body internal signals, external events not requiring arousal responses, etc. A system capable of disturbance-resistant tracking can be considered 'robust yet flexible'. Note that this robust flexibility has to exist in the real world, i.e. where neither noise/disturbance nor important inputs are completely predictable, i.e. the control system has to be uncertainty proof. This need to deal with uncertainty imposes important requirements (and thus constraints) on system architecture (see below). For more detailed discussions of control principles as applied to orexin/hypocretin networks, see [18].

Can the actions of orexin/hypocretin be considered to implement such robust-yet-flexible arousal? We believe the answer is yes, since without orexin/hypocretin, arousal becomes both flexible and less robust. For example, when orexin/hypocretin is knocked out, mice cannot respond to potentially dangerous intrusions by properly increasing blood pressure [116], and they cannot properly adapt to a fall in their energy levels by increasing locomotion [8]. Thus, a vital flexibility of arousal is lost without orexin/hypocretin. In terms of robustness, it is well known that without orexin/hypocretin arousal can dip to inappropriately low levels (unconsciousness) upon disturbances such as laughter in humans or sight of delicious food in animals [2, 117, 118]. Without orexin/hypocretin, there is no appropriate tracking/adjustment of arousal state to internal and external state.

If orexin/hypocretin actions implement arousal tracking, the understanding of arousal control will increase by viewing orexin/hypocretin system from general perspectives of robust-yet-flexible control systems. Such systems generally must contain autocorrecting feedback loops, since neither the world nor system performance can be precisely predicted [119, 120]. As a minimum, such a feedback loop circuit must contain at least three operationally different elements in order to be robust yet flexible, which we here call a comparator, a controller and a generator (Fig. 3). This error-based feedback system is a canonical engineering strategy to track a set point despite noise [119, 120]. Note that although artificial, stimulation of each of these elements would increase the final output of the system (e.g. arousal). However, this 'test' does not mean that the elements are redundant: their functions and dynamics are fundamentally distinct.

These distinct functions of the three components in this autocorrecting system architecture (Fig. 3) have been discussed in detail in control engineering literature [119, 121] and recently in arousal control literature [18, 58]. A summary of the latter discussions is that orexin/hypocretin neurons display functional hallmarks of comparators, some orexin type 2 receptor neurons (histamine cells) exhibit functional signatures of controllers, while some orexin type 1 receptor neurons (noradrenaline cells) have operational features of generators (for detailed arguments, see [18]). A particularly curious feature of some orexin type 2 receptor cells is that they appear to transmit a signal resembling a temporal integral of orexin/hypocretin neuron activity (Fig. 4) [18, 58]. This integration may enable them to function as integral controllers, engineering signals that are theoretically necessary and sufficient for robust-and-flexible control mediated by orexin/hypocretin in general and its type 2 receptors in particular [18]. Therefore, from an operational perspective, integral feedback is an important candidate mechanism for how orexin/hypocretin maintains appropriate behavioural state.

5 Explanatory and Predictive Value of Viewing Orexin/Hypocretin Actions as Control Computations

What is the scientific value, for orexin/hypocretin biology and clinical applications, of control engineering theories such as those shown in Fig. 3?

First, an important corollary is that these control schemes assign a clear operational reason for the hitherto puzzling diversity of seemingly redundant wake-promoting neurons in the brain. If brain wakefulness control was operating via integral control or a related feedback scheme, there would have to be several operationally nonredundant neural types (comparators, controllers, generators) cooperating together. Note that these neurons are nonredundant in the sense of operations they perform, for example in this case, addition, integration and amplification, respectively. However, the comparator, controller and generator neurons are redundant in the sense that they all promote wakefulness if separately stimulated (this follows mathematically from the scheme in Fig. 3). The latter 'redundancy', however, is a by-product of experimental manipulation – it could be useful clinically for achieving rapid arousal, but it does not mean that the wakefulness control architecture is redundant. Such considerations are attractive because they settle a long-standing enigma in the field – the diversity of wake-promoting neurons – and emphasize that wakefulness control/stability/flexibility/robustness is a separate process from wakefulness stimulation, which allows the terms redundant and nonredundant to be applied more precisely. Thus, a control view adds clarity and explanatory power to understanding how the complex biology of arousal control relates to the need for the brain to operate flexibly yet robustly in uncertain environments.

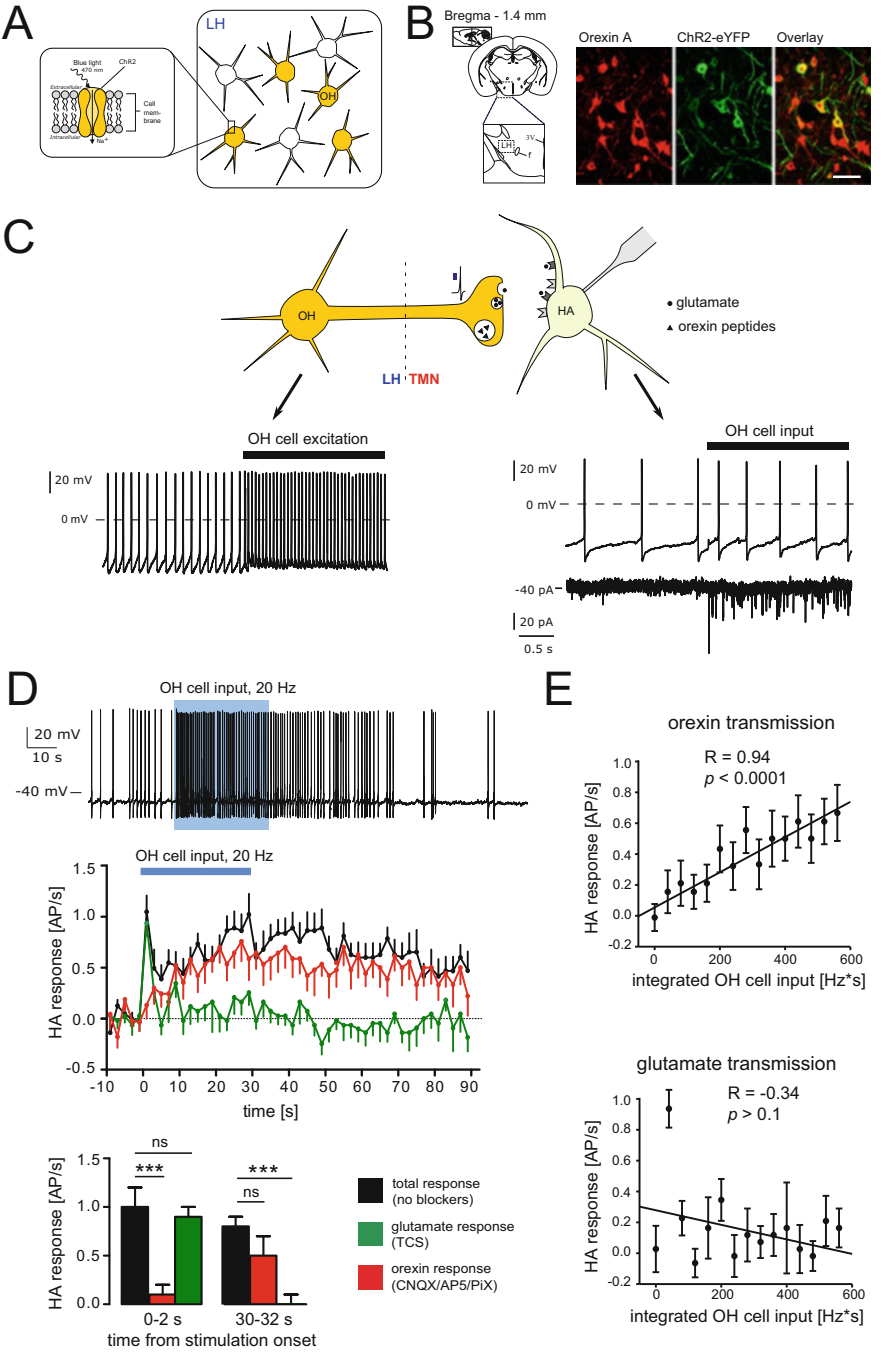

Fig. 4 Orexin/hypocretin and glutamate co-transmission enables fast and sustained control of histamine neurons. (**a**) Targeted expression of light-sensitive ion channel ChR2 enables selective control of excitatory membrane currents in orexin/hypocretin cells. (**b**) Cre-dependent expression of virally delivered ChR2-eYFP in orexin-cre mice allows selective expression in lateral

Second, control schemes such as those shown in Fig. 3 are formal mathematical theories that produce precise experimentally testable predictions about the temporal dynamics of distinct neurons. Such predictions of dynamics can be directly compared with real biological dynamics (measuring of temporal patterns of neuronal activity) and are thus essential for falsifying any theories of dynamic brain function. For example, when mathematically simulated, comparator, regulator and generator neurons produce different temporal signatures of activity in response to an input [18], and this can be experimentally tested. Furthermore, a mathematical control scheme such as that in Fig. 3 allows a proof-of-concept examination of whether a particular experimentally discovered neural operation is necessary to account for wakefulness stability. For example, if integration by orexin type 2 receptor cells is taken out of the model and replaced by a different computation (amplification), it can be mathematically demonstrated that both robustness and flexibility of the system are lost [18]. In contrast, more conventional (in biology) descriptions of arousal-implicated orexin/hypocretin biology that we gave earlier in this chapter (Fig. 1 and Table 1) are not mathematical theories and do not produce useful predictions and wakefulness dynamics. Thus, control engineering theories are useful for biology because they generate clearer predictions to guide experiments aimed at brain dynamics.

6 Overview, Omissions and Future Perspectives

In summary, we have reviewed postsynaptic orexin/hypocretin actions relating to arousal and presented a control theoretical view of these actions. This view theoretically accounts both for how orexin/hypocretin generates robust-yet-flexible arousal and for why multiple nonredundant types of arousal-promoting neurons exist in the brain.

We have omitted from this brief article many publications on the topic that have potential bearing on our interpretations. However, to the best of our knowledge, there are currently no experimental observations that invalidate our general argument. For example, under some behavioural manipulations, the actions of

Fig. 4 (continued) hypothalamic orexin-positive neurons. Data are from [61]. (**c**) *Left*: Light-activated action potential firing in ChR2-eYFP-expressing orexin/hypocretin cells recorded using whole-cell patch clamp. *Right*: Whole-cell recording of histamine neurons shows increased glutamate inputs (*bottom*) and action potential firing (*top*) upon light stimulation of adjacent orexin/hypocretin fibres. Adapted from [130], Fig. 2. (**d**) *Top*: Prolonged stimulation of orexin/hypocretin fibres for 30 s at 20 Hz produces fast and sustained increase in histamine cell firing. *Bottom*: Blockade of CNQX-sensitive glutamate currents blocks the fast rise in histamine firing, while a slow, long-lasting component remains. In contrary, orexin 2 receptor blockade (TCS) abolishes the slow component, leaving the fast component unaltered. Data from [58]. (**e**) Orexin-mediated increase in histamine firing integrates orexin/hypocretin activity (*top*), while glutamate-mediated increase in histamine firing does not (*bottom*). Data from [58]

noradrenaline on orexin/hypocretin cells in vitro have been reported to switch from inhibition to excitation [21, 122]. However, this does not mean that under such circumstances the negative feedback of arousal signals to orexin/hypocretin neurons does not exist; for example, it can be signalled by 5-HT. It would also be important to determine whether fast transmitters expressed by arousal-promoting neurons (e.g. GABA) affect orexin/hypocretin neurons. It has also been reported that some orexin/hypocretin neurons sense orexin/hypocretin themselves via orexin type 2 receptors [123], although this is controversial [19]. This opens up the possibility that orexin/hypocretin population may perform a dual function of regulators and comparators/error generators). Furthermore, we did not discuss the possible reasons for why different arousal-regulating neurons synthesize and use different transmitters, considering that activating or inhibiting downstream targets could be accomplished via glutamate, GABA and their many receptors. We speculate that transmitter diversity evolved to facilitate parallel signalling in a tight space [62], but it is beyond the scope of this review to discuss this in detail.

Overall, we feel that the results of our analysis provide some evidence that control-based logic is used by orexin/hypocretin system to dynamically control arousal. Furthermore, the integral feedback model provides a framework for studying wakefulness stability in both animal models and patients with narcolepsy. This model predicts transient responses of orexin/hypocretin neurons and more sustained responses of their downstream effector neurons to a change in arousal need [58]. These specific predictions can be tested in animal models with tools such as cell type-specific neural recordings, and this testing may aid the development of biomimetic medical robotics for patients with wakefulness disabilities.

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